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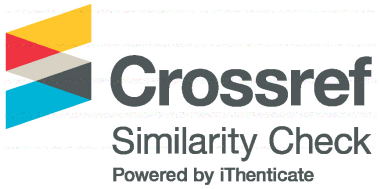

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Polyphenol component and antioxidant activity of *Thymus* spp.

Olena Vergun, Liudmyla Svydenko, Olga Grygorieva, Vladimíra Horčinová Sedláčková, Katarína Fatrcová Šramková, Eva Ivanišová, Ján Brindza

ABSTRACT

This scientific work was aimed to evaluate the antioxidant potential of aromatic plants of *Thymus* spp. in the East of Ukraine. These plants are known as medicinal and food around the world. All antioxidant parameters were investigated spectrophotometrically: total content of polyphenols (TPC), the total content of phenolic acids (TPAC), the total content of flavonoids (TFC), molybdenum reducing power of extracts (MRP), and antioxidant activity by DPPH method (DPPH). Investigation of ethanolic extracts demonstrated that TPC varied from 57.89 to 123.67 mg/g gallic acid equivalent (GAE) DW for *Th. pulegioides*, from 61.43 to 168.18 mg GAE/g for *Th. serpyllum*, and from 47.36 to 115.67 mg GAE/g for *Th. vulgaris*. TPAC ranged from 27.36 to 50.22 mg/g caffeic acid equivalent (CAE) DW for *Th. pulegioides*, from 28.58 to 59.62 mg CAE/g for *Th. serpyllum*, and from 22.95 to 53.82 mg CAE/g for *Th. vulgaris*. TFC was determined in a range from 29.88 to 61.23 mg/g quercetin equivalent (QE) DW for *Th. pulegioides*, from 36.0 to 82.43 mg QE/g for *Th. serpyllum*, and from 24.59 to 55.41 mg QE/g for *Th. vulgaris*. MRP was detected in the range of 94.65 – 204.76 mg/g Trolox equivalent (TE) DW for *Th. pulegioides*, 96.06 – 219.0 mg TE/g for *Th. serpyllum*, and 87.56 – 215.43 mg TE/g for *Th. vulgaris*. The antioxidant activity of extracts by the DPPH method was 6.34 – 9.23 mg TE/g for *Th. pulegioides*, 8.11 – 9.21 mg TE/g for *Th. serpyllum*, and 4.97 – 9.53 mg TE/g for *Th. vulgaris*. It was established that polyphenol accumulation depended on the growth stage and species. For all species was found a strong correlation between TPC and TFC (r=0.938, 0.908, and 0.854). Investigated *Thymus* spp. are a valuable source of antioxidants that can be used in pharmacological studies and the food industry.

Keywords: *Thymus*, polyphenol content, antioxidant activity

INTRODUCTION

Plant raw such as above-ground part **[1]**, leaves **[2]**, **[3]**, flowers **[4]**, pollen **[5]**, fruits **[6]**, **[7]**, **[8]**, **[9]**, roots **[10]** from different plant families, vegetables **[11]** and food products **[12]**, **[13]** is one of the most valuable sources of biologically active compounds with high antioxidant activity. The Lamiaceae family includes numerous species used in cosmetics, perfumery, food, and pharmaceutical industries worldwide. It is still a popular group of plants with increasing interest among aromatic cultures **[14]**. They have been used in traditional and non-traditional medicine to treat different diseases **[15]**. Among *Lamiaceae* representatives, *Thymus* spp. known for its therapeutic properties from ancient times, is a rich source of biologically active compounds, among which phenolic such as rosmarinic, salvianolic acids, luteolin glycosides with numerous biological activities **[16]**, **[17]**. Also, the antibacterial and cytotoxic properties of *Thymus* representatives are known **[18]**, **[19]**, **[20]**. One of the most widespread species of *Thymus* is *Th. vulgaris* L. **[21]**. This species is also known as thyme and belongs, like others, to aromatic and medicinal plants from the Mediterranean. Existing varieties of thymes are based on various chemotypes. The most studied raw material of *Th. vulgaris* last time is an essential oil component such as *p*-cymene, γ-terpinene, linalool, thymol, carvacrol that depends on plant origin or genotype **[22]**, **[23]**. *Th. serpyllum* is a perennial plant that has been extensively used in official and folk medicine. Essential oil from this plant contains (E)-nerolidol, caryophyllene oxide, myrcene, (E)-β-caryophyllene, germacrene D **[24]**. *Th. vulgaris* raw possesses antimicrobial **[25]**, medicinal, astringent, anthelmintic, disinfectant, tonic capacities **[26]**. The herb of this species is used to prepare natural remedies,

syrups, teas, etc. It is known antiseptic, ethnoveterinary usage of this plant, as remedies from bronchitis, bronchial catarrh, whooping cough, etc. Plant raw material of *Th. serpyllum* possesses antioxidant, antimicrobial, antitumor, cytotoxic activities **[27]**. The study of six *Thymus* species showed that flavonoid content was 0.15 – 0.42%, tannins 0.77 – 1.59%, and procyanidins 0.21 – 0.70% **[28]**.

The essential oil composition of *Th. capitatus* is carvacrol, p-cymene, γ-terpinene, Linalyl acetate, 1,8-cineole, β-myrcene, terpinene-4-ol, and α-terpinene. In total, the essential oil of this species was identified with 27 compounds **[29]**. According to Borugă et al. **[30]**, the major components of *Th. vulgaris* essential oil is *p*-cymene, γ-terpinene, and thymol. Thymol and carvacrol possess solid antiseptic activity. As reported by Verma et al. **[31**], the growth period significantly affects the quantity and quality of thyme oil composition. *Th. vulgaris* and its oil are a good source of vitamin A and ascorbic acid **[21]**. Essential oil from the thyme possesses antimicrobial **[30]**, **[32]**, antioxidant **[33]**, **[34]**, anti-inflammatory activities **[21]**. The highest oil components, such as thymol and carvacrol, were obtained under mineral nutrition **[35]**. *Th. pulegioides* is a less critical commercial species, such as *Th. vulgaris*, but antioxidant and antibacterial activities characterize its plant raw. It was found valuable essential oil components such as geranial, neral, geraniol, and linalool **[36]**. Aqueous and hydro-ethanolic extracts of *Th. pulegioides* demonstrated antioxidant and antiproliferative activities **[37]**.

However, numerous studies concerning the antioxidant capacity of *Thymus* spp. describe the effect of several factors on polyphenol composition in above-ground parts, first of all, growth conditions. This work aimed to assess the antioxidant capacity of three *Thymus* species grown in the East of Ukraine during vegetation and evaluate the peculiarities of polyphenol accumulation.

The specific aim was to evaluate polyphenol compounds accumulation in plant raw material of *Thymus* spp. during vegetation.

Scientific Hypothesis

Antioxidant properties of plant raw material of *Thymus* spp. during the vegetation from the East of Ukraine will depend on the plant part, the species, and the growth stage.

MATERIAL AND METHODOLOGY

Samples

The plant material of *Thymus pulegioides* L., *Thymus serpyllum* L., and *Thymus vulgaris* L. (Figure 1) took from a collection of aromatic and medicinal plants of Experimental Facility "Novokakhovska" of Rice Research Institute of Ukrainian Academy of Agrarian Sciences (EFN of RRI) (v. Plodove; 46°45′16.2″N 33°20′55.1″E) in 2018 – 2019. Plant raw material (leaves, buds, inflorescences, fruits, herb, i.e., all above-ground parts were prepared at the budding stage, flowering, and fruitage).

In this study, accumulation and distribution of polyphenols and antioxidant activity of ethanol extracts of *Th. pulegioides*, *Th. serpyllum* and *Th. vulgaris* from Ukraine during vegetation were studied. Plant samples were dried at 45 °C for three days. All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Resources, Department of Plant Storage and Processing.

Chemicals

All chemicals were of analytical grade quality and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Animals and Biological Material

The animal and biological materials weren't used in this research.

Instruments

The centrifuge Rotofix 32 A (Hettich, Germany), spectrophotometer Jenway, 6405 UV/Vis (England), vortex shaker (IKA VORTEX 3, Germany) were used in this research.

Laboratory Methods

Total polyphenol content (TPC) of extracts were measured by the spectrophotometric method with the Folin-Ciocalteu reagent **[38]**; the total content of phenolic acids (TPAC) was determined using Farmakopea Polska (1999) **[39]**; procedure of total flavonoid content (TFC) was conducted by a spectrophotometric method based on the formation of aluminum-flavonoid complex **[40]**, **[41]**; the reducing power of extracts (molybdenum reducing antioxidant power, MRP) was determined by the phosphomolybdenum method **[42]**; the antioxidant activity of samples was conducted by DPPH method **[43]**.

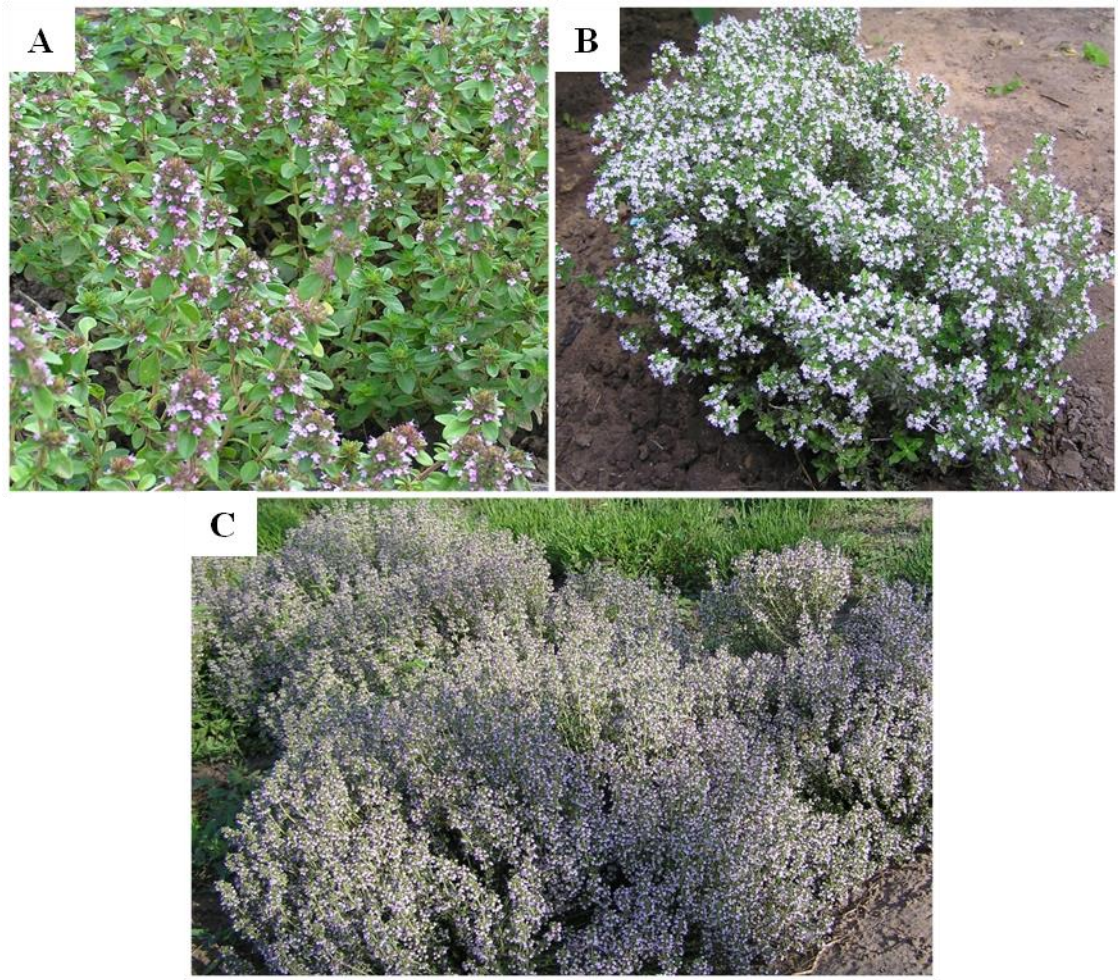

Figure 1 Plants of *Thymus pulegioides* L. (A), *Th. serpyllum* L. (B), and *Th. vulgaris* L. at the flowering period.

Description of the Experiment

Sample preparation: 0.2 g of dried plant raw material was extracted with 20 ml of 80% ethanol for 2 hours. After centrifugation at 4000 g for 10 min, the supernatant was used for the next measurements: antioxidant activity, polyphenols, and flavonoids. All data were expressed in mg of standard compound per gram of dry weight (DW).

Number of samples analyzed: we analyzed 27 samples.

Number of repeated analyses: all biochemical procedures were conducted in triplicate.

Number of experiment replication: 2 times.

Design of the experiment: Total polyphenol content of extracts was measured by the following procedure: 0.1 ml of each sample extract was mixed with 0.1 ml of the Folin-Ciocalteu, 1 ml of 20% (w/v) sodium carbonate, and 8.8 ml of distilled water. After 30 min. in darkness, the absorbance at 700 nm was measured using the spectrophotometer. Gallic acid (25 – 250 mg/l; R^2 = 0.996) was used as the standard, and the results were expressed in mg/g gallic acid equivalents. For phenolic acid content, 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v), and 0.5 ml of distilled water. Absorbance at 490 nm was measured using the spectrophotometer. Caffeic acid 1 – 200 mg/l (R^2 = 0.999) was used as a standard. The results were expressed in mg/g caffeic acid equivalents (CAE). The procedure of total flavonoid content determination was conducted the following way: 0.5 ml of sample extract was mixed with 0.1 ml of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 ml of 1 M sodium acetate, and 4.3 ml of distilled water. After 30 min. in darkness, the absorbance at 415 nm was measured using the spectrophotometer. Quercetin 0.01 – 0.5 mg/l (R^2 = 0.997) was used as the standard and the results were expressed in mg/g quercetin equivalents (QE). The reducing power of extracts was determined by the phosphomolybdenum method with slight modifications: the mixture of 1 ml of sample, 2.8 ml of monopotassium phosphate (0.1 M), 6 ml of sulfuric acid (1 M), 0.4 ml of ammonium heptamolybdate (0.1 M), and 0.8 ml of distilled water was incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer. Trolox 10 – 1000 mg/l (R^2 = 0.998) was used as the standard, and the results were expressed in mg/g TE. The antioxidant activity of samples was measured

using 2,2-diphenyl-1-picrylhydrazyl (DPPH): the ethanol extract (1 ml) was mixed with 4 ml of DPPH solution (0.025 g of radical in 100 ml of ethanol). The absorbance of the sample extract was determined using the spectrophotometer at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 10 – 100 mg/l (R^2 = 0.983) was used as a standard and the results were expressed in mg/g Trolox equivalents (TE).

Statistical Analysis

Significant differences (p<0.05) between means were evaluated by ANOVA and the Tukey–Kramer test. Correlation coefficients were calculated using Statistica version 13.0 software (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

In recent years, the interest in natural antioxidants has increased concerning phenolic compounds, including flavonoids and phenolic acids. Natural antioxidants are present in different plant raw materials **[44]**. Herbs from Lamiaceae exhibited the strong antioxidant potential and high content of polyphenol compounds in dry form as well as in fresh **[45]**.

The total content of polyphenol compounds (TPC)

The total content of polyphenol compounds in this study was from 57.89 to 123.67 mg GAE/g DW for *Th. pulegioides*, from 61.43 to 168.18 mg GAE/g DW for *Th. serpyllum*, and from 47.36 to 115.67 mg GAE/g DW for *Th. vulgaris* (Figure 2). As shown from Figure 2, the maximal content of total polyphenol compounds is determined in the buds for all species.

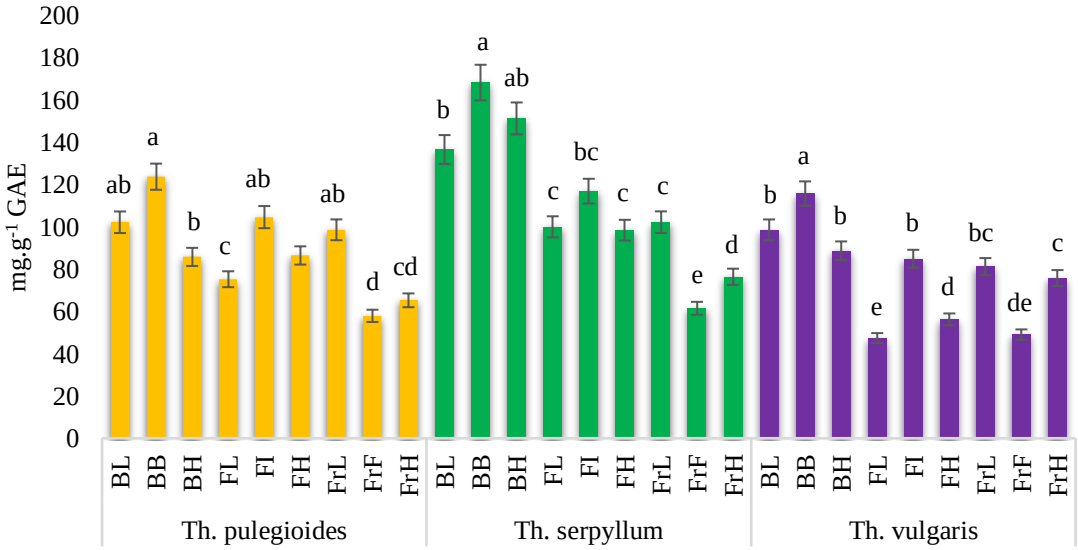

Figure 2 Content of polyphenol compounds in ethanol extracts of *Thymus* spp. depending on the stage of growth (Means in columns followed by different letters are different at p<0.05. Each value represents the mean of three independent experiments (±SD)).

Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; GAE – gallic acid equivalent.

As reported by Armatu et al. **[46]**, in methanol extracts of *Th. vulgaris* determined 32 mg GAE/g of TPC. The total polyphenol content of another species *Thymus capitatus* was 175.53 mg GAE/g **[29]**. Maximal value of TPC of Tunisian plants of *Th. capitatus* was 18.40 mg GAE/g DW in methanol extracts **[47]**. Plant ethanol extracts of other species *Th. riatarum* demonstrated 135.8 mg GAE/g DW of TPC **[48]**. According to Köksal et al. **[49]**, TPC of ethanol extracts of *Th. vulgaris* was 158 µg GAE/mg DW. However, some investigations demonstrated low content of TPC, namely 7.30 mg GAE/g DW of ethanol extracts **[50]**. Taghouti et al. **[37]** reported that *in vitro* hydro-ethanolic post-blooming extracts of *Th. pulegioides* demonstrated 155.38 mg GAE/g DW of TPC. Hydro-ethanolic extraction of freeze-dried raw showed TPC as 70.31 mg/g caffeic acid equivalent DW. Ethanol extracts of *Th. serpyllum* and *Th. vulgaris* from Slovakia showed 41.13 and 52.1 mg GAE/g DW of TPC, respectively **[51]**. The content of polyphenols and flavonoids in extracts of different *Thymus* spp. depended on the solvent. Methanol and acetone extracts contained higher polyphenol content than ethanol **[52]**. The methanol extracts of *Th. serpyllum* and *Th. vulgaris*, raw of which were shade dried at 25 °C, had 22.14 and 35.73 mg/g GAE of TPC, respectively **[53]**. Also, as reported in the study with *Th. vulgaris* and other

Lamiaceae herbs, the content of phenolic compounds and flavonoids depended on harvest time, so the best results were obtained at the first harvesting **[54]**.

As a result, it should be noted that maximal TPC accumulated at the budding stage for *Th. serpyllum* and *Th. vulgaris* (all parts of the plant), while for *Th. pulegioides* TPC accumulated unevenly.

The total content of phenolic acids (TCPA)

As reported in some studies, among phenolic compounds of *Thymus* spp. the most widespread are flavonoids and phenolic acids. The last group of polyphenols is represented by hydroxycinnamic or hydroxybenzoic structure **[55]**.

The total content of phenolic acids was in a range from 27.36 to 50.22 mg CAE/g DW for *Th. pulegioides*, from 28.58 to 59.62 mg CAE/g DW for *Th. serpyllum*, and from 22.95 to 53.82 mg CAE/g DW (Figure 3). TPAC for both investigated species was the highest in the bud's extracts (at the budding) and the lowest in the fruit's extracts (at the fruitage). This parameter decreased during vegetation in the above-ground part (herb) of *Th. serpyllum* and decreased from budding to flowering stage and increased from flowering to fruitage for *Th. vulgaris* plant extracts.

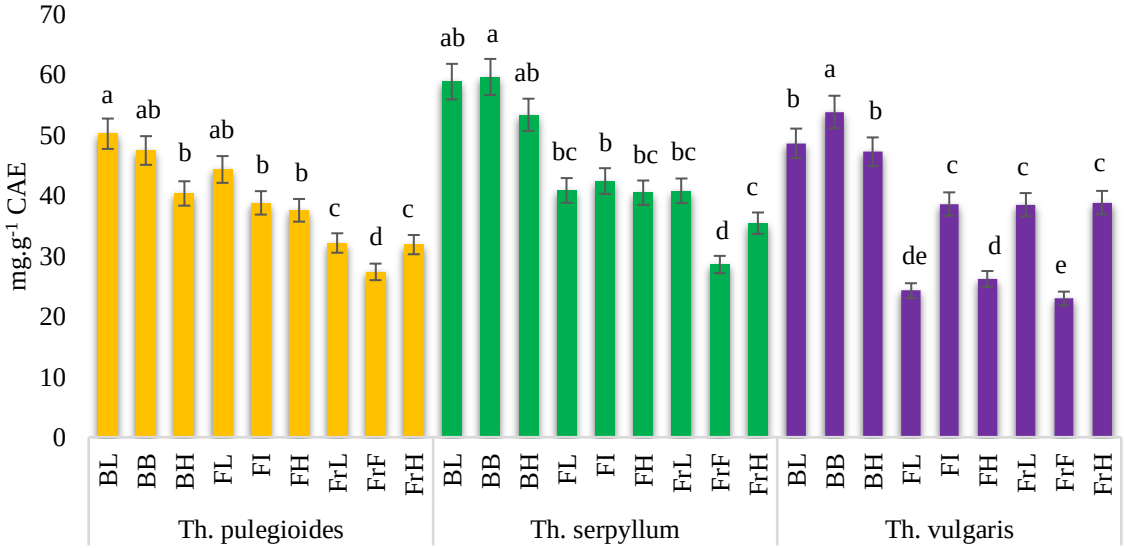

Figure 3 Content of phenolic acids in ethanol extracts of *Thymus* spp. depending on the stage of growth (Means in columns followed by different letters are different at $p<0.05$. Each value represents the mean of three independent experiments (±SD)).

Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; CAE – caffeic acid equivalent.

Taghouti et al. **[37]** reported that in vitro hydro-ethanolic extracts of *Th. pulegioides* exhibited 56.11 mg CAE/g DW of TPAC. Hydro-ethanolic extraction of freeze-dried post-blooming raw *Th. pulegioides* showed TPAC as 34.09 mg CAE/g DW. TPAC was the highest for *Th. serpyllum* and *Th. vulgaris* at the budding stage as well as TPC. Extracts of *Th. pulegioides* demonstrated maximal values at the budding besides herb extracts. In extracts of Slovakian samples of *Th. serpyllum* and *Th. vulgaris* from Slovakia showed 19.60 and 19.31 mg CAE/g DW, respectively, which was two times less than obtained result in this study for *Th. serpyllum* **[51]**.

The total content of flavonoids (TFC)

Flavonoids are secondary plant products and are an important component of the human diet due to their functions. From the polyphenol compounds, flavonoids received the most attention due to their biological activities, distribution in natural products **[56]**. The most important classes of flavonoids are flavones, flavanones, isoflavones, flavonols, anthocyanidins, etc. **[44]**.

The total content of flavonoids was in a range from 29.88 to 61.23 mg QE/g DW for *Th. pulegioides*, from 36.0 to 82.43 mg QE/g DW for *Th. serpyllum*, and from 24.59 to 55.41 mg QE/g DW (Figure 4).

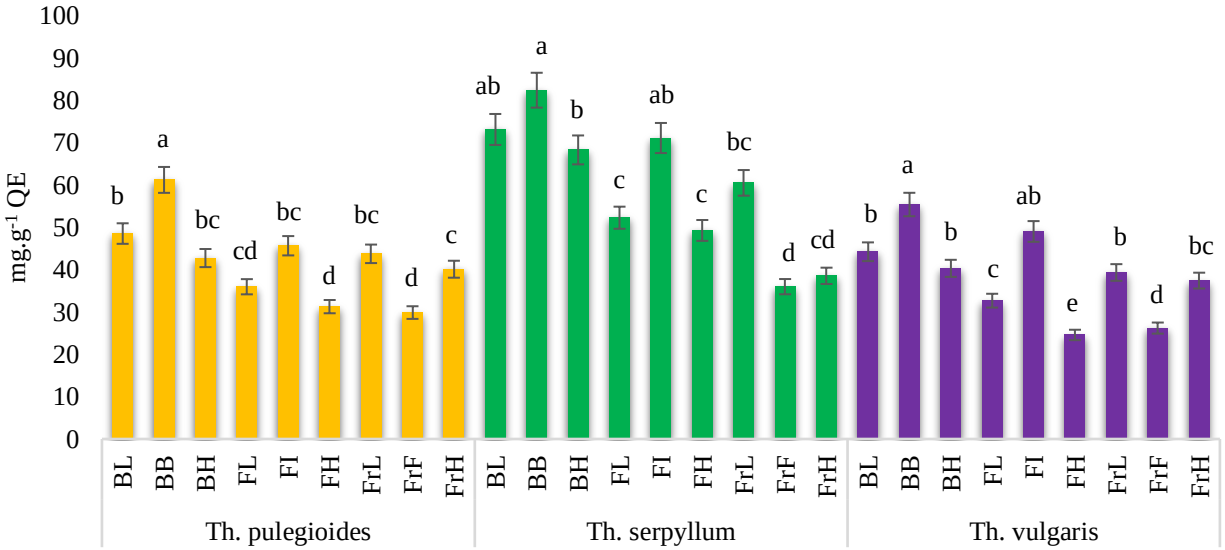

Figure 4 Content of flavonoids in ethanol extracts of *Thymus* spp. depending on the stage of growth (Means in columns followed by different letters are different at $p<0.05$. Each value represents the mean of three independent experiments (±SD)).
Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; QE – quercetin equivalent.

As reported Köksal et al. **[49]**, in the ethanol extracts of *Th. vulgaris* was determined 36 μg QE/mg DW of TCF. Taghouti et al. **[37]** reported that *in vitro* hydro-ethanolic post-blooming extracts of *Th. pulegioides* showed TFC as 61.75 mg/g catechin equivalent DW. After hydro-ethanol extraction of freeze-dried plant, raw TFC of this species was 36.22 mg/g catechin equivalent DW. According to Aouam et al. **[48]**, in ethanol extracts of *Th. riatarum* was detected 120.6 mg RE/g DW (rutin equivalent) of TFC. Also, Slovakian samples of *Th. serpyllum* showed two times less TFC than in our study **[51]**. Evidently, TPC, TFC, and TPAC of *Th. serpyllum* have depended on conditions of growth. Methanol extracts of *Th. serpyllum* and *Th. vulgaris* had TFC 4.36 and 8.70 mg QE/g, respectively **[53]**.

Considering peculiarities of TFC accumulation, it's seen that flavonoids distributed during vegetation unevenly, but for all species were characterized the highest value in the buds. The lowest TFC was found in fruit extracts of *Th. pulegioides*, *Th. serpyllum* and in herb extracts of *Th. vulgaris*.

The molybdenum reducing power of extracts (MRP)

Exist numerous assays to determine the antioxidant capacity of plant raw material, among which DPPH scavenging activity and the reducing power of extracts **[44]**. The phosphomolybdenum method of antioxidant activity determination is based on the reduction of molybdate ions **[57]**.

The molybdenum reducing power of ethanol extracts was from 94.65 to 204.76 mg TE/g DW for *Th. pulegioides*, from 96.06 to 219.0 mg TE/g DW for *Th. serpyllum*, and from 87.56 to 215.43 mg TE/g DW for *Th. vulgaris* (Figure 5). The study of MRP didn't show definite patterns in the manifestation of this activity during vegetation, but for *Th. vulgaris* extracts identified maximal values in the buds (at the budding), inflorescences (at the flowering), and herb (at the fruitage). *Th. serpyllum* extracts demonstrated the highest MRP in the leaves (at the budding and fruitage) and herb (at the flowering).

According to Armatu et al. **[46]**, methanol extracts of *Th. vulgaris* demonstrated significant antioxidant activity by phosphomolybdenum method at 5 mg/mL (ascorbic acid equivalent). Another *Lamiaceae* species *Scutellaria baicalensis* Georgi from the same region, demonstrated MRP as 260.24 mg TE/g DW **[58]**. According to Mňahončaková et al. **[51]**, MRP of ethanol extracts of Slovakian samples of *Th. serpyllum* and *Th. vulgaris* was 125.44 and 132.49 mg TE/g DW, respectively that differed from our result for both species.

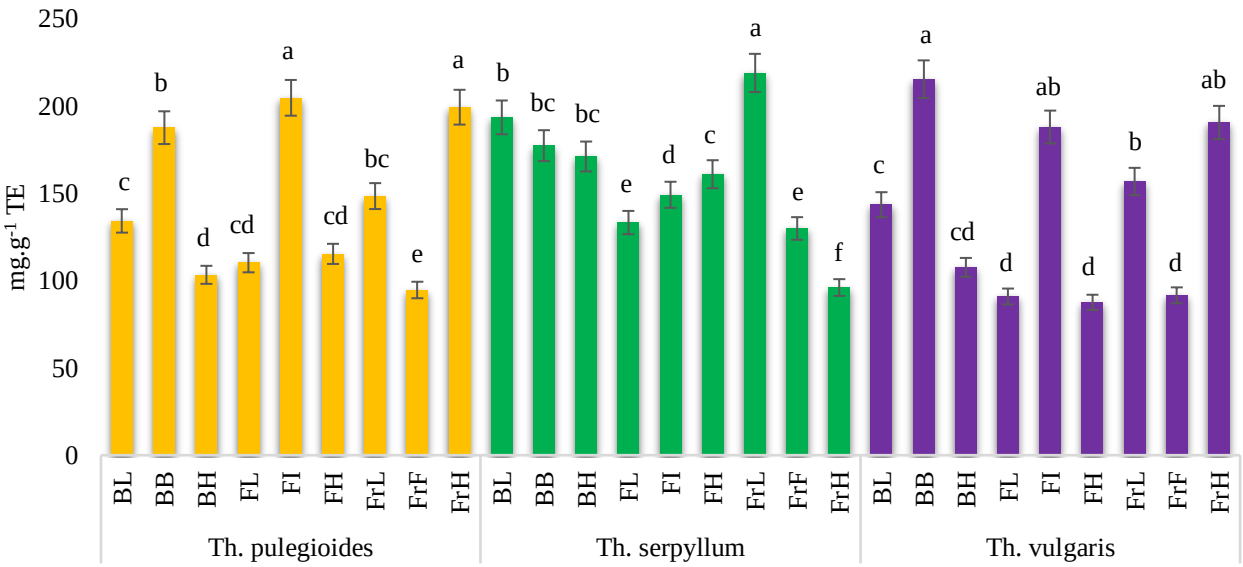

Figure 5 Molybdenum reducing power of ethanol extracts of *Thymus* spp. Depending on the stage of growth (The means in columns followed by different letters are different at $p<0.05$. Each value represents the mean of three independent experiments (±SD)).
Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; TE – Trolox equivalent.

The antioxidant activity of extracts by the DPPH method (DPPH)

One of the most widespread methods of antioxidant activity determination is the DPPH method, characterized by simple, accurate, and based on electron transfer that manifests on the discoloration of radical solution **[59]**. There are results not only about an investigation of antioxidant activity of medicinal plant extracts including Lamiaceae representatives such as *Thymus* spp. **[60]**, **[61]**, **[62]** but also products (juices) with herb addition **[63]**.

The antioxidant activity of ethanol extracts by the DPPH method was from 6.34 to 9.23 mg TE/g DW for *Th. pulegioides*, from 8.11 to 9.21 mg TE/g DW for *Th. serpyllum*, and from 4.97 to 9.53 mg TE/g DW for *Th. vulgaris* (Figure 6). These results demonstrated that maximal values of antioxidant activity by DPPH were found in the buds extracts of *Th. pulegioides*, *Th. vulgaris* and in leaf extracts of *Th. serpyllum*.

According to Chizzola et al. **[64]**, leaves of *Th. vulgaris* showed in 60 and 96% ethanol antioxidant activity by DPPH method 55.9 and 26.5 mg TE/g DW. *Scutellaria baicalensis* from the same region had antioxidant activity by DPPH method 8.83 mg TE/g DW **[58]**. Results obtained by Mňahončaková et al. **[51]** for *Th. serpyllum* and *Th. vulgaris* didn't differ from our concerning DPPH antioxidant activity and was 8.25 and 8.41 mg TE/g DW. The study of *Th. vulgaris* demonstrated that antioxidant activity was less in aqueous extracts than in ethanol extracts of leaves **[65]**. The antioxidant activity of *Th. serpyllum* extracts depended on extraction parameters and chosen assay of determination **[66]**, **[67]**.

The Pearson's correlation coefficients between investigated parameters of antioxidant activity are represented in Table 1. In the extracts of *Th. pulegioides* was found a very strong correlation between TPAC and antioxidant activity by DPPH ($r=0.840$, $p<0.01$), TPC and TFC ($r=0.854$, $p<0.01$).

We found a very strong positive correlation between TPC and the following parameters: TPAC ($r=0.963$, $p<0.01$) and TFC ($r=0.938$, $p<0.01$) for *Th. serpyllum* extracts. TPAC correlated with TFC also strong ($r=0.902$, $p<0.01$). A moderate correlation was found between molybdenum reducing the power of extract and polyphenol compounds, flavonoids, while between antioxidant activity by the DPPH method and all polyphenol compounds a negative correlation. A very strong correlation we determined between TPC and the following parameters: TPAC ($r=0.980$, $p<0.01$), TFC ($r=0.908$, $p<0.01$) and with MRP was found strong correlation ($r=0.758$, $p<0.05$) for *Th. vulgaris* extracts.

Also, a very strong correlation we found between TPAC and TFC ($r=0.865$, $p<0.01$), TFC and MRP ($r=0.834$, $p<0.01$). Compared with *Th. serpyllum* extracts, antioxidant activity by DPPH method of *Th. vulgaris* had a strong ($r=0.647$, $r=0.707$, $p<0.05$) or moderate ($r=0.521$, $p<0.05$) correlation between tested parameters. For all species found a weak correlation between MRP and antioxidant activity by the DPPH method. Antioxidant activity of *Th. serpyllum* by DPPH had a negative correlation with all polyphenol compounds.

Chizzola et al. **[64]** found a strong correlation between antioxidant activity by the DPPH method and TPC (r=0.946). Adámková et al. **[45]** determined a stronger correlation between antioxidant activity by DPPH and polyphenol compounds in dried herbs than in fresh. The reducing activity of extracts of *Th. sibthorpii* correlated with total polyphenols, as reported by Kontogiorgis et al. **[68]**.

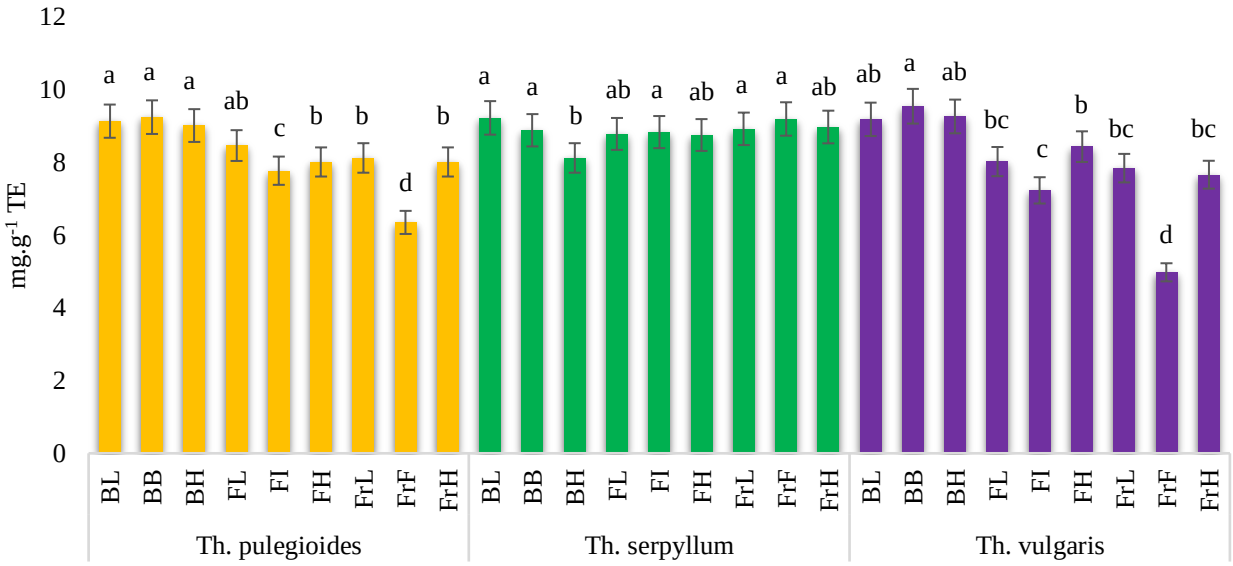

Figure 6 Antioxidant activity of ethanol extracts of *Thymus* spp. by DPPH method depending on the stage of growth (Means in columns followed by different letters are different at p<0.05. Each value represents the mean of three independent experiments (±SD)).
Note: BL – leaves, budding; BB – buds, budding; BH – herb (above-ground part), budding; FL – leaves, flowering; FI – inflorescences, flowering; FH – herb, flowering; FrL – leaves, fruitage; FrF – fruits, fruitage; FrH – fruitage, herb; TE – Trolox equivalent.

Table 1 The correlation coefficients between the different parameters of antioxidant activity of investigated plants of *Thymus* spp.

Parameters	Polyphenols	Phenolic acids	Flavonoids	MRP
		Thymus pulegioides		
Phenolic acids	0.656*	1	-	-
Flavonoids	0.854**	0.622*	1	-
MRP	0.456*	0.077*	0.601*	1
DPPH	0.657*	0.840**	0.707*	0.176*
		Thymus serpyllum		
Phenolic acids	0.963**	1	-	-
Flavonoids	0.938**	0.902**	1	-
MRP	0.561*	0.575*	0.657*	1
DPPH	-0.362*	-0.267*	-0.135*	0.375*
		Thymus vulgaris		
Phenolic acids	0.980**	1	-	-
Flavonoids	0.908**	0.865**	1	-
MRP	0.758*	0.688*	0.834**	1
DPPH	0.657*	0.707*	0.521*	0.266*

Note: MRP – molybdenum reducing power of extracts; DPPH – antioxidant activity by DPPH method; ** – correlation is significant at the level of 0.01; * – correlation is significant at the level of 0.05.

CONCLUSION

Plant raw material from *Thymus* spp. (*Th. pulegioides*, *Th. serpyllum*, and *Th. vulgaris*) from the Eastern region of Ukraine is a promising source of antioxidants, which can be used for their health properties in food products. The content of polyphenol compounds was statistically significant for all investigated species. It should be noted that an accumulation of polyphenol compounds, phenolic acids, and flavonoids was uneven and depended on the stage of growth, part of a plant, and species. The highest content of polyphenols (168.18 mg GAE/g), phenolic acids (59.62 mg CAE/g), and flavonoids (82.43 mg QE/g) was determined for *Th. serpyllum* extracts at the budding stage in buds. Molybdenum reducing power was maximal in ethanol extracts of *T. serpyllum* at the fruitage in leaves (219.0 mg TE/g) and antioxidant activity by DPPH in the buds of *Th. vulgaris* (9.53 mg TE/g). The minimal values of all investigated parameters were detected for *Th. vulgaris* extracts. So, the least polyphenol content (47.36 mg GAE/g) found in the leaf extracts at the flowering period, the least total phenolic acid content (22.95 mg CAE/g) found in the fruit extracts, the least content of flavonoids (24.59 mg QE/g) detected in the herb extracts of this species at the flowering period. Also, the fruit extracts and herb extracts (flowering) demonstrated the lowest values of antioxidant activity by the DPPH method (4.97 mg TE/g) and molybdenum reducing power (87.56 mg TE/g), respectively. A very strong and strong correlation was found between the accumulation of different polyphenol compounds for all species. A strong correlation between flavonoids and molybdenum reducing power of the extract was found for all investigation species (r=0.834 (*Th. vulgaris*), r=0.657 (*Th. serpyllum*), r= 0.601 (*Th. pulegioides*)). Obtained results can be used in further deep biochemical, pharmacological studies and selective work in nutritional, food, and horticultural practice.

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

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The intensification of dehydration process of pectin-containing raw materials

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ABSTRACT

The process of intensifying dehydration of pectin-containing raw materials by using centrifugation with simultaneous application of low-frequency oscillations to the working container creates an electroosmotic effect in unilateral diffusion to improve the filtration process. It is established that to reduce the technological resistance in the presented methods; it is necessary to create a fluidized bed of products due to the oscillating motion of the working capacity. An experimental vibration unit has been developed to determine the rational parameters of the vibrocentric moisture removal process using the electroosmotic effect. It is proved that the complex of the designed equipment provides consecutive carrying out of three-stage vibration filtration-convective drying of high-moisture production by an alternation of action of a stream of the heat carrier, an electromagnetic field, low-frequency fluctuations. According to the research results, the dependences of the kinetics of the moisture diffusion process on the electric field strength are obtained; frequency of electric current and duty cycle of pulses, which allowed to optimize the process parameters according to the criteria of minimizing energy consumption. It was found that the processing time to achieve the desired humidity with the application of vibration, filtration, and electroosmotic effect was twice less than for filtration drying in a fixed bed. In combination with the noted physical and mechanical factors, the proposed technology improves the technical and economic parameters of the studied process.

Keywords: vibratory-centrifugal moisture removal, electroosmotic effect, microcontroller system, drying kinetics, pectin-containing raw materials

INTRODUCTION

Pectin-containing raw material, in particular, beet pulp, which is a valuable raw material for processing, pharmaceutical, and food industries, was used as the object for processing. It is advisable to organize the production of beet pectin at sugar factories or near them **[1]**. One of the most promising areas of beet pulp use is dietary fiber production. Over the last decade, modern technologies have been developed to obtain clarified dietary fiber from beet pulp, which contains a large amount of pectin and can be used as an additive in manufacturing a wide range of foods. In addition, beet pulp dietary fiber has a lower moisture-holding capacity because it contains up to 10% of hydrated pectin. These properties allow using beet dietary fiber to produce dietary supplements for preventive nutrition **[2]**.

The process of dehumidification is one of the most complex and energy-intensive one in processing and food productions. It significantly increases the cost of production. Thus, the search for innovative technology and design solutions in the drying systems development, particularly with the use of mechanical and physicomechanical processing methods **[3]**, is becoming relevant. In modern technologies, mechanical pressing has become the most common method for removing excess moisture from the pulp-pectin raw materials. This operation is carried out mainly in screw-type machines, characterized by a reasonably high metal intensity and require significant energy consumption for their function **[4]**.

Considering that the Fiproductivity of any mass transfer process is inversely proportional to the diffusion resistance of the medium and directly proportional to the difference in concentrations of diffusion substance compared to equilibrium, physicomechanical actions, such as providing low-frequency oscillations to the working

container, increasing the osmotic pressure when applying an electromagnetic field in the technological environment, were used to intensify the process.

Vibrations of the working container cause both the general circulation of the loaded mass and the relative chaotic movement of the mixture components, which leads to the weakening of adhesion forces between the particles of the process medium, destruction of the formed conglomerates, changes in rheological characteristics of the material – viscosity, shear modulus, effective coefficient of friction, adhesion forces, which, taken together, create the effect of loosening the mass of the product, reducing the structural resistance and increasing the heat and mass transfer surface **[5]**. Electroosmotic processes in the highly humid layer of raw material, when creating a pressure difference in the working volume, allow increasing the filtration and dehumidification processes **[6]**.

The developed drying technology was evaluated using the technical and economic parameters of the dehumidification process, namely, the minimum possible energy consumption, minimal time for reaching the given humidity, and the most effective removal of free moisture.

Scientific hypothesis

The technological hypothesis assumes a reduction of energy and material consumption due to the combination of several types of physicomechanical and technological influence, namely centrifugal, filtration, vibratory, electroosmotic; creation of the most favorable conditions for technological processing of products different in their properties due to the choice of the required type of the combined physicomechanical action on raw materials.

MATERIAL AND METHODOLOGY

Samples

Sugar beet pulp (Alexandria) was used as an object of processing:

- triploid hybrid;
- created by Bila Tserkva DSS together with Ivanivska research and selection station;
- high yield;
- sugar content of 19 – 20%,
- sugar collection – 9 – 10 t/ha;
- resistant to cercosporosis;
- zoned in 1997;
- recommended for growing in the areas of Polissya and Forest-Steppe.

Edible seeds of melons, particularly pumpkin, zucchini, watermelon, and melon, were purchased from farms in Vinnytsia and Kherson regions, Ukraine.

Chemicals

Chemical reagents were not used for scientific research.

Biological Material

For experimental studies, used: pumpkin seeds, grade Volga gray 92 (supplier farm Sofia, Vinnytsia region, Ukraine); zucchini seeds, Diamond variety (supplier farm Sofia, Vinnytsia region, Ukraine); watermelon seeds, Giant variety (supplier Farm Taste of Summer, Kherson region, Ukraine); melon seeds, Crenshaw variety (supplier of the Taste of Summer farm, Kherson region, Ukraine).

Instruments

Frequency converter (Mitsubishi FR-E540-075EC, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Portable vibration analyzer (AGAT-M, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Accelerometer (LIS3DH, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Tachometer (UNI-T UT372, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Autotransformer (AOSN-20-220-75, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Electronic wattmeter (EMF-1, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Moisture meter (Wile-55, producer (Inter-Synthesis) Limited Liability Company, Ukraine)

Thermometer (Infrared Thermometer IT - 100, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Manual hydrometer (MS-13, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Micromanometer with an inclined tube ("MMN-240").

Stopwatch (SDSpr-1, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Technical scales (BTA-60, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Laboratory Methods

Studies of raw material drying processes were carried out according to the general method, which involved measuring the moisture content of products depending on the processing time. Products with 60% of initial humidity were placed in the drying chamber; appropriate modes were set, particularly the temperature of the drying agent, its feed rate, amplitude, and frequency of vibrations. The feed rate of the drying agent was measured with the anemometer.

A wireless power supply sensor for recording amplitude-frequency characteristics based on STMicroelectronics' LIS3DH accelerometer was used to evaluate the energy parameters.

The UNI-T UT372 wireless tachometer was used to record the drive shaft speed.

The AOSN-20-220-75 autotransformer was used to control and change the speed of the motor shaft.

An electronic wattmeter EMF-1 was used to determine the energy characteristics of the studied machine.

Electronic laboratory technical scales BTA-60 were used to study the dewatering parameters.

The temperature of the test raw material before drying and after was measured with a certified thermometer, "Infrared Thermometer".

A manual hydrometer MS-13 is used to measure the speed of air movement in the air duct.

To measure the airflow pressure in the air duct, a multi-limit micromanometer was used with an inclined tube MMN-240 TU-25-01-277-70.

Stopwatch SDSpr-1 was used to record the drying time.

A Wile-55 moisture meter was used to control humidity.

Description of the Experiment

Sample preparation: Samples of sugar beet pulp and pumpkin, zucchini, watermelon, and melon seeds were used for the research. Samples of sugar beet pulp were collected at Gaisinsky Sugar Plant, Vinnytsia Region, Ukraine.

Number of samples analyzed: During the experimental studies, 20 different samples of sugar beet pulp and 20 samples of pumpkin seeds were examined. zucchini, watermelon, and melons, which were purchased from farms on the territory of Ukraine.

Number of repeated analyses: All measurements of an 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: The microcontroller system allowed obtaining information about the main parameters of the process on the personal computer display in real-time, which made it possible to optimize the processing of highly humid materials, necessary for the dynamics of heating raw materials without heating them to maximum values, i.e., the loss of biologically active substances was limited. As a result, the current measurement of seed moisture in the process of filtration moisture removal during sealing of the drying chamber due to the possibility of measuring the relative humidity of the drying agent at the inlet and outlet, when sampling is technologically impossible, was performed. When the 10% value of the product moisture content was reached, the drying process was stopped.

Experimental studies were conducted in three stages. In the first stage, the drying chamber was provided mainly with vertical oscillations using an electromagnetic vibrator. At a constant oscillation frequency of 16 Hz, the amplitude values of 2, 4, and 6 mm were set, at which the duration of the filtration-convective drying process, which was carried out according to the above-mentioned method, was recorded. In the second stage of the research, the electromagnetic vibrator of horizontal oscillations was used in the previous modes, the operation of which was synchronized with the vibrator of vertical oscillations in terms of frequency and phase. In the third stage of the research, an electromagnetic vibrator of horizontal oscillations was used. With the help of an electronic electricity meter, power consumption per-process was measured, making it possible to compare specific energy consumption at different technological modes.

Statistical Analysis

The statistical evaluation of the results was carried out 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.

RESULTS AND DISCUSSION

Investigation of the dehumidification process was performed following the general method, which involved measuring the moisture content of pectin-containing raw materials depending on the processing time using a laboratory installation (Figure 1). Pectin-containing raw materials with 720% initial humidity were fed through the loading device and occupied 3/4 of its volume. The intensity of moisture removal was controlled by the difference in relative humidity of the drying agent at the inlet and outlet of the drying chamber. Processing was completed when the moisture of the product reached about 9 – 10%.

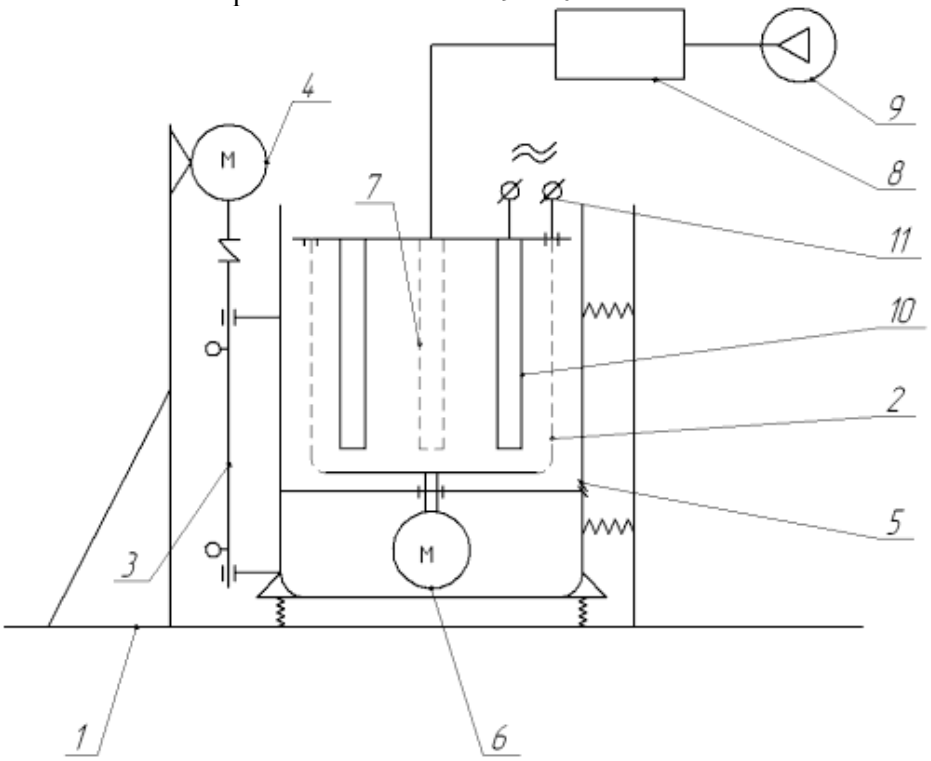

Figure 1 Vibratory centrifugal electroosmotic dehumidification.
Note: 1 – frame; 2 – perforated centrifuge rotor; 3 – vibratory drive; 4 – the electric motor of the vibratory drive; 5 – centrifuge housing; 6 – centrifuge motor; 7 – manifold; 8 – heat generator; 9 – compressor; 10 – electrodes; 11 – voltage converter.

Vibratory centrifugal electroosmotic experimental dehumidification provided a sequential three-stage filtration-convective dehydration of highly humid raw materials by changing the technological effects and parameters. At the first stage, filtration drying in a fixed bed was studied. In a stationary drying chamber, a heated drying agent was fed through a perforated cylinder. It passed through a layer of raw used, which allowed us to trace their effect on the time of moisture removal.

The set temperature of the drying agent was maintained automatically; it was also possible to quickly adjust it with a power regulator. Frequency and amplitude of vibrations were set independently using the electronic device and by the change of the vibration exciter balance weight setting angle.

In scientific works **[7], [8], [9], [10]** based on experimental studies of the drying process in the vibrofluidized layer with conductive heat supply for the range of vibration frequencies from 20 – 80 Hz and amplitude from 0.0005 – 0.0025 m, it was found that moisture removal occurs mainly in a period of constant drying speed, the efficiency of which is largely determined by the amplitude of oscillations at a vibration frequency of 40 – 60 Hz.

According to the results of using different combinations of physic mechanical factors of intensification of the studied process, it was found that vibration-filtration drying with the use of the electroosmotic effect, depending on the kinetic characteristics of the process, appeared to be the most effective (Figure 2).

Scientific works **[11], [12], [13]** describe the use of electro-osmosis for dehumidification of elastic-plastic masses in combination with electrothermal treatment with alternating current allowed to establish that electricity consumption does not exceed 60 – 75 kWh/m, efficiency compared to steam increases by 2 – 2.5 times.

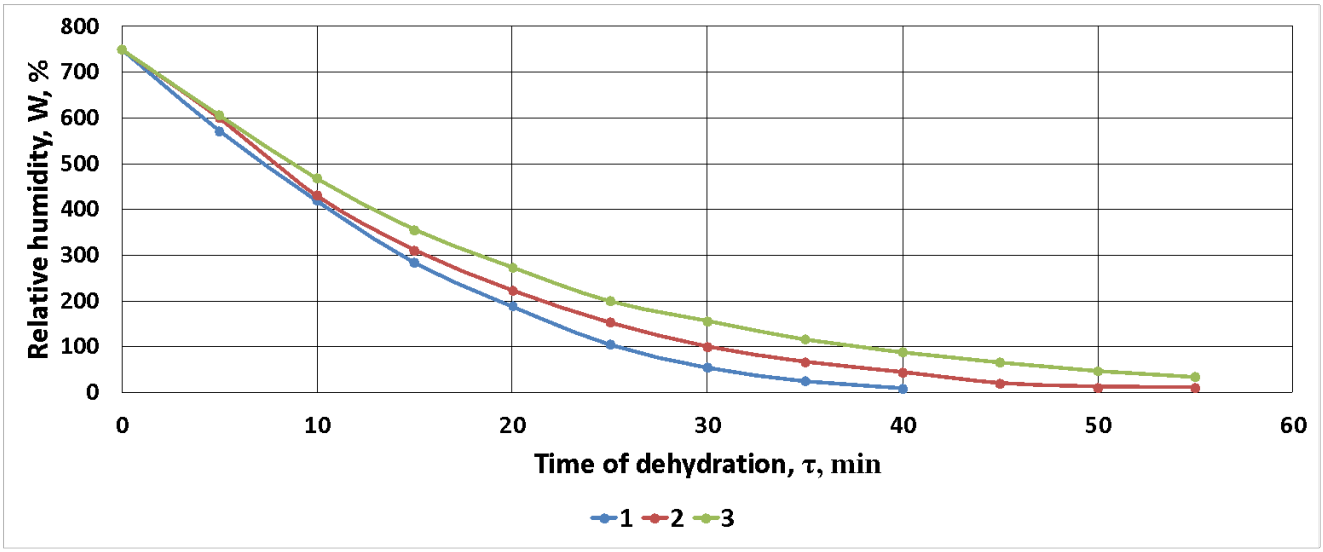

Figure 2 Kinetics of the pectin-containing raw materials drying with different methods of moisture removal. Note: 1 – filtration drying in a fixed bed at *Wn*=720%, *V*=3 m/s; 2 – vibratory filtration drying at *Wn*=720%, *V*=3 m/s, *A*=0.004 m; 3 – vibratory filtration drying using electro-osmotic effect at W_n=720%, *V*=3 m/s, *A*=4 mm, *E*=0.8 V/m, *F*=200 Hz, *Q*=3, *П*=0, 75.

Effective parameters of the electromagnetic field in terms of implementation of the electroosmotic effect were the following: electric field strength *E*=0.8 V/m (Figure 3); frequency of electric current *F*=300 Hz (Figure 4); pulse relative duration *Q*=3 (Figure 5). Variation of the specified electrotechnical parameters complex at an estimation of kinetic parameters of the investigated process of the pectin-containing raw materials dehumidification allowed optimizing the process parameters according to the energy consumption minimization criteria.

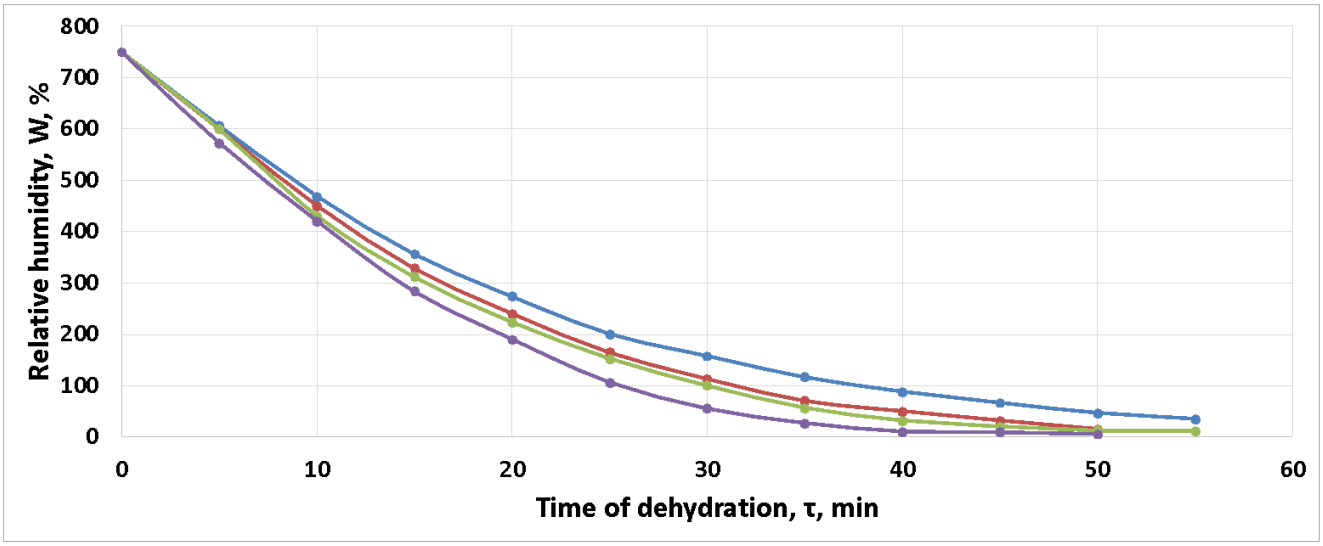

Figure 3 Effect of the electric field strength on the pectin-containing raw materials drying kinetics. Note: W_n=720%, *V*=3 m/s, *A*=0.004 m mm, *F* =200 Hz, *Q* =3, *П*=0.75.

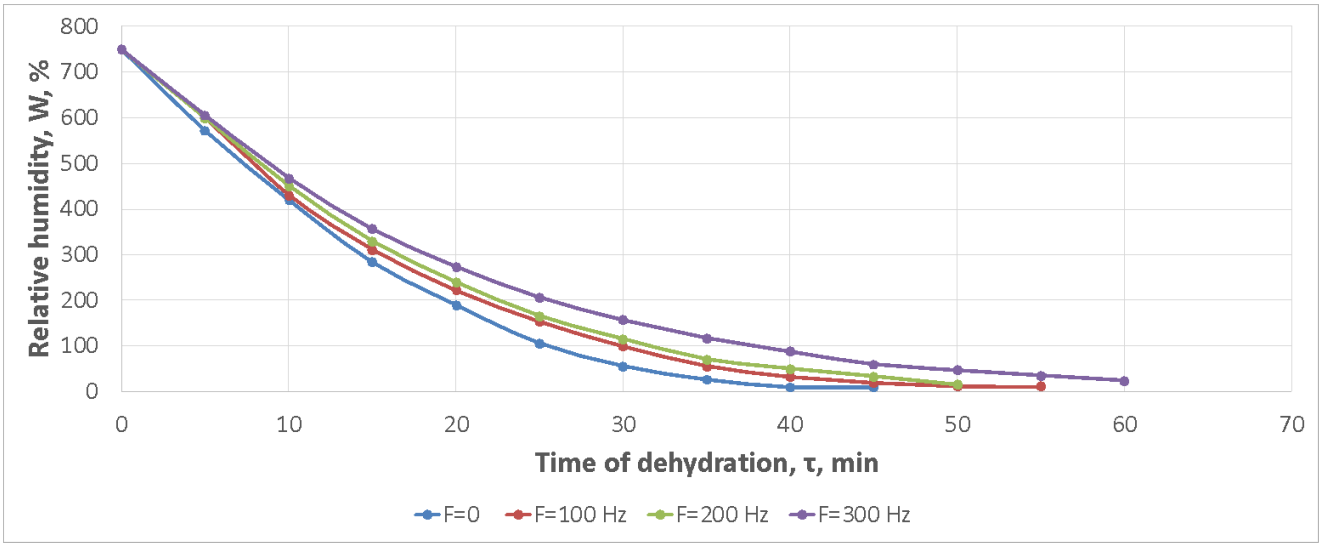

Figure 4 Influence of the frequency of electric current on the pectin-containing raw materials drying kinetics.
Note: W_n=720%, V=3 m/s, A=0.004 m, E=0.8 V/m, Q=3, Π=0.75.

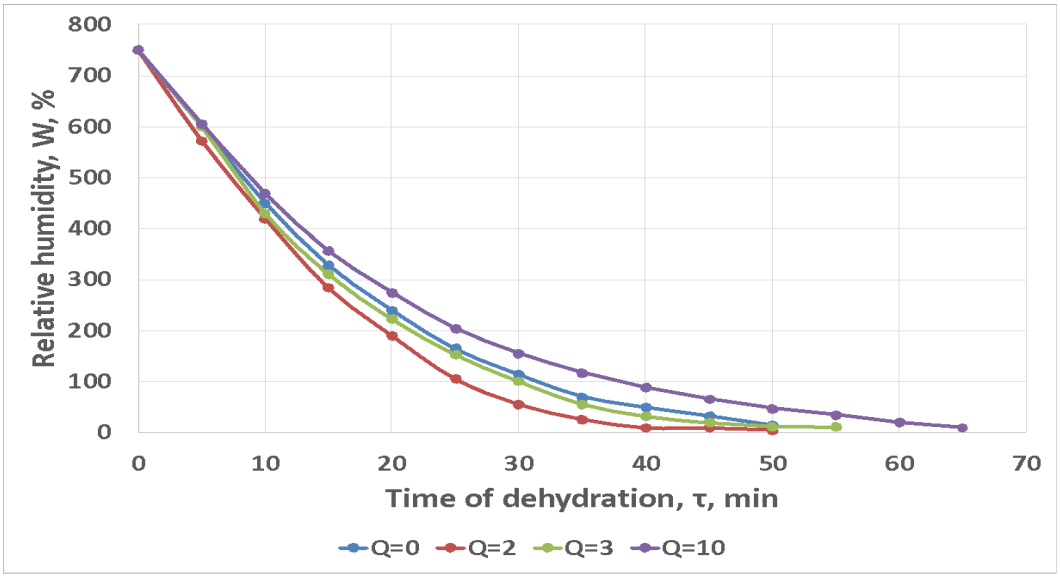

Figure 5 Influence of the pulse relative duration on the pectin-containing raw materials drying kinetic.
Note: W_n=720%, V=3 m/s, A=0.004 m, E=0.8 V/m, F=200 Hz, Π=0.75.

As a result of centrifugal filtration of two different materials in the same conditions, different contents of the liquid phase are often found in them, which is explained by their different moisture-holding capacity **[14]**, **[15]**, **[16]**, **[17]**.

Studies of the dynamics of the heating agent flow during dehumidification (Figure 6) revealed a slight decrease in processing time with increasing convective flow rate. Given that there is a slight increase in energy consumption for the process of dehumidification, we can consider insignificant the factor of increasing the speed of the heating agent.

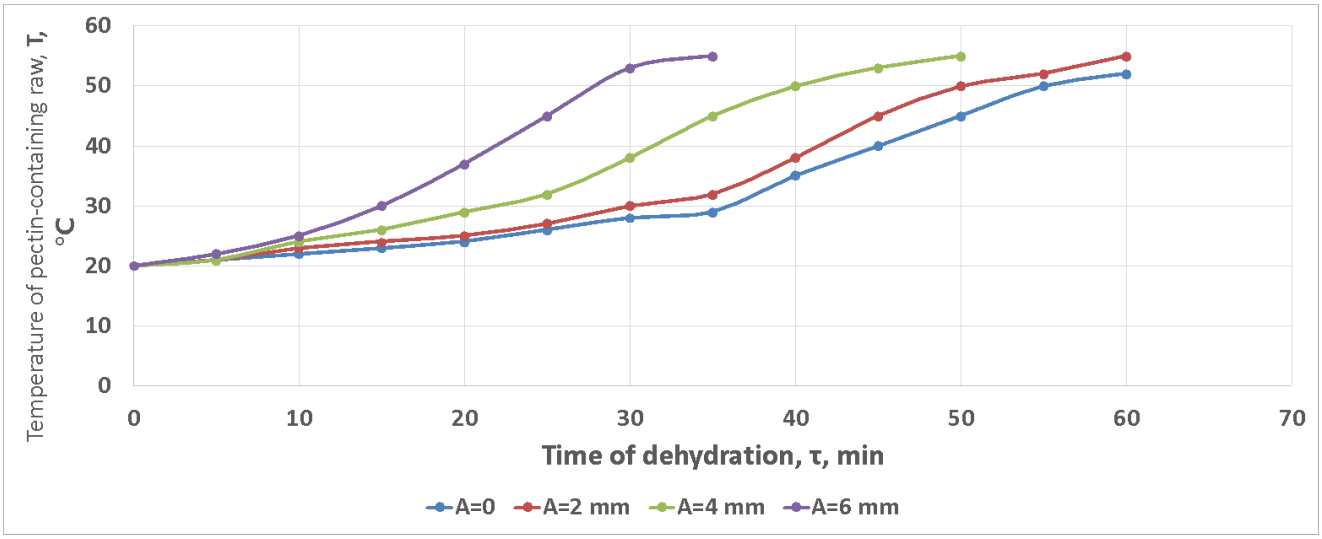

Figure 6 Dependence of the temperature of pectin-containing raw materials T °C on the dehydration time τ, min at different oscillations amplitudes.

When substantiating the use of a combination of physicomechanical factors for intensifying the process of the pectin-containing raw materials dehumidification, it was found that the use of vibratory filtration drying with electroosmotic effect reduces the moisture removal time by 1.44 times compared to filtration drying in a fixed bed (Figure 7 and Figure 8).

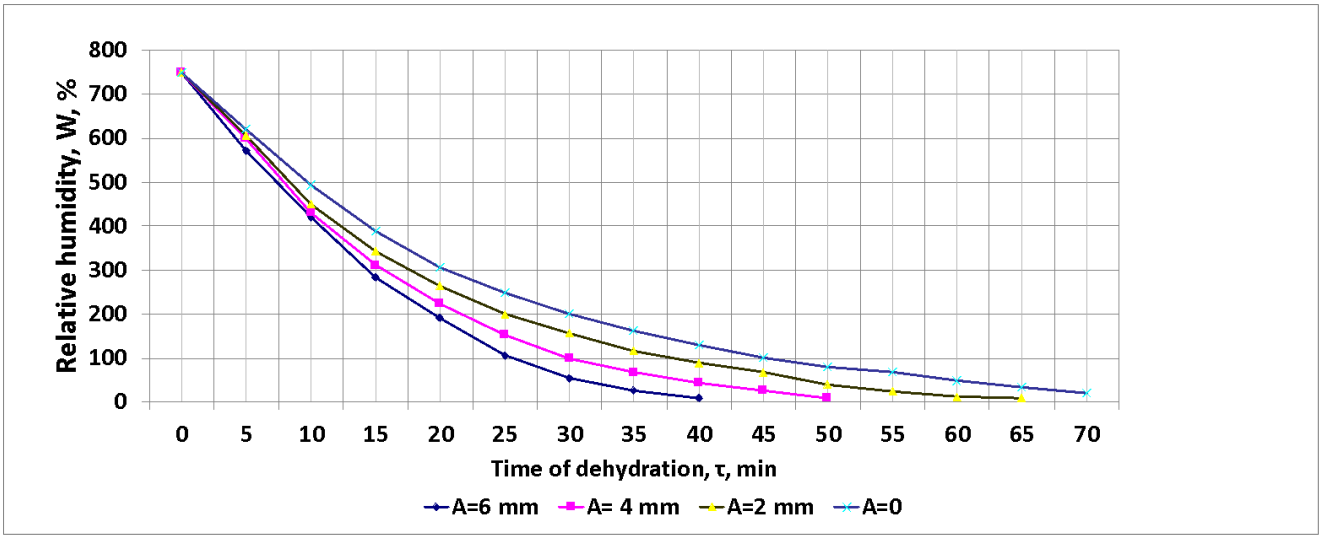

Figure 7 Dependence of the pectin-containing raw materials humidity W, % on dehydration time τ, min at different oscillations amplitudes.
Note: W_n=720%, V=3 m/s, Q=3, E=0.8 V/m, F=200 Hz, Π=0.75.

According to the results of scientific research **[18]**, **[19]**, **[20]**, it was found that the use of low-frequency oscillations in the second period of drying of beet pulp allowed to reduce the total duration of the process of heat treatment of products by 40 – 45%. But the authors of annealing works **[21]**. Proved that in the conditions of the vibrofluidized layer, the total surface area of the bulk material increases, resulting in an intensive removal of moisture and an increase in the drying rate.

Reducing humidity and increasing porosity can reduce energy consumption for further drying, which can significantly speed up the process, simplify the selection and maintenance of the dryer **[22]**, **[23]**, **[24]**.

The driving force of the dehumidification process is increased by vibration extrusion, centrifugation during rotor spinning, development of an electroosmotic effect when creating conditions for unilateral diffusion, and the process of filtering the medium through the perforation of the rotor.

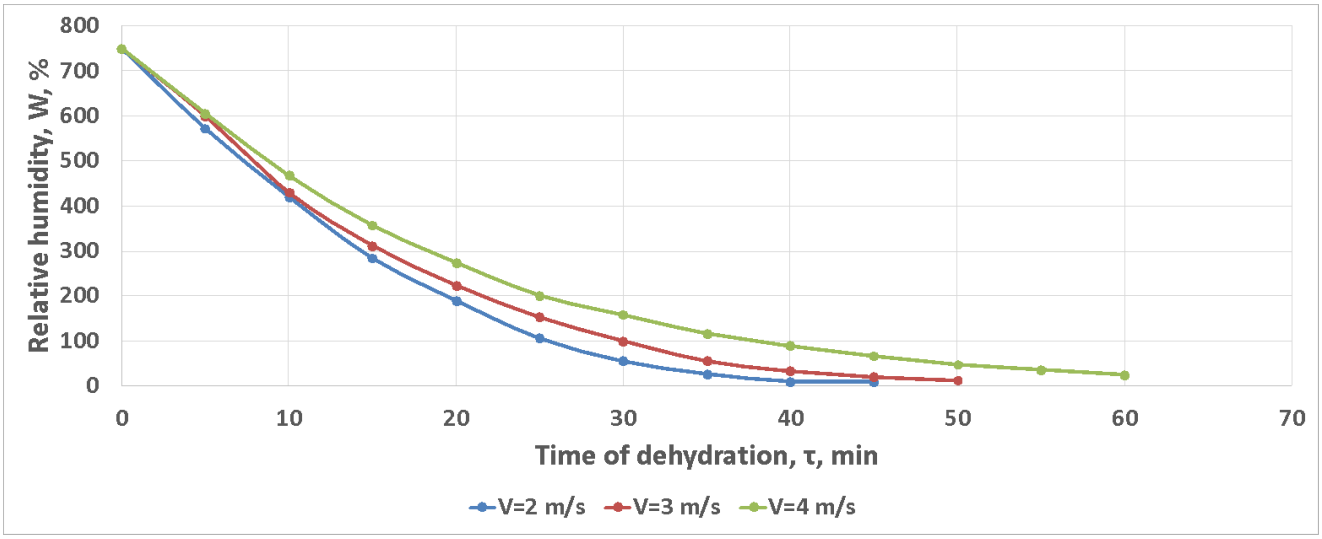

Figure 8 Dependence of the pectin-containing raw materials humidity *W*, % on dehydrating time τ, min at different airflow rates.
Note: W_n=720%, *A*=0.004 mm, *Q*=3, *E*=0.8 V/m, *F*=200 Hz, *П*=0.75.

However, the latter process leads to increased technological resistance, which worsens conditions for processing intensification. This disadvantage can be offset by creating a fluidized bed of the product by providing oscillating motion to the working container, which leads to a significant reduction in the forces of internal friction in the mass of the load. Thus, the action of these factors is aimed at increasing the intensification of the dehumidification process while minimizing energy consumption.

In his research **[25]**, **[26]**, **[27]** satisfied the factors of vibration action to intensify the drying process by increasing the heat transfer process, the speed of which in different frequency modes increases from 2.5 – 9 times.

A combination of the above-mentioned factors allows for effective dehumidification of pectin-containing raw materials with a specific set of physicomechanical properties and certain quality restrictions of the products. This allows the formation of an effective sequence of technological influence.

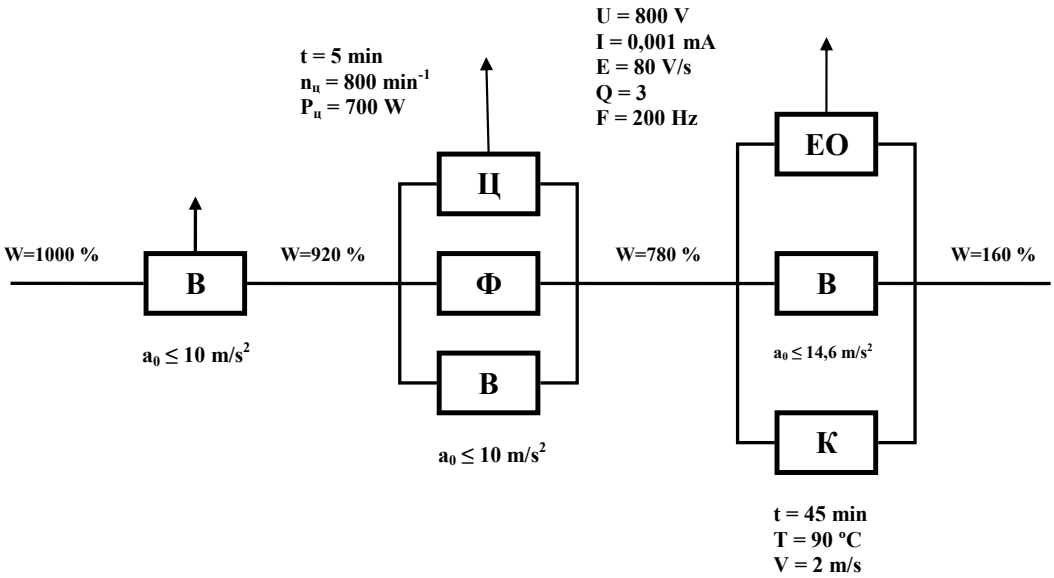

Figure 9 Scheme of the combined physicomechanical processing at implementing the modes of pectin-containing raw materials dehumidification.

The authors of the following scientific works **[28]**, **[29]**, **[30]** proved that in the conditions of the Vibro-liquefied layer, the total surface of the bulk mass of the material increases, as a result of which there is an intensive removal of moisture and an increase in the drying rate.

The vibration of actuators, as well as blowing coolant bulk materials with low friction between particles and a slight tendency to aggregation **[31]**, **[32]**, **[33]**, allow you to significantly reduce the speed of the drying agent, which reduces energy consumption for the process.

When processing the investigated pectin-containing products (Figure 9) at the 1st stage, vibration compaction is carried out to remove free moisture, which occurs due to the acceleration of the force field:

$$a_B \approx g = 9.81 \frac{m}{s^2}.$$

At the 2nd stage of processing, free moisture is squeezed out simultaneously by centrifugation, filtration, and vibration separation. These operations are performed when reaching the resonant mode of operation of the vibrator to prevent the negative effects of vibration when resonating on the structural elements of the filter centrifuge. At the 3rd stage, operations of electroosmotic pressing, vibration loosening of technological masses are implemented, which increases the efficiency of convective drying to achieve the desired humidity of the product.

The conducted research allowed to carry out the comparative analysis of the influence of various physicomechanical measures (Figure 9) and proved the efficiency of the vibratory centrifugal removal of free moisture at the 1st stage of processing, destruction of a continuous layer of the product under the action of a vibration field at the 2nd one and the subsequent convective diffusion with electroosmotic intensification at the 3rd stage of processing that allows reducing the energy consumption of the dehydration process by 2.7 times in comparison with traditional convective drying.

CONCLUSION

1. Taking into account the directions of the process of osmotic dehumidification improvement to determine the optimal design parameters of the equipment and mode parameters of the process, a functional diagram of a set of devices based on experimental vibration installation was developed.

2. The processing time to achieve the desired humidity when applying the vibratory, filtration, and the electroosmotic effect was twice less than filtration drying in a fixed bed.

3. The conducted research allowed determining the optimal parameters of dehumidification of thermolabile materials with the use of electroosmotic effect: electric field strength E = 0.8 V/m; electric current frequency F = 300 Hz; pulse relative duration Q = 3.

4. Technological scheme of electroosmotic vibration dehumidification was developed. Energy consumption for removing 1 kg of moisture is reduced by 2.7 times compared to traditional convective drying, given that the latter is quite destructive for thermolabile dispersed systems.

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The study of the intensification of technological parameters of the sausage production process

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ABSTRACT

One of the sources of sodium are meat products. Increased consumption of meat products and sodium intake leads to serious health problems. The task of reducing the dosage of sodium chloride in minced meat needs to be addressed. The partial replacement of table salt with sea salt will reduce the sodium concentration in products to 20%. It is established that this modification increases the moisture-binding properties of minced meat and lowers the dosage of salt in the mass of raw meat, which will reduce the level of harm to the body due to excessive consumption of sausages. It is proposed to introduce a bacterial preparation based on the strain *Staphylococcus carnosus*, which will reduce the amount of sodium nitrite in the finished products. Technology has been developed to regulate the composition of microelements in meat products by enriching them with a kelp extract. It was found that changes in the composition of minced meat can adversely affect the taste and physicochemical properties of the product, which is confirmed by expert studies. As a result of laboratory studies, it was found that a partial change of salt in the sea helps to improve the stability and physicochemical quality of minced meat (active acidity, water activity, moisture retention, and shear stress). According to the research results, the recipe of sausages recommended for implementation at the enterprises of the meat processing industry of Ukraine has been developed.

Keywords: sodium, concentration, sausage meat, kitchen salt, sea salt.

INTRODUCTION

Sodium is one of the elements critically essential for supporting the normal state of an organism, and the most source of its income is the table salt (sodium chloride, NaCl). The man's organism contains about 0.3 kg of this salt, and its most significant part is dissolved in blood and plasma **[1]**. Consumption of table salt assists in the system's normal functioning and oppresses the germination of putrescent microorganisms **[2]**.

The average sample of the table salt contains 94 – 99% of sodium chloride and small quantities of copper, iron, fluorine, magnesium, manganese, and potassium **[3]**. The positive functions of sodium ions that enter the organism with this salt in foods consist of normalising kidneys' functioning and activating absorption of amino acids and glucose in bowels. The presence of sodium is also retained in organism water, which assists in regulating water-salt metabolism and activation of state of albumen of angiotensin II, which normalises the level of arterial pressure **[4]**. The normal sodium concentration in plasma is 12 – 17 mEq/m^3, and its deflection of physiological norms leads to a severe increase in levels of morbidity and mortality **[5]**. The presence of sodium in normal concentrations assists in the normal functioning of the nervous system thanks to supporting the potential of nerve cells at the proper level. After they have received the signal, the electrochemical impulse perceived by the neighbouring cells is generated. Taking these facts into consideration and studying the effects of various doses of consumed sodium on the metabolism processes, the Linus Pauling Institute of the Oregon State University, USA, recommends the daily norm of consumption of sodium on the level of about 1500 mg of (0.0038 kg of table salt) **[6]**.

However, the factual level of sodium consumption is much bigger and composes 0.0082 – 0.0094 kg in the USA, 0.0094 kg in the UK, and 0.012 in the East-Asia countries. The excessive consumption of salt leads to worsening of the state of health, which appears in accumulation of water in the organism, spraining of muscular ligaments and deterioration of capability of muscles to contract, arising of inflammations in kidneys, nephropathy,

renal failures, and neutrality, impairment of transmission of impulses in the brain, dotting of blood and increasing of risks of insult, overactivity and excessive excitability **[7]**. It is recommended to abandon or decrease the consumption of spicy flavouring, canned foods, and sausages to decrease the organism's salt concentration; the prevalence of sodium in this kind of food leads, among others, to the appearance of neoplasms. For instance, the Japanese are often affected by stomach cancer because of excess salted and pickled products **[8]**.

At the same time, the significant part of the rations of Europeans consists of meat products. The typical structure of consumption of sodium with foods is as follows (Figure 1): cereals – 35%, meat and meat products – 26%, vegetables – 11%, dairy products 8%, other products – 20%.

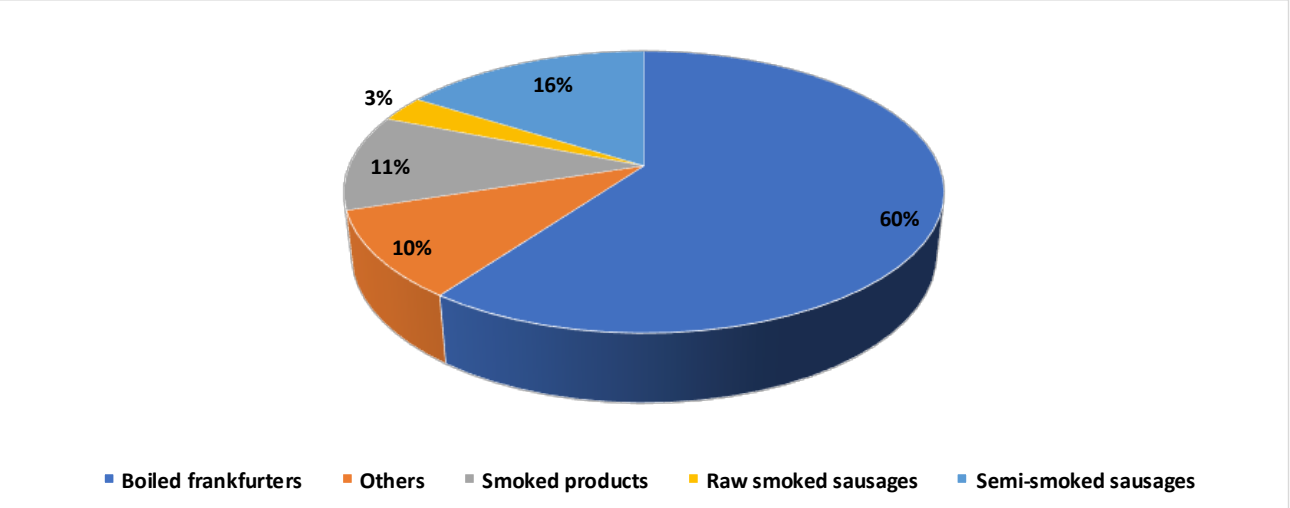


Figure 1 Structure of the market of meat products in Ukraine in 2020.

So, meat products, mainly sausages (60% of a full proposal of meat products on the market), are one of the organism's principal sources of sodium intake **[9]**.

Scientific hypothesis

The fabrication technology of frankfurters may be optimised in limiting sodium consumption. To reach this goal, we propose to replace the table salt used in the meat products formulations traditionally for the sea one and add to the minced meat mass the *Staphylococcus carnosus* bacterial culture. Such replacements should decrease the quantity of consumed sodium by a factor of 20 – 30% and cut the time of ripening of the mix. The iodine deficit in the ration may be solved if an additive of laminaria seaweed enriches the sea salt.

MATERIAL AND METHODOLOGY

Samples

The study was carried out using two samples, which composition is shown in Table 1. The design of basic ingredients of the control mix conformed to this one of frankfurters of "Liubytel'ski" produced by the standard of DSTU 4436:2005 **[10]**, which composition was modified by additives recommended for use to give the mix some medicinal properties.

The composition of the experimental meat mix was modified by replacing in traditional frankfurters formulation of kitchen salt for the sea one and enriching the basic formulation by *Staphylococcus* bacterial culture and extracts of rosemary and *laminaria* (Table 1).

Table 1 The basic ingredients of minced meat

Ingredient	Control mix	Experimental mix
Basic raw materials		
Beef	33	30
Semi-fatty pork	33	26
Fatty pork	34	34
Blood albumen	-	1.0
Water to hydrate the blood albumen	-	2.0
Cellular tissue (orange dietary fibers Citri-Fi 100)	-	0.5
Water to hydrate cellular tissues	-	6.5
In total	100	100
Spices and materials		
Kitchen salt	2.2	-
Sea salt with *laminaria*	-	2.1
Sugar	0.16	0.16
Sodium nitrite	0.0075	0.005
Bacterial preparation (Iprovit LRR)	-	0.05
Rosemary extract	-	0.015
Water	35.0	30.0

Chemicals

The components of the minced meat mixes, which masses were detected in this work, were as follows:

Petroleum ether (excise, AR grade, Khimlaborreaktyv LLC, Ukraine).

Nitric acid (A brand, CP, Khimlaborreaktyv LLC, Ukraine).

Potassium dichromate (AR grade, Khimlaborreaktyv LLC, Ukraine).

Hydrochloric acid (A brand, AR grade, Khimlaborreaktyv LLC, Ukraine).

Sodium hydroxide (A brand, AR grade, Khimlaborreaktyv LLC, Ukraine).

Sodium tripolyphosphate (technical, p 85%, Khimlaborreaktyv LLC, Ukraine).

Sulfuric acid (A brand, CP, Khimlaborreaktyv LLC, Ukraine).

Animals and Biological Material

The biological materials used in this work were beef muscle meat, semi-fatty pork muscle meat, blood albumen, and *Staphylococcus carnosus* bacterial culture.

Instruments

Drying oven (DC-300, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Muffle furnace (SNOL, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Laboratory press (Velp Scientifica, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Mettler Toledo analytical balances (producer (Inter-Synthesis) Limited Liability Company, Ukraine).

The analyser of fat (SOX 406, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Instrument of (Combo) for measuring the oxidative potential (producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Penetrometer (Ulab 3-31, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Laboratory centrifuge (producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Chemical cups (CC-100, CC-150, CC-200, CC-250, CC-500, producer (Laboratory equipment) Limited Liability Company, Ukraine).

Petri dish (producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Measuring flasks (MF-100, MF-150, MF-200, MF -250, MF-500, producer (Laboratory equipment) Limited Liability Company, Ukraine).

Muffle furnace (SNOL 8,2/1100, producer (Laboratory equipment) Limited Liability Company, Ukraine).

Measuring pipettes (MP-0,001, MP-0,002, MP-0,005, MP-0,01, MP-0,015, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Conical flask (CF-100, CF-150, CF-200, CF-250, CF -500, producer (Laboratory equipment) Limited Liability Company, Ukraine).

Burette for titration (producer (Laboratory equipment) Limited Liability Company, Ukraine).

Filters (producer (Laboratory equipment) Limited Liability Company, Ukraine).

Gas chromatograph (Kupol_55, Shimadzu Corporation, Japan).

Amino acid analyser (LC-2000, Biotronik, Khimlaborreaktyv LLC, Ukraine).

Laboratory Methods

Characterising of the chemical composition has been carried out according to the following methods: the mass fraction of moisture by drying the product sample down to a fixed weight at a temperature of 100 – 105°C according to DSTU 8029:2015 **[11], [12]**; the mass fraction of ash by weight method, after mineralisation of the product's sample weight in a muffle furnace at a temperature of 500 – 600°C according to DSTU 8718:2017 **[13]**; the mass fraction of lipids by Soxhlet method, which consists in the fact that fat is weighed after its extraction with a solvent from the dry sample weight in the Soxhlet apparatus, based on determining the change in the sample's weight after fat extraction with a solvent by DSTU 8718:2017 **[14]**; the mass fraction of protein by determining the total nitrogen by the Kjeldahl method. Cinefaction of samples was performed on Velp Scientifica DK6 series (Italy) with a vacuum pump (JP). Distillation was made on a steam distillation device Velp Scientifica UDK 129 (Italy), DSTU 8030:2015 **[15]**.

Determination of the fiber's mass fraction was carried out by removing acid-alkaline-soluble substances from the product and determining the residue weight, conventionally fiber by DSTU 8844:2019 **[15]**.

Determination of the fatty acid content was carried out by chromatographic method on the Kupol 55 chromatograph (Russia) GOST ISO 17764-1:2015 **[16]**.

The mineral composition (the content of potassium, calcium, magnesium, phosphorus, manganese, and so on) was determined by atomic emission spectrometry with inductive plasma, and the content of heavy metals (lead, cadmium, arsenic, mercury, copper, and zinc) was determined by atomic absorption spectrometry according to DSTU EN ISO 11885:2019 **[17]**.

Description of the Experiment

Sample preparation: The purpose stated in the experiment was the comparative determining of technological and functional properties of control and experimental mincemeat systems carried out after determining the composition of tested masses. The properties to control were as follows: active acidity, the activity of water, water holding capacity, and limiting shifting tension. The masses to control were prepared by the thorough mixing of ingredients in a special mixing vessel and storing of the mixed mass overnight before the experiment. Each property was determined threefold.

Number of samples analyzed: Four types of sausages with different moisture content and shelf life were used in the study of samples.

Number of repeated analyses: Each study was carried out five times, with the number of samples being four, which amounted to twenty repeated analyses.

Number of experiment replication: The study was repeated five times, with the experimental data processed using mathematical statistics methods.

Design of the experiment: The active acidity and oxidative potential of mixes were determined by measuring the difference of potential of glassy and reference electrodes poured in the mix by the method normalized by the standard of DSTU ISO 2917:2001 **[18]**. The water activities were determined in the indication of a moment of appearance of dew on the surface of the cooled indicator. The water-holding capacities and plasticity of tested samples were measured in the threefold pressing of the sample of 0.0003 kg mass by a strain of 1 kg and measuring of gain of mass of filtering paper and area of water spots on it. The limiting efforts of the shift were measured in measuring of depth of insertion of the measuring head of the penetrometer into the minced meat mass. The emulsifying capacity of homogenized mince meats was determined by adding a rated quantity of sunflower oil, centrifugation of the mix, and determining the volume of oil-free of weighed solid particles. The mass stability was determined by warming during 30 minutes of the sample prepared in the previous experiment to 80°C, cooling it for 15 minutes by flowing water, centrifugation for 5 minutes at 500 pm, and measuring the volume of emulsified oil. To obtain each property's real value, determining all properties was repeated in the same conditions once more.

The studied meat products are characterized by a relatively big sodium chloride content, which concentration varies in the diapason of 1.2 – 6%, even reaching in some cases 12% **[19]**. For instance, the content of NaCl in sausages is 1.3 – 3.5% (0.006 – 0.014 kg of sodium in one kilogram of the product) **[20]**. For instance, the content of sodium in 0.1 kg of the product in 10 types of sausages the most popular in Europe (the numbers in parentheses show its ratio to its recommended daily allowance – RDA) is as follows **[21]**:

Sausage, Polish, beef with chicken, hot: 1540 mg (96% RDA).

1. Sausage, summer, pork and beef, sticks, with cheddar cheese: 1483 mg (93% RDA).
2. Sausage, Berliner, pork, beef: 1297 mg (81% RDA).
3. Sausage, Italian, pork, cooked: 1207 mg (75% RDA).

4. Sausage, turkey, hot, smoked: 1196 mg (75% RDA).
5. Sausage, chicken, beef, pork, skinless, smoked: 1034 mg (65% RDA).
6. Sausage, chicken, and beef smoked: 1020 mg (64% RDA).
7. Sausage, Italian, turkey, smoked: 928 mg (58% RDA).
8. Sausage, smoked link sausage, pork, and beef: 911 mg (57% RDA).
9. Sausage, meatless: 888 mg (56% RDA).

Therefore, decreasing the quantity of sodium in sausages is one of the actual problems of the modern meat-processing industry **[21]**.

The existing fabrication methods of products, which contain lesser quantities of sodium, may be realized in different ways, including decreasing portions of added table salt, partial replacement of sodium chloride in it by other salts, etc. **[22]**. The components proposed to replace some sodium are chlorides of calcium and potassium. By information to WHO, the use of potassium chloride is the most efficient factor in decreasing the income of sodium in the organism, which assists in the normalization of blood pressure, normalization of the content of glucose in the blood, and decreasing the risk of the progress of cardiovascular diseases. Such a method is especially actual for Ukraine, where its level is the highest in Europe **[23]**. One more action in the betterment of the composition of consumed salt is its enrichment by iodine **[24]**.

Statistical Analysis

Experimental data were processed using mathematical statistics methods in the STATISTICA Microsoft Excel editor. 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 $p = 0.95$). Linear programming problems were solved using the MS Excel table processor's 'Search for a solution' setting (Excel Solver).

RESULTS AND DISCUSSION

The principal purpose of our investigation was a development of a formulation of sausage meat that would contain a decreased quantity of sodium. Similar scientific studies are described in the following scientific papers **[25], [26], [27], [28]**. Still, the authors of the above scientific papers used a different composition of raw meat and increased concentrations of sodium chloride. The assigned task was solved using a composition that contained a mix of table and sea salt. The basic indices of quality of both salts are cited in Table 2.

Table 2 Content of minerals of dry sea and table salts (%).

Mineral	Sea salt*	Kitchen salt of extra grade**
Sodium	30.6	38.7
Chloride	55.0	59.7
Calcium	1.2	0.024
Potassium	1.1	0.008
Magnesium	3.7	0.001
Iron	no data	0.005
Sulfate	7.7	0.16
Hydrocarbonate	0.45	no data
Iodine**	5×10^{-6}	-

Note: *(Wikipedia, 2020) ** (DSTU 3585:2015).

So, the partial replacement of table salt by sea one in salting of meat would permit to decrease sufficiently the content of sodium in it **[29] (WHO, 2007)**. Considering this fact, we exercised the compositions of sausage meats salted by usual and sea salts to find the level of acquiring by these of over-salted taste. There were used the solutions that contain 0.5 – 2.4 % of salts are as follows ***a)*** pure kitchen salt and ***b)*** pure sea salt. Scientific works **[30], [31], [32], [33]** describe studies using 3% table salt, which in our opinion may adversely affect the physicochemical composition of finished products. The expert method controlled the taste of salted meats, which results showed the practically identical feeling of salinity (Figure 2).

The experts found that the moderated taste of salinity of salted meat after its cooking is about 2%, whether it was the pure kitchen salt or the mix of kitchen and sea ones. Therefore, such concentration was recommended for the fabrication of sausage meat mixes. Scientific works **[34], [35], [36], [37]** describe studies using different concentrations of the table and sea salts in the range from 0.5 – 1.5%; as a result of similar studies, it was found that such concentrations hurt the organoleptic properties of the finished product. The one more recommendation

was to enrich them with iodine, preferably by the extract of *laminaria* to compensate its deficit, what technique is the best recommended for use **[38], [39], [40], [41]**.

One more source of income for the organism of cation of sodium with meat products is sodium nitrite $NaNO_2$ added to give them the stable red coloration. However, dietitians recommend limiting using of this salt because of its decomposition with forming of nitrogen oxide, NO, in acid environments, for instance, in the man's stomach, which leads to forming of mutagenic nitrosomioglobine in its interaction with the meat myoglobin **[42]**, **[43]**, **[44], [45]**. However, the result of preserving the red color of boiled sausages may also be reached in adding into the sausage meat of special cultures of microorganisms, which permits reducing the dosing of nitrite-ion.

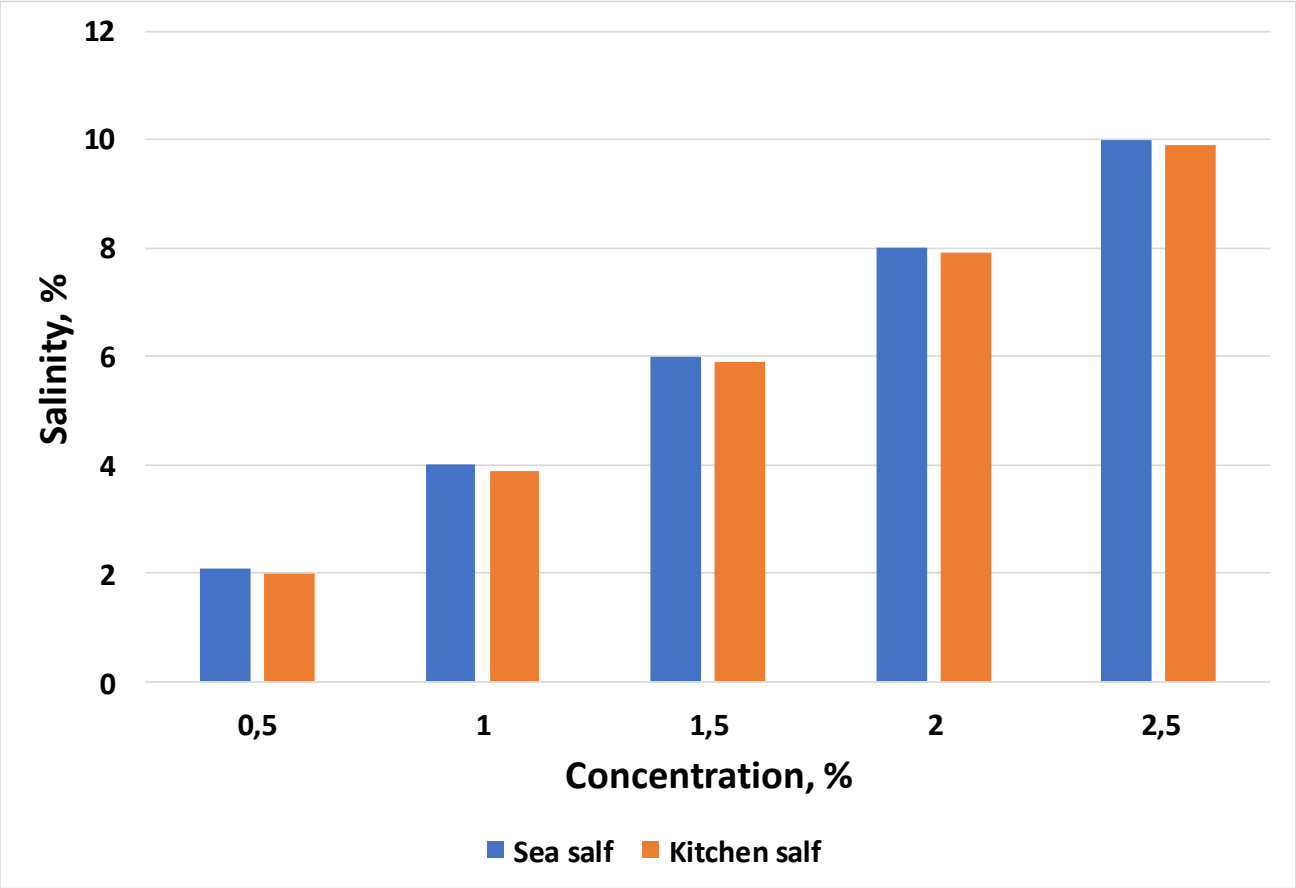


Figure 2 Results of organoleptic evaluation of salinity of meat systems.

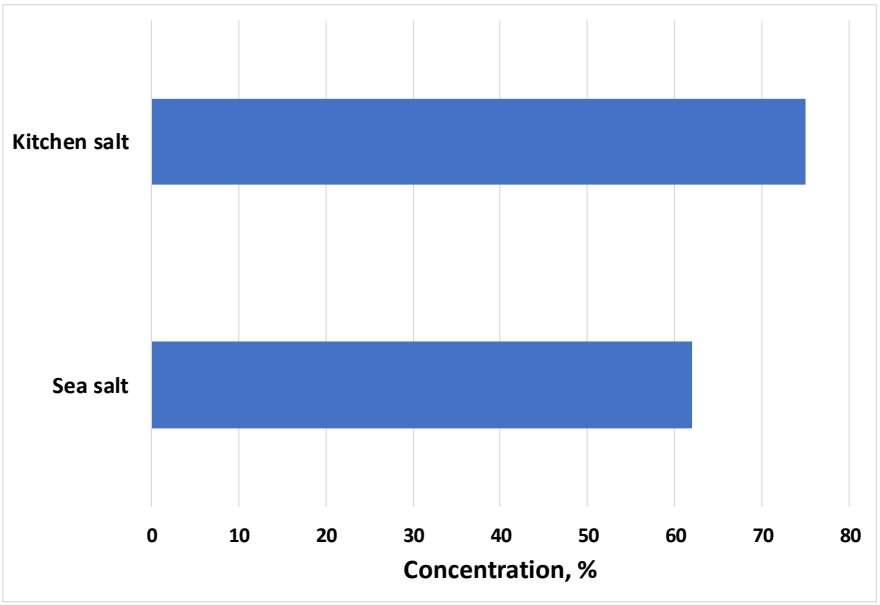
According to the research of leading scientists in meat production, it was proved that microflora reduces the residual amount of nitrites and preserves the basic physicochemical and organoleptic properties of meat products **[46], [47]**. To reach such goal, producers use the most often the specific culture of *Staphylococcus carnosus* **[48], [49]**. The special analysis of the kinetics of reduction of nitrate ion by this culture showed its completeness in boiled sausages **[50]**.

The results of studying of one of the principal functional properties to hold water by meats salted at +4°C during 24 hours in dosage of 0.0024 kg of pure kitchen and sea salts per 0.1 kg of meat showed that the use of sea salt permits to increase the quantity of retained water dramatically in use of sea salt instead of the kitchen one (Figure 3).

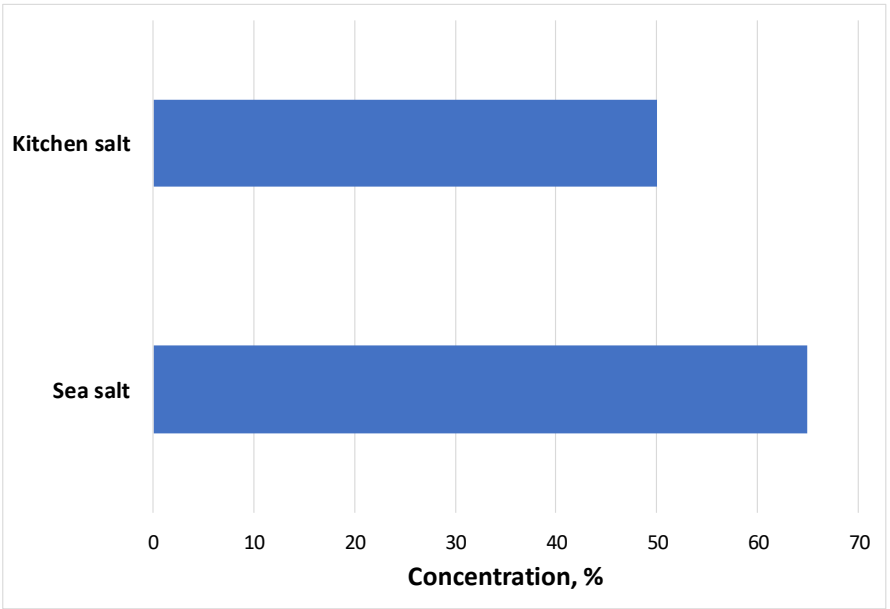
Because each ingredient of the mix influences on organoleptic and physicochemical properties of sausage meats, we studied the potential influence on such properties of mixes enriched by potassium chloride and preparation of "Vepro 75 PSC". There were studied the properties of control sausage meat, which composition conforms to the national standard of DSTU 3583:2015 **[51]**. "Kitchen salt. Specifications" and the experimental meat mixes salted by 20% solutions of kitchen salt (control sample) and by the mix of kitchen and sea salts enriched by extract of *laminaria*. The compositions of samples used in the experiment after their salting are shown in Table 3.

Table 3 Chemical composition of control and experimental samples of sausage meat, %.

Component	Designation of the sample	
	Control	Experimental
Albumen	12.3 ± 0.8	15.0 ± 0.7
Fat	9.4 ± 1.2	12.4 ± 1.0
Water	66.7 ± 0.7	70.1 ± 1.3
Sodium chloride	1.55 ± 0.1	1.2 ± 0.09
Sodium nitrite	0.0044 ± 0.0002	0.0012 ± 0.0002
Ash	0.97 ± 0.01	2.20 ± 0.01



a)



b)

Figure 3 Capability of salted meat to hold water: a) beef, b) pork.

Table 3 Chemical composition of control and experimental samples of sausage meat, %.

Component	Designation of the sample	
	Control	Experimental
Albumen	12.3 ± 0.8	15.0 ± 0.7
Fat	9.4 ± 1.2	12.4 ± 1.0
Water	66.7 ± 0.7	70.1 ± 1.3
Sodium chloride	1.55 ± 0.1	1.2 ± 0.09
Sodium nitrite	0.0044 ± 0.0002	0.0012 ± 0.0002
Ash	0.97 ± 0.01	2.20 ± 0.01

The organoleptic valuation of taste and physicochemical properties of tested products showed that all tested parameters of quality of the experimental product surpassed the ones of the control one (Figure 4).

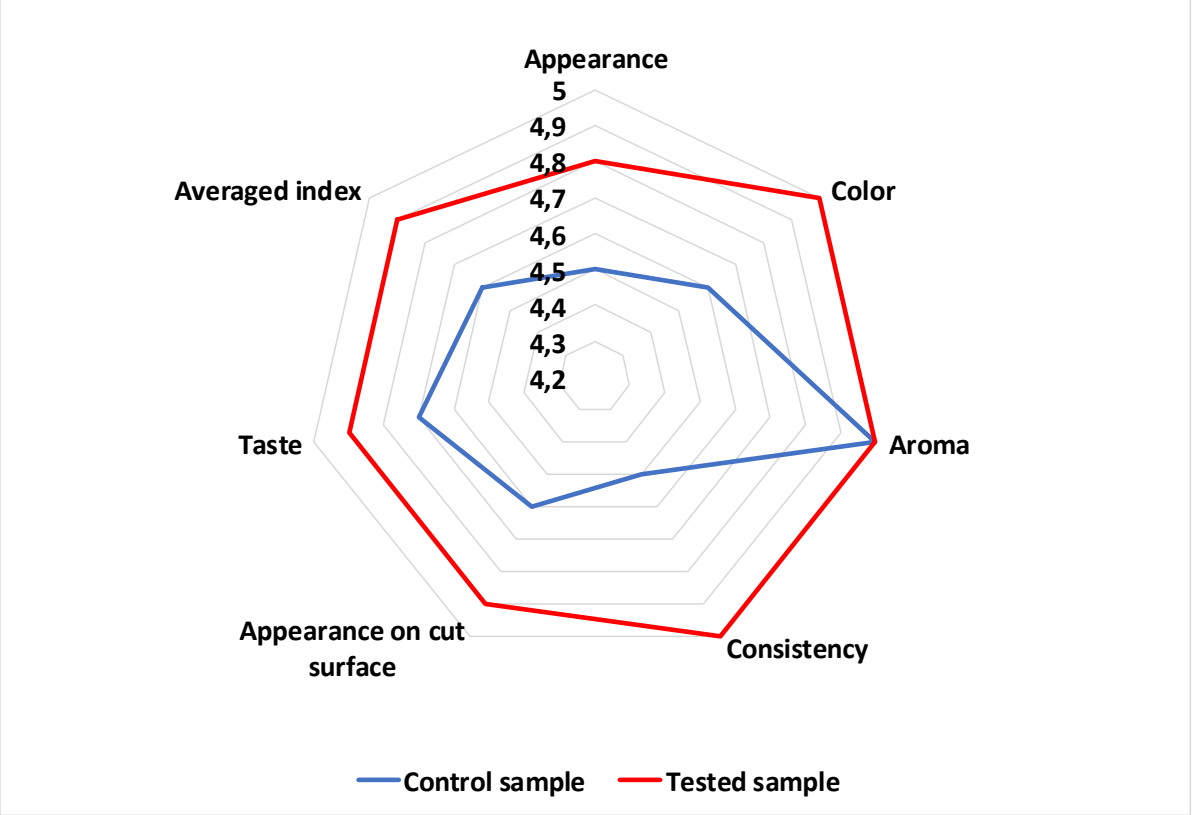


Figure 4 Results of organoleptic valuation of control quality and experimental mincemeat samples.

The physicochemical properties of sausage meats were controlled by indices of their active acidity, the activity of water, capability to hold water, and limiting shift effort (Table 4).

Table 4 Physicochemical properties of minces salted by kitchen salt and the mix of salts.

Index	Sample	
	Control	Experimental
Active acidity, pH	5.8 ± 0.1	5.5 ± 0.1
Activity of water, A_w	0.95 ± 0.05	0.96 ± 0.05
Limiting shift effort, Pa	605 ± 30	805 ± 39

It is clear from these data that replacing the usual salt for the mix of salts enriched by hydrophilic chlorides of magnesium and potassium permits an increase in the content of moisture in the mix and a decrease in the laying of meat in the mix.

Variation of the capability of the mincemeat in salting it by the sea salt shows Figures 5 and 6.

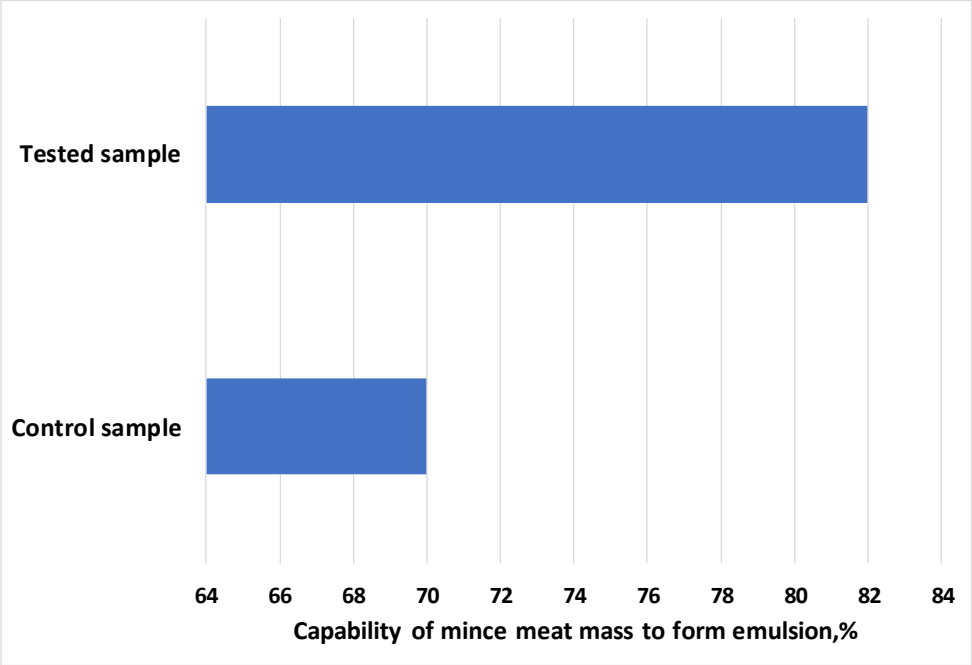


Figure 5 Capability of sausage meat systems to hold water.

Considering the results of this work, the formulation of the frankfurters character was developed by medicinal properties because of the decreased quantity of sodium in the mass.

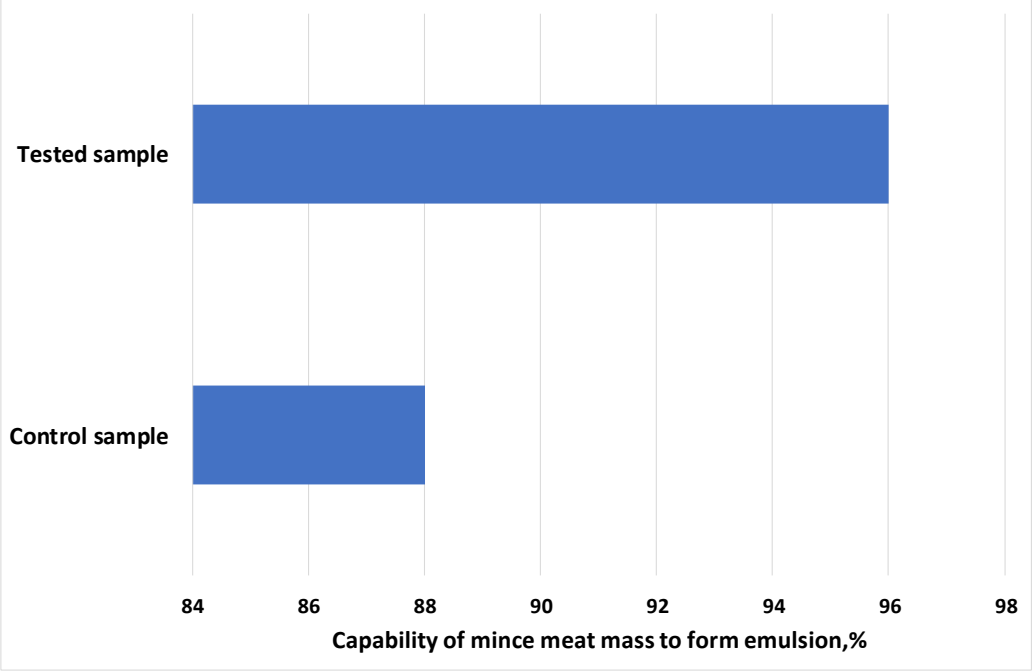


Figure 6 Stability of sausage meat systems.

Taking into account the results of experimental research, a recipe for sausages with health properties was developed sausages “Ozdorovchi” (Figure 7) by TU U 10.1-00493706-064:2019, which principal ingredients are milled beef and pork, bacterial preparation of “Iprovit-LRR”; preparation of porcine plasma blood of Vepro 75 PSC, sea salt of (Salty), citric fibers of Citri-Fi 100 and sugar.

The health properties of the product are related to several factors:

1) the product is characterized by an extended shelf-life;
2) the product is characterized by a reduced sodium content due to the use of sea salt.

Figure 7 Sausages “Ozdorovchi” by TU U 10.1-00493706-064:2019.

CONCLUSION

Based on a systematic analysis of domestic and foreign literature sources, as well as patent search, the problem of the limited range of food products enriched with digestible sodium compounds was revealed, which allowed justifying the prospects and relevance of use protein-bound forms of sodium to improve the quality of meat products.

Conducted market research, as well as consumer motivations and preferences when choosing meat products indicate expediency of launching new products with improved consumer characteristics and justify the choice of sausages for enrichment with sodium compounds.

Experimental studies have shown that replacing table salt with seaweed, which was enriched with kelp extract, can reduce the amount of sodium cation by 30%, enrich the mineral composition, maintain a feeling of sufficient salinity and enhance the health effects of the product.

It is proved that the addition of minced kelp extract can reduce the level of peroxide in the fat and meat mixture during storage for 10 days and makes it possible to slow down the growth rate of fatty acids present in the stuffing.

Therefore, there was realized the strategy of decreasing of consumption of excess sodium ions and giving the product of medicinal properties, which permits to recommend its introduction in serial production.

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Bromine in chicken eggs, feed, and water from different regions of Ukraine

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ABSTRACT

The purpose of these studies was to analyse and compare the content of bromine in samples of chicken eggs, feed, and water from different regions of Ukraine in the dynamics of 2016 – 2020: with an increased risk of bromine in products (Kharkiv, Poltava, Dnipropetrovsk and Mykolaiv regions) and outside the risk zone (Volyn, Vinnytsia and Zaporizhzhia). Studies of bromine content in eggs, feed, and water were performed in the laboratory of toxicological monitoring of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) using X-ray fluorescence analysis. As a result of the conducted researches, the increase of the bromine content in chicken eggs in the dynamics of 2016 – 2020 was established: the bromine content increased regardless of the region of the poultry farm location. The highest bromine concentration in chicken eggs was found in Kharkiv, Dnipropetrovsk, Mykolaiv, and Zaporizhia regions. Bromine source in poultry products is the excessive intake of bromine in the poultry body with alimentary environmental factors (feed and water). Bromine content in feed for chickens increased in the research dynamics (from 35.1% in the Poltava region to 2.5 times in the Zaporizhzhia region). It exceeded the established EFSA (4.4% of the total) and the average in Ukraine (51.2% of the total number of samples). In addition, the average bromine content in feed from poultry farms of the studied regions of Ukraine correlated with the number of registered and approved bromine-containing pesticides. The average bromine concentration in water sources in the studied regions of Ukraine had no significant differences compared to the beginning of the study but exceeded the maximum allowable concentration by 21.7% in 2016, 34.8% in 2018 and 39.1% in 2020. The maximum bromine concentration was in water sources in Mykolayiv, Kharkiv, and Dnipropetrovsk regions.

Keywords: eggs, bromine, feed, water, laying hens.

INTRODUCTION

Eggs are a valuable food product used directly for food or frozen egg products and dry powders production. Chicken eggs are one of the most useful products in the daily diet of a person, especially children. They contain all the necessary nutrients and biologically active substances easily absorbed by the human body **[19]**, **[20]**, **[28]**. In addition to protein, fats (fatty acids), enzymes, and vitamins, egg white and yolk also contain minerals (macro- and micronutrients). Chicken eggs are a source of iron, phosphorus, sulphur, calcium, chlorine, potassium, magnesium, and sodium. In small quantities contain silver, aluminium, boron, barium, bromine, cobalt, chromium, copper, fluorine, iodine, lithium, manganese, molybdenum, rubidium, selenium, silicon, strontium, titanium, vanadium, and zinc. Eggs may also contain heavy metals such as arsenic, bismuth, cadmium, mercury, lead, thallium and others in small concentrations. **[21]**, **[30]**, **[43]**.

However, inorganic elements that are necessary for the proper functioning of the body, as well as heavy metals, can become harmful to the human body by receiving them in high doses or in low, but for a long period **[16]**, **[33]**. It has been proven **[5]** that the introduction of such elements as silver, barium, beryllium, bismuth, cobalt, iron, gallium, mercury, potassium, magnesium, nickel, sulphur, antimony, silicon, zinc and zirconium into the diet of chickens causes increase in eggs of the following elements: bismuth, cobalt, nickel, sulphur, iron, potassium, antimony. When iodine is added to the diet of laying hens, the high content of the element in eggs is noted **[31]**,

[36]. The quality of eggs also depends on the sanitary conditions of productive poultry **[24]**. In recent years, scientists have been interested in an element such as bromine, as it is widely used in various industries (especially in agriculture) and can be included in the food chain **[6]**, **[10]**. It should be noted that today the mechanism of action of bromine on the body is insufficiently studied, and its positive physiological function is not fully proven **[25]**, **[38]**. Given that the WHO recommends human consumption of bromine in the amount of 0.4 $mg.kg^{-1}$ body weight per day **[47]**, we consider it necessary to ensure a safe level of the element in the human body, as eggs and products are widely used, and according to our previous studies **[14]**. When introduced into the diet of laying hens sodium bromide at a dose of 250.0 $mg.kg^{-1}$ of feed in egg white observed a significant increase in bromine (243.52 ± 4.39 $mg.kg^{-1}$) compared with the control group (9.06 ± 0.54 $mg.kg^{-1}$), the bird which received "background" amount of bromine 2.0 $mg.kg^{-1}$ of feed. Also, our previous studies **[13]**. found that bromine is a fairly common element in Ukraine. Its substantive content in the body of animals comes with both water and feed. Water sources with a bromine content of more than 1.8 $mg.dm^{-3}$ and feed concentrators of the element, which are components of feed for laying hens, are dangerous: barley and sunflower oilcake, the element content in which is 8 – 40 $mg.kg^{-1}$. In addition, the accumulation of bromine in feed has certain ecogeographical features: an increase in the content of bromine was mainly found in feed from the southern and eastern regions of Ukraine, especially in areas with developed industrial mining. Given the importance and scale of human consumption of eggs and egg products, this work aimed to analyse and compare the presence of bromine in samples of chicken eggs, feed, and water from different regions of Ukraine.

Scientific Hypothesis

The bromine content in chicken eggs does not significantly depend on the location of the poultry farm and its intake with feed and water gradually increases over time due to anthropogenic stress but does not exceed the WHO recommended dose of 0.4 $mg.kg^{-1}$body weight per day.

MATERIAL AND METHODOLOGY

Samples

Samples of eggs, feed and water were selected in poultry farms with increased risk of bromine contamination in four regions of Ukraine, namely Kharkiv, Poltava, Dnipropetrovsk and Mykolayiv. In addition, samples were collected in Volyn, Zaporizhzhia and Vinnytsia regions, which are outside the zone of this risk. These regions were chosen on the basis of the hydrogeological data from the State Service for Geology and Subsoil of Ukraine, as well as the cartographic data of the elevated bromine groundwater in Ukraine (Figure 1) **[35]**, **[13]**. Monitoring studies were conducted at two-year intervals in 2016, 2018 and 2020. Twelve egg samples (10 pieces per sample) and 12 samples of feed and water (once a month) were taken from each farm during the year. Thus, 252 egg samples, 252 fodder samples and 252 water samples were collected in total during the study period.

Chemicals

Standard sample of bromide ion SSSU 022.66-96, manufactured by SDTB RP IPC NASU, Ukraine;
Gallium (III) oxide, ABCR GmbH & Co, Germany, 99,999% (metals basis);
Silicium (IV) oxide, ABCR GmbH & Co, Germany, pure for analysis.

Animals and Biological Material

Laboratory and farm animals were not used directly during the studies.

Instruments

Studies of bromine content in eggs feed and water were performed in the laboratory of toxicological monitoring of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) on X-ray fluorescence spectrometer (XRF) "Spectroscan-Max-G" research and production facility Spectron" (St. Petersburg, Russia) according to the developed methodology: Determination of inorganic elements in biological substrates by X-ray fluorescence analysis (guidelines) **[12]**.

Laboratory Methods

The main parameters of the device for measuring spectral parameters: the first display - from 950 mÅ to 3150 mÅ, the second display – from 315 mÅ to 1575 mÅ. The step size of the device and the exposure time were 4.

The method use of X-ray fluorescence of elements with subsequent analysis of the spectra on the device "Spectroscan-MAX". When the sample is irradiated with X-rays, the sample, which is previously subjected to dry mineralization, begins to emit (fluoresce) in the X-ray range.

The spectrum of this secondary fluorescence adequately reflects the elemental composition of the analysed sample. The atom of each element has its characteristic spectral lines. The presence of certain characteristic lines in the recorded spectrum indicates the presence of the corresponding elements in the sample. The concentration of the element is determined by the change in the number of pulses along the characteristic line. The depth of penetration of X-rays into the irradiated sample (matrix) depends on its structure (material). The method provides

measurements with a relative error not exceeding 15% with a confidence level of 0.95. The limit for determining the bromine content by this method is: for feed – 0.27 mg.kg^{-1}, biological material (including egg yolk and egg white) – 0.18 mg.kg^{-1}, for water – 0.014 mg.mL^{-1}.

Description of the Experiment

Sample preparation: To prepare samples of eggs and feed for analysis, porcelain crucibles were selected with the volume 5 times exceeding the sample volume. For the analysis of chemical elements by X-ray fluorescence method, we took the average sample of eggs 25.00 – 30.00 g and feed weighing 10.00 – 15.00 g (with an accuracy of weighing up to 0.01 g). Water samples were prepared by 300 – 400 mL evaporation and porcelain crucibles.

Number of samples analyzed: 252 egg samples (10 pieces per sample), 252 feed samples and 252 water samples.

Number of repeated analyses: 12

Number of experiment replication: 3

Design of the experiment:

We incinerated the test material (ashing to black or gray ash) for X-ray fluorescence analysis. The crucibles were placed in a muffle furnace with an adjustable and controlled heating system. When burning samples, the optimal amount of ash is formed at a temperature of 350 – 400 °C for 4 – 6 hours, and from water samples, the dry residue is formed in 2 – 3 hours at a temperature of 250 °C.

To correct the individual fluorescence intensity of each matrix, we applied an internal standard in the form of gallium silica powder to each ash sample.

The weight of the internal standard was selected taking into account the weight of ash and the concentration of the gallium element in the standard. It was weighed to the nearest 0.01 g (usually 0.07 – 0.08 mg of the internal standard – gallium concentration of 5.0 mg.g^{-1} was added to the ash samples of feed and eggs).

The mixture of ash and internal standard was stirred with a glass rod and ground in a mortar. The mass of ash was then determined in the sum of the weight of the introduced standard by weighing to 0.001 g. The crushed material was transferred to glass vials with a volume of 10 – 20 cm^3, closed with stoppers and stored for analysis on the device.

We performed measurements on the device "Spectroscan-MAX", following the instructions for its use. The ash samples prepared for the study were placed in the cuvette of the instrument and sealed. The radiation intensity of an element in the sample depends on each specific test sample's qualitative and quantitative composition. Therefore, this shortcoming was eliminated when quantifying the element by introducing the coefficient (K1) into the basic formula, calculated by the fluorescence intensity of the internal standard (gallium) in each sample and the fluorescence intensity of gallium in the standard on silicon oxide. The total calculation of bromine in the samples was performed by formula (1):

$$X = \frac{ME_{\text{Inst}} \times \text{M}_{stGa} \times 5000}{\text{Ga}_{\text{Inst}} \times \text{M}} \quad (1)$$

Where:

X is the amount of the element in the test product, mg.kg^{-1}; ME_{Inst} - the concentration of the element in the test sample, obtained using the device, mg.kg^{-1}; M_{stGa} - mass of the internal standard in the sample, g; 5000 – concentration of gallium in the internal standard, mg.kg^{-1}; Ga_{Inst} - the concentration of gallium in the test sample obtained by the device, mg.kg^{-1}; M is the mass of the sample of the test material, g.

Statistical Analysis

The obtained results were processed by methods of variation statistics using the software package for analysis of variance (ANOVA) StatPlus 5 (6.7.0.3) (AnalystSoft Inc., USA). Correlations between the data groups were evaluated by the Pearson coefficient, the probability of the obtained results was evaluated by the Tukey criterion (HSD mean difference) at a probability level of 95.0% (p <0.05).

RESULTS AND DISCUSSION

It should be noted that the data on the content of bromine in chicken eggs is not enough: the content of bromine from 4 different regions of China, found its average content (5.51 mg.kg^{-1}), the minimum value was 1.66 mg.kg^{-1} and a maximum of 10.7 mg.kg^{-1}, and the average bromine content in chicken eggs from Pakistan was of 7.3 ± 0.5 mg.kg^{-1} **[26]**. However, in our opinion, it is best to interpret the data obtained by us in relation to the European data given in the EFSA technical report, hereinafter EFSA (max 2.6 mg.kg^{-1}) and established in Ukraine, hereinafter UA (max 4.79 mg.kg^{-1}) **[23]**. The content of bromine in chicken eggs from Kharkiv region in 2016 and 2018 exceeded EFSA by 43.5% and 54.6%, but was within the UA indicator, while in 2020 the content of

bromine in eggs exceeded both indicators: EFSA – almost 2 times, and UA – by 7.9%. Compared to the beginning of the research, the content of bromine in chicken eggs from the Kharkiv region tended to increase (by 7.8%) in 2018, while in 2020 it reliably exceeded both the initial indicator by 38.6% (p <0.05) and the indicator in 2018 – by 28.6% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Kharkiv region in 2016 were 3.23 – 4.21 mg.kg^{-1}; in 2018 – 3.45 – 4.56 mg.kg^{-1} and in 2020 – 4.86 – 5.54 mg.kg^{-1}.

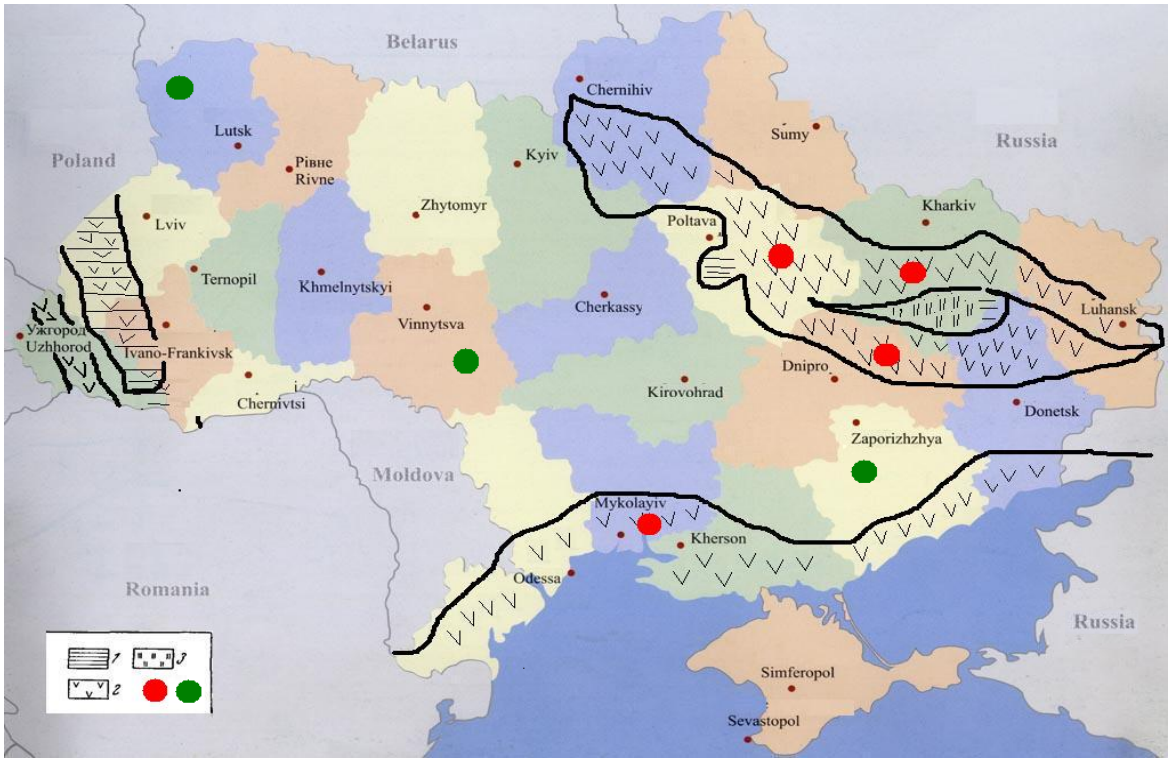

Figure 1 Schematic map of groundwater in Ukraine **[35]**, **[13]**. Note: 1 – 3 – areas of distribution of waters with industrial content (1 – Iodine, 2 – Bromine, 3 – Potassium, red (with the risk of increased bromine) and green (without risk) circle – sampling areas).

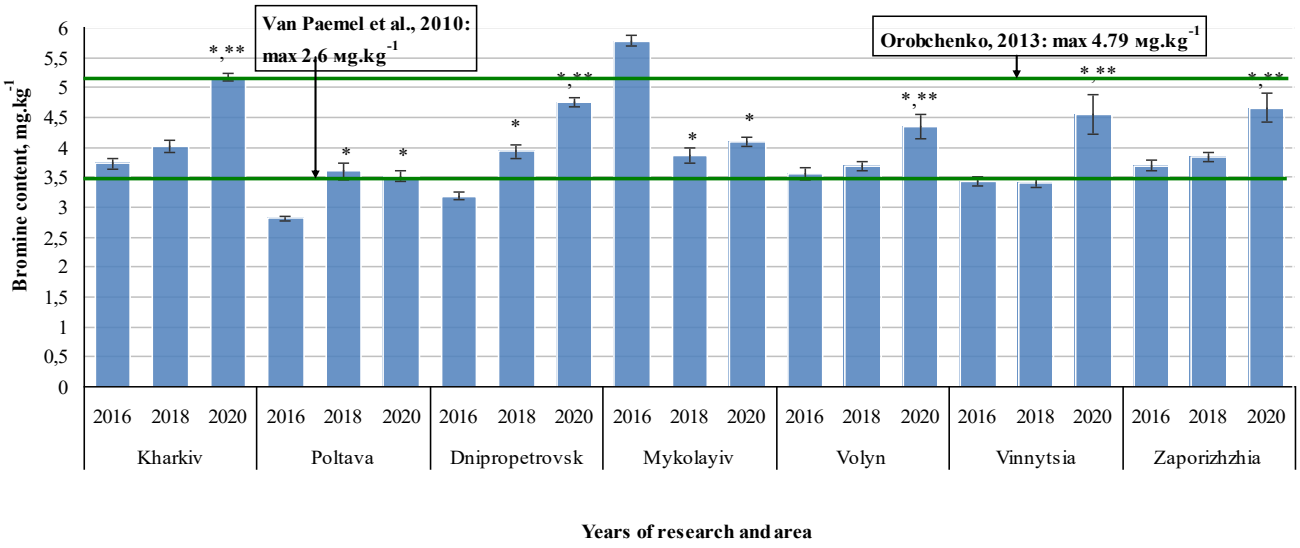

Figure 2 The results of the study of chicken eggs from different regions of Ukraine for bromine content in 2016, 2018 and 2020 (M ± m, n = 12). Note: * – p <0.05 – relative to 2016, ** – p <0.05 – relative to 2018.

Bromine content in chicken eggs from the Poltava region in 2016, 2018, and 2020 exceeded the EFSA technical report by 8.1%; 38.5% and 35.4%, respectively, but in all 3 terms of the research was within the established UA indicator. Relative to the beginning of the research, the content of bromine in chicken eggs from Poltava region in 2018 and 2020 exceeded the initial indicator by 28.1% and 25.3% (p <0.05) (Figure 2). Fluctuations in the

bromine content in chicken eggs from the Poltava region in 2016 was 2.51 – 3.04 mg.kg^{-1}; in 2018 – 2.98 – 4.19 mg.kg^{-1} and in 2020 – 2.91 – 4.01 mg.kg^{-1}.

Bromine content in chicken eggs from the Dnipropetrovsk region in 2016, 2018 and 2020 exceeded the EFSA technical report by 22.7%, 51.2% and 83.1%, respectively, but in all 3 terms of the research was within the established UA indicator. Compared to the beginning of the research, the content of bromine in chicken eggs from the Dnipropetrovsk region in 2018 and 2020 exceeded the initial indicator by 23.2% and 49.2% (p <0.05). In addition, in 2020 the bromine content in eggs exceeded the indicator of 2018 by 17.4% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Dnipropetrovsk region in 2016 was 2.82 – 3.70 mg.kg^{-1}; in 2018 3.45 – 4.71 mg.kg^{-1} and in 2020 4.27 – 5.13 mg.kg^{-1}.

The content of bromine in chicken eggs from the Mykolayiv region in 2018 and 2020 exceeded the EFSA indicator by 48.8% and 57.3%. Still, it was within the UA indicator, while in 2016, the bromine content in eggs exceeded both indicators: EFSA – 2.2 times and UA – by 20.7%. Compared to the beginning of the research, the content of bromine in chicken eggs from the Mykolayiv region decreased by 33.0% in 2018, and in 2020 the decrease was by 29.2% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Mykolayiv region in 2016 were 5.24 – 6.25 mg.kg^{-1}; in 2018 – 3.12 - 4.47 mg.kg^{-1} and in 2020 – 3.84 – 4.54 mg.kg^{-1}.

Bromine content in chicken eggs from the Volyn region in 2016, 2018 and 2020 exceeded the EFSA technical report by 36.9%; 41.9% and 67.3%, respectively, but all three research terms were within the research established UA indicator. Compared to the beginning of the study, the content of bromine in chicken eggs from the Volyn region tended to increase (by 3.7%) in 2018, while in 2020, it reliably exceeded both the initial indicator by 22.0% (p <0.05) and the indicator in 2018 – by 15.2% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Volyn region in 2016 were 3.06 – 4.09 mg.kg^{-1}; in 2018 – 3.35 – 4.13 mg.kg^{-1} and 2020 – 3.45 – 5.62 mg.kg^{-1}.

Bromine content in chicken eggs from the Vinnytsia region in 2016, 2018 and 2020 exceeded the EFSA technical report by 31.9%; 31.2% and 74.6%, respectively, but in all 3 terms of the research was within the established UA indicator. Compared to the beginning of the research, the content of bromine in chicken eggs from the Vinnytsia region did not have significant deviations in 2018, while in 2020 it reliably exceeded both the initial indicator by 24.4% (p <0.05) and the indicator in 2018 – by 24.9% 0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Vinnytsia region in 2016 were 3.02 – 3.82 mg.kg^{-1}; in 2018 – 3.16 – 3.91 mg.kg^{-1} and in 2020 – 3.07 – 5.96 mg.kg^{-1}.

Bromine content in chicken eggs from the Zaporizhzhia region in 2016, 2018 and 2020 exceeded the EFSA technical report by 41.9%; 47.7% and 79.2%, respectively, but in all 3 terms of the research was within the established UA indicator. Compared to the beginning of the research, the content of bromine in chicken eggs from the Zaporizhzhia region did not have significant deviations in 2018, while in 2020 it reliably exceeded both the initial indicator by 26.3% (p <0.05) and the indicator in 2018 – by 21.4% (p <0.05) (Figure 2). Fluctuations in the bromine content in chicken eggs from the Vinnytsia region in 2016 were 3.12 – 4.11 mg.kg^{-1}; in 2018 – 3.44 – 4.27 mg.kg^{-1}, and 2020 – 3.56 – 5.91 mg.kg^{-1}.

From research results, we can see the tendency to increase bromine content in chicken eggs in almost all areas (except the Mykolayiv region), even in those which are out of a risk zone. It should also be noted the higher concentration of bromine in eggs from Kharkiv, Dnipropetrovsk, and Zaporizhzhia regions, which is due to more developed industrial activities and, accordingly, the technogenic load on the ecosystems of the areas. Despite the above, the average bromine content in eggs from poultry farms of the studied regions of Ukraine had no significant differences compared to the beginning of the study and in 2016 was 3.74 ± 0.10 mg.kg^{-1}, in 2018 – 3.77 ± 0.04 mg.kg^{-1}, and in 2020 – 4.44 ± 0.09 mg.kg^{-1}, i.e., there was a tendency to increase by 18.7% and 17.8%, respectively, compared to 2016 and 2018).

The ingress of inorganic elements (including bromine) in products can occur mainly due to excessive entry into the body of animals (poultry) with alimentary environmental factors (feed and water) **[11]**, **[37]** which became the subject of our further research.

In Ukraine, the bromine content in the feed is not regulated. The monograph **[1]** gives the maximum allowable level of inorganic bromides in feed, 35.0 mg.kg^{-1}. In the EFSA technical report **[41]**, the bromine content in complete feeds is up to 9 mg.kg^{-1}. The maximum tolerable level of bromine in poultry feed, according to **[22],** is 2500.0 mg.kg^{-1}, which also contributes to the accumulation of bromine in the products, as there are no symptoms of poisoning due to large amounts of the element in the body of poultry.

The EFSA report **[41]** in addition to the content of bromine in the product also indicates its content in feed (max 9.0 mg.kg^{-1}), but our previous studies showed that the average content of bromine in feed for poultry in Ukraine is 3.65 mg.kg^{-1} **[13]**.

Thus, compared to EFSA data, no excess bromine content was found in the feeds of all poultry farms in the studied areas, with the exception of bromine content in feeds from Kharkiv region for the period of 2020, which exceeded EFSA data by 18.7% (Figure 3).

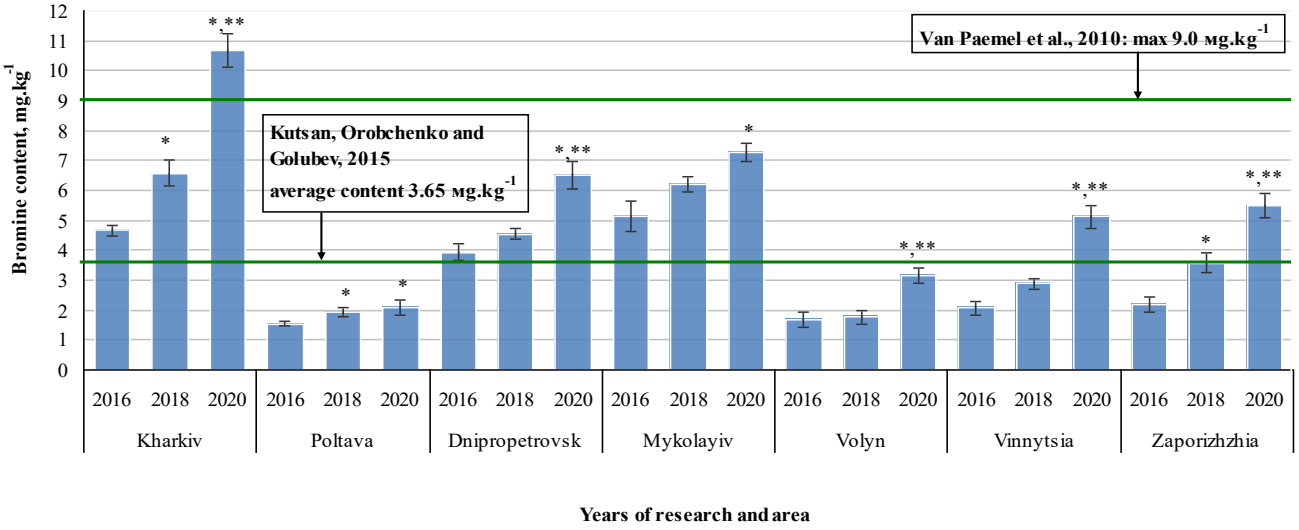

Figure 3 The results of the study of feed for chickens from different regions of Ukraine for bromine content in 2016, 2018 and 2020 (M ± m, n = 12). Notes: * – p <0.05 - relative to 2016, ** – p <0.05 - relative to 2018.

Compared to the average indicator in Ukraine, the content of bromine in feed from Kharkiv region exceeded it by 27.7% in 2016, by 80.3% in 2018, and in 2020 the excess was 2.9 times (Figure 3). Fluctuations in the content of bromine in feed for chickens from the Kharkiv region in 2016 was 4.04 – 5.75 mg.kg^{-1}; in 2018 – 5.04 – 9.18 mg.kg^{-1} and in 2020 – 7.89 – 13.49 mg.kg^{-1}.

In Poltava and Volyn regions, the content of bromine in feed did not exceed the average in Ukraine. Fluctuations in the bromine content in compound feeds for chickens in 2016 were 1.02 – 1.84 and 0.84 – 3.16 mg.kg^{-1}; in 2018 – 1.24 – 3.05 and 0.89 – 2.98 mg.kg^{-1} and in 2020 – 1.01 – 3.62 and 1.67 – 4.89 mg.kg^{-1}, respectively.

In the Dnipropetrovsk region, the bromine content exceeded the average in Ukraine by 7.9%; 24.4% and 78.1% in 2016; In 2018 and 2020, respectively, a similar situation was found in the Mykolayiv region: the average indicator was exceeded by 40.5%; 69.9% and 99.5% respectively. Fluctuations in the bromine content in compound feeds for chickens in 2016 were 3.08 – 6.12 and 3.29 – 8.69 mg.kg^{-1}; in 2018 – 3.73 – 5.78 and 4.44 – 7.47 mg.kg^{-1} and in 2020 – 4.43 – 9.62 and 5.27 – 8.59 mg.kg^{-1}, respectively.

In Vinnytsia and Zaporizhzhia oblasts, the bromine content exceeded the average in Ukraine only in 2020 by 40.5% and 50.1%, respectively (Figure 3). Fluctuations in the bromine content in compound feeds for chickens in 2016 were 0.89 – 3.33 and 1.05 – 3.81 mg.kg^{-1}, in 2018 – 2.14 – 3.85 and 2.30 – 6.78 mg.kg^{-1} and in 2020 – 3.87 – 8.26 and 3.20 – 8.23 mg.kg^{-1}, respectively.

When comparing the content of bromine in feed relative to the beginning of research (2016) it was found that in the Kharkiv region in 2018 the content of bromine increased by 41.2% (p <0.05), and in 2020 – 2.3 times, which compared to 2018 was 62.3% (p <0.05).

In the feed from Poltava region in comparison with 2016, the bromine content reliably (p <0.05) increased by 24.0% and 35.1% in 2018 and 2020, respectively.

In compound feeds from the Dnipropetrovsk region in 2018, relative to the beginning of research, only a tendency to increase the bromine content (15.2%) was established, while in 2020 the bromine content in feeds exceeded the initial indicator (p <0.05) by 65.0%, which compared to 2018 was 43.2% (p <0.05).

In compound feeds for poultry from the Mykolaiv region in 2018, a tendency to increase the bromine content (20.9%) was established, while in 2020 the bromine content in the feed exceeded the initial indicator (p <0.05) by 41.9%.

In compound feeds from Volyn region in 2018, relative to the beginning of the research, only a tendency to increase the bromine content (6.0%) was established, while in 2020 the bromine content in feeds exceeded the initial indicator (p <0.05) by 89.2%, which compared to 2018 was 78.5% (p <0.05).

In feed for poultry from Vinnytsia region in 2018 compared to the beginning of the study only a tendency to increase the bromine content (39.6%) was found, while in 2020 the bromine content in the feed exceeded the initial indicator (p <0.05) by 2.5 times, which compared to 2018 was 77.5% (p <0.05).

In the Zaporizhzhia region in 2018 the bromine content in compound feeds for poultry increased by 63.5% (p <0.05), and in 2020 – 2.5 times, which compared to 2018 was 53.1% (p <0.05).

The average content of bromine in compound feeds from poultry farms of the studied regions of Ukraine had no significant differences from the beginning of the study, but there was a tendency to increase: in 2016 the content was 3.03 ± 0.18 mg.kg^{-1}, in 2018 – 3.92 ± 0.22 mg.kg^{-1}, and in 2020 – 5.76 ± 0.32 mg.kg^{-1}, which was 29.4% and 90.1%, respectively, compared to 2016 (Figure 3).

It should be noted that excessive amounts of bromine can be released into the environment with brominated flame retardants. They are widely used in the manufacture of electronics, cars, furniture, building materials, etc. to slow down the ignition of combustible materials in case of fire.

Thus, in 2000, 38% of world production of bromine was used as flame retardants. During operation or after the end of service life and improper disposal of such products, brominated substances may leach or evaporate and thus contaminate the environment and food **[4]**, **[45]**, **[48]**. Due to the detection of these compounds in samples of various environmental objects on the recommendation of the European Commission **[2]**, Member States of the European Union should monitor their presence in food. According to Fernandes AR et al. **[6]** the highest ranges of flame retardant concentrations were found in fish, fish products and fish feed. Similar data were obtained by other authors **[3]**, **[7]**, **[27]**. Wang et al. **[42]** noted in their studies the content of polybrominated biphenyl ethers in feed and meat of various Chinese producers. In the 1980s, bromine compounds were widely used as pesticides, while today most European countries have abandoned the use of bromine-containing pesticides due to their negative impact on the Earth's ozone layer **[39]**.

In Ukraine, on the contrary, the number of such drugs is growing: according to **[18]**, at the beginning of 2012 in Ukraine, only three drugs were registered and allowed to use; at the beginning of 2016 – already 23, at the beginning of 2018 – 40, while at the beginning of 2021 – 52 drugs with active substances that contain bromine (mainly diquat dibromide, bromadiolone, bromoxynil octanoate, metobromuron, bromuconazole).

It should be noted that the average bromine content in compound feeds from poultry farms of the studied regions of Ukraine correlated with the number of registered and permitted for use bromine-containing pesticides (r = 0.96). Our data can be confirmed by the fact established on the example of methyl bromide in 1981, the possibility of accumulating about 10 – 30% of the pesticide in the soil with subsequent cleavage into bromide and inclusion in vegetable raw materials for the manufacture of feed **[44]**.

In Ukraine, there are no regulations on the rationing of bromine in water used for watering animals and poultry. In contrast, bromine's maximum permissible concentration (MPC) in human drinking water is 0.23 mg.L^{-1} [34]. The data analysis showed that in the sources from the Kharkiv region, the bromine content exceeded the MPC during the whole research period: in 2016 by 17.4%, in 2017 by 1.9 times and in 2020 by 2.5 times (Figure 4).

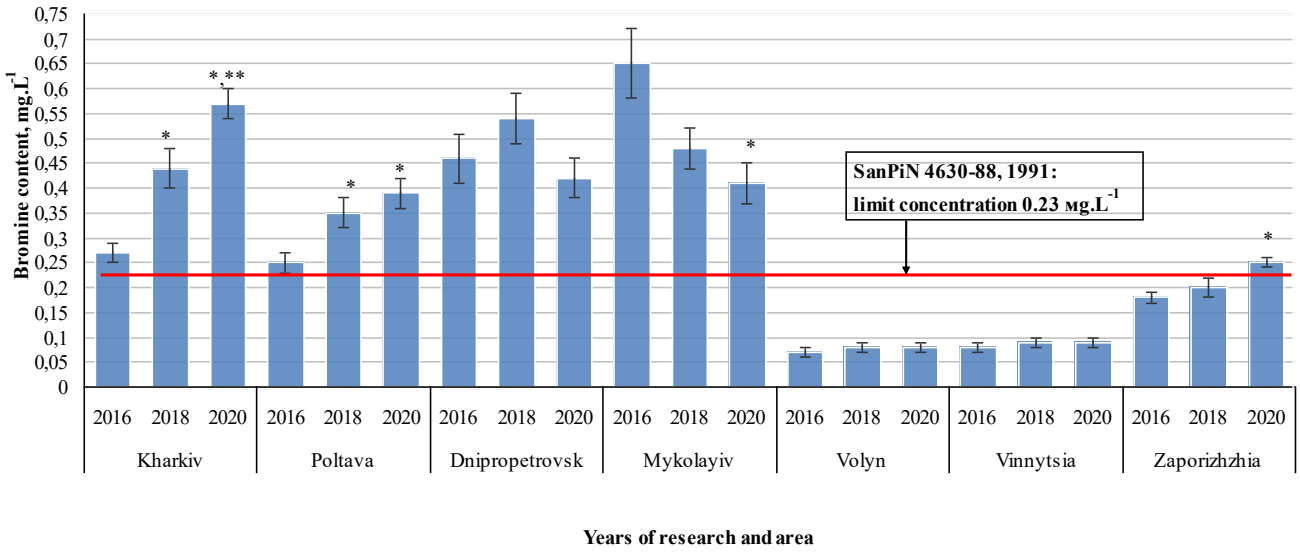

Figure 4 The results of the study of water for chickens from different regions of Ukraine for bromine content in 2016, 2018 and 2020 (M ± m, n = 12). Note: * – p <0.05 – relative to 2016, ** – p <0.05 – relative to 2018.

Compared to the beginning of research (2016), the concentration of bromine in water increased by 63.0% (p <0.05) in 2018 and 2.1 times in 2020 (p <0.05), which exceeded the indicator of 2018 by 30.0% (p <0.05)

(Figure 4). Fluctuations in the content of bromine in water from poultry farms in the Kharkiv region in 2016 was 0.17 – 0.35 mg.L^{-1}; in 2018 – 0.19 – 0.61 mg.L^{-1} and in 2020 – 0.44 – 0.82 mg.L^{-1}.

In sources from poultry farms of the Poltava region, the bromine content exceeded the maximum concentration limit throughout the study period: in 2016 by 8.7%, in 2018 by 52.2% and in 2020 by 69.6% (Figure 4). Compared to the beginning of research (2016), the concentration of bromine in water increased by 40.0% (p <0.05) in 2018 and by 56.0% in 2020 (p <0.05) (Figure 4). Fluctuations in the bromine content in water from poultry farms in the Poltava region in 2016 were 0.14 – 0.33 mg.L^{-1}; in 2018 – 0.15 – 0.51 mg.L^{-1} and in 2020 – 0.24 – 0.52 mg.L^{-1}.

The concentration of bromine in water sources for poultry from the Dnipropetrovsk region was consistently high throughout the study period (2016 – 2020) and had no significant deviations from the start of research: in 2016 the maximum concentration limit was exceeded 2 times, in 2018 – 2.3 times and in 2020 year – 1.8 times. Fluctuations in the content of bromine in water from poultry farms in the Dnipropetrovsk region in 2016 was 0.23 – 0.67 mg.L^{-1}; in 2018 – 0.25 – 0.78 mg.L^{-1} and in 2020 – 0.20 – 0.62 mg.L^{-1}.

In the sources of water for poultry from the Mykolayiv region, the reverse dynamics of bromine concentration was established: in 2018 there was a tendency to decrease (by 26.2%), and in 2020 a decrease was reliable (p <0.05) and amounted 58.5%, despite this, the concentration of bromine was higher than the MPC by 2.8; 2.1 and 1.8 times respectively in 2016; 2018 and 2020. Fluctuations in the bromine content in water from poultry farms in the Mykolaiv region in 2016 were 0.41 – 1.13 mg.L^{-1}; in 2018 – 0.24 – 0.73 mg.L^{-1} and in 2020 – 0.23 – 0.56 mg.L^{-1}. We connect the received data in the Mykolayiv region with commissioning of a new well in the poultry farm **[13]**.

In water sources from Vinnytsia and Volyn regions, the bromine content did not exceed the MPC nor differ statistically in research dynamics. Fluctuations in the bromine content in water from poultry farms in the Volyn region in 2016 were 0.02 – 0.12 mg.L^{-1}; in 2018 – 0.04 – 0.11 mg.L^{-1} and in 2020 – 0.04 – 0.12 mg.L^{-1}. Fluctuations in the content of bromine in water from poultry farms in Vinnytsia region in 2016 was 0.05 – 0.15 mg.L^{-1}; in 2018 – 0.05 – 0.12 mg.L^{-1} and in 2020 – 0.04 – 0.15 mg.L^{-1}.

In sources from poultry farms in the Zaporizhzhia region, the concentration of bromine in 2016 and 2018 did not exceed the MPC, while in 2020 the excess was 8.7%, which was 38.9% relative to the beginning of the study (p <0.05) (Figure 4). Fluctuations in the bromine content in water from poultry farms in the Zaporizhzhia region in 2016 were 0.12 – 0.28 mg.L^{-1}; in 2018 – 0.12 – 0.28 mg.L^{-1} and in 2020 – 0.18 – 0.31 mg.L^{-1}.

The average concentration of bromine in water sources from poultry farms of the studied regions of Ukraine had no significant differences from the beginning of the study, but there was a tendency to increase: in 2016 the concentration was 0.28 ± 0.02 mg.L^{-1}, in 2018 – 0.31 ± 0.02 mg.L^{-1} and in 2020 year – 0.32 ± 0.02 mg.L^{-1}, which was 10.7% and 14.3%, respectively, relative to the beginning of the study (Figure 4). It should also be noted that the average concentration of bromine in sources from the studied poultry farms of Ukraine exceeded the MPC by 21.7% in 2016, by 34.8% in 2018 and by 39.1% in 2020.

Excess bromine can get into drinking water from open sources of water supply (rivers, lakes) as a result of pollution by waste from thermal power plants (coal) and products of incinerators, disinfectants, flame retardants, herbicides **[8]**, **[17]**, **[40]**, **[46]**. However, poultry farming is a complex closed process, and each farm has its own water supply system mainly due to drilling wells, so in our opinion, there is no such tendency to increase bromine in water as for feed. However, drilling a well is not a guarantee as there is a possibility of bromine contamination of groundwater due to the extraction of minerals (especially oil and natural gas). **[9]**, **[29]**, **[32]**, **[35]** which is confirmed by the increase of bromine in the waters of Kharkiv, Poltava, Dnipropetrovsk and Zaporizhzhia regions, where gas fields are being developed.

The consumption rate of chicken eggs in Ukraine is 275 – 310 eggs per person per year, which is an average of 17.5 kg **[15]**, and in EU countries the consumption rates of chicken eggs in some countries – members of the community have significant differences: from 9.3 kg in the Czech Republic and Ireland to 29.3 kg – in the Netherlands. Based on the results of our research, the average estimated amount of bromine consumption per person (with an average weight of 60 kg) with eggs is: in the Kharkiv region - from 0.0026 mg.kg^{-1} in 2016 to 0.0044 mg.kg^{-1} in 2020, in the Poltava region – from 0.0020 mg.kg^{-1} in 2016 to 0.0032 mg.kg^{-1} in 2020, in the Dnipropetrovsk region – from 0.0023 mg.kg^{-1} in 2016 to 0.0041 mg.kg^{-1} in 2020, in the Mykolayiv region – from 0.0050 mg.kg^{-1} in 2016 to 0.0036 mg.kg^{-1} in 2020, in the Volyn region – from 0.0024 mg.kg^{-1} in 2016 to 0.0045 mg.kg^{-1} in 2020, in the Vinnytsia region – from 0.0024 mg.kg^{-1} in 2016 to 0.0048 mg.kg^{-1} in 2020 and in the Zaporizhzhia region – from 0.0025 mg.kg^{-1} in 2016 to 0.0047 mg.kg^{-1} in 2020.

The obtained data indicate that human consumption of bromine from different regions of Ukraine with chicken eggs increased 1.6 – 2.0 times compared to 2016 but did not exceed the WHO recommended human consumption of bromine 0.4 mg.kg^{-1} body weight per day. However, if the trends we have established continue, then approximately in 150 years the intake of bromine only with eggs will be half of the recommended dose.

Research perspectives: In the future, we plan to study the impact of products with high bromine content on the body of laboratory animals, as well as residual amounts of organic bromine in feed and poultry products.

CONCLUSION

The bromine content increased regardless of the region of location of the poultry farm, and the bromine content in chicken eggs from all surveyed farms at all study dates exceeded the established EFSA (in 99.6% samples of the total quantity) and the average in Ukraine (17.1% of the total quantity). Bromine enters poultry products mainly due to excessive entry into the body of birds with alimentary environmental factors (feed and water). The bromine content in feed for chickens increased in the dynamics of research (from 35.1% in the Poltava region to 2.5 times in the Zaporizhzhia region) and exceeded the established EFSA (4.4% samples of the total quantity) and the average in Ukraine (51.2% samples of the total quantity). The average content of bromine in compound feeds from poultry farms of the studied regions of Ukraine correlated with the number of registered and permitted for use bromine-containing pesticides ($r = 0.96$). The average concentration of bromine in water sources from poultry farms in the studied regions of Ukraine did not have significant differences compared to the beginning of the research, but exceeded the MPC by 21.7% in 2016, 34.8% in 2018 and 39.1% in 2020. Consumption of bromine with chicken eggs increased 1.6 – 2.0 times compared to 2016 but did not exceed the WHO recommended human consumption of bromine 0.4 $mg.kg^{-1}$ body weight per day.

Recommendations: Excess bromine in poultry diets can lead to iodine deficiency due to antagonism of these elements, as well as to excessive accumulation in poultry products, which may be one of the causes of iodine deficiency in humans. To solve this problem, it is necessary to conduct more extensive research, which would include: a comparative study of the content of bromine and iodine in soils, water, feed used in Ukraine to establish the so-called biogeochemical provinces; determination of toxicodynamics and kinetics of various bromine compounds in animals and the behaviour of the element in the environment; establishment of a scientifically based maximum permissible level of bromine in water, soil, feed and products of animal origin; determination of biological "markers" of the effect of bromine on the body and the establishment of physiological values of the element in the organs and tissues of animals; and depending on the results of research to create drugs for the correction of pathological conditions caused by excessive intake of bromine in the body.

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Entrepreneurial development in the production sphere of the regions of the Republic of Kazakhstan

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ABSTRACT

In recent years, in connection with the rise of prices for resources the aggravation of the competition, the concept of enterprise performance management has begun to be actively used. The process of financial diagnostics and assessment of the security level in economic entities' activities is one of the most debatable in the scientific community. This research aimed to study the entrepreneurial development in the milk production sphere in Kazakhstan and to find the most effective tools for ensuring the financial security of enterprises. The databases of the Bureau of national statistics of the Agency for strategic planning and reforms of the Republic of Kazakhstan and the Eurasian Economic Commission were used as the study materials. The main methods were the resource and functional approach, integrated, and financial risk. Within the framework of this study, the dairy industry of Kazakhstan was investigated. Industrial enterprises operate in challenging economic conditions. Therefore, the managerial decisions should be directed towards strengthening the financial and property status and developing strategic potential capable of adapting to unfavourable environmental factors. The scientific novelty of the study lies in the fact that the presence of an organic link between the economic situation in the state and the profit margins requires an increase in the efficiency of using the production capabilities of the enterprise. The study's practical significance lies in using the findings and developments as recommendations for improving the economic aspects of dairy enterprises.

Keywords: production efficiency, dairy industry, regional economy, strategic potential, financial security

INTRODUCTION

The modern financial system of Kazakhstan has not yet acquired the features inherent in a market economy; the disorganization of this system, which goes alongside its criminalization, poses a significant threat to the country's financial security **[1]**. The imbalance of the state budget, which is the main destabilizing factor of the crisis of public finances, and the malpractice of its untimely annual adoption, the payments crisis, leads to the establishment of the so-called debt economy. The principal debtor is the state. The development of the banking system of Kazakhstan is taking place in the context of constant changes in the economic environment and shortsightedness, which affects the instability of many banks the inability of the national currency to accumulate **[2]**. Government policy has chosen to criticise predecessors and appease the population with social payments and promises rather than solving real problems that threaten economic security in general and the financial security of milk processing plants as its component **[3]**. Thus, the internal political aspects at the present stage have a decisive influence on the critical state of the economy. Therefore, there is a constant threat to the entire national security system and the very independence of Kazakhstan and its territorial structure **[4]**.

In 2020, Kazakhstan produced 598.5 thousand tonnes of cream and processed drinking milk, 7.5% more than in 2019. By the end of the year, 189.1 thousand tonnes of processed milk and cream (about 32% of the total production in the Republic of Kazakhstan) was produced in the North Kazakhstan Region. The second place in production was Almaty Region (98.9 thousand tonnes), and the third was Akmola Region (94.9 thousand tons). For example, in the North Kazakhstan Region, more than eighty organized farms are engaged in milk production,

including eighteen modern dairy units. Moreover, new modern dairy complexes are being built in the region, and the share of animal husbandry in farms is increasing.

At present, 15 enterprises of the Akmola Region are engaged in dairy production. Their total production capacity is 146.8 thousand tonnes of products per year. The main share in the production of processed milk is occupied by Tselinograd District (LLP AF Rodina, LLP Maksimovski Molochny Kombinat, JSC Astana Onim) – 42.4%, Zerendinsky District (LLP Milk Project) – 24.9%. The leader in butter production in the city of Kokshetau (LLP Gormolzavod) – 46.3% and Zerendinsky District (LLP Milk Project) – 25%. Also, an important region in milk production is the Kostanay Region. It produced 56.5 thousand tonnes of milk **[5]**.

Based on the estimates above, it can be concluded that the essence of financial security is primarily associated with the environment of the business entity, that is, the relationships that the entrepreneur establishes in the course of business activities **[6]**. Each author gives their interpretation, but the overwhelming group of scientists considers the financial security of an enterprise as a component of economic security. But the complete definition of financial security was given by I.O. Blank: "The financial security of an enterprise reflects the protection of its activities from the negative influences of the external environment, as well as the ability to quickly eliminate various threats or adapt to existing conditions, which does not adversely affect its activities" **[7]**. Financial security, as an economical category, can be considered a set of socio-economic and legal relations that provide such an economic condition in which the stability of the enterprise to external threats and risks is revealed with the rational use of its financial resources **[8]**. The production of cream and processed drinking milk in Kazakhstan is shown in Figure 1.

Figure 1 Production of cream and processed drinking milk, 2020.

The best practices of ensuring the financial security of enterprises at the micro- and macro- levels will be the object for considering the experience of the most economically developed countries **[9]**. Due to their own national, religious, cultural factors, business entities in developed countries have their specific means of achieving financial security **[10]**. They have in common that they operate based on the following postulates: scientific knowledge is the key to the future; technology is the driving force for the development of financial security of enterprises; the responsibility of leaders is to foster the advancement of technology and science **[11]**. In developed countries, the main focus is on creating theoretical, applied foundations for increasing the financial security of an enterprise and the favourable environment for activity **[12]**. The most successful in this area were enterprises from the USA, Japan, Germany, France **[13]**. The technological revolution is one of the factors that provided these countries with stable development **[5]**.

Financial security can counteract existing risks and threats that could inflict financial losses, change the capital structure, or liquidate the enterprise **[14]**, **[15]**, **[16]**. The economic security of an enterprise is an essential component of national security. It acts as an important condition for the further operation and development of entrepreneurship in the economy. It can secure the vital interests of an economic entity from actual and potential sources of danger or economic threats **[17]**. The financial security of milk processing plants in the narrow sense is their ability to function as economic entities with the current resource provision level and the chosen production specialisation **[18]**. The financial security of milk processing plants in a broad sense is their ability to achieve an appropriate level of competitiveness at any degree of influence of possible risks and threats. Ensuring financial security at the enterprise level depends on economic, organizational and other government measures to maintain it properly **[19]**. There is a close connection between market transformations in the banking and finance sector of

the country and an increase in the level of financial security of enterprises. The financial security of the enterprise acts as:
– the degree of integration of the financial system of the enterprise into the national economic and credit sphere;
– to a certain extent, as an entity independent from the banking and financial sector of the country.

This dual role of the financial system of the enterprise has the following manifestations:
– the ability to conduct own financial policy within the framework of the current legislation;
– ability to implement financial measures for urgent financial situations at the enterprise associated with local financial miscalculations at the central level;
– ability to consistently maintain the compliance of the existing financial standards at the enterprise with the generally accepted world's practice;
– ability to respond to crisis changes in the banking and financial sector of the country.

A threat to financial security is a potential or actual action of individuals or legal entities that violate the security of a business entity and could lead to the termination of its activities or financial and other losses **[20]**. Threats to financial security include components of the external and internal environment and their relationships. They are determined through the number of failures that lead to a decrease in the economic potential of the enterprise **[21]**. In a general sense, security is a state of an object (enterprise) that can maintain development under conditions of adverse internal or external influence. Security is a degree of protection from the negative impact of any internal and external factors. The ability to fully counteract adverse effects from the external environment without attracting additional funds and personnel **[22]**. A threat to the financial security of an enterprise is an existing or potentially possible phenomenon or factor that creates a danger for the implementation of the economic interests of an enterprise and disrupts its work at the proper level.

Scientific Hypothesis

The authors aimed to analyse the best practices of the world's major economies and study the entrepreneurial development in the milk production sphere of the regions of the Republic of Kazakhstan. The expected results are that the most effective tools for ensuring the financial security of enterprises are the improvement of the legal framework, implementation of constant measures to prevent threats, a policy of effective use of personnel, using innovations in all areas of economic activity.

MATERIAL AND METHODOLOGY

Description of the experiment

The management system of milk processing plants combines various aspects: goals, functions, methods, principles, technologies, which is directed at the enterprise to achieve the established quantitative and qualitative parameters. The effectiveness of the management system of milk processing plants depends on the goals set in managing the enterprise. Therefore, the structure of the management system should be changed so that the management process promotes the maximum level of security and a sufficient level of fulfilment of specific goals. In managing financial security, it is crucial to select the necessary methods for assessing the level of efficiency, which must first meet the criteria of efficiency and reliability. This is because the management, at any time, must-have information on the current status of financial security, which is the key to the successful operation of the enterprise in the short term, and the possible risks and threats that impede the achievement of its financial interests in a long time. Accordingly, an enterprise with an unsatisfactory financial condition and a low level of financial security is limited in choosing business partners, attracting investments and loans, and the like.

The dairy industry is one of the leading sectors of the food industry in the Republic of Kazakhstan. In the North Kazakhstan, Almaty, Akmola and Kostanay regions, many enterprises are engaged in the production of milk and dairy products. Since 2010, dairy producers have constantly been expanding their distribution capabilities and adhering to a marketing strategy to reduce sales to wholesale consumers, increase sales through retail outlets and supermarkets, and attract them to cooperation.

The process of financial diagnostics and assessment of the security level in economic entities' activities is one of the most debatable in the scientific community. In various areas of the enterprise's activities, a comprehensive assessment of the level of financial security is rather complicated from a methodological standpoint and always raises controversial questions among scientists and practitioners. The resource and functional approach involve calculations using economic and mathematical modelling. It allows predicting the effectiveness and consequences of the decisions of governing bodies to make the best possible decision. An integrated approach provides for calculation using an integral indicator, expert assessment, cluster analysis, and the theory of artificial neural networks, allowing to review of the state of financial security using various approaches, which, when combined, give an optimal result. The financial risk approach analyses multilateral conflict situations considering their

mutual influence **[23]**. Actual due processes and their development are modelled when using this method to assess the level of business activity's financial security.

After analyzing the literature sources, it may be concluded that financial security was first studied at the state level. The indicators by which financial security was studied were not sufficiently formed. Kazakhstani scientists assess the financial security of the state by the following indicators: national output and income; the state of national budget execution; internal and external debt; independence from foreign capital; security with money supply; investment activity; the volume of reserve and insurance funds; development of the banking sector, stock and insurance markets.

Scientists from the United States were among the first to suggest such indicators for determining an enterprise's level of financial security. These indicators were: production index (not less than 1); revenue trends (no fluctuations); the amount of receivables and payables (to ensure solvency); market share (taking into account demand, but not decreasing); profitability (positive, at the level of 10 – 50%); investment (constant increase); share of loans **[12]**, **[13]**, **[14]**. Among these indicators, there are only two indicators that reflect financial condition. This is the amount of debt that can be used to conclude about the solvency of the enterprise, and the share of long-term loans, according to which it is possible to conclude about the structure of the company's capital and its independence from short-term financing. There are, of course, few such financial indicators for a complete assessment of the level of financial security of an enterprise, the level of its solvency, capital structure. The reflection of such indicators as production index, market share, and investment activity in the composition of indicators for determining the level of financial security is positive since these indicators allow assessing the potential that the company has for economic activities in the future **[16]**, **[17]**, **[18]**. If these indicators are growing, the share of the company in the market does not decrease; it means that the company has correctly defined its development goals and has every chance to increase the amount of profit received.

Data

We have used the database of the Bureau of national statistics of the Agency for strategic planning and reforms of the Republic of Kazakhstan and the Eurasian Economic Commission.

Samples

The activities of factories engaged in milk processing in Kazakhstan were analysed. Among them are the following: LLP AF Rodina, LLP Maksimovski Molochny Kombinat, JSC Astana Onim, LLP Milk Project, LLP Gormolzavod, Burnenskaya Molochnaya Kompaniya LLC, Agrofirma-Tau company.

Statistical Analysis

The risks of the enterprise are assessed based on the statistical approach, which compares the losses with levels of risk. The maximum unacceptable level of risk appears when an enterprise risks its funds, that is, all its property. Analysis of the research results using descriptive statistics Microsoft Excel produced the statistical analysis data.

RESULTS AND DISCUSSION

Dairy production enterprises were selected to assess the status of development. To analyze the external production environment of milk processing plants in Kazakhstan, it is necessary to study the industry's level and dynamics of action **[3]**. The main task of the industry is to provide the population with high-grade food products. In the system of agro-industrial production, the manufacturing of dairy products is closely related to agriculture as a producer of raw materials, which indicates that the situation in the agricultural sector significantly affects the activities of milk processing plants. In recent years, the negative trends in the country's economy have had a particularly tangible effect on the processing industries, including dairy products. Dairy production refers to the food processing industries. This food group includes milk processing, butter, and cheese production. Among the food produced, milk and dairy products occupy a leading place in Kazakhstan; since dairy products are important sources of proteins, vitamins, minerals, micro- and microelements, they are widely used in nutrition, especially by certain groups of the population (children, older adults). Several milk processing products (casein, milk sugar) are used in other production industries **[1]**, **[2]**, **[3]**, **[4]**.

For the period 2013 – 2019, the activity of milk processing plants was accompanied by various crisis phenomena, such as a shortage of raw materials, an increase in prices for dairy products, a decrease in population demand, and competition from foreign producers. In Kazakhstan, dairy production is significantly lower than the volume of consumption required by the population **[15]**. This affects the quality of food for the citizens of the country. Dairy production itself cannot grow rapidly since the profitability of animal husbandry is low, and the country's population is unable to buy the required number of dairy products due to low purchasing power and high prices for dairy products.

Dairy production in the past decade has been characterized by a slight increase in production volume, a lack of expansion of the range of products, a high wear rate of fixed assets, and the absence of a favourable credit policy

in the country. The priority task of researching the market for milk processing plants is to study the demand for dairy products from the population, study the purchasing power of the people, study the possibility of reducing the production cost, and state regulation of prices for manufactured products as a strategically important. Such a survey will allow effective product development programs, quickly respond to the market situation, and effectively counter the competition **[16]**, **[20]**, **[23]**. To make rational decisions, reliable information on the market needs, consumer groups, existing competitors, and production volumes is necessary. Depending on demographic indicators, groups are distinguished by choice of dairy products. The analysis of milk yield in the Western Region of Kazakhstan for the period from 2013 to 2019 allows us to draw the following conclusions:

–For the analyzed period, milk yields in the considered region decreased much slower than the average in Kazakhstan. As a result, almost a third of its volume is currently produced there, and, therefore, there is a significant raw material potential for milk processing plants;

–the share of the Western Region in the volume of milk production is almost 33% and exceeds the percentage of dairy products, which barely reaches 10%. It can be concluded that local milk processing plants are not using their raw material potential enough, and raw materials (milk) are exported outside the region.

For dairy production in Kazakhstan, the most pressing issue is the provision of dairy raw materials (Figure 2). When supplying raw milk to milk processing plants, the following problems can be distinguished:

1. Changes in the structure of milk supply for industrial processing. At the moment, the largest suppliers are private farms. This indicates the need to improve cooperation with farmers since it is more profitable for the population to sell manufactured products locally or in other regions.
2. With the decrease in milk yields, the competition for the supplier of raw materials has intensified. This indicates the need to improve the pricing policy of milk processing plants and install modern equipment for storing and processing raw materials.
3. A decrease in the number of cattle is a threat from the external environment to the financial security of enterprises. This indicates the need to create subdivisions for raising cattle or merge such enterprises with farms that raise cattle to increase their number.

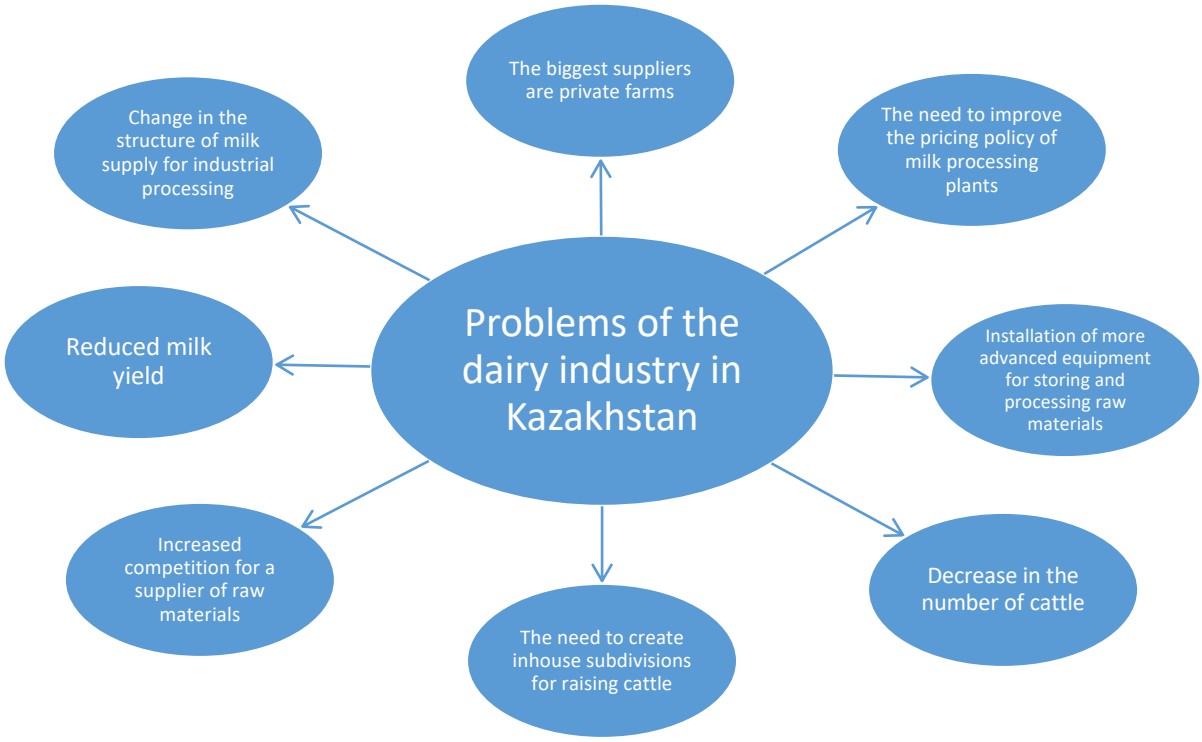

Figure 2 Problems of the dairy industry in Kazakhstan.

More than 300 factories are engaged in milk processing in Kazakhstan; however, about 80% of these enterprises are united in groups. Today, the Terra Food company is the most influential in milk and dairy products, with total revenue of KZT 3.435 billion in 2015. The company includes 19 whole milk products, cheese, vegetable-butter spreads, and butter. In 2016, the company accounted for a sixth of cheese and cheese products produced in the country. Terra Food company supplies its products to more than 40 countries, including the Middle East, North Africa, the Balkans, China, the USA and North Korea. The Burnenskaya Molochnaya Kompaniya LLC is the leader in milk sales in Kazakhstan (22% of the market). Six enterprises of the company produce and export

dairy products, cheeses, dry milk whey. The company exports its products to 35 countries, including Arab-Muslim countries. In December 2015, it received certificates for the export of dairy products under the Merkenskiy Syrzavod trademark to China, and since January 10, 2016, the right to export to EU countries. In 2016, the Burnenskaya Molochnaya Kompaniya exported 550 tonnes of butter, 140 of which to the EU, 382 to Morocco and Egypt, and 25 tonnes to the UAE. Total revenue in 2015 amounted to KZT 3.3 billion. The Agrofirma-Tau company is a division of the French food company.

In Kazakhstan, the company processes 150 thousand tonnes of milk annually. It specializes in producing yoghurts, cottage cheese, and baby food. The facilities of Agrofirma-Tau are located in Aktau. Now, these products account for 5% of the total production in Kazakhstan. The total revenue has amounted to KZT 2.1 billion. The JLC-SUT company is one of Kazakhstan's largest cheese and cheese producers. It occupies 23% of the market. The company includes seven enterprises located in Almaty with a total processing capacity of over 620 thousand tonnes of milk per year. In addition, the company is a leader in the production of butter and spreads with a 16% share. The company supplies its products to more than 50 countries, including the CIS countries and Africa, the Middle East, the USA, Mexico, and Japan. The total revenue amounted to KZT 1.986 billion. The Sairam Sut company includes eight enterprises that produce whole milk products and cheese under the Sairam trademark. The total revenue amounted to KZT 1.780 billion.

There is a tendency to displace smaller milk processing plants and their absorption by larger enterprises in the dairy industry. If this trend continues in the future, only a few enterprises may remain in the milk processing market, which will belong to several companies. To characterise the external environment of milk processing plants, it is necessary to highlight the critical success factors, including raw materials, technology, and the ability to sell. The company's successful activity is based on correct and timely strategic decisions. These decisions have a decisive impact on the competitiveness of products and the enterprise. By overcoming the crisis at milk processing plants, access to foreign economic markets and increased export of products can be achieved **[7]**, **[12]**, **[14]**. When analyzing the external environment of enterprises, it is necessary to consider the foreign financial market of dairy products.

The stability of the milk processing industry depends on the strength of the work of each business entity. In turn, the stability of the business entity depends on the efficiency of the financial security management system **[11]**, **[18]**. The functioning of the financial security management system for milk processing plants should provide for the interconnection of goals and objectives of each level, choosing the best ways to implement decisions. The primary purpose of the functioning of the financial security management system is to ensure financial and economic balance, achieve specific performance efficiency set goals and objectives for the further development of the enterprise, create and implement conditions that provide the financial security of the enterprise. These conditions are determined based on the criteria for assessing the level of financial security. The essential conditions that are considered in the financial security management system structure are minimization of enterprise expenditures and adaptation to innovations. These conditions can significantly impact the company's profit, thereby ensuring its financial security (Figure 3).

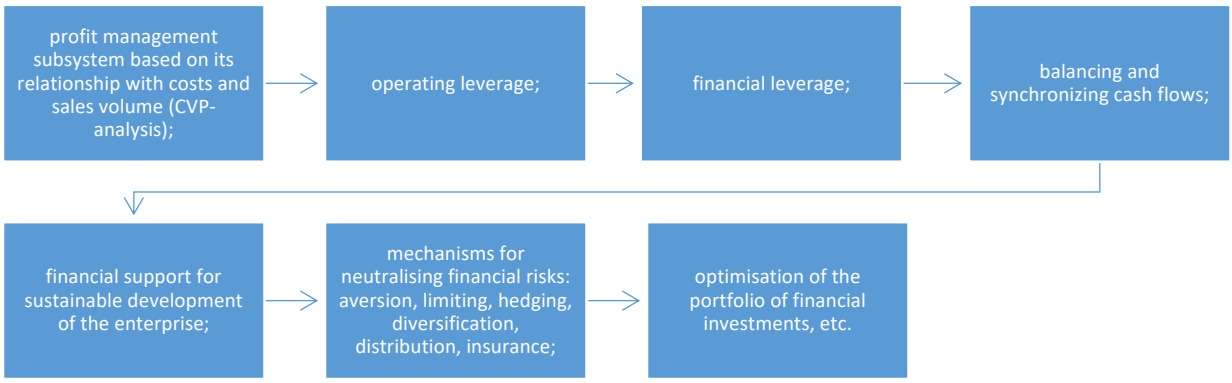

Figure 3 Financial security management system of a milk processing plant with mandatory functions.

From the standpoint of the process and functional approach, financial security management is defined as a phased, continuous process of performing management functions that describe its content. The main functions of

financial security management of a milk processing plant are defined as follows: organization, analysis, planning, motivation, and control. In 2020, Kazakhstan was supposed to switch to a new regulation to assess milk quality **[3]**, **[17]**. However, at the very end of last year, by the decision of the EAEU Council, the transition was postponed for five years. This does not mean that Kazakhstani milk is of poor quality – it meets all the standards of the Eurasian Economic Union, except for microbiological ones **[9]**, **[22]**. But to solve these microbiological problems, the dairy industry requires a severe restructuring at the macroeconomic level.

The dairy products market of Kazakhstan experienced significant difficulties in 2019. On December 31, the deadline expired, after which processing plants had to start accepting raw milk of uniformly high quality for processing. Such rules are dictated by the Technical Regulations of the Customs Union 033-2013, which regulates the safety of dairy products in the EAEU. According to this regulation, quality grades of milk are abolished, and one quality is introduced – the highest. Any milk that goes for processing must be entirely safe for humans. To achieve this, Kazakhstan needs to bring milk production standards back to normal and eliminate the microbial environment in milk.

According to the Dairy Union of Kazakhstan, the milk market amounts to 5 million tonnes. Only 1 million tonnes of this are marketable milk, suitable for processing. According to the Ministry of Agriculture of Kazakhstan, currently, 164 enterprises are engaged in milk processing. Thirty-five milk plants have their dairy farms and do not depend on suppliers. The remaining 129 factories (80%) buy raw materials on the open market. The total processing capacity of the factories is about 2 million tonnes of raw materials per year. The factories are half loaded. In winter, due to a shortage of raw materials, the load drops to 20%, which causes some factories to close **[5]**, **[6]**. The market is currently dominated by small milk producers, which significantly complicates the transformation of the dairy industry. Competitiveness in the market suffers due to the difference in prices for milk production by different factories. Because of this, the profit margin is entirely different for each company.

Upon analyzing the financial condition of milk processing plants in Kazakhstan for 2013 – 2019, it was concluded that it is critical. In the considered milk processing plants, financial security management is carried out by structural, organizational units: the financial and economic department (diagnostics of the financial condition, neutralization of financial risks, etc.); marketing department (monitoring the external environment, competitive intelligence, etc.) and the legal department (legal protection), HR department (personnel selection) and others. A particular unit was not created under such management, which led to critical financial conditions. These problems and an increase in the number of threats, risks, and dangers in economic activities necessitate the creation of a specialized unit (department, service) of financial security at the enterprise **[13]**, **[20]**.

The first step describes the financial strategy of the dairy processing company (the establishment of long-term tasks of economic activities) and depends on the features of the implementation of the company's financial security management. The second step in the implementation of monetary policy is to formulate a strategy for managing financial security, namely: in marketing research, making pricing decisions, focusing on the money market sectors, working capital and return capital, providing financial resources, balancing the timing of the receipt of money, ensuring profitability, and so on. In the third step, a financial department is created, whose functions include determining the monetary tactics of the company and its implementation. In terms of size, such a department can be represented by management, a department, or the department’s functions are assigned to a staff unit of the company (manager, assistant, chief accountant). This general model of the company's tactics and the tactics of managing financial resources is updated by the globalization of the economy the internationalization of markets.

Financial security management of milk processing enterprises is defined as a purposeful activity, which consists of the constant process of making and implementing management decisions to reduce the negative impact of risks and threats and achieve the maximum level of economic security of milk processing enterprises. We will divide the logical-structural scheme of financial security management of dairy processing enterprises into current and prospective management. In turn, we will divide the assessment of the level of financial security of an enterprise into evaluations of the actual level of economic instability, assessments of the forecast level and determining the probability of establishing the predicted level of financial instability, which determines the optimal strategy for ensuring the financial security of dairy enterprises. The proposed conceptual approach to the establishment of a financial security management system for milk processing enterprises provides for the implementation of a mechanism for ensuring and assessing the level of financial security, as well as a set of measures aimed at using the capabilities and resources of milk processing enterprises, providing systemic-synergistic effects of protecting its economic interests from identified actual and potential threats of external and internal nature, as well as the achievement of stable and effective functioning and the set goals and objectives for the further development of milk processing enterprises.

The developed approach includes five sequential stages. It consists of the substantiation of the goal and objectives of financial security management, development of the concept of financial security management,

implementation of a mechanism for ensuring financial security management, assessment of the level of financial security, and formation of a set of measures to ensure the financial security of milk processing plants. The financial security management of milk processing plants should be carried out, considering the influence of both individual factors of the external and internal environment and their synergistic effect on activity and development. Protection from the external environment, where factors of a macroeconomic nature operate, includes the state and directions of development of the general economic interests of the state, the conjuncture of the financial and stock markets, the development of the financial and credit system of the state, the impact of international financial and economic institutions. Developing financial institutions' infrastructure requires creating a system monitoring and attracting qualified financial analysts for objective analysis and assessment of trends and consequences of macroeconomic processes. Moreover, it is essential to determine the issues that the enterprise analysts can solve, which is worth attracting external experts.

The protection from the internal environment factors is defined by the problems of interconnection and coordination of the general strategy of activity and development of enterprises with the possibilities of ensuring their investment and financial potential. To ensure the achievement of strategic goals in the activities of enterprises, it is necessary to periodically compare the results of the analysis of external and internal factors and, if necessary, adjust both strategic and tactical intentions. Given the multifaceted nature of the financial security category, the complexity of the interrelationships and interdependencies of its various elements, it is impossible to identify all problematic issues immediately and propose specific measures to eliminate them. In this case, it is advisable to determine the indicators that have significant importance and influence on the support of activity and sustainable development of an enterprise.

In the economic literature **[1]**, **[2]**, **[3]**, **[8]**, **[10]**, there are various approaches to the formation of a system of indicators of economic security, while the criteria for financial security are the least studied. This study suggests that these criteria should include, first of all, essential resource and performance indicators. They must define the stability of the state's economic system (and even its structure) as a whole and its regions – as characteristics of the development of socio-economic processes at the macro level. Such features are of significant importance for business entities in the form of financial and credit institutions and entrepreneurial structures of the production direction since they characterize the dynamism of the development of the financial and economic mechanism of the state, its adaptability and effectiveness of influence on inflationary processes, the stability of the national currency, the development of foreign economic relations in the legal and trade areas. The system of indicators that are formed at the level of business entities should include those that are documented in the system of financial, managerial, statistical, and operational accounting organized at the enterprise, as well as the predicted values of private and integral indicators obtained using economic and mathematical modelling and forecasting **[19]**. At the same time, in the financial management system of enterprises, special attention should be paid to and tracked the dynamics of the financial condition of financial institutions, counterparties in the scheme of technical and technological relations, intermediaries, and the like.

The financial security strategy should clearly define the facilities and the level of threats caused by the actions of the external and internal environment and how to ensure each facility's security. It should be noted that it is impossible to establish the threat level's quantitative characteristics for all objects. This is because the classification of threats has qualitative attributes of the feature. These pairs of threats should be referred to them: explicit – implicit; real – virtual; external – internal; objective – subjective; spontaneous (random) – those that are characterized by purposefulness; those that can be eliminated – those that cannot be eliminated yet; single-aspect (simple) – multi-aspect (complex); based on symmetric or asymmetric information; brief – constant (that is, short – or long term); progressive – degressive; those that require the development of measures and the organization of continuous monitoring – those that need periodic diagnostics **[21]**.

The difficulty in determining the nature of threats is because they result from the motives that cause their occurrence and the action of various subjects: the state, financial institutions, counterparties – business entities. Based on the nature of threats, it is necessary to determine the main consequences of their influence. In addition to that, promising and latent threats should be given qualitative and, if possible, quantitative predictive characteristics of the probability of their occurrence. For the attributes of threats that may affect the level of financial condition and financial results in the current calendar period, variable (within the coordinates: maximum-minimum) quantitative estimates should be determined. Ensuring the financial security of the activity and development of dairy enterprises should not be considered a problem of one functional unit of the enterprise. Financial security is influenced by decisions made at all management system levels (along with the system of vertical-horizontal and functional-horizontal links). Therefore, when organizing an effective financial security system, the specifics of the organizational and managerial management structure should be taken into account and promptly changed.

Considering the multiplicity and diversity of factors affecting the financial security of enterprises and their situational nature, each organization should create its bank of possible threats in the context of objects and a bank of possible options for measures to eliminate them. Especially effective is developing a bank of proactive and preventive measures. There is no doubt about creating such a bank, taking into account the provision of prompt decision-making in the current response system to a certain financial or production and economic situation. The main conditions for applying the extrapolation method are the invariability of the development trends of business entities (indicator), a clear manifestation of such trends and their positive character, the correspondence of the nature of the indicator. This method is applied when developing a plan for income and expenses. Thus, having considered the content of the above methods of strategic planning, the following conclusions can be drawn: the choice of a method is determined by many factors, for example, the duration of the planning period, the goals of the plan, the availability of the necessary information, the possibility of using software products, the qualifications of specialists carrying out strategic financial planning. Therefore, before deciding on a method of strategic financial planning, a business entity must approach it carefully and take into account the factors that affect its development. Forecasting milk production in Kazakhstan by extrapolation is presented in Figure 4, using time series data from 2011 – 2019.

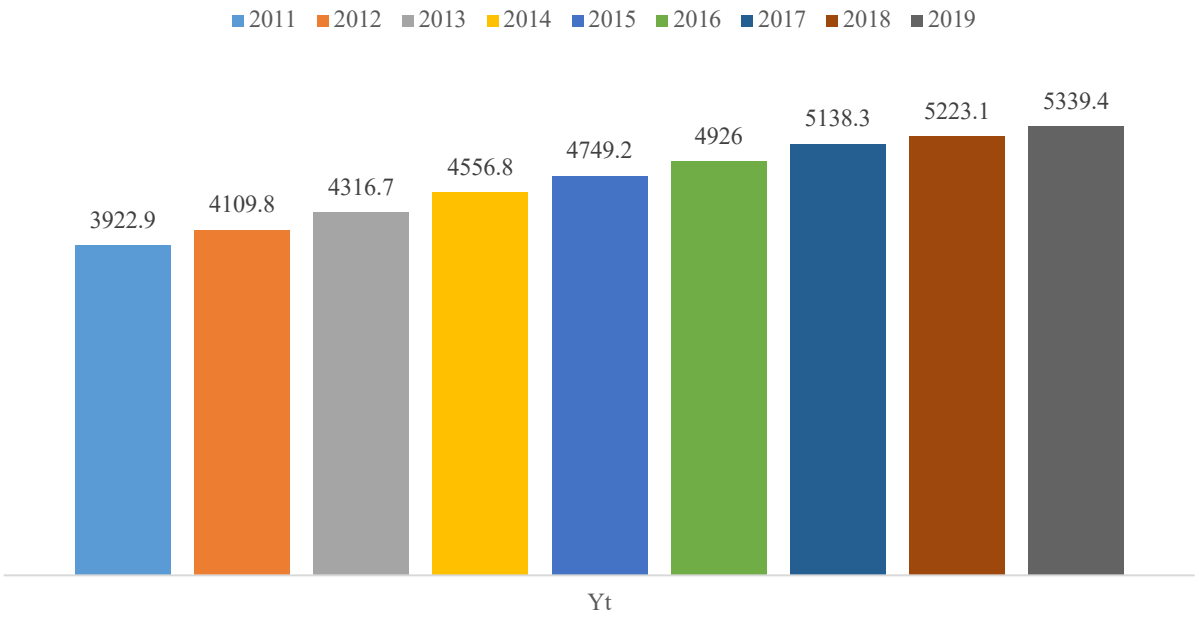

Figure 4 Milk production in the Republic of Kazakhstan for the period 2011-2019, thousand tonnes. Note: Source **[23]**.

Using the trend dependence equation (1), time series smoothing is obtained as one of the main methods of regression analysis – the least-squares method **[9]**:

$$\hat{Y}t = 3323.393 + 201.657t \quad (1)$$

The extrapolation method consists in substituting the trend equation for the value of the independent variable t, which corresponds to the value of the forecast period (Figure 5). The regression model assumes that the predicted values fall within the upper and lower bounds interval based on which the forecast was made, with a confidence level of 0.9 (or 90%) (Figure 6).

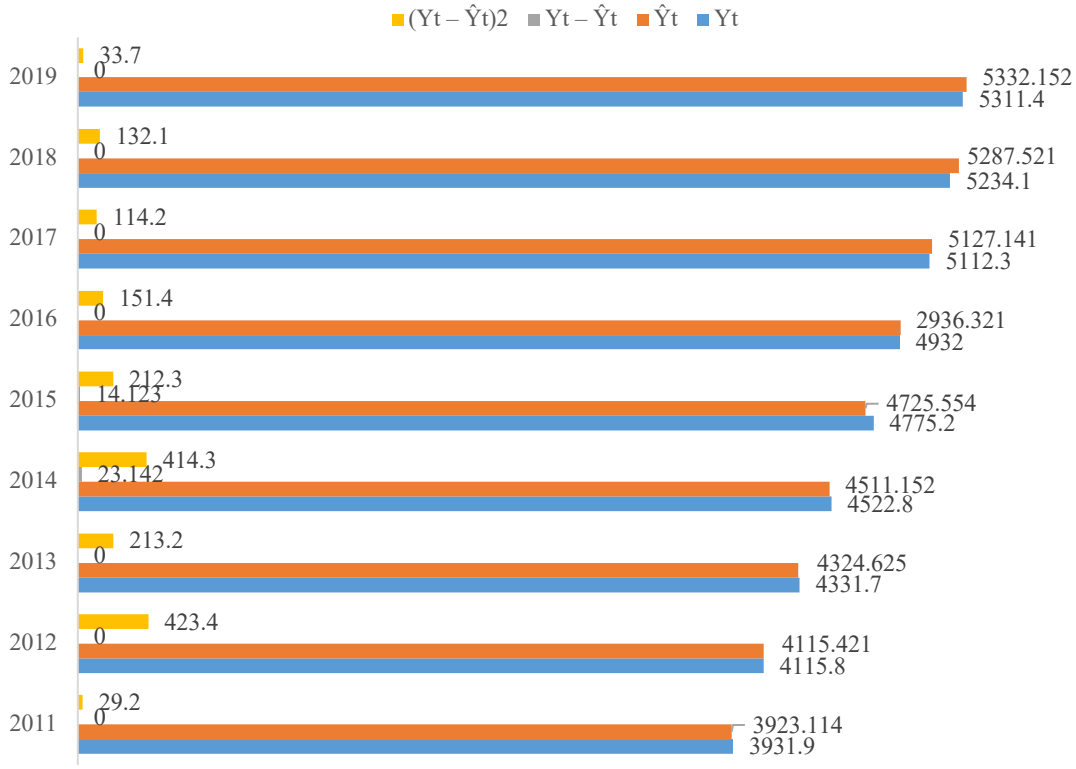

Figure 5 Estimated parameters of the model.

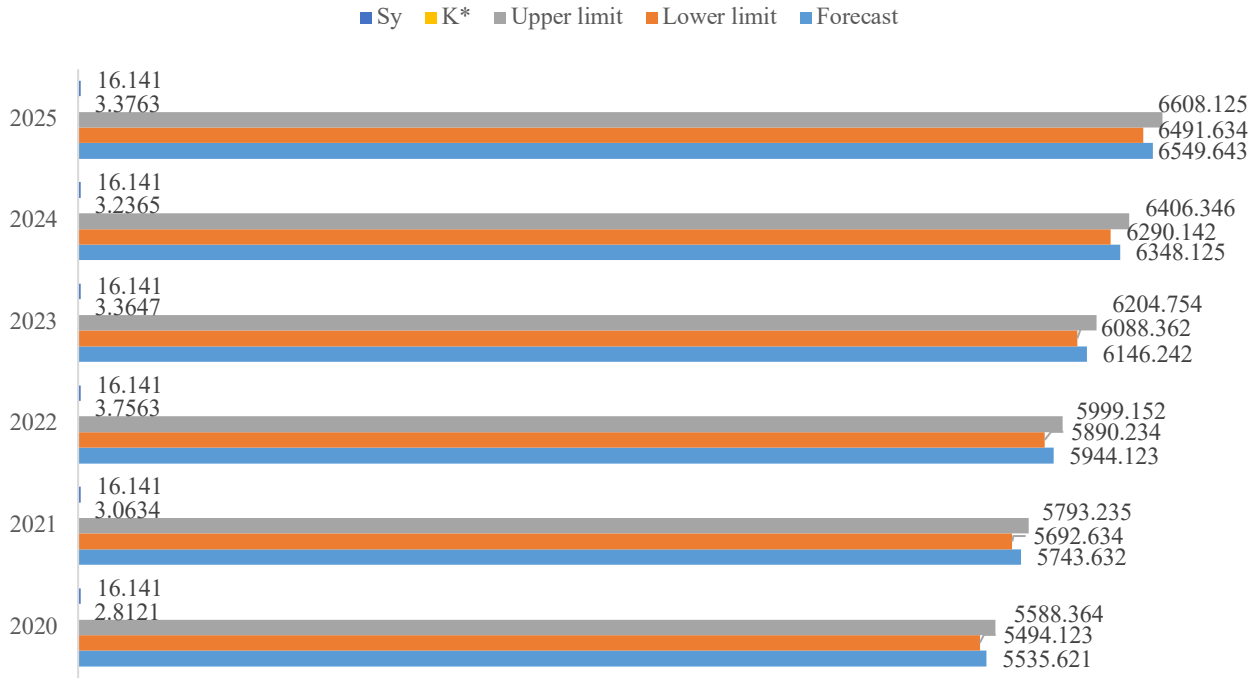

Figure 6 Forecast of milk production in the Republic of Kazakhstan for the period 2020 – 2025, thousand tonnes.

The study suggests that strategic financial planning can be called long-range or advanced, but not every long-range financial plan can be strategic. The horizon (and the range) of planning is the period it spreads. On this basis, long-range (1 – 5 years ahead), medium-range (3 – 12 months in advance) and short-range (1 – 3 months) planning are distinguished. In some cases, other ranges are also used – for example. A long-range plan is drawn up for ten years in advance or for the current coming week. Studying the standard features of financial planning and forecasting reveals the unity of goals and objectives. Forecasting creates the basis for planning management decisions generates development options based on possible directions.

The differences between them are due to the time factor, and the level of uncertainty since forecasting is the research base for planning through the anticipatory nature of the forecast relative to the plan. In addition, forecasting has a variant content, and planning is an unambiguous decision, even if developed on a variant basis. The projected indicators of milk production in Kazakhstan for 2020 – 2025 are presented in Figure 7, taking into account the upper and lower boundaries of the interval. Extrapolation allows seeing the trend with the exponential smoothing method. The comparison of the projected values for each aspect of dairy production is presented in Figure 8. Statistical information on cattle and average annual milk yield was used to calculate **[23]**.

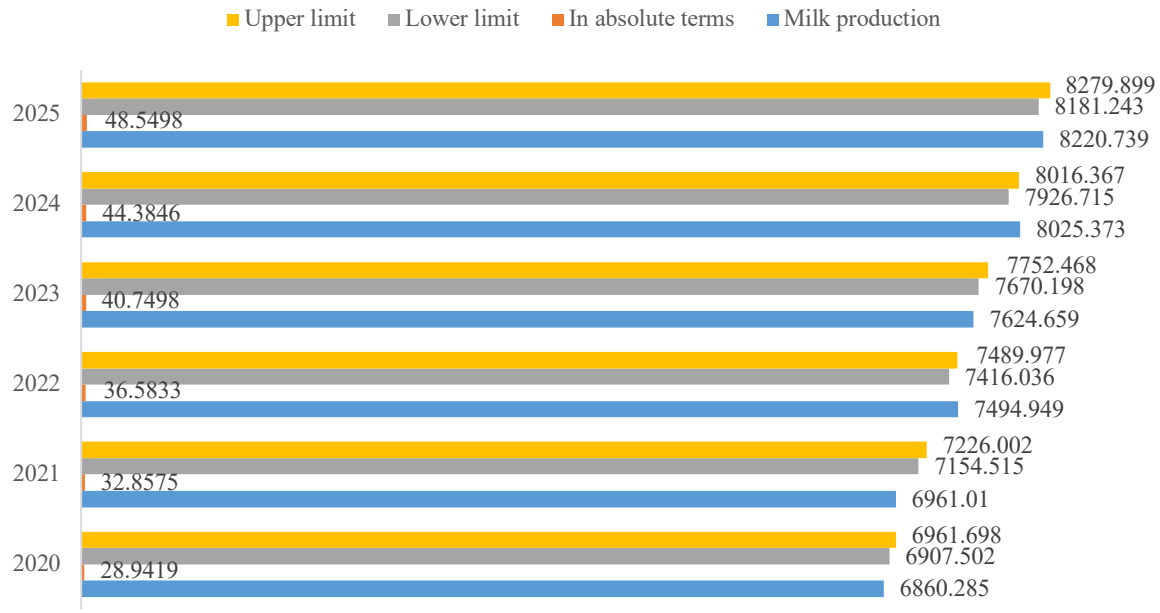

Figure 7 Forecast of milk production in the Republic of Kazakhstan for the period 2020-2025, thousand tonnes.

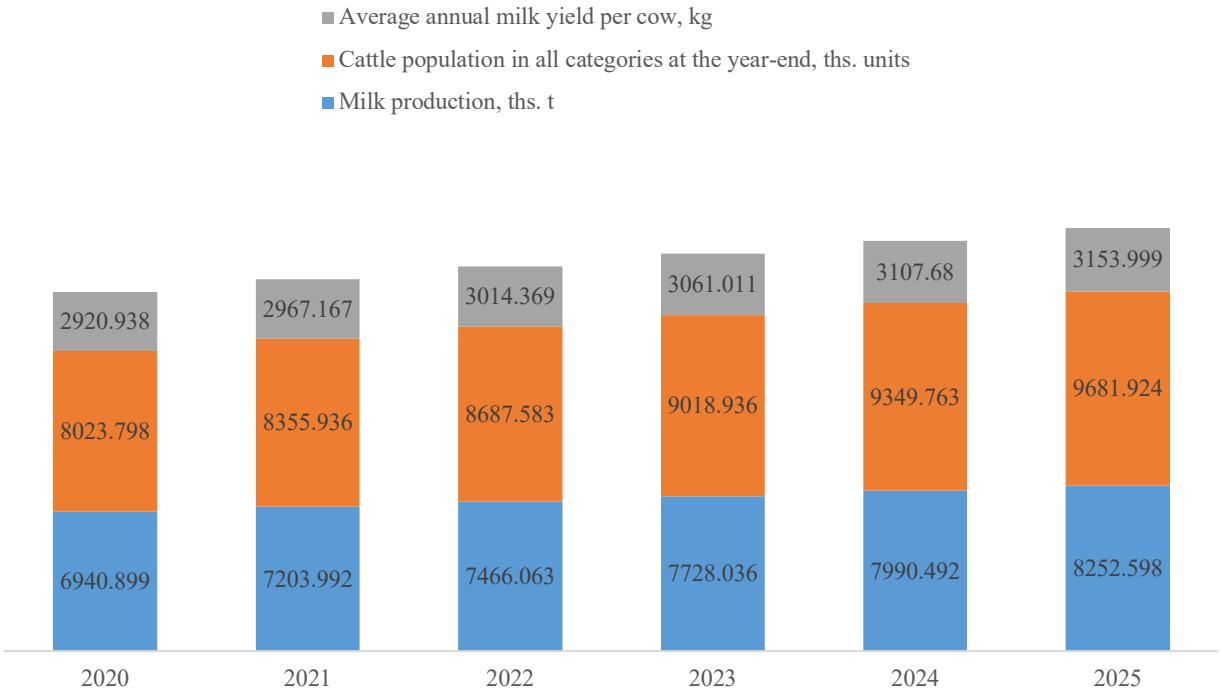

Figure 8 Forecasted values of dairy products in the Republic of Kazakhstan according to the given forecasting methods.

Using the extrapolation method, a 19% increase in milk production in Kazakhstan is expected by 2025. This is directly related to the increase in cattle population by 21.2%. The expected milk yield per animal is likely to be in the range of 250 litres per year, which is also a significant increase in the baseline values.

CONCLUSION

Financial security, as an integral part of the economic security of an enterprise, should be considered and solved based on an interdisciplinary approach. It includes, first, aspects of a legal, financial and economic, informational, socio-psychological nature. All methods and organizational forms of protection should not go beyond the limits of the current legislation. In the last case, non-compliance with this requirement leads to protracted litigation and arbitration processes that undermine not only the financial and economic but also the moral and ethical positions of the enterprise in the business space. Of note is that the financial and economic security of the activity and development of economic entities is inextricably linked with the general strategy and tactics of the action and development of the enterprise. It is formed, first, by such components of their policy as marketing, investment and innovation, personnel, information, etc., the efficiency of which is provided by their inherent methods and tools.

In this study, the dairy industry in Kazakhstan has been investigated. Problems of the dairy industry in Kazakhstan were analysed and their possible solutions were suggested. Forecasting of the projected indicators of milk production in Kazakhstan for 2020 – 2025 was presented. It can be stated that in managing investment and financial resources, it is necessary to adhere to a policy of strategic consistency, which should be based on the principles of adaptive and aggressive behaviour of a business entity. Such a sequence should consider the aspects of the enterprise's adaptability to changes in the external economic conditions while not excluding the introduction of innovative, aggressive measures within the framework of compliance with the current legislation. Combining these two approaches does not contradict the policy of forming a conservative and progressive policy of managing financial resources of market environment entities, adopted in the world practice of financial management: the state, credit and financial institutions, individual enterprises and their associations.

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Analysis of qualitative and quantitative indicators of milk production and processing at the enterprises of the Akmola region

Zhanara Nurtayeva

ABSTRACT

The primary tasks are the development of the agro-industrial complex of the Republic of Kazakhstan and the provision of high-quality products to the population. The Akmola region is one of the country's most developed agricultural and industrial regions. The assessment of qualitative and quantitative indicators of milk production and processing in the Akmola region reflects the development dynamics of the dairy industry of the country and the main problems that need to be solved; therefore, research on this issue is relevant. The purpose of the study was to analyse the quantitative and qualitative indicators of milk production and processing at the enterprises of the Akmola region, review research on the development of formulations based on the raw materials of the area, and study factors affecting the quality of dairy products. The main method used in the study was the analysis of the suitability of milk of the Akmola region as a raw material for producing cheese fermented milk. It was found that, in general, the quality of milk of the Akmola region in terms of fat, protein, minerals, and lactose, the fatty-acid composition of milk fat, the content of somatic cells is good. The specificity of the region, which consists in the fact that most of the raw materials for milk processing plants are supplied by private farms, leads to the variability of microbiological indicators of milk and the content of somatic cells, which limits the use of milk in the production of certain types of cheese. It was concluded that there is a necessity in the development of new recipes, considering the characteristics of raw and powdered milk produced in the Akmola region, which will increase the range of high-quality dairy products, which will be distributed within the country and for export.

Keywords: cow's milk, protein, fat content, fermented milk products, agriculture, milk yield

INTRODUCTION

An indispensable component of economic security is food security. In food supply, import substitution covers a complex of industries related to agriculture **[1]**. For the development of agriculture in the Akmola region, subsidies are allocated in livestock breeding and improving the productivity and quality of livestock products, reducing the cost of milk production **[2]**. In 2020, 5047 heads were purchased at the expense of the republican and local budgets, and breeding work was underway. Subsidising the production and sale of milk in 2020 amounted to 29,337.56 tons, 398,296 tons of mare's milk, while in 2019 – 18,297.1 and 314.6 tons. The growth in milk production to the level of 2019 with the plan of 1.2% was 1.7% (141.7%). The indicator was exceeded due to an increase in the commissioning of new commodity dairy farms and an increase in existing production farms. Subsidising the costs of processing enterprises for the purchase of milk covered 19 thousand tons of milk, for the production of butter – 809.9 tons, hard cheese – 65.1 tons **[3]**.

Agriculture is one of the leading and dynamically developing sectors of the regional economy. Work is underway to attract investment in the industry. In 2020, more than 62 billion tenges were allocated to agriculture, which is 18.1% higher than in 2019. In addition to that, all funds are private investments. The modernisation of the dairy processing plant LLP (Limited Liability Partnership) "Gormolzavod" (total capacity – 150 tons per day), the products of which are the brand of the region, the creation of a feed mill LLP "MMK "Ayan" Arshaly district, with a capacity of 28 thousand tons per year. In the Birzhan-sal area, the construction of a breeding reproducer

for 4.5 thousand heads of cattle has been completed – Burabay Astyq LLP, in Kokshetau – the structure of a fattening site of Bibord LLP, with a capacity of 5 thousand heads. Eighteen dairy farms have been established. As a result, 56 specialised enterprises with total livestock of 12.5 thousand heads are currently engaged in milk production in the region. The capacity of fattening sites for the one-time maintenance of cattle has been increased up to 36.5 thousand heads **[4]**. There are 15 dairy production enterprises in the Akmola region, with a production capacity of 146.8 thousand tons of milk per year (Table 1).

Table 1 Milk processing enterprises of Akmola region.

Name of the district	Enterprise name	Capacity, tons/year
Akkol	Eco milk LLP	25000
Arshaly	Izhevskiy PC (production cooperative)	1800
	Voskhod 2004 LLP	3600
Burabay	Yesil-Agro LLP	3500
Esil	Esilskiy butter-making plant LLP	3050
Zhaksy	Aybat LLP	3050
Zharkain	Molprodjarkaın APC (agricultural production cooperative)	900
Zerendi	Milk Project LLP	18000
	Agrofirma Rodina LLP	16000
Tselinograd	Maksimovsky dairy plant LLP	2100
	Astana Onim JSC (Joint Stock Company)	3050
Shortandy	Molochny 2 APC	3600
Kokshetau	Gormolzavod LLP	7300
	Moloko Sinegorye LLP	45800
	Natizhe LLP	10000

Note: Source **[5]**.

Considering that about 80% of the total milk production takes place in households, to develop the region's dairy industry, the Yrys programme is being implemented in the region within the framework of the Agribusiness 2020 programme for the development of a network of family dairy farms. Farmers had the opportunity to purchase a breeding stock of dairy cattle, small cattle (goats), the necessary machinery and equipment. Work on organizing the purchase of milk from the population is being carried out to load the production capacities of existing milk processing enterprises **[6]**.

Scientific Hypothesis

The development of dairy production in the Akmola region is a priority and contributes to ensuring the food security of the Republic of Kazakhstan. The purpose of the study was to analyse the quantitative and qualitative indicators of milk production and processing at the enterprises of the Akmola region, review research on the development of formulations based on the raw materials of the region, and study factors affecting the quality of dairy products. The hypothesis of research work hinged on the assumptions that the quality of milk of the Akmola region in terms of fat, protein, minerals, and lactose, the fatty-acid composition of milk fat, the content of somatic cells is good.

MATERIAL AND METHODOLOGY

Data Collection

This article is an overview of the research on the production of milk and dairy products in the territory of the Akmola region. The review includes statistical reports provided by the Department of Agriculture of the Akmola region. The analysis of the suitability of milk of the Akmola region as a raw material for producing cheese fermented milk products was conducted. References are given to the studies on developing formulations of dairy products in the region using cow's milk and its substitute – goat's milk. To produce high-quality dairy products, processors need high-quality raw milk, which is defined as:

- full in composition (protein and fat content within the norm);
- without foreign tastes and smells;
- without detectable residues of medicinal substances, added water, or other impurities;
- with a low total number of bacteria;
- with a low number of somatic cells **[7]**.

Typically, the processor monitors the milk supply to the dairy plant to ensure that quality raw milk will be produced. The quality of raw milk is most often considered in terms of the potential impact on the quality of the processed product, which depends on the number of somatic cells and the total number of bacteria. High levels of somatic cells and bacteria are associated with increased activity of enzymes that damage milk components and lead to product defects. The enzymes related to increased content of somatic cells and bacteria to influence the quality of dairy products depends on several factors. These include the amount of enzyme, specificity, heat resistance, processing and storage temperature, pH (pondus Hydrogenii), humidity, and the presence of inhibitors and activators. The potential effect will vary depending on the enzyme, product, and conditions. In the United States of America, the regulatory limits on the number of somatic cells for class A milk production is 750000 CFU/mL and on the total bacterial content – 100,000 CFU (colony-forming units)/mL **[8]**.

Description of the Experiment

A brief overview of the main factors that affect the quantitative and qualitative indicators of milk is also conducted. These include the breed of animals, maintenance conditions, seasonality, conditions for the delivery of milk to processing plants. The leading indicators of milk that affect its technological quality for different types of dairy products are considered.

Most milk producers strive to meet stricter requirements, which is often associated with promoting quality and the payment of "allowances" offered by cooperatives or other buyers of raw milk. The increased payment by processors of higher-quality raw milk is the ability to process such milk more efficiently, obtaining products of higher quality and with a longer shelf life. Since the product's characteristics ultimately depend on the quality of milk, there is a great interest in identifying the fundamental relationship of the factors of milk production that influence its composition. The overall purpose of such studies is to increase the profitability of milk processing operations **[9]**. Since many milk and dairy producers are concentrated in the Akmola region, the analysis of qualitative and quantitative indicators of milk and dairy products of the region and factors that affect these indicators is relevant to obtain an overall picture of the state of the dairy industry of the country.

Statistical Analysis

All determinations were conducted in triplicate or more, and all results were calculated as mean ± standard deviation (SD). The threshold of reliability of the obtained data was designated as $p < 0.05$. Microsoft Excel produced the statistical analysis data.

RESULTS

Milk production in the Akmola region has a steady tendency to increase (Table 2).

Table 2 Cow's milk production in all categories of farms of Akmola region (thousand tons).

Year	2015	2016	2017	2018	2019	2015 – 2019	2019, %	
							2015	2018
Gross milk yield, total	359.0	377.0	383.8	385.8	396.1	1901.7	110.3	102.7
Agricultural enterprises	41.1	66.9	71.3	70.5	77.0	326.8	187.5	109.3
Individual entrepreneurs, peasant or husbandry farms	14.3	17.2	18.9	19.3	19.9	89.6	138.9	103.2
Households of the population	303.6	292.9	293.6	296.0	299.2	1485.3	98.5	101.1

For five years (2015 – 2019), 1901.7 thousand tons of milk were produced in all categories of farms in the Akmola region, including in agricultural enterprises – 326.8 thousand tons (17.2%), peasant or husbandry farms – 89.6 (4.7%), households – 1485.3 thousand tons (78.1%). During the analysed period, milk production increased by 10.3%, including in agricultural enterprises – 87.4%, individual entrepreneurs and peasant or husbandry farms – by 39.2%, in households, milk production decreased by 1.5%. The largest volume of milk for the analysed period was produced in all categories of farms in 2019. It amounted to 396.1 thousand tons, in agricultural enterprises in 2019 – 77 thousand tons, in individual entrepreneurs and peasant or husbandry farms in 2019 – 19.9 thousand tons. The main milk producers for the analysed period are the farms of Zerendi – 257.2 thousand tons

or 13.5% of the total production, Ereymentau – 182.8 thousand tons (9.6%), Burabay – 177.4 thousand tons (9.3%), Tselinograd – 166.6 thousand tons (8.8%) districts. The main producers of milk are the households of the population. For five years, their share in the structure of milk production averaged 78%, in agricultural enterprises – 17%, in individual entrepreneurs and peasant or farm farms – 5% **[10]**.

During the analysed period, there was an increase in the average milk yield per dairy cow for all categories of farms, which was 3050 kg. In 2019, the average milk yield per dairy cow was 3118 kg, 2.2% higher than the analysed period. The highest level of milk yield in five years – 4158 kg was observed in agricultural enterprises of the region. This is explained by the fact that agrarian enterprises have the opportunity to purchase highly productive dairy cattle, which most peasant or husbandry farms do not yet have. Over the past five years, high milk yields per dairy cow have been noted in all categories of farms in Tselinograd (3665 kg), Akkol (3400 kg), and Burabay (3211 kg) districts **[10]**. In 2020, milk production in the Akmola region increased by 1.7%, to 402.7 thousand tons, dairy products – by 6.4% (to 94.9 thousand tons) **[4]**. The workload of milk processing enterprises for the period from January to July 2021 amounted to 66.4% (in 2020 – 68.3%), the volume of processed milk production amounted to 48.7 thousand tons, there was a decrease of 2.8% to the same level in 2020, 599 tons of cream butter produced with an increase of 12.8%, cheese and cottage cheese – 684 tons, fermented milk products – 3.2 thousand tons with an increase of 0.03% **[11]**.

An analysis of the state of the dairy industry in Kazakhstan shows that the main problem remains the underdevelopment of the raw material base. To create a raw material base, processing enterprises in the region are opening stationary milk reception points to increase the volume of milk purchases in private subsidiary farms. One of the main factors influencing the change in dairy production is the number of cows for dairy purposes. It is impossible to ensure sustainable development of processing production without a stable raw material base **[8]**. The number of cows in the Akmola region is increasing. As part of the development of dairy farming this year, it is planned to create 19 dairy farms with the acquisition of 4.1 thousand heads, while seven farms for 911 heads were created **[11]**. Cattle of the best breeds have been imported into Kazakhstan and continue to be imported. The study of imported animals' adaptation and descendants is of great scientific and industrial importance – the object of the study **[12]** was the first-calf heifers of the Holstein breed of individual generation, obtained from Canadian Holsteins in the conditions of the Agrofirm “Rodina” LLP of the Akmola region. The animals were in a facility with loose keeping, mobile distribution of feed mixture, and milking on a Carousel-type installation. The animals’ diet consisted of a feed of individual production (corn silage, wheat hay) mixed feed for cattle. It was designed to receive six thousandth milk yields of first-calf heifers.

The milk productivity of the Holstein first-calf heifers of individual generations was studied compared to their mothers imported from Canada. The milk yield of the first-calf heifers was 6488 kg, which is 139 kg less than mothers. The content of fat, protein, and minerals in the milk of offspring is slightly higher than that of mothers (3.64% and 3.60% fat content, 3.08% and 3.03% protein, respectively). The resulting offspring is characterised by an increased haemoglobin content (148 $g.L^{-1}$ versus 136 $g.L^{-1}$ in their mothers), β-globulins. The resistance of farm animals to harmful environmental factors, particularly to infectious diseases, was evaluated. Cellular defence factors were at the level of 55%, with the aggressiveness of neutrophils at 8.8%, in mothers — 54%. Humoral protection factors were higher in first-calf heifers: bactericidal activity 74.3%, lysozyme activity 9.3%, in mothers these indicators were 71.5% and 7.4%. The data obtained indicate a satisfactory adaptation of Holstein animals in the conditions of the north of Kazakhstan **[12]**. The study **[13]** examined the availability of protein and energy in the diet of dairy cows of the Akmola region and its impact on productivity. As an indicator of the quality of cow feeding, the urea content in milk was used, which generally should be within 15 – 30 mg%. A high level of urea concentration can indicate an excess of protein and a lack of easily digestible carbohydrates necessary for the vital activity of the microflora of the rumen.

Conversely, a decrease in urea in milk can be caused by low protein levels and an excess of carbohydrates in the diet. Low urea content in milk can cause a decrease in productivity, problems with animal fertility, the occurrence of acidosis. The increased content of urea, in turn, increases the risks of diseases of reproductive function, metritis, lameness, ketosis, liver diseases. The indicators of milk productivity and milk composition of dairy herds of the Akmola region of the Republic of Kazakhstan obtained in the study **[13]** are presented in Table 3. The urea content in the milk of the dairy herd of Agrofirma Rodina LLP was 34.25 ± 0.29 mg%, which indicates that the upper threshold value was exceeded. It was confirmed that the amount of protein exceeded 7.9% compared to the standards of feeding dairy cattle. In Yesil-Agro LLP, the urea content in milk was 11.7 mg%, with a lower threshold value of 15 mg%. This was a consequence of an excess of carbohydrates and a lack of protein in the diet of dairy cows, which in the future could lead to a decrease in productivity and impaired reproductive function.

Table 3 Indicators of milk productivity and milk composition of dairy herds of the Akmola region of the Republic of Kazakhstan.

Name of the farm	Daily milk yield, kg	Mass fraction of fat, %	Mass fraction of protein, %	Urea, mg%
Agrofirma Rodina LLP	7446 ± 56.9	4.44	3.63	34.25
Yesil-Agro LLP	7456 ± 86.0	3.54	3.39	11.7

The study by A. Zh. Khastaeva, V. S. Zhamurova, L. A. Mamaeva, A. T. Kozhabergenov, N. Zh. Karimov, K. M. Muratbekova **[14]** investigated the influence of animal breed and season on the physicochemical characteristics of milk. The milk of the Holstein breed of cows of Astana Onim JSC of the Akmola region of Kazakhstan was analysed. Holstein cows in autumn had the highest fat content – 3.8% and the highest protein content – 3.3%. Physicochemical indicators of milk quality for the lactation period of 305 days are presented in Table 4.

Table 4 Physicochemical and microbiological indicators of milk by season.

Season	Mass fraction of fat, %	Mass fraction of protein, %	Somatic cell content, thousand/cm^3	Acidity, ◦T
Spring	3.70	3.18	260.11	16.9
Summer	3.73	3.17	167.96	18.0
Autumn	3.80	3.30	282.77	17.0
Winter	3.64	3.22	343.92	16.9

Note: Source **[14]**

The seasonal dynamics of the mass fractions of 16 major fatty acids in milk fat is presented in Table 5. The most common fatty acids were C16:0, the sum of the isomers C18:1, C18:0, and C14:0. At the expected levels, short- and medium-chain saturated fatty acids (SFA), such as C4:0, C6:0, C8:0, C10:0, and C12:0, were present at the expected levels. In spring and summer, relatively high content of C4:0 butyric acid was observed in milk, which creates the taste of dairy products. Among monounsaturated fatty acids (MUFA), C18:1 isomers were the main components. In general, the remaining percentage of monounsaturated and polyunsaturated fatty acids (PUFA) in the total milk fat of Holstein cows was considerably higher than in the milk of Simmental cows. The percentage of 3-polyunsaturated fatty acids was higher in the samples of the Simmental breed (1.06% and 1.17% versus 0.97% and 0.8%) in summer and winter compared to the Holstein breeds; however, the percentage of 3-polyunsaturated fatty acids in the spring and autumn periods in the Holstein breed was 1.29% and 1.01% compared to 0.42% and 0.67%. In spring, summer and autumn, the percentage of 6-polyunsaturated fatty acids (3.84%; 3.84%; 3.6% vs 3.04%; 2.49% and 2.46%) was higher in the Holstein breed than in the Simmental breed **[14]**.

The study **[15]** developed a technology for cheese production by thermal acid deposition from the milk of the Akmola region. Product developments were conducted at Milk Project LLP. The composition of milk, the absence of antibiotics, chemical and microbiological stability are essential requirements for the raw materials in cheese production by thermal acid deposition. The rennet capacity of milk for thermoacid coagulation cheese is not as important as for hard cheese. According to the chemical composition, milk with a mass fraction of protein of at least 3% and a sufficient mass fraction of fat was selected. The stability of milk in protein was about 91%, and in fat – 75%. Milk of this quality may well be used for the production of cheese by thermal acid deposition. At LLP "Scientific and Production Association "Innovator" Kosshi, Akmola region, formulations of fermented milk products with extruded grain bases from buckwheat, millet, lentils, and chickpeas with two versions of the milk base – milk with a fat content of 2.5% and 3.2% were developed **[16]**. According to physical and chemical parameters, all samples met the standard requirements for fermented milk products ST RK 1733-2015 "Milk and dairy products. General technical conditions" **[17]**, according to safety indicators – within the requirements of the Technical Regulations of the Customs Union "On the safety of milk and dairy products" **[18]**.

The study **[19]** developed a technology for producing fermented milk products from powdered milk produced at the enterprises of the Akmola region using sour whey. Milk powder and sour whey were combined in certain proportions; the resulting raw materials were exposed to ultrasound with simultaneous heating. Then fermentation was carried out using different types of starter cultures. The optimal technology was chosen to analyse the physicochemical and organoleptic characteristics of the resulting yoghurt. In the Akmola region, research is underway to develop recipes for products from other types of milk, for example, goat's milk.

Table 5 Fatty acid composition of milk (%) of Holstein breed of cows of Astana Onim JSC of Akmola region, depending on the season.

Fatty acid	Spring	Summer	Autumn	Winter
C4:0	3.29	3.66	3.27	2.83
C6:0	1.78	1.88	1.58	1.78
C8:0	1.16	1.48	1.23	1.27
C10:0	3.05	2.95	2.08	2.93
C10:1	0.29	0.34	0.26	0.30
C12:0	3.11	3.11	2.71	0.31
C14:0	9.99	11.29	10.03	11.03
C14:1	1.23	1.18	1.29	1.12
C16:0	29.29	28.06	28.27	27.76
C16:1	2.28	1.99	2.09	2.08
C18:0	9.84	11.32	9.02	9.94
C18:1	25.14	23.61	29.25	26.17
C18:2	3.84	3.84	3.60	2.91
C18:3	1.29	0.90	1.01	0.80
C20:0	0.20	0.15	0.13	0.22
C22:0	0.09	0.10	0.07	0.09
Other	4.13	4.16	4.09	5.47

Note: Source **[14]**.

The analysis of preferences of dairy consumers in the Akmola region showed that 81.5% of 417 respondents prefer dairy products from cow's milk, 10.1% – from mare's milk (koumiss), 6% prefer goat products (fermented milk, cheese), 2.4% – from sheep's milk. In addition to that, the majority of respondents in rural areas independently produce dairy products **[20]**. The use of goat's milk in the production of fermented milk drinks offers broad prospects for reducing the shortage of cow's milk, using existing technological equipment, and developing new fermented milk products. The use of ozonation of goat's milk as a modern processing method allows eliminating tastes and odours and increasing the shelf life.

The study **[21]** includes formulations of a fermented milk drink made from goat's milk with various berry fillers, while goat milk produced in the Akmola region at the Zerenda farm was used, which was ozonated for 10 minutes at an ozone concentration of 80 mg/m3. In the study **[22]**, goat's milk obtained at the Zerenda farm was used to develop a recipe for yoghurt enriched with amaranth flour. The study **[23]** investigated regional features of mare's milk of the Republic of Kazakhstan. According to its physicochemical and biological properties, mare's milk has a number of features that characterise it as a valuable medicinal product. The physicochemical parameters of mare's milk considerably depended on the region, the time of year, and the conditions of keeping animals. Mare's milk of Akmola district was characterised by a relatively high-fat content (1.7%), the protein was 1.87%. The lactose content in the milk of mares differed slightly by region and amounted to 5.37% for the Akmola region. Mare's milk of the Akmola region had a relatively high vitamin A content – 30.8 micrograms, whereas, in other regions, this indicator was considerably lower (18.3 – 26.3 micrograms). An advantageous difference between the mare's milk samples is that they had a high PUFA content with mainly ω-6 and ω-3 fatty acids. All the samples studied were collected from mares of the Jabe breed. According to the owners, the animal's diet consisted of wild and forage plants; after milking, the milk was stored at room temperature. In general, in all regions, the productivity of dairy mares was 3.6-8.5 litres per day. Thus, samples of mare's milk collected from all regions of Kazakhstan can be considered suitable for processing.

DISCUSSION

Milk producers are primarily interested in increasing the mass of milk produced by a cow per unit of time. Nevertheless, a commercial milk processor is primarily interested in the percentage composition of milk, since deviations in the composition of milk must be compensated for in the production of dairy products, for example, cheese, since a constant ratio between certain components of milk must be observed in the finished product. The focus should be on the technological properties of milk, namely the possibility of its effective use for processing into certain products: fermented milk products, cheese, butter **[24]**. The composition of milk may vary considerably. A great practical interest in the composition of milk has led to many studies aimed at clarifying the various factors responsible for differences in the composition. Variations in the fat content in milk have been studied to a greater extent than other components due to the greater economic value of fat. The variability of milk

composition among cows of the same breed depends partly on hereditary differences and environmental factors. When studying the physiological and environmental factors affecting milk composition, it is necessary to consider possible changes in milk yield. An increase in fat content may reflect a decrease in milk yield rather than an increase in fat secretion itself. The picture may be incomplete if the percentage of components will be analysed without considering milk yield **[9]**.

It is known that the chemical composition of milk varies inversely with the amount of milk produced. Thus, seasonal changes in the fat content in milk can be considered a dilution of fat due to an increase in milk production by a cow. However, the protein content in milk is subject to fewer seasonal variations **[25]**—the study **[24]** also revealed an inverse relationship between milk yield and its fat and protein content: the lower the milk yield, the higher the fat and protein content in it. According to the presented results of the work performed in the Akmola region, it is also possible to note a tendency to increase milk's fat content with a decrease in milk yield. Studies show a reduction in feed per 1 litre of milk in highly productive cows. In addition to that, there is no clearly expressed relationship between the volume of feed consumed and the amount of milk produced; an increase in concentrate consumption does not lead to a rise in the volume of milk produced. The effect of total cow feed intake on the composition and volume of milk produced without changing the diet was investigated **[25]**. In general, overfeeding does not lead to a change in milk composition. The main result of overfeeding is the fattening of the animal. In addition to that, underfeeding leads to a decrease in milk yields and depletion of the accumulated fat reserves of the animal.

In some cases, a decrease in milk yield may be accompanied by an increase in fat content, although the overall secretion of fat remains constant. Often underfeeding occurs at the beginning of lactation. This is accompanied by a noticeable increase in fat percentage in milk, especially if the cow is fat. The yield of milk fat increases. In certain instances, there is a slight decrease in the content of skimmed solids in milk. There is a decrease in the content of protein and lactose with underfeeding. A lot of work has been done to determine the effect of the cows' diet on milk composition. In particular, the effect of the introduction of coarse feed into the diet on the content of fat, protein, and minerals in milk was investigated. It was discovered that a considerable decrease in the fat content in milk (about 0.5%) could be observed due to small changes in milk yield when feeding cows with diets with a low content of a coarse feed. The reduction in fat content is not entirely consistent; it is more pronounced in the early stages of lactation and depends on the type of carbohydrates in the concentrate. The low content of coarse feed in cows' diet changes the processes occurring in the rumen so that less fat is synthesised. The content of skimmed milk substances did not change much with a decrease in the consumption of coarse feed **[9]**.

The cow's body synthesises milk fat from carbohydrates with a fat-free diet, but a small increase in milk and fat yields can be obtained by directly including fat in the diet. The protein content in the diet does not have a noticeable effect on the composition of milk, although some articles indicated a slight decrease in the protein content in milk from cows on low-protein diets. In addition to that, it should be considered that in the long term, increased and reduced protein content in the diet of dairy cows leads to diseases and reduced productivity **[13]**. There are pretty pronounced and characteristic differences in milk composition from cows of different breeds, which is especially clearly reflected in the fat content of milk. Differences in the fat content in milk are more pronounced between Guernsey and Jersey cows, to a lesser extent between the Holstein and Ayrshire breeds. Data on other milk components are not as extensive as data on fat; however, the available information indicates that the protein and lactose content of different breeds of cows varies in the same way, but to a lesser extent than the fat content (Table 6).

Table 6 The composition of milk of different breeds of dairy cows.

Breed	Number of cows	Number of samples	Mass fraction of fat, %	Mass fraction of protein, %	Mass fraction of lactose, %	Mass fraction of ash, %	Mass fraction of dry substances, %
Holstein	19	268	3.55 ± 0.57	3.42 ± 0.51	4.86 ± 0.27	0.68 ± 0.04	12.50 ± 1.04
Brown Swiss	17	428	4.01 ± 0.60	3.61 ± 0.53	5.04 ± 0.39	0.73 ± 0.05	13.41 ± 0.95
Ayrshire	14	208	4.14 ± 0.54	3.58 ± 0.34	4.69 ± 0.47	0.68 ± 0.04	13.11 ± 0.96
Jersey	15	199	5.18 ± 0.80	3.86 ± 0.43	4.94 ± 0.36	0.70 ± 0.04	14.69 ± 1.11
Guernsey	16	321	5.19 ± 0.71	4.02 ± 0.46	4.91 ± 0.30	0.74 ± 0.03	14.87 ± 1.10

Note: Source **[9]**.

Seasonal fluctuations in the composition of milk are pretty pronounced. Seasonal changes in the fat content in milk are pronounced; in winter, the fat content is higher than in summer. The content of the dry fat-free substance shows the same trend, although to a lesser extent and with much greater unevenness. The protein and mineral content are higher in winter than in summer. In the study **[24]** the highest dry matter content, skimmed milk powder, fat, and protein in milk was observed in winter, while in summer these indicators were the lowest. This is due to a change in the diet, namely, replacing part of the feed with greens and increasing milk yield. In the study **[14]** analysis of the milk of Holstein cows showed, on the contrary, a slight decrease in the fat content in milk in winter, which can be explained by the influence of diet or other factors. For example, ambient temperature has been suggested as one of the factors responsible for seasonal fluctuations in milk composition. Convincing evidence of the influence of temperature is the results of experiments in which cows were kept in facilities with controlled temperature and a diet of constant composition. These studies have shown that fluctuations in ambient temperature from 1 °C to 24 °C do not considerably affect the milk yield or milk composition of cattle of European origin. With temperatures between 29 °C and 41 °C, the milk yield decreased, the fat content increased, the content of fat-free solids, total nitrogen, and lactose decreased. The decrease in the content of fat-free dry matter was more pronounced in the Holstein breed than in the Guernsey breed. Ambient temperatures below four °C caused an increase in fat content, an increase in the content of fat-free solids and total nitrogen **[9]**.

Cow's milk contains an average of 4% fat, which 97 – 98% consists of triacylglycerols **[7]**. Milk fat can contain up to 400 different fatty acids, which are usually grouped by the saturation of their carbon chain into saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Cow's milk usually contains 70% SFA, 25% MUFA, and 5% PUFA. From the standpoint of human health, attention has recently been paid to fatty acids C12:0, C14:0, and C16:0, which increase the deposition of fat on the walls of blood vessels, along with fatty acids with odd and branched chains which are capable of suppressing cancer. Dietary intake of fats rich in cis-monounsaturated fatty acids and long-chain polyunsaturated fatty acids plays a role in preventing heart disease. As a result, there is an increased interest in changing the fatty acid composition of milk fat. Many factors can influence the fatty acid composition of cattle milk, including breed, lactation stage, maintenance conditions, diet composition, season, geographical location, access to fresh pastures, type of pasture, feeding with grain and oilseeds, the addition of oil to feed. Studies have shown the possibility of changing the fatty acid profile of milk fat **[26]**. The analysis of the fatty acid profile of milk fat from Holstein cows of the Akmola region was conducted in the study **[14]** showed an increased content of monounsaturated and polyunsaturated fatty acids in comparison with the milk of Simmental cows.

A very important indicator of quality for milk processors is the content of somatic cells and the total number of bacteria. In addition to that, this indicator of milk quality is difficult to maintain in conditions when most of the milk coming for processing is produced in private farms. The quality of milk in households is generally poor due to the lack of necessary sanitary conditions for keeping livestock and veterinary measures. In addition to that, the availability of demand and a good purchase price, along with control, should encourage private households to improve their quality **[8]**. An increase in the number of somatic cells in raw milk is associated with mastitis, an inflammatory reaction of the breast, most often due to bacterial infection. The total number of somatic cells in the collected milk is usually used to indicate milk quality, herd health. Mastitis infection directly affects the composition and milk yield of infected cows. From an economic standpoint, an increase in the number of somatic cells in the milk is associated with a decrease in herd yields and farmers' profits. From the standpoint of product quality, an increase in the number of somatic cells is associated with an altered milk composition, increased enzyme activity, and an increased risk of product defects. The native milk protease plasmin plays an essential role in this process. Plasmin actively hydrolyses β-casein and α-caseins. Casein is the main milk protein involved in the coagulation process in producing cheese and fermented milk products.

Hydrolysis of β-casein by plasmin leads to the formation of γ-casein and proteosopeptones, which are lost with whey during the production of quarg and cheese. Both plasmin and plasminogen are heat-resistant and can withstand pasteurisation (72 °C/15 s); after treatment (138 °C/2 s), up to 40% of activity remains, and only a temperature above 147 °C leads to complete inactivation. Although plasmin is considered the main cause of milk protein breakdown, other proteases in milk have been identified as having activity against caseins. In addition to proteolysis, increased lipolysis in milk, presumably due to lipoprotein lipase activity, is also associated with mastitis and a high content of somatic cells in raw milk. An increase in the content of free fatty acids due to lipolysis directly affects the taste of milk and dairy products (for example, rancidity and related defects) **[7]**. With a high content of proteolytic enzymes in milk, the reported protein content in milk is increased since the protein content in milk is mostly determined by the Kjeldahl method by multiplying the total nitrogen content in the sample by a coefficient equal to 6.38. This analysis may show a high protein content in milk since part of the nitrogen is in milk in non-protein nitrogen. This is due to the gradual decomposition of proteins in raw chilled milk by native milk proteases and proteolytic enzymes of extraneous microflora during storage at low

temperatures (from 3 °C to 5 °C). An increase in the content of γ-casein and the protease peptone fraction negatively affects rennet coagulability, synergetic properties of protein clots, the thermal stability of milk, and its other technological properties. Typically, γ-casein is 3% of the casein content in milk, but its content can greatly increase (up to 10%) with late lactation and mastitis **[27]**.

In the study **[24]** milk in terms of somatic cell content and bacterial contamination had the lowest quality in spring, which was associated with climatic changes, that is, with the temperature and humidity regime. The content of somatic cells in the milk of Holstein cows of the Akmola region was analysed in the study **[14]** ranged from 344 thousand/cm^3 up to 260 thousand/cm3, the highest value was in the winter, while the norm is not more than 7.5 thousand * 10^5 for raw milk and not more than 5 * 10^5 for milk intended for the production of baby food, cheese, and autoclaved milk, according to the Technical Regulations of the Customs Union "On the safety of milk and dairy products" **[18]**. There are increased requirements for milk intended for processing into cheese. According to the method of coagulation of milk protein, three main groups of cheese can be distinguished: rennet coagulation (up to 75% of all cheeses produced), acid coagulation, and combined thermal acid coagulation. Milk for processing into cheese by rennet coagulation must be microbiologically pure. This is because during rennet coagulation, the use of harsh pasteurisation regimes is not allowed. Usually, the pasteurisation mode is 72 °C in 15 s. An increase in milk exposure at temperatures above 70 °C causes a deterioration in the rennet coagulation. The cheese obtained from pasteurised milk usually has an increased moisture content, a crumbly consistency, and high losses of protein and fat with whey. Milk for processing into cheese must also have a certain concentration of fat and casein; the ratio of these components is an important parameter affecting cheese quality **[27]**.

Because there is a shortage of raw milk in the Republic of Kazakhstan, O. V. Koltyugina et al. **[15]** used the method of thermal acid deposition for the production of cheese. At the same time, several farms were selected as milk suppliers to ensure proper primary processing of milk and stability of the chemical composition. A common feature of the technology of all acid coagulation products is that an acid-induced gel is formed at the initial stage, which is then further processed. Heat treatment of milk is one of the most important processes affecting the texture of acid coagulation gels. Milk for certain fresh sour cheese, such as quarg, undergoes considerable heat treatment. The inclusion of whey protein in fresh cheese is an important aspect of its production due to the increased yield **[28]**. Another important test that should be conducted for milk intended for the production of cheese and fermented milk products is the presence of acid-inhibiting substances. While minor changes in the chemical composition can affect the quality of the final product and the economy of the process, the presence of antibiotics in a batch of milk intended for fermentation with sourdough leads to a complete loss of this batch.

CONCLUSION

One of the priorities in ensuring the country's food security is milk production and its processing. Akmola region is one of the main agricultural and industrial regions of the Republic of Kazakhstan. The government pays great attention to the development of rural industry, allocating subsidies for livestock breeding and reducing the cost of milk and dairy food production. Milk production in the Akmola region in 2020 reached 402.7 thousand tons. The main problem of the dairy industry remains the lack of a raw material base. The main producers of milk are the households of the population. This problem is associated with the inconstancy of the bulk milk quality coming to the Akmola region's enterprises in terms of microbiological quality and the number of somatic cells. Other countries solve this problem using a system of incentives for enterprises that supply milk of consistently good quality according to these indicators to processing plants.

To increase the workload of the Akmola region dairy enterprises with raw materials, the number of dairy cows is increased, and recipes for dairy products using other types of milk – goat and mare's milk are developed. While milk producers are interested in obtaining high milk yields, processors, firstly, evaluate the technological qualities of milk, its suitability for the production of certain types of goods. Milk indicators such as the content of fat, protein, and lactose, the absence of antibiotics and adulterating substances, low levels of somatic cells and microbiological indicators are crucial. The analysis of milk samples produced in the Akmola region confirms its suitability for processing in terms of protein and fat content somatic cell content. Analysis of the fatty acid composition of milk fat from Holstein cows of the Akmola region showed an increase in the proportion of mono- and polyunsaturated fatty acids in the composition of milk fat triglycerides, which is beneficial to human health.

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The effect of transportation and pre-slaughter detention on quality of pig meat

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ABSTRACT

This research aimed to determine the influence of stress of various etiologies in pigs caused by transportation and pre-slaughter conditions on meat quality. For this purpose, pigs were divided into 11 groups within two meat processing enterprises, depending on the duration of transportation (short, long without breaks and long with breaks) and the conditions of keeping animals before slaughter. Also, within the two groups were created two subgroups with pigs of different breeds. A total of 156 pigs were studied. Blood was collected from all pigs to determine cortisol and lactate levels, and a sample of meat from the longest back muscle. The pH of the meat was determined at different stages of its maturation, and the weight loss of the sample was determined. As a result of the research, it was found that the highest quality pork was obtained from pigs that experienced lower levels of stress before slaughter. Keeping pigs for 10 – 14 hours before slaughter without access to water and food resulted in higher stress levels, which were probably expressed in higher blood concentrations of cortisol and lactate. Pigs' access to food and water during pre-slaughter retention allows for high-quality meat by reducing the influence of stress. Pigs' access to water before pre-slaughter does not affect the stress level but positively affects the loss of meat weight during maturation. The higher the concentration of lactate in pigs' blood, the faster the pH of the meat decreases after slaughter, which negatively affects its quality and moisture retention. If there is a long-term transport of pigs, there is no rest stop that can significantly reduce stress levels in pigs. Duration of transportation of pigs does not correlate with stress levels, as the conditions before slaughter content.

Keywords: pork, meat quality, pig stress, cortisol, lactate

INTRODUCTION

According to the concept of "The Only Health", the quality of life of people depends on the safety and quality of the food they consume. For the safety and quality of meat to meet consumer expectations, one should relentlessly control it at all stages of production and circulation, as the final result depends on many factors. In particular, the conditions of keeping animals, feeding, veterinary treatments, pre-slaughter **[1]**, **[2]**, **[3]**, **[4]** and post-mortem factors **[5]**, **[6]**. Much remains unknown despite the significant amount of research on various factors directly or indirectly affect pork quality. Today it is known that the quality of pork depends on the amount of stress he had placed before the slaughter of pigs **[7]**, **[8]**, **[9]**, **[10]**, **[11]**. In particular, muscle carbohydrates (glucose and glycogen) under anaerobic oxidation are metabolised to lactate, which lowers the pH of the meat. Under stress, the lactate level in the blood increases significantly, and muscle glycogen content decreases. In this case, lowering the pH of the meat during its maturation will be too fast. As a result of fast hardening, muscle fibers are damaged in their structure. There is excessive loss of moisture and discolouration, thus decreasing the quality and expiration date of the meat **[10]**, **[12]**. Depending on the geographical location of the farm and technologies of preparation of pigs for slaughter, various methods of transportation and pre-slaughter keeping are practised. If the farm is located at a considerable distance from the slaughterhouse, it can be practised as transportation without stops and with breaks (long or short) for the rest of the animals. Also, in some cases, the

animals are transferred to a starvation diet; in others – the animals are fed and given access to water. There is also a different approach to the pre-slaughter keeping of animals.
In some cases, the slaughter of animals is practised immediately upon arrival; in others – animals are sent to quarantine of various durations – from several hours to one or several days. Animals are often accumulated during the day and slaughtered in the morning. In this period, at some slaughterhouses, animals are given access to water; on others to food and water, third animals are not watered or fed. In addition, they sometimes practise an approach in which they try not to mix animals from different farms or even different piggeries of one farm for transportation and pre-slaughter **[13]**. In others, cases are neglected, especially when forming a kept pig batch on small household farms.

Scientific Hypothesis

Theoretically, we assume that all of these factors can affect the stress experienced by animals. The level of stress will be different depending on the animal's conditions. In addition, we assume that the breed of pigs will directly influence stress. Hence the quality of pork will be different. This work includes the research results on the pig meat quality, depending on transport conditions, before slaughter maintenance and breed, which was our goal.

MATERIAL AND METHODOLOGY

The study was performed at the Department of Veterinary and Sanitary Inspection laboratory of S. Z. Gzhytskyi National University of Veterinary Medicine and Biotechnologies in Lviv.

Samples

The material for the research was pig blood and meat. In general, it was samples taken from 156. Blood samples were taken during the bleeding of the carcass in sterile tubes, and the meat was up to 30 minutes after the slaughter of pigs. One meat sample was taken from each carcass (weighing 450 – 500 g) from the longest back muscle in the area of 9 – 12 thoracic vertebrae. Meat samples were stored in a refrigerator at 4 ±2 °C. To obtain plasma, the blood was immediately centrifuged at three thousand rpm.

Chemicals

The cortisol content was determined in blood plasma by enzyme-linked immunosorbent assay using DRG (Germany) test kits. Hydrazine-glycine buffer pH 9.0, NAD+ (0.3 molar solution, pH 6.0) and lactate dehydrogenase solution (protein content 2 $mg.mL^{-1}$) were used to determine lactate (Khimreaktiv, Ukraine).

Animals and Biological Material

Samples were taken from two slaughterhouses located in the Lviv region of Ukraine. The experiment was performed from July – to September 2021. Before sampling, the attendants studied documents and conducted the initial inspection and weighing of pigs. Total experimental pigs were divided into 11 groups, as shown in Table 1. In 1 and 8 experimental groups, the number of studied animals was twice as large as in others because, in these groups, pigs of two breeds were included – Ukrainian Steppe White and Landrace. In the article, comparisons of the indicators received from pigs of the Ukrainian steppe were carried out white breed except for the part where the data of breed features are given.

Table 1 Division of pigs into groups, depending on the duration of transportation and detention conditions until slaughter.

Group No.	Number of samples, n	Transportation time		Breaks for transportation		Shutter speed		Diet		
		6 – 8 hours	0.5 – 1 hour	Without breaks	0.5 hours every 2 hours	up to 1 hour	10 – 14 hours	Without water and food	Only water	Food and water
1.	24	+		+		+		+		
2.	12	+		+			+	+		
3.	12	+		+			+		+	
4.	12	+		+			+			+
5.	12	+			+	+		+		
6.	12	+			+		+		+	
7.	12	+			+		+			+
8.	24		+	+		+		+		
9.	12		+	+			+	+		
10.	12		+	+			+		+	
11.	12		+	+			+			+

Before slaughter, all animals were analogues by sex (pigs), live weight kg and clinically healthy. All animals were kept unattended until the slaughter pig keeping system.

Transportation of pigs was carried out with the help of a specialised transport at the rate of 0.8 m^2 area per animal at a temperature environment not higher than 28 °C, for unloading animals used a bridge, which was installed so that there were no cracks and the angle of inclination to the surface did not exceed 20 °C. Upon arrival, the pigs crossed a corridor about 8 m long to the pre-slaughter room. To increase efficiency in unloading and moving pigs within the enterprise, employees' meat sticks are used by meat processing companies.

Before slaughter, all experimental animals were stunned with the help of an electric current.

Instruments

Stat-Fax analyzer (model 4300 ChroMate; USA).

Electronic laboratory scales (TBE-0.5, producer (Inter-Synthesis) Limited Liability Company; Ukraine).

pH meter TESTO 205 (Germany).

Centrifuge MICROmed (China).

Spectrophotometer ULAB 102 (China).

Laboratory Methods

The cortisol content was determined in blood plasma by enzyme-linked immunosorbent assay. Whole blood determined lactate content **[14]**. Meat samples were tested for pH at the first, third, and 12^{th} hours of storage using a pH meter. The quality of the meat was visually inspected and palpated, dividing it into three categories: 1) NOR (close to optimal quality indicators); 2) PSE (pale, soft, exudative); and 3) DFD (dark, solid, dry).

Sample preparation

Blood samples were taken during the bleeding of the carcass in sterile tubes, and the meat was up to 30 minutes after the slaughter of pigs. One meat sample was taken from each carcass (weighing 450 – 500 g) from the longest back muscle in the area of 9 – 12 thoracic vertebrae. Meat samples were stored in a refrigerator at 4 ±2 °C. To obtain plasma, the blood was immediately centrifuged at three thousand rpm.

Number of samples analysed: we analysed 156 blood samples and 156 samples of meat.

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: The experiment was performed from July to September 2021. Before sampling, the attendants studied documents and conducted the initial inspection and weighing of pigs. Research teams were formed based on the information received from suppliers of live pigs and available in the documents. The period of pigs' transportation (long or short) to the meat processing plant and the transportation conditions (with breaks for rest or without stops) were considered. In addition, we paid respect to the requirements of pre-slaughter keeping of pigs, access to feed and water, length of stay before slaughter. To ensure the purity of the experiment, the sex, breed and live weight of pigs were taken into account. In total, 11 experimental groups of pigs with different transport and pre-slaughter conditions were formed, as shown in Table 1. A total of 11 experimental groups of pigs were formed with different combinations of transport and pre-slaughter conditions, as shown in Table 1. Blood samples and the samples of the longest back muscle were taken after slaughter. After the preparation of the samples, studies were performed on the content of cortisol and lactate in the blood as markers of pre-mortem stress and the pH of meat and its natural weight loss. An organoleptic assessment of the quality of the meat was also performed.

Statistical Analysis

The ChroMate Manager software was used. The obtained digital data were processed in Excel (2010 professional +), determining the average arithmetic value (M), statistical error of the arithmetic mean values (m), the probability of the difference between the arithmetic means of the two variations series and correlation coefficient (r). The difference between the comparable values was significant for $p < 0.05$. The statistical reliability of the research results was provided by analyzing samples with the number from 12 to 24.

RESULTS AND DISCUSSION

As can be seen from the data in Table 2, the highest concentration of cortisol in the blood plasma of slaughter pigs was after their quarantine for 10 – 14 h at the slaughter point under starvation conditions. Thus, compared with blood samples taken from pigs slaughtered shortly after arrival, cortisol levels were higher by 36 – 41.4% ($p < 0.05$).

Table 2 The content of cortisol in pigs' blood, depending on the pre-slaughter conditions of storage and transportation.

Terms		Transportation					
		long without breaks		long with breaks of 0.5 hours every 2 hours		short	
Before slaughter		Slaughter on arrival	Slaughter in 10 – 14 hours	Slaughter on arrival	Slaughter in 10 – 14 hours	Slaughter on arrival	Slaughter in 10 – 14 hours
Without water and food	M ±m	245.2 ±21.31	333.4 ±22.84	217.2 ±22.94		208.6 ±13.14	294.9 ±14.97
	Min	122.1	190.4	78.6		107.9	155.2
	Max	369.0	408.8	310.0		250.4	355.7
Only water	M ±m		314.1 ±23.44		252.5 ±16.2		247.8 ±19.78
	Min		162.3		108.2		94.5
	Max		410.8		321.8		305.4
Food and water	M ±m		180.8 ±17.25		170.5 ±20.10		143.2 ±12.27
	Min		102.1		75.4		77.6
	Max		302.1		295.4		210.4

Note: lactose concentration (mmol.L^{-1}); n = 12.

In the group of pigs with free access to before slaughter water, the plasma cortisol concentration was slightly lower than in the blood of pigs without such access. However, the difference was in incredible values. The lowest hormone levels were found in the blood plasma of pigs that had access to water and food before slaughter. Yes, compared to animals, who were without water and food, the figure was lower by 45.8 – 51.4% (p <0.05), and compared to animals that had access only to water, by 42% (p <0.05).

We found that the time of transportation of pigs to the slaughter point slightly affected the level of cortisol in the blood plasma of pigs. In particular, comparing the average level of cortisol in the blood of transported pigs to the slaughterhouse for 6 – 8 hours and scored in a short period on arrival, we found a lower (15%) content compared to the figure obtained from pigs that were delivered to slaughter within 1 hour, but this difference was statistically unlikely.

Our research showed (Table 2) that the transportation of animals for an extended period with the use of pauses for rest allowed to reduce the level of cortisol in the blood of slaughter animals (by 5.7 – 19.6%), but probably a significant result (p <0.05) was recorded only when comparing groups in which they kept the exposure for 10 – 14 h at the slaughterhouse and free access of pigs to water. Unfortunately, it was impossible to form a group of pigs, transported for a long time with breaks for rest and kept based on pre-slaughter keeping without access to water and food.

In general, the cortisol level in the blood plasma of slaughter animals ranged quite widely (from 75.4 to 408.8 nmol.L^{-1}), depending on transportation conditions and before slaughter.

Studies of the lactate content in the blood of slaughter pigs showed (Table 3) that the highest concentration was found in pigs that were quarantined for 10 – 14 hours at the slaughterhouse. Thus, the content of lactate in the blood of such pigs was higher by 34.7 – 37.9% (p <0.05) compared with its content in the blood of pigs that were slaughtered immediately upon arrival. Notably, in the blood of pigs that had access to water and food during quarantine, the lactate concentration was 2 – 3 times lower (p <0.05). The access of pigs to water did not significantly reduce the lactate level in the blood.

Analyzing the lactate level in the blood of pigs, depending on the duration of their transportation to slaughter, we can say about the ambiguity of the results. Among the groups of pigs slaughtered immediately upon arrival at the slaughterhouse, the highest rate was obtained in the blood of animals after short-term transportation and the lowest among those given rest during long-term transport. However, the difference was incredible. There was also no significant difference in the lactate level in pigs' blood when comparing the two approaches to long-term transportation: with rest breaks and without them.

The obtained results showed that the level of lactate in pigs' blood ranged from 2.9 to 24.5 mmol.L^{-1}, which is above the upper limit of physiological fluctuations for this species. Statistical analysis showed a positive correlation between medium (r = 0.4) and high (r = 0.9) levels between the concentration of cortisol and lactate in the blood of pigs.

Table 3 The lactate content in pigs' blood, depending on the pre-slaughter conditions and transportation.

Terms		Transportation long without breaks		long with breaks of 0.5 hours every 2 hours		short	
Before slaughter		Slaughter on arrival	Slaughter in 10 – 14 hours	Slaughter on arrival	Slaughter in 10 – 14 hours	Slaughter on arrival	Slaughter in 10 – 14 hours
Without water and food	M ±m	12.4 ±1.70	16.7 ±1.09	11.4 ±1.50		13.2 ±1.20	18.2 ±1.04
	min	4.3	9.5	5.5		6.5	12.4
	max	24.5	23.8	20.1		19.1	21.6
Only water	M ±m		16.6 ±0.91		16.1 ±1.08		15.8 ±1.08
	Min		9.7		9.8		8.5
	max		20.5		21.0		20.1
Food and water	M ±m		8.3 ±0.97		7.5 ±1.23		5.8 ±0.95
	min		6.1		3.2		2.9
	max		18.5		19.5		13.4

Note: lactose concentration (mmol.L^{-1}); n = 12.

Figure 1 shows that the highest pH level of the longest back muscle was found in the group of pigs with lower levels of cortisol and lactate in the blood immediately after slaughter. The pH of pig meat with medium and high cortisol and lactate levels in the blood was more acidic (0.3 units; p <0.05). A similar trend persisted at other stages of meat maturation. From the first to the twelfth hour of meat maturation, the pH of the longest back muscle of pigs with medium and high levels of cortisol and lactate in the blood was more acidic (p <0.05), but the most pronounced difference was (0.7 – 0.8 units) one hour after slaughter. At 12 hours of meat maturation, the difference was 0.4 pH.

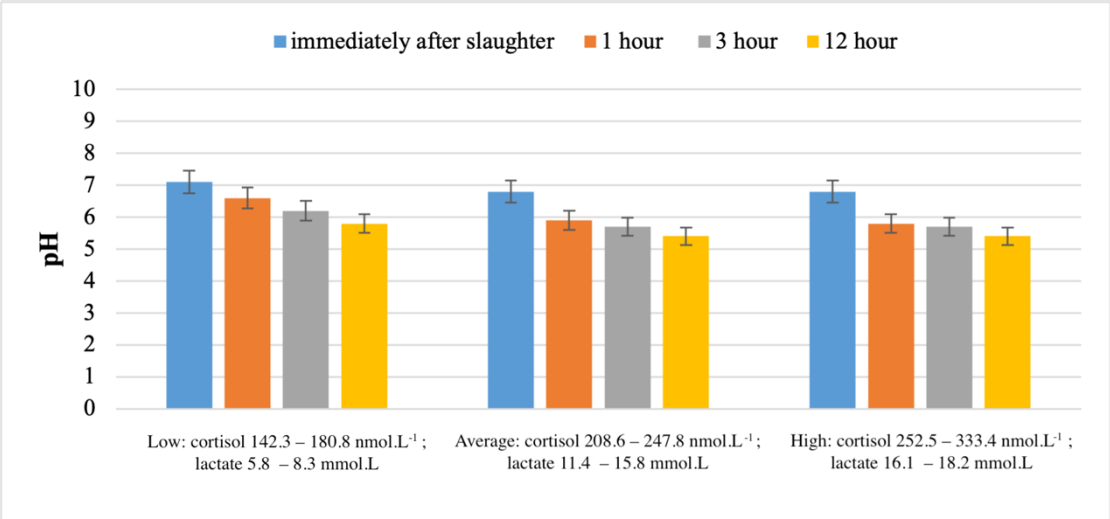

Figure 1 pH of the longest back muscle during maturation, depending on the level of cortisol and lactate in the blood. Note: n = 12. When dividing pigs into groups according to the level of cortisol and lactate into low, medium and high, our results were taken into account, not the physiological norm of these substances.

In meat selected from pigs with lower cortisol and lactate levels, a gradual decrease in pH to 12 hours after slaughter was observed (Figure 1). In contrast, in pig meat with medium and high levels of cortisol and lactate, there was a sharp decrease in pH during the first hour of maturation and a gradual up to 12 hours. No significant results were found between pigs with high and medium cortisol and lactate levels.

Table 4 shows data comparing the stress level in different breeds of pigs. The blood of Landrace pigs showed slightly lower cortisol and lactate concentrations than Ukrainian steppe white pigs, but the difference was unbelievable. The pH of the meat of both breeds of pigs at different stages of its maturation was also similar. Only the probably lower pH values (p <0.05) of the meat of pigs transported to the slaughterhouse within 0.5 – 1 h compared to those transported 6 – 8 h are noteworthy. Thus, one hour after slaughter, the meat's pH difference ranged from 0.6 to 0.7 units. However, the difference in performance is probably due to the peculiarities of the delivery of pigs, not their breed.

Table 4 The content of cortisol and lactate in the blood and pH of pig meat of different breeds.

Indicator		Transportation			
		6 – 8 hours without breaks		0.5 – 1 hour	
		Breed			
		Ukrainian white steppe	Landras	Ukrainian white steppe	Landras
Cortisol nmol.L^{-1}	M ±m	245.2 ±21.31	207.5 ±25.49	208.6 ±13.14	185.9 ±14.80
	min	122.1	81.4	107.9	103.6
	max	369.0	305.4	250.4	278.6
Lactate mmol.L^{-1}	M ±m	12.4 ±1.70	11.3 ±1.74	13.2 ±1.20	12.5 ±1.57
	min	4.3	3.2	6.5	4.1
	max	24.5	20.1	19.1	20.5
pH of meat after slaughter	M ±m	6.8 ±0.07	6.9 ±0.08	6.8 ±0.10	6.8 ±0.04
	min	6.4	6.1	5.7	6.5
	max	7.1	7.1	7.0	7.1
pH of meat 1 hour	M ±m	6.4 ±0.10	6.4 ±0.11	5.7 ±0.07	5.8 ±0.08
	min	5.9	5.9	5.3	5.1
	max	7.0	7.0	6.0	6.2
pH of meat 3 hours	M ±m	6.0 ±0.06	5.9 ±0.10	5.7 ±0.10	5.7 ±0.03
	min	5.84	5.4	5.3	5.3
	max	6.35	6.3	6.7	5.8
pH of meat 12 hours	M ±m	5.3 ±0.05	5.4 ±0.09	5.5 ±0.06	5.5 ±0.07
	min	5.1	5.0	5.2	5.1
	max	5.8	6.0	5.8	5.7

Note: n = 2.

Figure 2 shows the natural weight loss results of the longest back muscle sample during 12 hours of maturation. The most pronounced decrease in the mass of the longest back muscle samples was found in the groups of pigs that experienced the most significant pre-slaughter stress and had high cortisol and lactate levels in the blood. Thus, during the 12-hour maturation of pig meat with a lactate level in the blood above 11 mmol.L^{-1}, the samples lost an average of 6.12 g, which was 1.15%, and samples of pig meat with a lactate level in the blood above 16 mmol.L^{-1} – 6.44 g, which was 1.23%. Instead, a similar weight loss of the sample taken from pigs with low blood lactate levels (up to 8.3 mmol.L^{-1}) was 0.97%, respectively. In addition, a more uniform natural weight loss of samples taken from pigs with low cortisol and lactate levels is noteworthy.

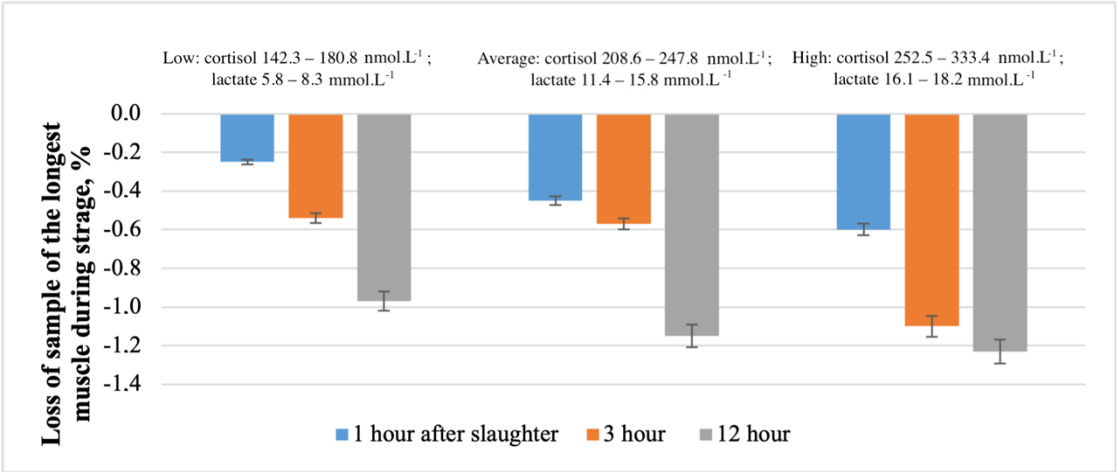

Figure 2 Loss of sample of the longest pig back muscle during 12 hours of storage, depending on the level of cortisol and lactate in the blood, %. Note: when dividing pigs into groups according to the level of cortisol and lactate into low, medium and high, our results were taken into account, and not the physiological norm of these substances.

As shown from the data shown in Figure 3, pigs' access to water during the quarantine period of slaughter allowed to reduce (p <0.05) the natural weight loss of samples compared to samples obtained from animals

without access to water and food. The difference was 0.36% after one hour of storage, 0.59 on the third and 0.23 on the twelfth. Compared to pigs that received water and food, the difference was unlikely.

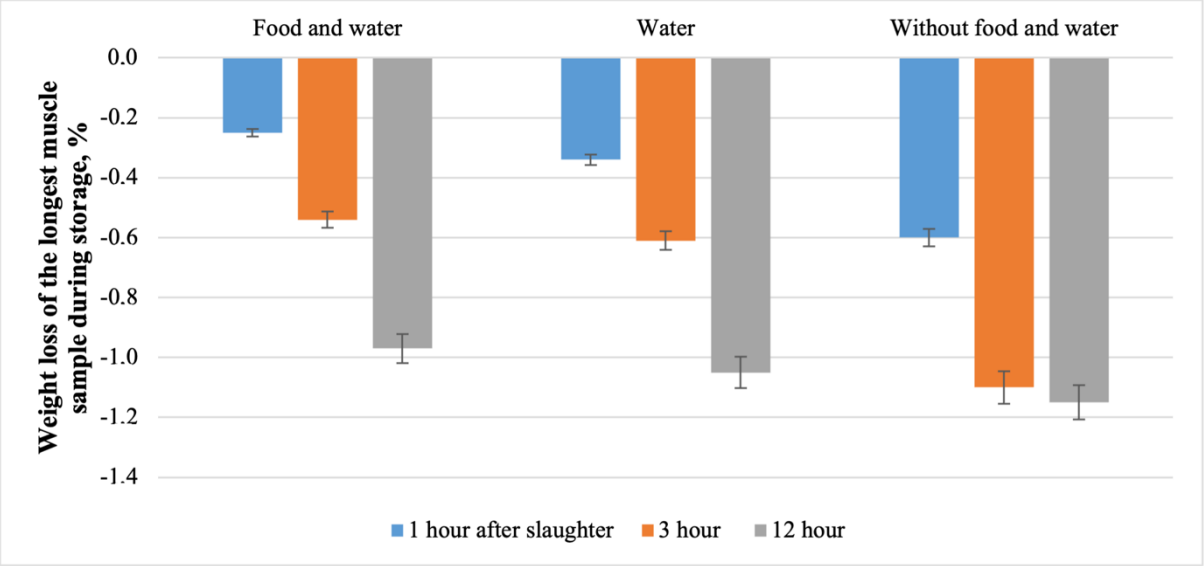

Figure 3 Weight loss (%) of the longest back muscle sample during 12 hours of storage, depending on the conditions before slaughtering keeping pigs.

After 12 hours of maturation, meat obtained from pigs with high lactate levels and more acidic pH was paler, softer, and exudative than meat obtained from lower lactate levels and less acidic pH (Table 5). Thus, most meat samples (75 – 83%) classified as low quality (PSE) were obtained from pigs slaughtered after 10 – 14 hours in a slaughterhouse without access to food. Meat obtained from pigs that experienced lower stress levels was pink, soft and non-exudative (NOR). As a result, the percentage of high-quality meat samples (NOR) in the groups of pigs that had access to water and food ranged from 67 to 75%. The quality to poor quality meat ratio in the animals with access to water ranged from 25:75 to 33:67%. It is noteworthy that in the groups in which the pigs were slaughtered before one o'clock on arrival, the ratio of quality to low-quality meat ranged from 58:42 to 50:50%.

Table 5 Quality of pig meat, depending on transport conditions and before slaughter; several samples.

Meat quality category	Conditions	Transportation: Long without breaks		Transportation: Long with breaks 0.5 hour. every 2 hours		Transportation: Short	
	Before slaughter	Slaughter on arrival	Slaughter in 10 –14 hours	Slaughter on arrival	Slaughter in 10 – 14 hours	Slaughter on arrival	Slaughter in 10 – 14 hours
DFD	Without water and food	1	0	0		1	0
NOR		6	3	6		7	2
PSE		5	9	6		4	10
DFD	Only water		0		0		1
NOR			4		3		4
PSE			8		9		7
DFD	Food and water		0		2		1
NOR			9		8		9
PSE			3		2		2

It should be noted that individual meat samples obtained from pigs of different groups were classified as DFD (dark, hard, dry).

Given that cortisol is a glucocorticoid hormone, which is the trigger for the development of a chain of biochemical stress reactions, it can be said that the most significant stress was experienced by pigs, which were kept for 10 – 14 hours without food and water after delivery to the meat processing plant. In our opinion, this stress factor has several components. First of all, it is a change of habitual location, transport in which the animal can not feel at ease because in a very limited space it does not find a safe place, the use of electric sticks to keep pigs indoors. In addition, the new model of the day adds its influence because the animal does not receive food at the usual time in the usual place. Mixing animals from different farms also increases stress. There is evidence in

the literature that this is also a significant stressor **[13]**. In particular, hungry pigs in a state of stress become aggressive and conflict with each other. Also important is the number of animals in the same group **[15]**.

In pigs slaughtered immediately upon arrival, cortisol levels were significantly lower, indicating that the magnitude of the stress factor that occurs during ante-mortem maintenance without food is higher than the level of "transport" stress. The lowest cortisol level was found in the blood plasma of pigs that had access to water and food during their stay at the slaughterhouse. This suggests that food is a calming factor for pigs. It can be assumed that releasing endorphins from meeting physiological needs can slow down the cascade of stressful biochemical reactions. It should be noted that cortisol was high in pigs with access to water while kept at the slaughterhouse—watering the animals before slaughter did not significantly reduce stress. Data in the literature indicate the optimal pre-slaughter time of pigs in reducing stress and improving the quality of meat. In particular, according to researchers **[16]** – it is three hours.

Significant fluctuations in the concentration of cortisol in the blood plasma of pigs in one experimental group indicate the individual characteristics of the response to the stress factor. In particular, there are data in the literature on the dependence of the amount of stress, and therefore the quality of meat, on the nature of the animal, its age and sex **[10]**, **[17]**.

Under stress, the activity of metabolic processes in the body increases significantly. Carbohydrates are used to meet the needs of energy metabolism. Muscle carbohydrates (primarily glucose and glycogen) are metabolized to lactate by anaerobic oxidation. Accordingly, the greater the amount of stress, the higher the lactate level in the body and the lower the level of muscle carbohydrates. Our studies have shown a moderate to strong positive correlation between cortisol and lactate levels in pig blood. Thus, the lactate concentration was the highest in the blood of pigs kept for 10 – 14 hours in the slaughterhouse without access to water and food.

In contrast, the lowest lactate levels were in pigs' blood with lower cortisol levels. These were pigs that had access to water and food. The lactate level in the blood of pigs that were slaughtered immediately upon arrival had an intermediate position. These patterns support the assumption of researchers that measuring the lactate content in the blood of slaughter pigs can predict the quality of the meat obtained **[18]**, **[19]**. We are also inclined to think about the high informativeness of this indicator. Other researchers point to the high diagnostic value of lactate dehydrogenase **[20]** and glucose **[21]** as prognostic markers of meat quality.

The pH of meat is one of the main indicators of its quality. In the case of high concentrations of lactate in pigs, the pH of the meat will shift to the acidic side. However, if the pH of the meat decreases too quickly during maturation, the quality of the pork will decrease. Rapid hardening of muscle fibers damages their structure causes excessive moisture loss and discolouration, and thus reduces the quality and shelf life of meat **[17]**, **[22]**. We found that when the lactate concentration in the blood of slaughter pigs is above 11 $mmol.L^{-1}$, there is a sharp decrease in the pH of the longest back muscle during the first hour after slaughter. In addition, the higher the blood lactate content, the more acidic the starting pH of the meat. The lower the content of cortisol and lactate in the blood, the more evenly the pH of the meat decreases as it matures. Similar results have been established by other researchers **[23]**.

As a result of a sharp decrease in the pH of the meat of pigs, which, judging by the high concentration of cortisol in the blood, experienced a high level of stress before slaughter, the products were of low quality. The review showed that the meat obtained can mainly be classified as PSE (pale, soft, exudative) with some exceptions when individual meat samples corresponded to the NOR category (close to the optimal quality indicators). In contrast, the predominant number of meat samples (9 – 11 samples) obtained from pigs with blood concentrations of cortisol and lactate in the range of 142.3 – 180.8 $nmol.L^{-1}$ and 5.8 – 8.3 $mmol.L^{-1}$, respectively, corresponded to the NOR quality category, and fewer samples (2 – 3 samples) of PSE category. In this case, single samples of meat in different groups of pigs were classified by us as DFD (dark, hard, dry). In our opinion, the main reason for this is the chronic stress experienced by pigs in the place of rearing. Accordingly, with low amounts of muscle carbohydrates, the pH of meat during maturation will be high, protein denaturation is inactive, water is tightly bound, and there is little or no exudate formation, which leads to dry, hard and dark meat (DFD) **[10]**, **[24]**, **[25]**.

After 12 hours of maturation of the meat, the highest percentage of weight loss was found in those samples in which there was a sharp decrease in pH and assigned to low-quality categories.

Interestingly, the pigs' access to water did not reduce the stress level but prevented significant weight loss of the sample during 12 hours and obtained products of slightly higher quality than on a completely starving diet—watering pigs during quarantine at the slaughterhouse. Similar results have been established by other researchers **[26]**.

The highest levels of stress and, consequently, the lowest quality of meat are characteristic of pigs kept in quarantine before slaughter without access to water and food. Feeding and watering pigs before slaughter positively affects the quality of the meat, but the positives of this scheme have their negatives. In particular, the animal's intestines filled with food can adversely affect the safety of the meat, as careless handling can

contaminate the contents of the intestines. The more filled the gastrointestinal tract during slaughter, the higher the risk of rupturing these tissues during ingestion and contamination of the carcass. It is known that the rate of salmonella secretion in animals increases both with the time of food withdrawal and with stress **[27]**, **[28]**, **[29]**. Equally important is that after ingestion, the food will be absorbed in the small intestine in four to eight hours, and most of the nutrients will be absorbed into the bloodstream in nine hours **[29]**. In addition to the above, it should be borne in mind that the filled intestine creates problems for the technological processing of the intestine itself. For example, for its picking and making a natural sausage casing. In addition to contaminating the product's contents, such intestines will be well filled with blood, not allowing us to obtain high-quality products. Lower levels of stress are experienced by pigs slaughtered immediately upon arrival. Still, such a scheme makes it impossible to observe animals before slaughter for clinical signs of infectious or non-infectious diseases.

Our research results indicate no dependence of stress levels on the breed of pigs. However, in our research, we selected material only from pigs of two breeds: Ukrainian steppe white and landrace. Perhaps other breeds of pigs have more pronounced stress resistance. In particular, some researchers point to this, associating it with the presence of a specific genotype that exhibits a high sensitivity to halothane **[7]**, **[30]**, **[31]**.

The duration of transporting pigs to the slaughterhouse probably did not affect the stress level. It also did not significantly reduce the stress level of alternating rest and transportation during long trips. During transport, the main stress factor for pigs is not the feeling of "road", but being in an unusual and limited space where the animal does not find a safe place. However, convincing evidence can be found in the literature that the amount of stress during pig transport depends on the season **[11]**, **[32]**.

In conclusion, feeding pigs before slaughter reduces the negative impact of stress on meat quality. To date, there are no clear time limits for a starvation diet for pigs before slaughter. But pork must be safe first and foremost, so the logistics of determining the duration of a starvation diet for pigs should consider many factors: the remoteness of the farm from the meat processing plant, feed digestion time, technical capacity of the enterprise; season and many others.

CONCLUSION

The highest quality pork was obtained from pigs that experienced lower stress levels before slaughter. Keeping pigs before slaughter without access to water and food caused higher stress levels, probably expressed in higher blood concentrations of cortisol and lactate. The higher the lactate concentration in pigs' blood, the faster the pH level of meat after slaughter, which adversely affects its quality and moisture retention. Access to pigs to food and water during pre-slaughter keeping allows them to obtain high-quality meat by reducing the impact of stress factors. Pigs' access to water during pre-slaughter ageing does not affect the stress level but positively affects the weight loss of meat during maturation. For the needs of long-term transportation of pigs, rest stops do not significantly reduce the level of stress in pigs. The duration of transportation of pigs does not correlate with the level of stress, as the conditions of pre-slaughter keeping have a decisive influence.

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

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The hypoglycemic and regenerative effect of the pancreas using instant porridge mix of pumpkin and brown rice flour on diabetic rats

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ABSTRACT

Diabetes is a congenital disease resulting from inefficiencies in insulin production and activities. Instant porridge mixed with pumpkin and brown rice (instant porridge mix) can be a functional food to lower blood sugar. This study aimed to determine the hypoglycemic activity and the ability of instant porridge mix to regenerate pancreatic beta cells in diabetic rats. Diabetes was induced by Streptozotocin (STZ). Instant porridge mix was used to substitute the standard feed AIN-93 at 0, 10, 20, and 30% levels. The hypoglycemic activity test used 30 Sprague Dawley rats assigned to five groups with six each. The groups were (1) normal rats fed with standard feed AIN-93, (2) DM/diabetes mellitus rats fed with AIN 93 feed, (3) DM rats fed with 10% instant porridge mix, (4) DM rats fed with 20% instant porridge mix, and (5) DM rats fed with 30% instant porridge mix. The treatment was carried out for twenty-eight days, and blood sampling was carried out at seven-day intervals for blood analysis to determine glucose levels. At the end of the study, the levels of MDA (malondialdehyde) and blood glucose in the liver of the rats were also analyzed. A histopathology test was also done on the pancreas. The results showed that feed substitution (20%) with instant porridge mix significantly (p <0.05) reduced the level of blood glucose from 271.81 to 99.66 mg.dL^{-1} in DM rats. In conclusion, DM rats fed with 20% instant porridge mix were the best treatment for hypoglycemic and regenerative effects of the pancreas.

Keywords: instant porridge mix, pumpkin, brown rice, pancreatic β cells, hypoglycemic, diabetic rats.

INTRODUCTION

Diabetes mellitus (DM) is a chronic degenerative disease resulting from low insulin production by the pancreas or poor insulin performance. DM is characterized by chronic high blood sugar (hyperglycemia) due to impaired insulin production, secretion, or insulin resistance. The increasing number of people suffering from DM and the accompanying complications have made DM a very concerning disease. In 2014, 12 million people in Indonesia suffered from DM, reportedly **[1]** the third most deadly disease after stroke and heart disease. Another study defines DM as a chronic, multi-factorial metabolic disorder generally characterized by disorders of carbohydrate, protein, and lipid metabolism resulting from damage to the action of insulin, secretion of insulin, or both **[2]**. The prevalence of world diabetes in 2004 was 2.8% and is estimated to reach 4.4% by 2030 **[3]**. In 2014, WHO reported a 9% diabetes prevalence among the human population, and therefore, diabetes must be treated immediately. Various kinds of antidiabetic medicine have been used to treat diabetes, including oral antidiabetic drug, which reportedly causes side effects such as liver problems, diarrhoea, and lactic acidosis **[4]**. Today, there has been considerable research on the potential natural ingredients for safer treatments of diabetes. Vegetable bearing hypoglycemic potentials such as pumpkin (*Cucurbita pepo* L) has been made into flour and fed to rats to reduce blood sugar levels **[5]**. Pumpkin contains bioactive compounds such as phenolic, proteins, peptides, sterols, terpenes, and polysaccharides **[6]** and hypoglycemic properties and antidiabetic effects **[7]**. Ju and Chang **[8]** found that pumpkin flour as feed successfully increased plasma insulin and reduced glucose levels

significantly. Various pumpkins contain pharmacological characteristics, including antioxidant, hepatoprotective, and lipid-lowering abilities **[9]**, antidiabetic abilities **[10]**, antimicrobial and anti-carcinogenic **[11]**. The mechanism of blood sugar depletion is through the accelerated release of glucose from circulation, which is closely related to the work of the heart, and by accelerating the filtration and excretion of the kidneys. By this, urine production increases, and the rate of glucose excretion through the kidneys increases, thereby decreasing glucose levels in the blood. Rice is food and a source of energy, and the source of protein, vitamins, and minerals that are beneficial for human health. There are several types of rice in Indonesia based on colors, such as white rice, black rice, glutinous rice, and brown rice. Brown rice is generally sold without brushing but ground into cracked rice with the husk still attached to the endosperm. While brown rice bran is rich in natural oils, essential fats, and fiber **[12]**, organic brown rice contains 6.5% moisture, 1.8% fat, and 73.8% starch **[13]**.

Pumpkin and brown rice instant porridge (instant porridge mix) is the potential functional food to lower blood sugar in people with diabetes. This study was conducted to determine the effect of different levels of pumpkin and brown rice instant porridge on antidiabetic activity and the capacity of the porridge mix to regenerate β-pancreatic cells in rats.

Scientific Hypothesis

This study hypothesizes that pumpkin and brown rice instant porridge could serve as a novel functional food to control DM by depleting blood glucose and generating β-pancreatic cells in diabetic rats.

MATERIAL AND METHODOLOGY

Chemical substances

All chemical substances used in this study were analytical grade. The Streptozotocin (STZ) was purchased from Sigma-Aldrich (Germany), and the reagents included GOD-PAP (Glucose Oksidase-Phenol Amino peroxidase) and Nicotinamide (Na). The insulin kit (Rat) Elisa was manufactured by DRG Catalog No EIA 2048. We used AIN-93 standard feed, which consisted of casein, fiber, soybean, choline bitrate, AIN 93 MX, corn starch, L-cystine, and AIN 93VX.

Description of the Experiment

Sample preparation: The pumpkin was peeled, seeded, and cut to a size of 2 x 2 x 2 cm. Then, the pumpkin was mixed with brown rice (75:25 w/w) in a blender, added with 50 mL of distilled water, and blended. The mixture was oven-dried at 160 °C for 15 minutes to make instant porridge **[14]** that would substitute the feed for the Sprague Dawley rats.

Preparation of feed formulation: The feed formulation used is present in Table 1.

Table 1 Feed formulations for experimental rats.

Composition	Feed treatment (g)			
	Standard and 0%	10% Instant porridge	20% Instant porridge	30% Instant porridge
Maizena	620.69	520.69	420.69	320.69
Instant porridge*	0	100	200	300
Casein	140	140	140	140
Sucrose	100	100	100	100
Oil	40	40	40	40
Fiber	50	50	50	50
AIN 93 MX	35	35	35	35
AIN 93 VX	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5

Note: DM – Diabetes mellitus.

Animal experiment

We used 30 male Sprague Dawley rats aged two months and weighed 230 grams to be reared in stainless steel cages. Before the experiment, the rats were adapted to standard feed AIN-93 for three days, and drinking water was provided ad libitum. AIN-93 was the standard feed used in this study **[15]**. The rats were reared in a room with natural lighting at room temperature. After the adaptation period, the rats were fed with high cholesterol feed

for seven days by substituting the maizena with 20 g of cholesterol and 2 g of cholic acid. After seven days, 24 Sprague Dawley rats were induced with diabetes intravenously using Streptozotocin/STZ dissolved in 3 mL citrate buffer administered at a dose of 65 mg.kg^{-1} body weight of the rat **[16]**. Five days after the administration, blood samples were drawn from the rats for day 0. All 30 rats were rationed to 5 treatment groups, namely (1) normal rats fed with standard feed AIN-93, (2) 0% DM rats fed with AIN 93 feed, (3) DM rats fed with 10% pumpkin and brown rice instant porridge mix (4) DM rats fed with 20% instant porridge mix, and (5) DM rats fed with 30% instant porridge mix. Food and water were provided ad libitum, the treatment was carried out for twenty-eight days, and blood samples were collected at seven days (days 0, 7, 14, 21, and 28) for blood glucose analysis using Insulin Elisa Kit (U-Cloud-Clone Corp). Before abdominal surgery, the rats were anaesthetized using diethyl ether to obtain the liver and pancreas. The liver was subjected to analysis using the thiobarbituric acid reactive substance (Cayman, USA) to determine MDA levels.

Histological and immunohistochemical analysis (IHC)

The pancreas was cleaned and fixed in 10% formalin solution and dehydrated and dipped in a paraffin solution before slicing to 7 µm thick using a microtome. The incised portion was stained with hematoxylin-eosin, and the Island of Langerhans found on the stained pancreas was subjected to IHC analysis using the Histofine Mouse Stain Kit. Langerhans' dark brown islets indicate insulin presence, whose intensity was analyzed semiquantitatively.

Statistical Analysis

The statistical analysis was performed using SPSS version 24 (SPSS Inc., Chicago, Illinois, USA), and significant differences were tested using the Duncan Multiple Range Test (DMRT) at the 95% confidence level (p <0.05).

RESULTS AND DISCUSSION

Weight of rats and feed intake

The average body weight of the rats at the start of the experiment was 185.99 ±7.98 grams (Figure 1), then increased to 209.50 ±7.21 g after the adaptation period. However, after diabetes treatment, the body weight of control diabetic rats (DM rats fed on standard feed AIN-93 or 0% instant porridge mix) decreased from day 0 to day 28. Thus, the control diabetic rats experienced the most weight loss throughout the experiment. Meanwhile, increased body weight was observed among normal rats with standard feed and not significantly different (p <0.05) body weight among DM rats fed with 10, 20, and 30% instant porridge mix on the 28th day. This study revealed that pumpkin and brown rice instant porridge mix could improve metabolism in the rat compared to DM rats fed on standard feed or 0% instant porridge mix.

The average feed intake of the rats during 28 days showed that feed intake was not significantly different (p <0.05) between normal rats (11.78 ±0.36 g) and DM rats fed on instant porridge mix at 0% (13.7 ±0.19 g), 10% (11.21 ±0.25 g), 20% (11.36 ±0.31g) and 30% (11.70 ±0.59 g). The highest feed intake was in DM rats fed with 0% instant porridge mix. Therefore, in diabetic rats, standard feed (0% instant porridge mix) could not improve body weight and diabetes conditions because the rats' body metabolism was affected, which increased feed demand but lowered weight gain.

Blood glucose levels of rats

The blood glucose levels are shown in Table 2 and Figure 2. The initial blood glucose level ranged from 87.26 to 88.96 mg.dL^{-1}, suggesting an average serum glucose level before the induction of diabetes with STZ. According to **Nichols [17]**, blood glucose level for normal rats ranges between 62 and 175 mg.dL^{-1}. After the adaptation period, DM rats fed on 0, 10, 20, and 30% instant porridge mix were induced with diabetes. Three days after the induction, the blood glucose level increased significantly (p <0.05) from 265.48 to 276.84 mg.dL^{-1}, whereas in the normal rats, significant differences (p <0.05) were not observed. Treatment with STZ resulted in diabetic conditions in the rats and could damage the β-pancreatic cells. The glucose transporter (GLUT2) enables the transfer of STZ into β-pancreatic, rendering necrosis in the insulin-secreting cells **[16]**.

The effects of pumpkin and brown rice instant porridge mix on the level of blood glucose in DM rats are shown in Figure 2. During the 28-day experiment, the glucose levels of the blood serum decreased across treatment except for the control DM rats (rats fed with 0% instant porridge mix). Treatment with pumpkin and brown rice instant porridge mix generally reduced the blood glucose levels in which the higher percentage of treatment, the lower the glucose level (Table 2). In other words, the intake of pumpkin and brown rice instant porridge can improve diabetic conditions, and the antidiabetic capacity increases with the intake level. Aukanit and

Sirichoworrakit **[18]** reported that feeding rats with 2 g.kg^{-1} pumpkin flour could better reduce glucose levels (from 104.2 to 98.75 mg.dL^{-1}) than 1 g.kg^{-1} pumpkin flour.

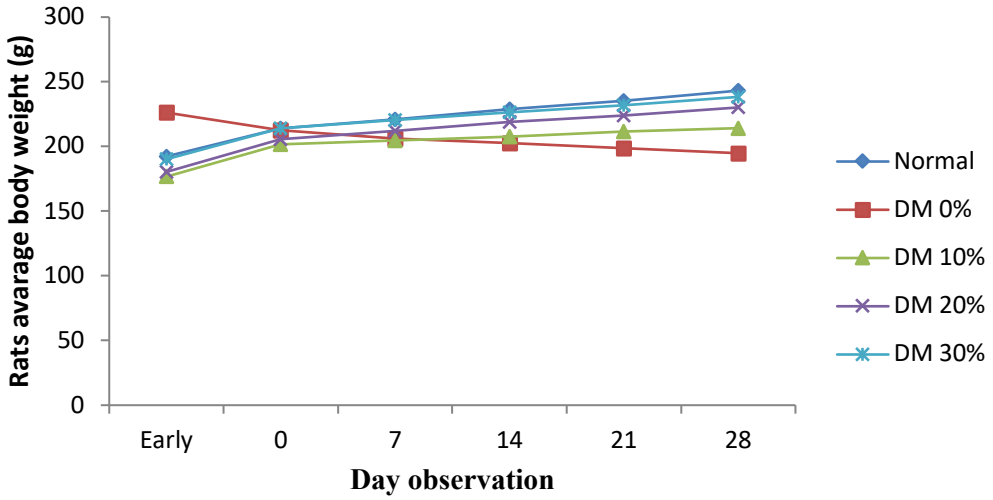


Figure 1 Body weight (average) of rats used in this study.

In this study, the blood sugar level of the DM rats fed with 0% instant porridge mix was 271.81 mg.dL^{-1}. We found that pumpkin and brown rice instant porridge mix significantly reduced blood sugar levels. In DM rats, the blood glucose levels were highest in DM rats fed with 0% instant porridge mix, followed by the 10%, 30%, and 20% treatments. The decrease in blood glucose level was because feeding DM rats with 10, 20, or 30% instant porridge mix reduced sugar levels in the blood compared to the 0%. Sharma and Rao **[19]** reported that pumpkin contains reducing sugar and total sugar of 77.30 and 90.13 mg.g^{-1}, respectively.

Table 2 The levels of blood insulin, glucose, and liver malondialdehyde in rats.

Treatment/rats group	Blood glucose levels (mg.dL^{-1})	Blood insulin levels (IU.dL^{-1})	Liver MDA levels (nmol.g^{-1})
Normal rats (standard feed)	89.97 ±3.34^{a}	17.25 ±0.06^{e}	0.97 ±0.01^{a}
DM 0% (standard feed)	271.81 ±10.76^{e}	5.94 ±0.03^{a}	6.74 ±0.03^{e}
DM 10%	146.37 ±10.43^{d}	10.12 ±0.05^{b}	4.76 ±0.02^{d}
DM 20%	99.66 ±1.84^{b}	14.31 ±0.05c	2.22 ±0.02^{c}
DM 30%	111.93 ±1.76^{c}	15.47 ±0.11^{d}	1.41 ±0. 01^{b}

Note: Values with the same letter within a column show no significant difference at p <0.05 and *vice versa*.

The effects of polysaccharides in a pumpkin on rats with type 2 diabetes include increased insulin tolerance and HDL and decreased blood glucose, total cholesterol, and LDL **[20]**. Polysaccharides in pumpkin consist of xylose, arabinose, glucose, rhamnose, galactose, and glucuronic acid that reportedly exhibit good scavenging function against hydroxyl radicals and hydroxyl anions. In addition, polysaccharides in pumpkin function as antioxidants **[21]**. Treatment with chayote juice at a dose of 1 mL.100g^{-1} of body weight of rats per day decreased the triglycerides, total cholesterol, and LDL and increased HDL **[22]**. Pumpkin porridge and arrowroot starch at a 5:1 had a resistant starch content of 11.97% **[23]**, affecting blood glucose levels in rats because resistant starch is not easily digested into glucose.

Insulin levels of blood in rats

The insulin level of the blood in Table 2 shows that the DM rats fed with 0% instant porridge mix exhibited the lowest level of insulin (5.94 ±0.03 IU.dL^{-1}). In contrast, DM rats fed with 10, 20, and 30% instant porridge mix had higher blood insulin levels. We found that the higher the substitution level of instant porridge mix, the higher the insulin levels in the blood of the DM rats. In contrast to the blood glucose level (except rats fed with 20% instant porridge mix), the higher the blood insulin, the lower the blood glucose. This further strengthens the notion that treatment with pumpkin and brown rice instant porridge mix can improve diabetes conditions by increasing insulin production. This study demonstrated a positive possibility of insulin production for DM rats.

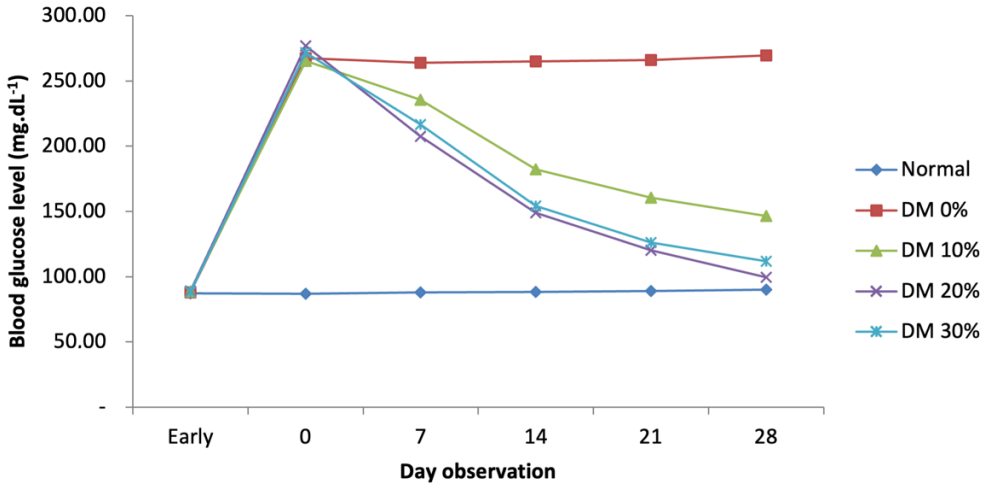


Figure 2 Rats blood glucose levels during the experiment.

Malondialdehyde (MDA) levels in rats

Diabetes is associated with oxidative stress. In the current study, STZ induction was used to trigger oxidative stress to cause diabetes in rats. It has been reported that oxidative stress can lead to type 2 diabetes, cell dysfunction, impaired glucose tolerance, and resistance to insulin **[24]**. The determination of malondialdehyde (MDA) in biological materials is widely used as an indicator of the presence of free radicals and oxidative damage, especially in unsaturated fatty acids that have more than one double bond. Malondialdehyde is an end-product of lipid peroxidation after free radicals attack the lipid membranes, which are rich in polyunsaturated fatty acids (PUFA). Analysis of free radicals was carried out by determining the levels of MDA in the liver of the rat (Table 2).

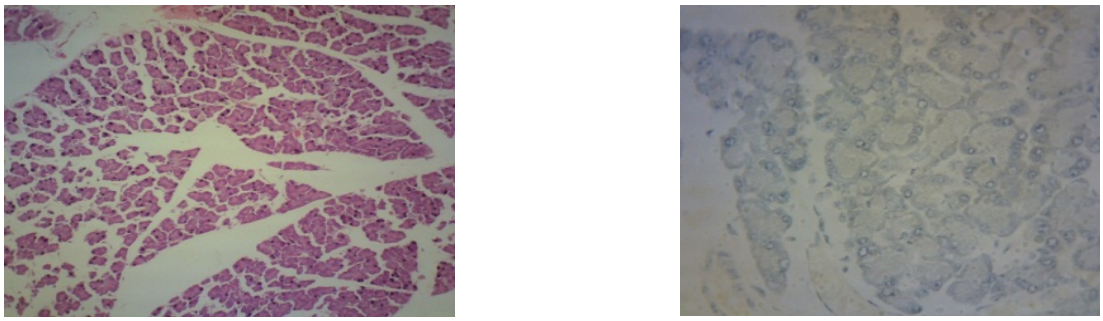
From Table 2, DM rats fed with 0% pumpkin and brown rice instant porridge mix had higher levels of MDA than the normal rats, and DM rats fed with 10, 20, and 30% instant porridge mix. Therefore, the treatment of DM rats with instant porridge mix can reduce MDA levels. The MDA levels in DM rats fed 30% instant porridge mix was 1.41 nmol.g^{-1}, and closer to the MDA level (0.97 nmol.g^{-1}) of rats fed on the standard feed, compared to DM rats fed with 10 and 20% instant porridge mix. Diabetes can increase the activity of fatty acyl-coenzyme A oxidase, which initiates the oxidation of fatty acids and results in lipid oxidation. STZ can trigger an increase in blood glucose and free radicals. Also, an increase in blood glucose levels can promote oxidative stress. Advanced glycation end products, peripheral nerve polyol, protein kinase activation, oxidative phosphorylation, and glucose automation pathways, which increase oxidative stress due to increased blood glucose levels.

Table 2 and Figure 2 show that the reduced blood glucose level in rats fed with instant porridge mix can decrease MDA level and increase blood insulin. The level of MDA in DM rats fed 10, 20, and 30% instant porridge mix were lower than that in 0% treatment. Animals, including rats, possess an internal oxidative stress defence system that can be increased by fortifying external antioxidants that are needed by the pancreas because it has a limited defence system against oxidative stress **[25]**. Pumpkin and brown rice instant porridge mix has high antioxidative activity and can function as an antidote against oxidative stress.

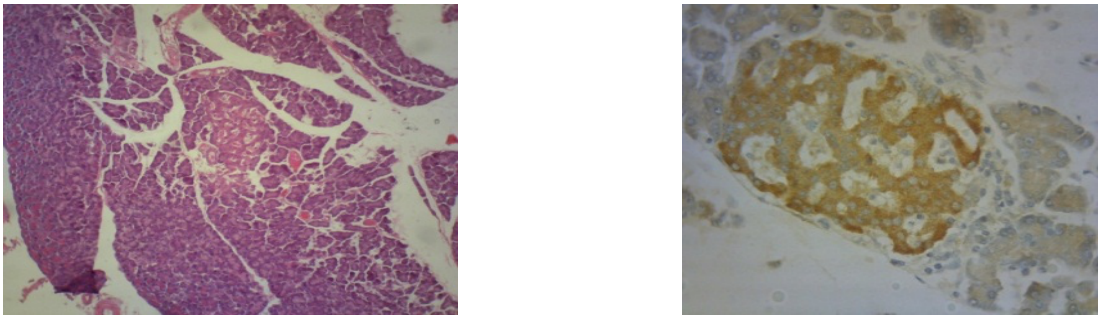
A higher ethyl acetate extract dose corresponds with the lower level of MDA in the liver of DM rats. Pumpkin and brown rice instant porridge contain 0.19% phenol **[23]** which acts as antioxidants, and therefore, the higher the dose, the greater the oxidative activity and the lower the MDA level. The antioxidant activity exhibited by phenol is associated with the balance of oxidation-reduction reaction. The electron is donated to the aromatic ring, thus increasing the speed of the oxidation inhibition reaction by antioxidants. Natural plant antioxidant compounds are generally phenolic compounds that are multifunctional and act as antioxidants because of their ability to act as reducers, free radical scavengers, metal binders, or triplet oxygen made from the singlet form **[26], [27]**.

Histopathology of the pancreas

The histopathology results are presented in Figure 3A through 3E. Figure 3a is a histological picture of the pancreas for the control group (DM rat given standard feed or fed on 0% instant porridge mix). The results of hematoxylin-eosin (HE) staining in these samples showed swollen cells and purple color (but not intense brown color). The brownish color indicates the presence of β-pancreatic cells, while the purple ones are not β-pancreatic cells.

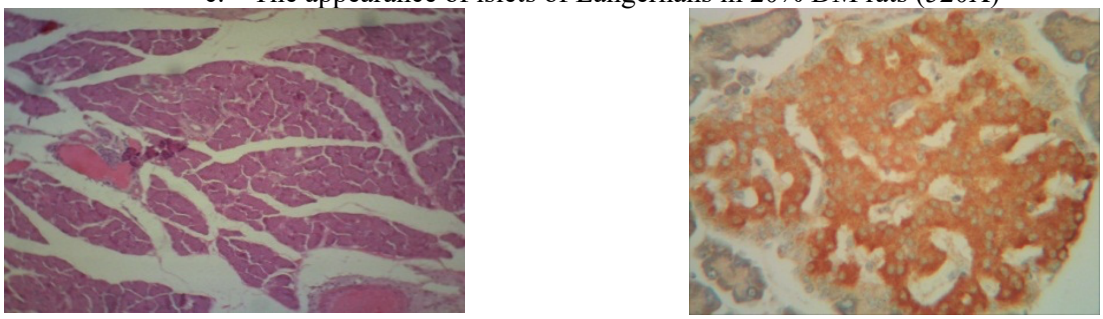


a. The appearance of islets of Langerhans in 0% DM rats (520X)



b. The appearance of islets of Langerhans in 10% DM rats (520X)

c. The appearance of islets of Langerhans in 20% DM rats (520X)



d. The appearance of islets of Langerhans in 30% DM rats (520X)

e. The appearance of islets of Langerhans in normal rats with standard feed (AIN 95) (520X)

Figure 3 Histology of the pancreas stained with HE.

This is very different (Figure 3a) from the staining results in the normal rats (Figure 3e), which showed a high brown color intensity with a very dense nucleus. The brown color indicates that insulin production occurs in the pancreas. Also, the control rats showed no β-pancreatic cells but necrosis and degeneration of the islands of Langerhans. Necrosis is characterized by empty spaces in the middle of the islands of Langerhans. According to **Szkudelski [16]** and **Rajeswari, Kesayan, and Jayakar [28]**, this observation supports the notion that STZ induction results in damage to β-pancreatic cells, decrease in the production of insulin, and an increase of glucose, all of which lead to hyperglycemia.

Based on the semi-quantitative observational data (Table 3), the intensity of insulin-positive cells in the control DM rats was negative (-), but in the DM rats fed 10, 20, and 30% instant porridge mix was increased intensity of insulin-positive cells. Feeding or treating DM rats with pumpkin and brown rice instant porridge mix can increase the intensity of insulin-positive cells, regenerate pancreatic beta cells and increase the ability of insulin secretion, as shown by the increased insulin and reduced glucose levels in the blood in this study.

Table 3 The intensity of insulin-positive cells in the islands of Langerhans in rats.

Parameter	Treatments				
	Normal rats (standard feed)	DM 0% (standard feed)	DM 10%	DM 20%	DM 30%
Insulin-positive cell intensity	+	-	++	+++	+++

Note: DM: diabetes mellitus; negative: -; weak intensity: +; moderate intensity: ++; high intensity: +++

CONCLUSION

Taken together, pumpkin and brown rice instant porridge mixed can reduce blood glucose and liver malondialdehyde levels while increasing blood insulin levels of diabetic rats. The higher the intake of instant porridge mix, the lower the blood glucose level and the less liver damage due to the reduced oxidative stress and higher insulin level in the blood. Feed substitution treatment at 20% was the best in reducing blood sugar and regeneration of pancreatic cells in diabetic rats.

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The authors declare no conflict of interest.

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Acclimatization of fish to the higher calcium levels in the water environment

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ABSTRACT

It is established that calcium concentration changes (variations) in the water environment significantly influence its intake and distribution in tissues and organs of hydrobionts. The decrease in calcium concentration in water from 100 to 60 mg.L^{-1} significantly reduces its content in fish liver. In the gills glandular apparatus of fish acclimated to the environment with lower calcium level (in comparison with control one), its concentration on the first day of the acclimation period slightly exceeded the initial level, thus testifying to its possible excretion of endogenous calcium by gills. The increase of calcium excretion through the renal and digestive systems in fish acclimates to the higher water level, and specific changes in phosphates excretion dynamics accompany oral intake. Long keeping fish in water with 100 mg.L^{-1} calcium is accompanied by the increase of total phosphorus in urine (by 2 – 2.5 times), and its day excretion increases by 1.9 – 2.4 times. During fish acclimation to higher calcium levels in the water environment, the excretion of total phosphorus with faecal matter increases. The increase of calcium in the water environment to 100 mg.L^{-1} leads to a temporary increase in total phosphorus excretion with faecal issues. The rise in cation concentration to 200 mg.L^{-1} increases significantly during long-time fish stay in such an environment.

Keywords: *Cyprinus carpio L.*, Ca^{2+} concentration, water environment, regulation, diuresis, excretion.

INTRODUCTION

Calcium and phosphorus are the most plastic elements in bone and other tissue structures, both terrestrial and water animals. Due to its chemical properties, calcium is one element that makes strong connections with proteins, phospholipids, organic acids, and other substances. These properties play an important role and influence many physiological and biochemical processes ongoing in animal organisms **[9]**, **[10]**. It plays an important role in cell membrane permeability since calcium has a considerable stabilizing effect on their structural and functional properties **[1]**. In freshwater fishes, the direct dependency between gill epithelium permeability for Na^+ and Ca^+ and Calcium concentration in the environment is established **[5]**, **[39]**. Under chronic heavy metals' lethal concentration influence, nutrition depression occurs, causing substantial growth retardation in water animals **[40]**, **[41]**. There are literature data on calcium concentration growth with other ions in fishes during their temperature acclimation **[2]**, **[6]**, **[15]**. In fish, the calcium content grows with a temperature rise from 24 to 36 °C **[30]**, thus testifying to metabolism intensification in fish organisms when environmental temperature increases.

According to Grynevych et al. **[7]**, Lall, and others **[16],** this property makes us consider the increase of calcium level in juvenile Atlantic salmon before migration into seawater and in adults during migration to freshwater to spawning grounds as the phenomenon helping to increase moving ability during these periods in salmon's life. Calcium ions decrease the ability of tissue proteins to link water. Under its concentration increase in water environment, the increase in water content in the tissues of brown trout and decrease in marked water turnover and water absorption rate during drinking and its excretion with urine **[23]**, **[36]**. At the same time, in fish in

seawater without only calcium, no changes in water outlet through the gills are registered, but the simultaneous absence of both Ca^{2e} and Mg^{2e} increases water outlet through gills by 33% **[35]**, **[42]**.

The introduction of manganese sulfate salts, zinc, and magnesium into artificial intelligence granular feed increases fish growth and makes significant changes in phosphorus-calcium exchange **[21]**, **[26]**.

Changes in water environment calcium concentration can influence acid-base balance in the organism. Researchers **[3]**, **[38]** proved that the increase of calcium level in water from 2.5 to 5 $mg.L^{-1}$ has a short time influence on acid-base balance in the carp's blood, thus decreasing buffer base concentration to 1/3. The animal organism normalizes the ionized calcium concentration with blood pH shift **[31]**.

The role of calcium as a mating factor in muscle fibres contraction and stimulation can be explained by its influence on acetylcholine intracellular distribution **[14]**, **[20]**, **[33]**. As a result, the fish mobility (guppies) quickly changes depending on calcium salts concentration in the environment **[8]**. And the decrease of cation level from 10 to 1 mg makes short, less than a second, stop in cilia beating of mussel's gills **[17]**, **[32]**.

Calcium decreases the nervous system's excitability, and the decrease in its content in the blood leads to its overexcitation. It is considered **[4]**, **[11]** that calcium's role in brain cells metabolism is determined by its activating effect on mitochondrial phospholipids hydrolysis. After removing calcium from the environment, the restoration of neurons' ionic composition in invertebrates doesn't occur **[22]**, **[29]**. In solutions without Ca^{2+}, nerve cells of gigantic neurons in mollusks lose their ability to generate action potentials as the inactivation of Na^{+} transfer takes place, and sometimes, the whole system providing delayed straightening becomes broken. In nerve cells in such conditions, the transport of labeled proteins along axons decreases by 40 – 60% **[25]**, **[37]**.

Scientific Hypothesis

The introduction of hydrobionts into water bodies with high calcium content in the aquatic environment leads to an increase in excretion with metabolic products and total phosphorus.

The results of our studies showed that when fish acclimate to an increased level of calcium in the aquatic environment, along with changes in its content in glandular tissues, the intensity of excretion with urine and faeces, the excretion of total phosphorus with faecal masses also increases.

MATERIAL AND METHODOLOGY

The research of phosphorus calcium metabolism in fishes required developing a unique systematic approach allowing to study tissue, cell, and organ mechanisms in metabolic regulation, taking into account peculiarities of aquatic habitat. We should consider the possibility of calcium and phosphorus intake into fish organisms through the digestive system and directly from the water and the influence of temperature factor in these elements absorption significantly changing the intensity of metabolic process in poikilothermic animals such as fishes.

Samples

The object of investigation is one year and two-year carps *(Cyprinus carpio L.)* with an average mass of 21.0 ±1.6-34.2 ±2.1 and 255 ±9.7 g. Before the experiment, the fish taken from Kyiv region fisheries in the autumn period were kept in stationary capacities of 4 m^3 each. Then 5 specimens of 2-year-old fish and 50 specimens of 1-year-old fish were put in 100 – 130 L aquariums equipped with programmed systems regulating temperature, photoperiods, water gas contents. After the preliminary week acclimation period, the experiments were carried out. The aquariums were filled with aged tap water with the following base mineral components concentration: Na^{+}: 11.7; K^{+}: 6.4; Ca^{2+}: 50 – 100; Mg^{2+}:120.0.

Depending on targets and goals, different experiments on the influence of calcium in different concentrations in the water environment on phosphorus-calcium metabolism in fish and during this cation oral intake were carried out.

Chemicals

The influence of calcium content in a water environment equal to 60, 100, 200, 400 $mg.L^{-1}$ on fish phosphorus-calcium metabolic indices was researched. The preset calcium concentration was reached by adding the calculated amount of calcium chloride to water. The water temperature during the experiment was maintained at 18 – 20 °C; O_2 content fluctuated within 7.59 – 9.76 $mg.L^{-1}$, CO_2: 2.32 – 3 32 $mg.L^{-1}$, HCO_{3^-}: 3.6 – 5.3 $mg.L^{-1}$, pH level – from 7.64 to 7.85.

Animals and Biological Material

One year and two-year carps *(Cyprinus carpio L.),* gills, and kidney glandular tissues

Instruments

The study of calcium ions in higher environmental concentration influences phosphorus-calcium metabolism was carried out on gills and kidney glandular tissues after 1, 3, and 7 days of their exposure. In some cases, the investigations were carried out on the experiment's 11 – 14 and 24th days. The tissue homogenate was prepared

on 0.2 sucrose (dilution 1:10). The researched tissues were quickly extracted after fish decapitation, weighed, and homogenated in a homogenizer (HG15A, Daihan, South Korea).

Statistical Analysis

The received digital data are processed using standard methods of variation statistics and special computer programs MS Excel and Statsoft Statistica 6.0.

RESULTS AND DISCUSSION

It is common knowledge there is a constant exchange between environment ions and hydrobionts organisms. As for some mineral substances, freshwater hydrobionts have clearly expressed the ability to concentrate them, and the level of these elements (calcium included) greatly exceeds their level in the environment **[34]**. The high calcium content in the extracellular fluid determines its specific role in key organism reactions, including phosphate metabolism. The last one is the base for many structural and functional units of living organisms. Also, they are the most labile component of the main energetic cell-substrate – adenosine triphosphoric acid. In warm-blooded animals, there is a relation between calcium transport and the chemical breakdown of organophosphorus compounds rich in energy **[12]**, **[19]**.

It is important to establish close connections between phosphorus and calcium metabolism to research the effect of calcium in different concentrations on its accumulation in fish functionally different tissues. Also, it is important to research the peculiarities of its excretion from the organism taking into account this cation's high biological activity and its possible influence on tissue phosphatase activity and phosphorus metabolism in glandular and other fish tissue structures.

After investigating calcium tissue metabolism during fish acclimation to its higher content in water and peroral intake, it is established that changes in calcium concentration in the water environment significantly influence its intake and distribution in the tissues and organs of hydrobionts. Thus, the decrease of calcium concentration in water from 100 to 60 $mg.L^{-1}$ results in a significant decrease in the fish liver content (Table 1). The level of general calcium in carp liver decreased more than by three times and didn't return to the initial one even after a 7-day stay of fish in such an environment.

Table 1 The influence of different calcium concentrations in water on its content in fish tissues (mg% of dry tissue) and blood (mg%), M±m.

Days	Calcium concentration in water, $mg.L^{-1}$	Gills	Calcium content in fish tissues and liquids	
			Hepatopancreas	Blood
1	100	2246.6 ±38.1	116.21 ±3.85	-
	60	2439.55 ±145.2	104.16 ±15.08	-
3	100	1994.00 ±109.1	118.21 ±5.99	14.00 ±0.22
	60	2121.40 ±226.5	32.16 ±2.10*	13.00 ±0.14
7	100	2713.44 ±81.08	103.12 ±11.3	18.90 ±0.20
	60	2547.40±176.34	90.39 ±9.03	12.00 ±0.15*
1	100	650.00 ±14.52	129.00 ±10.00	-
	200	1042.00 ±31.00*	131.00 ±9.00	-
3	100	664.50 ±15.10	146.00 ±0.12	14.00 ±0.32
	200	900.00 ±54.00*	225.40 ±15.10*	16.50 ±0.15
7	100	815.96 ±33.13	124.32 ±11.60	18.90 ±0.20
	200	991.18 ±62.12	98.21 ±14.20	12.10 ±0.13*
1	100	650.00 ±14.52	129.00 ±10.00	-
	400	980.70 ±21.00*	143.00 ±15.00	-
3	100	707.00 ±16.00	166.00 ±2.00	14.00 ±0.22
	400	1117.86 ±90.80*	163.60 ±17.00	16.00 ±0.12
7	100	654.50 ±15.10	146.00 ±12.00	18.90 ±0.13
	400	852.00 ±40.00*	114.00 ±10.00*	13.60 ±0.16*

Note:* the reliable result.

This tendency in general calcium quantity is observed in the fish blood. It is worth noting that in glandular gills apparatus of fish acclimated to the environment with lower, in comparison with control one, calcium level, its concentration in first days of acclimation period slightly exceeded the initial one, thus possibly testifying to the excretion of calcium with gills. But during the 7-day acclimation period in such conditions of the environment, the calcium content in fish gills becomes lower than in control ones. The described changes in general calcium

content in gills, glandular tissues, and liver and also in fish blood can be explained by the constant loss of cation in the process of vital fish activity in conditions of reduced intake from water with lower concentration.

The data received by Smart **[28]** testify to possible calcium loss in hydrobionts while keeping them in water with a low level. The author showed that ion excretion into the environment makes up to 10% of the total calcium content in the organism. When the calcium level increases in water to 200 $mg.L^{-1}$, its increase in gills and liver is registered in the fish organisms. Thus, in fish kept in water with 200 $mg.L^{-1}$ calcium level, its quantity in gills increased by 50 – 60% on the first day and stayed at this level during the whole acclimation period. The calcium content in the liver reached its maximum after 3 days in the water with a 200 $mg.L^{-1}$ cation concentration.

If in fish tissues calcium stayed at a higher level during the whole 7-day period of acclimation to its higher content in water, in the blood, it exceeded its level only on the third day. Then its concentration stayed lower than in the control group level. Registered changes in calcium levels in fish can testify that this organ plays a significant role in calcium metabolism regulation in animals and fish.

Another regularity in calcium accumulation in carp's tissue was registered after its increase to 400 $mg.L^{-1}$ in the environment. Suppose gill tissue accumulates a significant quantity of calcium during the 7-day acclimation period to these conditions, then in the fish liver and blood. In that case, its level increases only at the beginning of the experiment (1 – 3 days), and then it even decreases. Data on calcium content in glandular organs and blood in high cation concentration (400 $mg.L^{-1}$) suggest that this quantity may cause changes in gill cells membranes permeability, thus, reducing its intake in fish blood and hepatopancreas.

It is known **[24]**, **[27]** that calcium has a significant stabilizing effect on biological membranes' structural and functional properties.

Based on the data provided, we can conclude that calcium level in glandular tissue of gills and liver and biological liquids of carp depends on its concentration in the environment. Thus, after a decrease of calcium concentration from 100 $mg.L^{-1}$ to 60 $mg.L^{-1}$, hepatopancreas glandular tissue, and the blood loss it. In the gill tissue of the experimental fish, the calcium content increases at the beginning of the experiment, which can testify to endogenic calcium excretion with the gill glandular apparatus. After increasing calcium concentration in water to 200 $mg.L^{-1}$, its accumulation in gills and hepatopancreas is observed. 7-day acclimation period normalizes processes in fish organisms, and the calcium level in blood decreases. Calcium content in glandular organs and blood of fish from the environment with relatively a high level (400 $mg.L^{-1}$) let us suppose that this cation concentration can change the permeability of gill cells membranes, thus reducing its intake in blood and hepatopancreas.

Along with non-organic admission into fish organisms directly from water, a significant part of mineral substances, calcium included, enter orally with feed. Depending on ways of calcium intake into fish organisms, peculiarities of its tissue distribution are registered.

The analysis of obtained data showed that in one day after oral calcium intake in 75 $mg.kg^{-1}$ of fish kept in water with 40 $mg.L^{-1}$ cation concentration, the hypercalcemic reaction was observed and characterized with its content increased blood serum from 11.73 ±0.56 to 20.62 ±0.37 mg%. More long (7 days) calcium intake was accompanied by increased its content in blood to 30.39 ±1.03 mg%, almost by 3 times compared with control fishes. The observed rapid growth of calcium content in blood serum testifies its significant intake into the organism orally (Figure 1).

The oral calcium intake is accompanied by its significant accumulation in fish glandular organs (Figure 1). Thus, its amount in the liver during the 7 days exceeded control indices by 3.4 – 3.9 times, in kidneys by 1.5 – 2.1 times. At the same time, the calcium content in gills was lower than in control fishes by 24.8 – 200%. In the researched period, significant accumulation (by 2.1 – 2.2 times) of calcium was registered in fish muscle tissue after oral intake.

Analyzing calcium tissue distribution in fish glandular organs and muscle tissues after its oral intake, it is worth noting the observed effects depended on salt load impact duration. At the same time, the high calcium level in the liver, kidneys, and muscles tissue can testify to their significant depositing possibilities. Its content decrease in gills can result from calcium excretion increase in this way **[13]**.

We also researched renal and extra renal mechanisms of calcium metabolism regulation in fishes. Considering the interrelation in calcium content in water and its intake in general blood flow after absorption with glandular gill cells, it is important to establish the role of renal and digestive systems in its metabolism regulation.

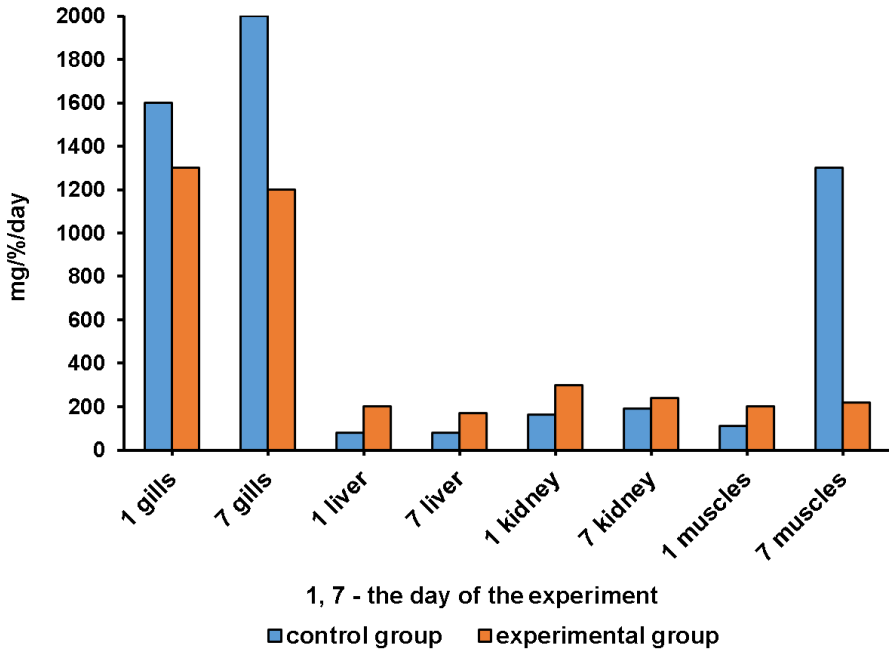

Figure 1 The influence of oral calcium intake on its distribution in carp tissues.

During experiments, it was established (Table 2) that the daily amount of diuresis depends on calcium concentration in water. Thus, in fish kept in water with 40 mg/l calcium concentration, diuresis was 34.12 – 29.2 mg.L^{-1} mass/day with the increase of calcium concentration in water to 100 mg and 200 mg.L^{-1}, the day diuresis in carps decreases.

The higher the calcium concentration in water, the more pronounced its antidiuretic effect is. Thus, with 100 mg.L^{-1} calcium content in water, the diuretic function of carp kidneys decreased by 25.7% and stayed at this level during a short time (1 day) period of fish keeping in such conditions. With the increase of calcium content in water to 200 mg.L^{-1}, the day diuresis in fish decreased by 25.68 – 51.59% and reached the initial level only in seven days. From the literature data **[18]**, we know that calcium significantly influences cell membrane permeability for water, and salts cause the excretion of urine in a lower amount but in higher concentrations.

With the decrease of kidney diuretic function in carps kept in an environment with higher calcium concentration, its amount is registered in urine (Table 2). Thus, in fish acclimated in water with low calcium concentration (40 mg.L^{-1}), its content in the urine was 63.16 – 68.66 mg.L^{-1}, whereas, under 100 mg.L^{-1} concentration, this index increased by 18.3 – 25.8%. Under higher (200 mg.L^{-1}) calcium level in the water, its excretion in the fish urine in one day increased by 40.4% and in 3-7 days by 1.6 – 1.7 times compared to the initial level.

It is known that day calcium excretion with urine is determined both by its concentration and the diuresis level. As the result of the conducted investigation, no direct correlation in calcium concentration increase in water and its day excretion with urine was registered. Thus, on the last day of fish keeping in an environment with higher (100 mg.L^{-1}) calcium concentration, its excretion with urine didn't increase, caused by a rapid decrease in kidney diuretic function. After 3 days, both diuresis and day calcium excretion with urine significantly increased, staying at a higher level in the following 7 days of the experiment. Herewith more direct correlation in day calcium excretion with urine and its level in the water environment was registered during carps acclimation to 200 mg.L^{-1} cation concentration. The obtained results correspond to the results obtained by Buda and others **[2]** which showed the increase of calcium excretion after injection with calcium-chloride during fish acclimation to its higher level in the water, proving the dependence on environmental ionic content and water animals organisms.

The faeces' excretion changes during fish acclimation to higher calcium concentration in the water environment. It should also be noted that calcium faeces excretion in fish acclimation to 100 mg.L^{-1} cation concentration decreased, whereas it increased under 200 mg.L^{-1} concentration (Table 2). It should also be noticed that the most explicit changes in faeces excretion of carps from 2 environmental groups occurred during their long keeping in water with a higher calcium content.

Changes in calcium faeces excretion are closely connected with liver biliary function and thus with metabolic processes levels in fish being acclimated to higher calcium levels in the water. Data on calcium excretion with bile prove it. Therefore, in fish being kept in an environment with 40 mg.L^{-1} calcium level, its concentration in bile fluctuated from 34.04 ±0.73 to 40.67 ±2.60 mg.L^{-1}. In contrast, in 100 mg.L^{-1} Ca^{2+} concentration, it increased in 1 day of acclimation by 28.4% and in 7 days – by 43.3%. The same changes in calcium content in fish bile

were registered in fish being acclimated to 200 mg.L^{-1} calcium level in the water environment. It should be noted that fish were kept in an environment with a 200 mg.L^{-1} calcium concentration for a long time. Its excretion with bile increased by 2.1 times compared to the initial level. The minimal calcium loss with faeces in fish was observed on the third day of the experiment under both concentrations in water. As we showed earlier, higher tissue calcium accumulation in fish glandular organs can be caused by higher tissue calcium accumulation.

Table 2 The influence of calcium concentration in the water environment on kidney diuretic function and its excretion with urine and feces in carps, M ±m.

Ca^{2+} concentration in water mg.L^{-1}	**Days of the experiment**					
		% to control		**% to control**		**% to control**
Diuresis mL/kg/day						
40 (control level)	34.12 ±1.68		29.60 ±2.12		29.20 ±2.56	
100	25.32 ±2.92*	-25.79	28.00 ±2.36	-5.41	29.08 ±2.92	-0.41
200	16.52 ±1.28*	-51.58	22.00 ±1.16*	-25.68	32.40 ±1.64	+10.96
Calcium concentration in urine mg.L^{-1}						
40 (control level)	63.13 ±4.18		80.67 ±3.78		68.66 ±3.76	
100	74.67 ±4.08	+18.28	80.67 ±3.55*	+25.83	78.86 ±8.01	+14.86
200	88.66 ±3.87*	+40.44	106.00 ±10.58*	+65.34	117.33 ±10.39*	70.89
Calcium day excretion with urine mL/kg/day						
40 (control level)	2.15 ±0.072		1.90 ±0.060		2.00 ±0.096	
100	1.89 ±0.056*	-12.09	2.26 ±0.144*	+18.95	2.29 ±0.088	+14.50
200	1.95 ±0.048*	-37.21	2.34 ±0.124*	+23.16	3.80 ±0.253*	+90.0
Calcium day excretion with feces						
40 (control level)	4.32 ±0.072		4.92 ±0.272		6.60 ±0.128	
100	2.96 ±0.068*	-31.48	1.64 ±0.104*	-66.67	2.76 ±0.100*	-54.0
200	5.40 ±0.70*	+25.0	1.04 ±0.170*	-78.86	9.00 ±0.150*	+50.0

Note: * the reliable result

It should be noted that during fish acclimation to extremely high (400 mg.L^{-1}) calcium concentration in water, its excretion with bile doesn't increase, but vice versa decreases from 184.00 ±2.4 to 135.00 ±2.1 mg%. It can be explained by the rapid decrease in calcium absorption from water by the gill glandular apparatus and its intake into the organism.

Oral calcium injections caused, together with its tissue distribution, changes in excretion of organs activity. In this case, the urine output in fish after oral calcium injection in the 75 mg/kg/day dose decreased during a short time (1 – 2 days) period by 23.7 – 30.9% (Figure 2.), reaching control indices after 7 days of the exposition. During oral calcium injection, its excretion with urine increased in the first day from 2.15 ±0.072 to 4.16 ±0.16 mg/kg/day or by 1,9 times, on the third day – from 1.90 ±0.060 to 5.26 mg/kg/day (by 2.7 times), on the seventh day – from 2.00 ±0.006 mg/kg/day to 3.92 ±0.224 (by 2 times) in comparison with its excretion in fish without calcium oral injections.

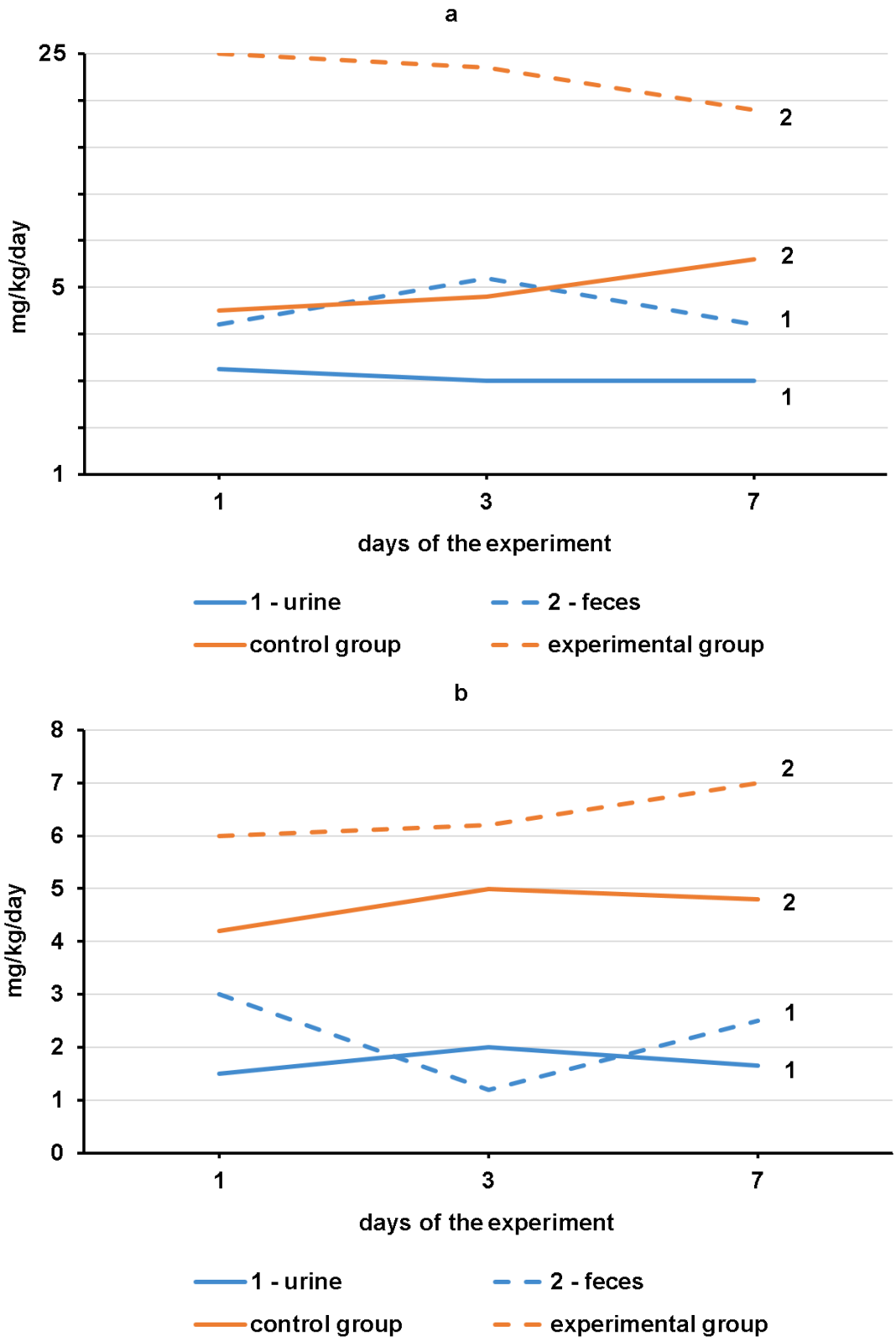

Figure 2 The influence of oral calcium injections on calcium excretion (a) and phosphorus (b) excretion with the fish urine and faeces.

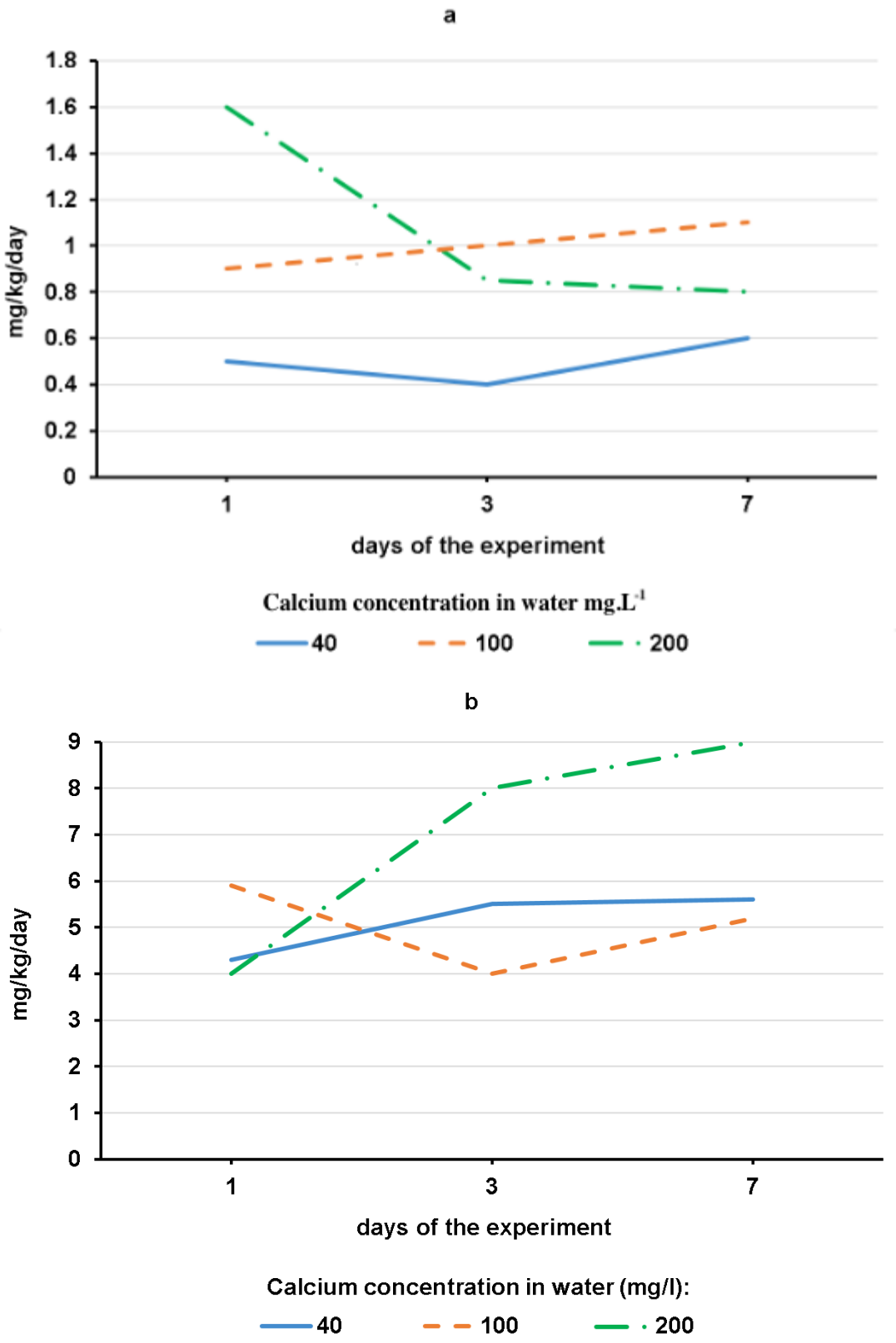

Figure 3 The influence of calcium higher level in water environment on phosphorus day excretion (mg/kg/day) with urine (a) and faeces (b) by carp.

At the same time, its feces excretion sharply increased. It increased after 1, 3 and 7 days of calcium injection from 4.32 ±0.072 to 26.4 ±0.33 mg/kg/day; from 4.92 ±0.272 to 23.5 ±1.60 mg/kg/day; from 6.00 ±0.13 to 16.92 ±2.90 mg/kg/day accordingly.

The increase of calcium excretion through the renal and digestive system of fish being acclimated to its higher level in the water and oral intake is accompanied by certain changes in phosphate excretion dynamics. Thus, in

fish being kept in water with 40 mg.L^{-1} calcium level, the phosphate concentration in urine fluctuated from 14.54 ±0.63 to 20.0 ±0.45 mg.L^{-1} and day excretion from 0.496 to 0.584 mg/kg/day (Figure 3). The long-time fish exposure in water with 100 mg.L^{-1} calcium level was accompanied by the increase of total phosphorus level in urine (by 2 – 2.5 times), and its day excretion increased by 1.9 – 2.4 times.

The total phosphorus faeces excretion increases during fish acclimation to high calcium levels in a water environment (Figure 3).

The increase of calcium level in the water environment to 100 mg.L^{-1} causes only a short increase in total phosphorus excretion with faeces. Under the cation, concentration increases to 200 mg.L^{-1} significantly increase during the fish's long stay in such an environment.

It should be noted that during oral calcium intake to fish organism phosphorus day excretion with urine during 7-day exposition exceeded control level by 44.5%. In contrast, it sharply increased during the short time (1 day) (Figure 2).

Phosphate excretion with faeces in fish getting calcium orally during 1, 2, 7 days of the experiment exceeded by 35.4; 15.3 and 26.7% its excretion in fish without cation intake but kept in the same experimental conditions (Figure 2).

So, calcium and phosphorus excretion in fish is determined by the calcium level in the environment and its intake through the digestive tract. The importance of the digestive system in calcium and phosphorus excretion grows with the increase of Ca^{2+} intake into fish organisms. This proves the important role of this system in their metabolism.

CONCLUSION

The investigation results showed that during fish acclimation to increased calcium level in the water environment and the changes of its content in glandular tissues, the intensity of excretion with urine and faeces the excretion of total phosphorus with faeces also increases. The increase of calcium content in the water environment to 100 mg.L^{-1} caused only a short time increase of total phosphorus excretion with faeces. In contrast, cation concentration increases to 200 mg.L^{-1} significantly increases during the long stay of fish in such an environment.

It should be noted that during oral calcium intake into the fish organism, the day phosphorus excretion with urine under 7-day exposition exceeded the level in control fish by 44.5%. In contrast, it was sharply increased in a short time (1 day). The phosphorus excretion with faeces in fish getting calcium orally during 1, 2, and 7 days of the experiment exceeded by 35.4; 15.3 and 26.7% its excretion in fish without cation intake but kept in similar experimental conditions. So, fish's calcium and phosphorus excretion is determined both by their content in the environment and their intake through the digestive tract. The meaning of the digestive system in calcium and phosphorus excretion grows with calcium intake increase in fish organisms, thus indicating the important role of this system in their metabolism.

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

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Development and shelf-life assessment of soft-drink with honey

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ABSTRACT
This scientific work describes research that aims to determine the physicochemical parameters of homogenized honey and its safety indicators based on the determination of toxic metals and radionuclides. A series of experimental studies were conducted to develop and study recipes for honey water based on different types of honey collected in the Lviv region of Ukraine, namely acacia, buckwheat, sunflower, coriander, goldenrod, linden, and weeds. According to the results of experiments, it was found that the studied honey meets all the requirements presented in the standard for natural honey. And the results obtained to determine the dry matter content and pH allowed to blend different types of honey and get honey drinks, which will expand the range of non-carbonated products, which is very popular, especially in summer, and drink this drink during the year. To prolong the shelf life of honey drinks, it is recommended to add citric acid in an amount of 1% by weight of the drink and sodium benzoate as a preservative in an amount of 0.1%. The quality of the obtained honey water samples was assessed using organoleptic evaluation and physicochemical parameters. The resulting beverages have good organoleptic characteristics and can be offered for products in the industry.

Keywords: honey, water, beverage, dry matter content, pH value, formulation

INTRODUCTION
Beverage preparation is a component of food culture. And this is an integral part of national culture in general. Ukrainian drink "drinking honey" has a rich history, based on the knowledge of many generations of our ancestors **[1]**. Such beverages belong to the products of alcoholic fermentation of aqueous solutions of natural honey **[2]** and contain biologically active substances of honey and the use of only natural raw materials **[3]**. The need to form a culture of beverage consumption creates a search for the production of natural and environmentally friendly raw materials (fruit and berry, honey). This allows you to expand the range of buyers and the range of beverages made from natural raw materials **[4]**, **[5]**. Both ancient recipes for fermented honey drinks and drinks created thanks to modern scientific achievements are known **[6]**, **[7]**. The production of beverages based on natural raw materials should solve the interconnection and balance of ingredients, which form the taste and aromatic basis of the drink **[8]**. Usually, soft drinks consist of water, sweetener, and flavouring. Sugar sweeteners are sugar, glucose-fructose syrup, or other sweeteners (in the case of diet drinks). Drinks may also contain caffeine other components **[9]**. Use in the production of soft drinks based on honey can be promising. According to the Food and Agriculture Organization of the United Nations (FAO), honey exports from Ukraine for 11 months of 2020 reached an absolute record – 69.8 thousand tons with a total value of $ 117.5 million. In Figure 1, you can see how rapidly honey exports from Ukraine over the past 9 years **[10]**.

The previous record was set in 2017 and amounted to – 67.8 thousand tons ($ 133.9 million). Volumes of honey production in Ukraine on the European market take the first place, and on the world - the third **[11]**.

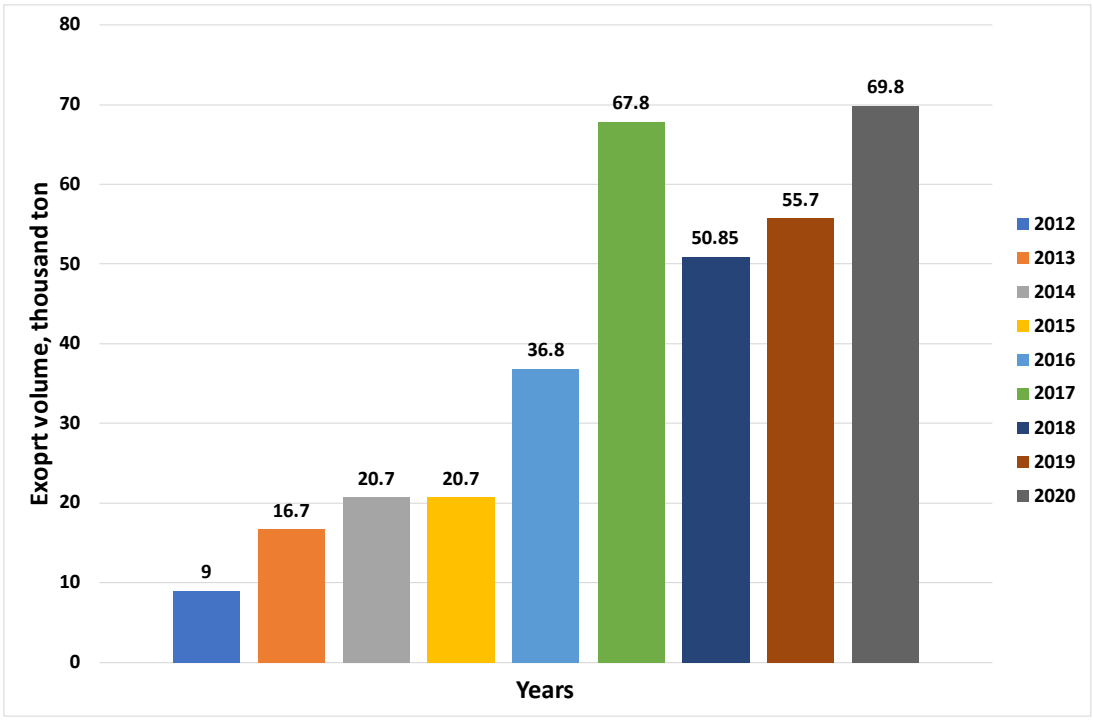

Figure 1 Dynamics of honey exports from Ukraine.

So, both honey and its products are a great way to rejuvenate and revitalize the body. That is why specialists abroad are very interested in honey.

Scientific hypothesis

There is a technology of fermented honey beverages, for which it is necessary to ferment the wort to carry out additional technological operations for its filtration. Our scientific hypothesis is that creating recipes with the addition of different types of honey to honey and preservatives in the form of citric acid and sodium benzoate can reduce the production process and obtain a non-alcoholic product with extended shelf life. The cost of producing such a product will be lower compared to fermented beverages. Therefore, the low cost of the product will allow its use to different segments of the population.

MATERIAL AND METHODOLOGY

Samples

For the study, we used selected samples of honey of seven types and homogenized honey, obtained at the plant engaged in processing and exporting honey to the EU and Canada of the Lviv region.

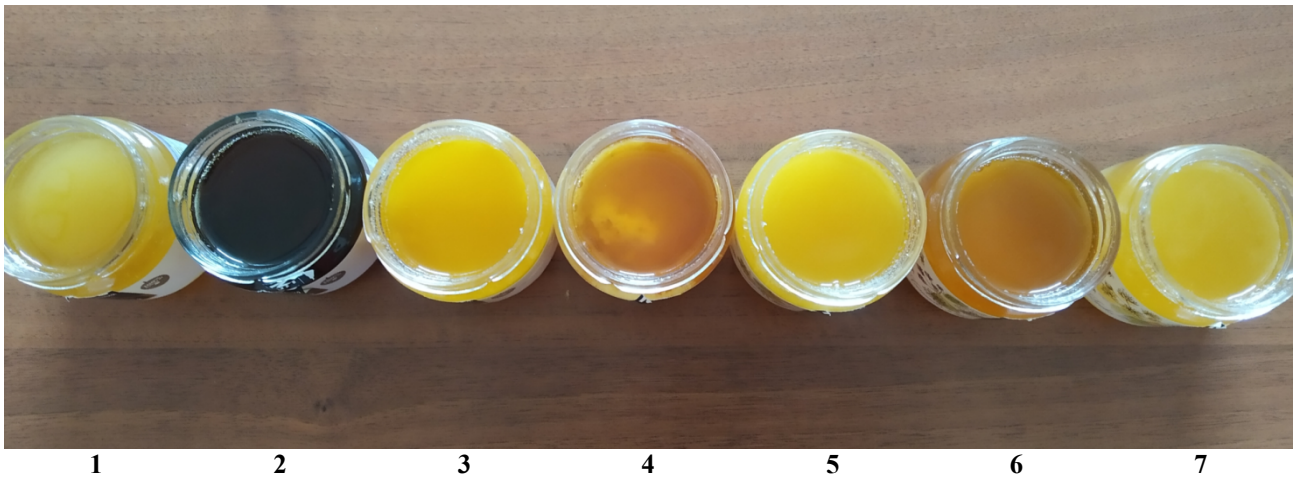

Figure 2 Types of honey for research: 1 – acacia; 2 – buckwheat; 3 – sunflower; 4 – weeds; 5 – goldsmith; 6 – coriander; 7 – linden.

Physico-chemical indicators and indicators of honey safety were determined according to the methods set out in DSTU 4497: 2005 and SOU 01.25-37-373.2005 **[12, 13]**.

Chemicals

Starch ($(C_6H_{10}O_5)_n$, (VYMAL), Chernihiv, Ukraine)
Sodium chloride (NaCl, Artemsil State Production Association, Donetsk Region, Ukraine)
Barbituric acid ($C_4H_4N_2O_3 \cdot 2H_2O$, (INTERKHIM), Kharkiv, Ukraine)
Paratuloidin (C_7H_9N, Merck KGaA, Germany)
Isopropanol ($CH_3CH(OH)CH_3$, Ineos Solvents, Germany)
Potassium hexacyanoferrate **(**$K_4[Fe(CN)_6]$, China)
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$, (INFRAKHIM), Yaroslav, Russia)
Acetic glacial acid (CH_3COOH, (LOST LTD), Ivano-Frankivsk, Ukraine)
Sodium hydroxide (NaOH, (Novokhim), Kharkiv, Ukraine)
Hydrochloric acid (HCl, (Novokhim), Kharkiv, Ukraine)
Sulfuric acids (H_2SO_4, (Novokhim), Kharkiv, Ukraine)
Copper sulfate ($CuSO_4 \cdot 5H_2O$, (Novokhim), Kharkiv, Ukraine)
Fermented salt ($KNaC_4H_4O_6 \cdot 4H_2O$, (Novokhim), Kharkiv, Ukraine)
Sodium thiosulfate ($Na_2S_2O_3$, (NOVOSIBKHIMFARM), Novosibirsk, Russia)
Glucose ($C_6H_{12}O_6$, (Novokhim), Kharkiv, Ukraine)
Fructose ($C_6H_{12}O_6$, (Novokhim), Kharkiv, Ukraine)
The citric acid ($C_6H_8O_7$, (Smilyansky sugar factory), Smila, Ukraine)
Sodium benzoate (NaC_6H_5COO, Eastman, Kohtla-Järve, Estonia)

Animals and Biological Material:

For research used: acacia honey; buckwheat honey; sunflower honey; herbal honey; goldenrod honey; coriander honey; linden honey (sold by various manufacturers of Lviv region, Ukraine).

Instruments

Refractometer (IRF-454 B2M, manufacturer, open joint-stock company "KOMZ", Kazan, Russia). To determine the water content in honey.

Laboratory thermometer (TLS-200, manufacturer LLC "Inter-Synthesis", Ukraine).

Photo colourimeter (KFK-3, Altavir Limited Liability Company, Belgorod, Russia).

Flame spectrophotometer (Saturn-4, manufacturer "Inter-Synthesis" Limited Liability Company, Ukraine). The content of toxic elements and radionuclides was determined.

Laboratory Methods

All physicochemical parameters were determined according to DSTU 4497:2005 Natural honey. Specifications.

The mass fraction of reducing sugars was determined using a photocolorimeter using a calibration graph with different concentrations of inverted sugar.

A combined reagent was prepared to determine the diastasis number, which included 0.2 M acetate-buffer solution (pH 5.0), solutions of starch, and sodium chloride. The optical density of the samples was measured on a photocolorimeter at a wavelength of 590 nm against water in a cuvette 10 mm thick.

Solutions of barbituric acid, paratuloidin, and Kerres reagent were prepared to determine hydroxymethylfurfural content. At a wavelength of 550 nm, the optical density of the honey solution was measured relative to the control solution every minute for 6 minutes.

The acidity of honey was determined by titration with sodium hydroxide solution to a pH value of 8.3.

A refractometer was used to determine the water content in the honey.

Toxic elements (lead, cadmium, arsenic) and radionuclides (caesium and strontium) were determined by atomic absorption spectrometry with electrothermal atomization.

Sensory analysis of honey drinks based on a scale was performed.

An analysis of the shelf life of ready-made beverages based on the addition of citric acid and sodium benzoate.

Description of the Experiment

Sample preparation: 7 types of action honey, buckwheat, sunflower, coriander, herbs, linden, and goldenrod were used for the study.

Number of samples analyzed: During the experimental studies, 8 samples were used, 7 of which were described and one sample of homogenized honey, i.e. blended in equal quantities from all species and heated to 35 ºC to determine physicochemical and safety indicators of honey.

Number of repeated analyzes: 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: to determine the physicochemical and toxic elements and radionuclides in homogenized honey, equal amounts of honey were mixed and burned to 40 °C, then in chilled honey to 20 °C by known methods determined the indicators presented in Table 1, Table 2 and Table 3. Depending on the amount of added honey, indicators of dry matter content pH values in honey drinks were determined using a refractometer and a pH meter, respectively. 4, 6, 8, and 10 g of honey were added to the samples. During the development of honey drink recipes, the formation of microorganisms in honey samples was visually observed.

Statistical analysis

Mathematical and statistical processing of experimental data was performed to determine the criteria of Cochren, Fisher, and Student. The accuracy of the data was determined using the Cochren test, the adequacy of the mathematical model was checked using the Fisher and Student criteria. Statistical processing was performed in Microsoft Excel 2016. Values were evaluated using mean and standard deviations and subsequently calculated in the statistical program XL Stat. When testing the hypotheses, if the value of p is below a significant level, in the case of XL Stat software from Addinsoft (version 2019.3.2), this value was 0.05, the null hypothesis was rejected, and the alternative hypothesis was confirmed.

RESULTS AND DISCUSSION

Studies of physicochemical and safety indicators of homogenized honey were conducted at the State Research Institute for Laboratory Diagnostics and Veterinary Sanitary Examination, which confirmed compliance with standards and documents presented for honey.

According to the authors **[14]**, the homogenization of natural honey according to the current technological regimes slightly changes its quality indicators and the content of hydroxymethylfurfural. Still, it improves the consumer properties of the product.

As can be seen from Table 1, the content of hydroxymethylfurfural, mass fractions of sucrose, and water in homogenized honey are lower by 22, 23, and 15%, respectively, than the norm.

Table 1 Physico-chemical parameters.

Name of indicator and unit of measurement	Permissible level according to normative documents	Results	Deviation	Conformity mark
The content of hydroxymethylfurfural, $mg.kg^{-1}$	No more than 40.0	29.5	±3.31	Confirmed
Diastasis number, Gotte units	No more than 8.0	29.6	±1.20	Confirmed
Acidity, mEq. NaO /dm^3	No more than 50.0	22.8	±1.32	Confirmed
Mass fraction of sucrose (to anhydrous substance),%	No more than 5.0	3.7	±0.41	Confirmed
Mass fraction of reducing sugars (to anhydrous substance),%	No more than 60.0	80.0	±1.59	Confirmed
Mass fraction of water for temperatures of 20 °C,%	No more than 20.0	17.0	±0.22	Confirmed

In scientific works **[15]**, similar studies were conducted to determine the mass fractions of sucrose and water in honey, and the following results were obtained, the figures were lower by 18, 13, and 10%, which in our opinion, may reduce the shelf life of the final product.

In the following manuscripts **[16], [17],** similar studies were conducted to determine the mass fractions of sucrose and water in honey. The following results were obtained, the figures were lower by 20, 10, and 15%.

The diastasis number is 29.6 Gotte units, which indicates the naturalness of honey and its longevity. Some scientific works **[18], [19]** described methods for determining the quality of honey and ways to extend its shelf life and found that at 20 °C, the period of reduced diastase activity is 1480 days, at 25 °C – 540 days, and at 80 °C – only 1.2 hours. However, scientific works **[20], [21]** describe the methods and ways of storing honey and found that the maximum shelf life is only 8 months, after which honey loses its useful properties.

Further research on the development of honey water recipes based on the honey of different types, namely monofloral - acacia, sunflower, buckwheat, coriander, linden, goldenrod, poly flora - weeds, were devoted to finding the optimal amount and different ratio of honey with drinking water. Honey drinks that have shown previous good results. For long-term storage of such beverages, we conduct research with the addition of natural preservatives to prevent the use of substances of chemical origin.

In determining toxic elements and radionuclides in honey, they are either not detected, or the amount is much less than the standard of these substances (Tables 2 and 3).

Table 2 Toxic elements.

Name of indicator and unit of measurement	Permissible level according to normative documents	Results	Deviation	Conformity mark
Mass fraction of lead, $mg.kg^{-1}$	No more than 0.1	0.046	±0.008	Confirmed
Mass fraction of cadmium, $mg.kg^{-1}$	No more than 0.03	<0.005	-	Confirmed
Mass of arsenic adol, $mg.kg^{-1}$	No more than 0.5	<0.01	-	Confirmed

Table 3 Radionuclides.

Name of indicator and unit of measurement	Permissible level according to normative documents	Deviation	Indicator of compliance with radiation safety criteria*	Conformity mark
The content of radionuclides Cs-137, Bk/ kg	No more than 200	<6.34	0.09	Confirmed
The content of radionuclides Sr-90, Bk/kg	No more than 50	<5.84		Confirmed

Note: * – the compliance indicator meets (≤1) radiation safety criteria.

Therefore, from the obtained results, we can say that bee honey homogenized for human consumption on the content of radionuclides meets the State standards "Permissible levels of radionuclides Cs-137 and Sr-90 in food and drinking water" approved by the Ministry of Health of Ukraine, order from 03.05 .2006, No 256 with changes; in terms of physicochemical parameters complies with the Directive of the Council of the European Union 2001/110/EC, 396/23/EC of December 20, 2001; complies with the content of toxic elements following "Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and products of animal origin"; DSTU 4497:2005.

Many scientific manuscripts **[22], [23]** have conducted studies on the comparative analysis of honey samples, but the authors only determine organoleptic indicators. In our opinion, this method can not be effective, and the next step should be to create an ISO standard for honey from Europe or USA.

A series of similar experiments are described in the following works **[24], [25]**. Still, the authors of the above scientific works conduct a comparative analysis of honey samples according to GOST 19792-2017 **[26]** and TU 01.49.21-077-37676459-2017 **[27]**. Still, in our opinion, these methods can not be used for honey from Europe, Asia or Africa.

To develop recipes for honey water, we used various blends of honey to obtain a pleasant drink. In figure 3, you can see the change in dry matter content in honey water depending on the type of honey that was added to a drink and the amount of honey in 100 g of water.

From the diagram, you can see the increase in dry matter content in samples of beverages with a higher content of honey but different amounts of dry matter content within the same amount of honey. Based on physicians' recommendations on the use of the daily norm of honey, we selected samples corresponding to 6 g of honey in 100 ml of water. For this variant, the highest honey content corresponds to a mixture of honey from sunflower and coriander, which indicates a smaller amount of water in them compared to the samples of acacia + buckwheat and herbs + goldenrod + linden.

Similar scientific researches which are connected with the preparation of honey drinks with various combinations of samples of honey are described in the following scientific works **[28]**, **[29]**. Some combinations of samples can be incompatible, and their combination will be of no use.

Studies related to the preparation of honey drinks with different samples of honey-based on fruit juices are described in the following scientific papers **[30]**, **[31]**. Still, in our opinion, some combinations of samples may be incompatible, and their combination may lead to the formation of harmful microflora in the finished product.

Drinking water without additional treatments was used to prepare drinks. The pH of this water was 7.20 and distilled – 7.55. Similar studies have been conducted in scientific works **[32]**, **[33]**, but various fruit juices, milk, dairy products, and pre-purified water were used to prepare beverages.

A similar series of experiments were conducted by the authors of the following manuscripts **[34]**, **[35]**. Still, for the preparation of honey drinks used a variety of dairy products, these are quite interesting experiments. They have no recommendations for shelf life and no organoleptic evaluation.

Since honey has an acidic environment accordingly, the pH value of beverage samples decreased due to the addition of honey to water. Figure 4 shows that the minimum pH value, regardless of the amount of honey added to the water, corresponds to honey samples from sunflower and coriander. The maximum pH value is obtained by adding a mixture of goldenrod herbs and linden.

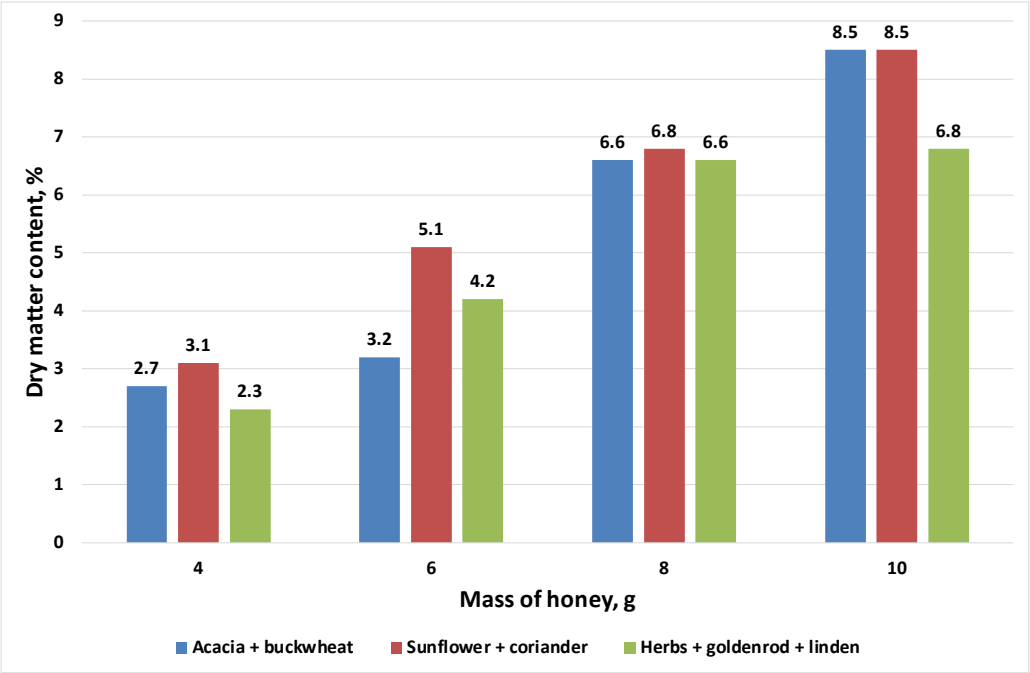

Figure 3 Change in dry matter content in different samples of honey water.

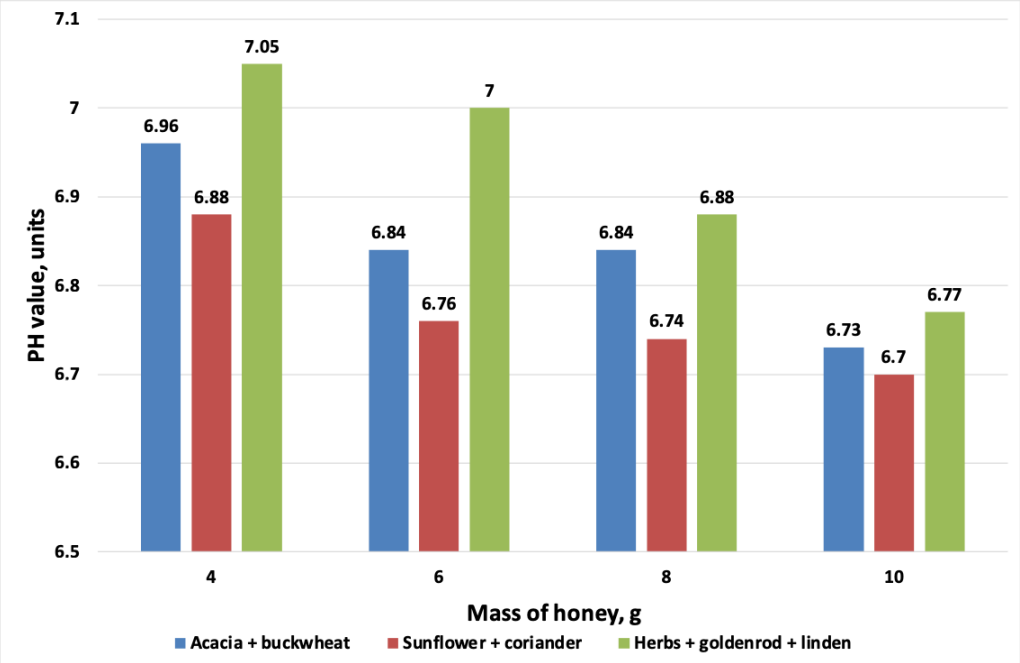

Figure 4 The effect of pH on the amount and types of honey.

The smaller the pH value, the better the honey water will be stored because the acidic environment harms the development of microorganisms. Therefore, our further research was experimenting to find the shelf life of such honey water without additional components at room temperature and with the addition of citric acid.

Black mould appeared after the storage of honey water samples at a temperature of 18 ºC on the seventh day (Figure 5). However, as can be seen from the photo, when adding 6 g of honey in 100 mL of water, this mould is absent, and for other samples of honey, visible colonies of microorganisms.

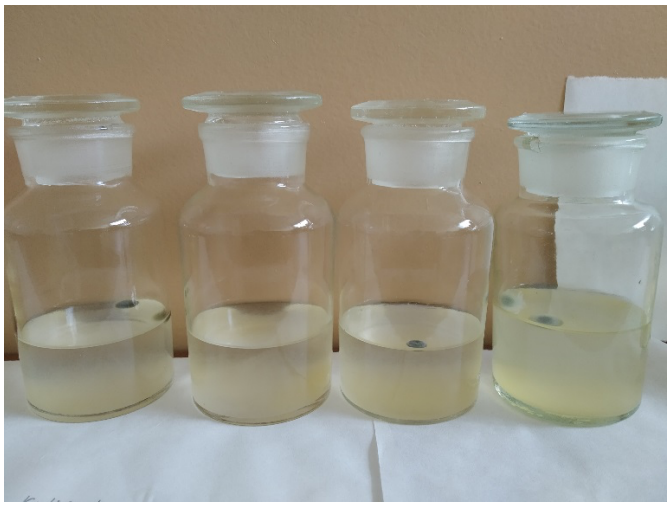

Figure 5 Presence of black mould in honey water samples.

When added to the samples of honey water, which contained 6 g of honey, 0.5 and 1.0 g of citric acid (Figure 6) compared with the control test on the tenth day, yeast appeared (Figure 7), which indicates the elongation shelf life of honey drink for three days longer than without the use of citric acid. Moreover, more yeast can be seen visually with less added citric acid.

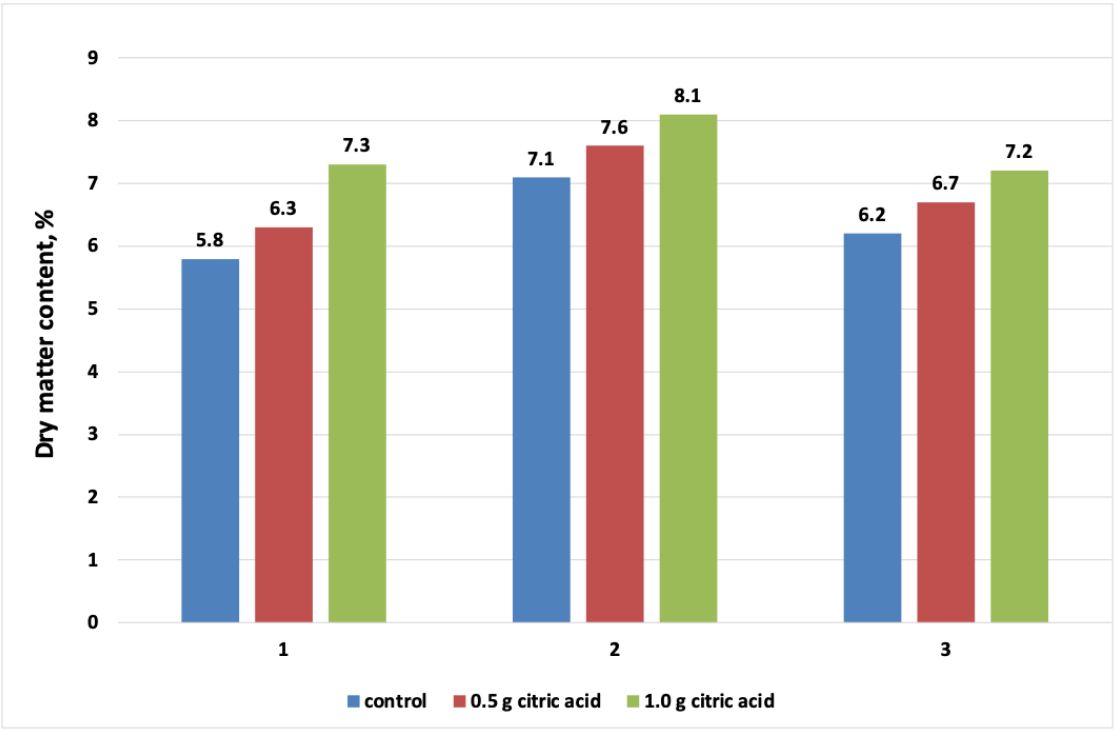

Figure 6 Change in dry matter content with the addition of citric acid: 1 – acacia and buckwheat; 2 – sunflower and coriander; 3 – weeds, goldenrod, and linden.

The citric acid in the drink acts as a regulator of acidity and, therefore, extends its shelf life. But this is not enough to increase the shelf life of the drink. Consequently, it is impossible to produce such honey water without using a preservative because, in addition to the formation of microorganisms, there is also a fermentation process due to free glucose in honey.

The authors of the following scientific works **[36], [37]** conducted many similar studies, which developed recommendations for extending the shelf life of honey and products with its components through the use of various storage methods, which we believe should be better studied.

Scientific works **[38], [39]** describe a series of experiments that investigated various technologies and equipment to extend the shelf life of honey and products with its components using a variety of preservatives that can inhibit the beneficial properties of the final product.

Figure 7 Formation of yeast during storage of honey water.

Formulations of honey water based on water, honey, and preservative - sodium benzoate in the amount of 0.1% by weight of the drink to extend the shelf life was developed.

Figure 8, Figure 9 and Figure 10 show 4 samples of honey drinks with the same amount of honey from 4 to 10 g (4, 6, 8, and 10 g) per 100 g of water (from left to right). Thus, in Figure 8 you can show samples of honey drinks using acacia and buckwheat, in Figure 9 – sunflower and coriander, and in Figure 10 – a mixture of honey from herbs, goldenrod, and linden. As can be seen from Figure 8, Figure 9 and Figure 10, the colour of honey drinks is different because the colour itself is different for different types of honey. The most intense colour corresponds to the honey drink "Fantasy" because buckwheat honey has a dark brown hue. Linden and goldenrod honey are the lightest, so the drink "Honey Mixture" is also not rich in colour.

Figure 8 Development of the recipe for the drink "Honey Fantasy" based on acacia and buckwheat honey.

Figure 9 Development of the recipe for the drink "Honey Delight" based on sunflower and coriander honey.

Figure 10 Development of the recipe for the drink "Honey Mix" with the addition of honey from herbs, goldenrod, and linden.

Organoleptic evaluation of the created recipes of honey drinks is carried out. According to the results, the obtained drinking honey fully meets the standard, is characterized by transparency, developed light, characteristic of this type of honey, smell and taste, pleasant aftertaste, the bouquet is tender and developed (Table 3).

Table 3 Tasting evaluation of honey water.

Indicators	Characteristic	Rating (points)
Transparency	Crystal clear	0.5
Colour	Full compliance with the default	0.5
Bouquet	Thin and developed	4
Taste	Very thin and developed	4
Default	Full compliance with the default	1
Overall rating	High-quality drink	10

According to research, it is established that for 1000 kg of finished honey drink, you need 993 kg of water, 6 honey, and 1 kg of sodium benzoate.

The drink can be made on an industrial scale and stored in a closed container for one year when using such a formulation.

This research does not end this work because there are still some questions about the use of water for drinking - what should be this water, whether it is possible to use spring water, what other ingredients it is desirable to add to the drink to use a useful product and so on.

CONCLUSION

Studies have been conducted to determine the physicochemical, toxic, and radionuclides in homogenized honey, a mixture of acacia, buckwheat, coriander, sunflower, weeds, goldenrod, and linden. These studies have confirmed that honey meets the state standard requirements for natural honey. Based on the types mentioned above of honey, studies were conducted to determine the dry matter content and pH value when adding different amounts of honey to create recipes for honey drinks. Studies with the addition of citric acid in amounts of 0.5 and 1.0% by weight of the drink did not give positive results in extending their shelf life. It is recommended to add the preservative sodium benzoate in 0.1% by weight to the beverage to extend the shelf life.

Based on seven types of honey, recipes for honey drinks have been developed. The production of which on an industrial scale will expand the range of non-carbonated products and reduce the duration of the technological process and the cost of finished drinks.

It is recommended to add the preservative sodium benzoate in 0.1% by weight to the beverage to extend the shelf life.

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The prolonged effect of GLUTAM 1M biologically active preparation on dairy productivity and milk quality of cows

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ABSTRACT

We have studied the effect of biologically active preparation of metabolic-neurotropic action "Glutam 1M" on milk productivity of cows and quality indicators of raw milk. This preparation was used for dry cows in the last trimester of pregnancy. Studies were performed in the private agricultural enterprise "Savertsi" of Popilnyansky district of Zhytomyr region on cows of Holstein breed. The biologically active preparation "Glutam 1M" has been administered to the cows of the experimental groups under the skin behind the shoulder blade in an amount of 20 ml, starting from 270 and 265 days of gestation, once a day for three consecutive days. Cows of control groups were injected with saline in the same dose. Using the biologically active preparation "Glutam 1M", a milk yield decreased slightly by 2% (91.9 kg). The milk yield increased by 2.9% (141.5 kg) in the control group. 305-days milk yield in the control group of cows was almost the same as in the previous lactation period. During the experiment, the experimental group of cows – has decreased by 2.9% (136.5 kg). A similar situation has been observed during the biologically active preparation "Glutam 1M" on the 270 – 272th days of pregnancy. Milk yield in the experimental group of animals for the previous lactation and after the use of preparation remained almost at the same level in the control group – decreased by 4.7% (207.1 kg). 305-days milk yield in the control group of cows for the previous and post-lactation experiment period was almost the same. In the experimental group of animals, there was an increase in this indicator by 2.7% (128.7 kg). The use of the "Glutam 1M" preparation did not affect milk quality, namely the mass fraction of fat and protein; fluctuations of the above indicators stayed within the error.

Keywords: raw milk, milk productivity, biologically active preparation, lactation, milk yield

INTRODUCTION

Due to cattle infertility, considerable attention of scientists and specialists has always been to analyze the reproduction problems of these species. In addition, as for all mammals, the birth of an offspring causes lactation for cows, which causes many morphofunctional changes in the neuroendocrine regulation of metabolic processes in the female body, which can negatively affect reproductive ability. However, there is another problem that most scientists pay little attention to – the impact of the restoration of the reproductive cycle of cows on milk yield, especially in the first months of lactation, and the quality of raw milk, namely protein and fat content **[1]**, **[2]**.

The level of the reproductive capacity of animals is closely related to their milk productivity. At a high level of milk productivity, the deterioration of the reproductive ability of cows is naturally traced **[3]**, **[4]**. As the reproductive capacity of cows increases, the hormonal background in the body of females causes the emergence of the so-called sexual dominant, i.e. all hormonal systems are set up to restore sexual cycles and the appearance of signs of the first sexual desire. Many studies in the literature show that a high and artificially prolonged lactation increases the hormonal function of the pituitary gland, its anterior part, which is responsible for the secretion of prolactin, which stimulates lactation **[5]**, **[6]**. The release of prolactin causes inhibition of the gonadotropic function of the pituitary gland, which is interrelated with lactogenic function. Thus, increased prolactin secretion reduces the release of gonadotropins, which stimulate sexual function. Kochuk-Yashchenko OA. and co-authors have found

that an increase in the service period leads to a rise in milk productivity– milk yields for 305 days of lactation of animals with an extended period are by 1190 kg higher than of animals with a short service period. It is proved that the duration of the service period directly affects the reproductive capacity of cows. Still, in contrast to the improvement of quantitative indicators of milk productivity, reproduction indicators deteriorate significantly – the reproductive capacity decreases with a long service period down to 0.77. Economically advantageous for the farm and physiological for animals is the expected duration of the service period (average 125, 3 days), in which animals most effectively combine high milk productivity with satisfactory reproductive performance **[7]**. As a result of research **[8]**, the inverse relationship between milk productivity and fertility of the first-born cows of the Ukrainian black-spotted dairy breed has been found. As the fertility index per unit increases, a milk yield will increase by 148.5 kg of milk. An increase in the duration of lactation and the deterioration of fertility are associated with an increase in the service period from 123.1 to 158.3 days. According to data **[9]**, **[10]**, the milking of cows depends on their live weight. The most productive were cows with a live weight of 540 kg and more after the first calving, after the second one – 590 kg and more, and after the third one – 640 kg and more. A positive correlation coefficient was established between the live weight of animals and milk productivity: for the first, second, and third lactation between milk yield and body weight, it was in the range of 0.413 – 0.551 between fat content in milk and live weight – in the range of 0.037 – 0.113, between milk yield fat and body weight – in the range of 0.414 – 0.537. The effect of live weight on milk yield depending on lactation was 18.8 – 32.3, on the fat content in milk – 2.1 – 3.6, and on the input of milk fat – 18.7 – 30.8%.

Therefore, the study of the relationship between the reproductive capacity of cows and the intensity of milk productivity during different periods of lactation and the chemical composition of milk is relevant.

The study aimed to investigate the milk productivity of cows and their milk quality after using the biologically active preparation “Glutam 1M” in the last decade of pregnancy.

Scientific Hypothesis

With the introduction of a biologically active drug (Glutam 1M), we expect improvements in cow reproductive ability. However, the introduction of drugs may affect the dairy performance of cows after calving. Therefore, it was decided to check the influence of the drug “Glutam 1M” on dairy productivity and milk quality, with its introduction by experimental cows in the last trimester in the period 265 – 267 and 270 – 275 pregnancy days.

MATERIAL AND METHODOLOGY

Samples

The productivity of cows and high-quality rates of milk was determined by the results of control milking and on actual records in accounting magazines. Samples for studies were milk samples (Figure 1), which were selected in the morning and determined fat and protein content.

Figure 1 Samples of experimental milk.

Chemicals

The components of the minced meat mixes, which masses were detected in this work, were as follows:

Sodium glutamate (Khimlaborrektiv LLC, Ukraine);

Isotonic sodium chloride 0.9% (Khimlaborrektiv, LLC Ukraine);
A concentrated sulfuric acid(Khimlaborrektiv, LLC Ukraine);
Isoamyl alcohol(Khimlaborrektiv, LLC Ukraine);
Calcium chloride(Khimlaborrektiv, LLC Ukraine).

Animals and Biological Material

The research has been conducted on the Holstein black-spotted breed cows in the conditions of the private agricultural enterprise "Savertsi" of Popilnyansky district of Zhytomyr region.

Instruments

Butyrometerfor milk (0 – 6%), (Khimlaborrektiv, LLC Ukraine);
Pipet-dispenser single-channel (TopPette, Khimlaborrektiv, LLC Ukraine);
Dispenser for isoamyl alcohol and acid(Khimlaborrektiv, LLC Ukraine);
Centrifugal (OPN-3.01, Khimlaborrektiv, LLCUkraine);
Water bath(1012.2 Labexper, Khimlaborrektiv, LLCUkraine);
Refractometer for milk (VMK1, Khimlaborrektiv, LLCUkraine).

Laboratory Methods

The content of fat (acid method) and protein (refractometric method) in milk has been studied by standard methods in the Department of Processing Technologies and Quality of Livestock Products of Polissya National University laboratory.

The mass fraction of fat in milk was determined by an acidic Gerbera method according to GOST 5867-90 (Figure 2).

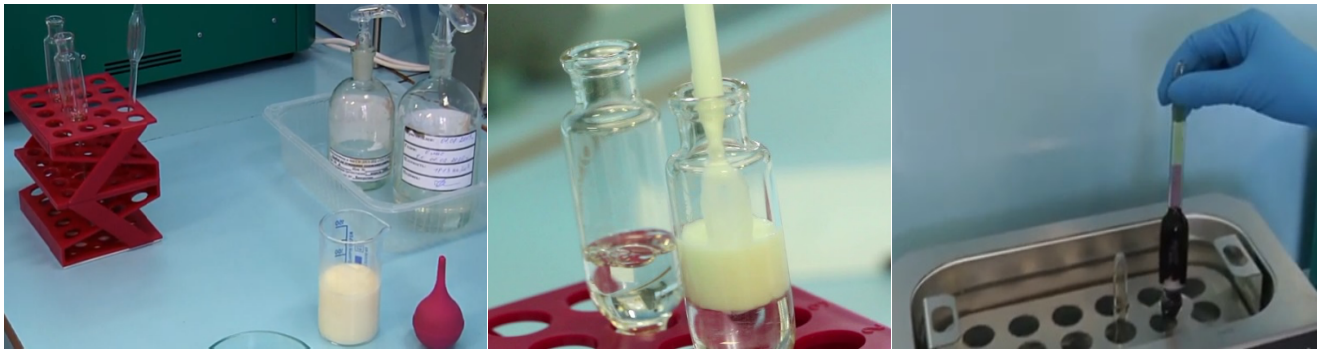

Figure 2 Determination of fat content in milk.

The refractometric method determined the protein content in milk (Figure 3). This method is based on determining the difference in the refractive index of light after its passage through the milk and without protein serum derived from it.

Figure 3 Determination of protein content in milk.

In a glass tube to 5 cm^3 milk, add six drops of calcium chloride solution. The test tubes are closed by rubber cork, mixed thoroughly, and placed on a water bath for 10 minutes. It is necessary to destroy the protein clot by energetic shaking. The test tube is centrifuged for 10 minutes. Then, 1 – 2 drops of transparent serum are taken away with a pipette and applied to the refractometer's measuring prism. Observing the eyepiece of the refractometer, with the help of a correction, clean the coloured lights and shadows. On a scale of "protein", at least three observations were carried out. Then, with the prisms of the refractometer, the serum is removed, and

two drops of studied milk are dripped, and on the "protein" scale are carried out at least five observations, since the sharpness of the light limits and the shade of milk are worse than serum.

The mass fraction of protein in the milk X_1 (%) is calculated by the formula (1):

$$X_1 = X_2 - X_3 \qquad (1)$$

Where:

- X_3 is the average arithmetic value of the observation results on the scale "protein" for milk and serum, respectively.

Description of the Experiment

Sample preparation: The research was conducted within 30 calendar days. At least 5 – 8 different milk samples were taken daily from 4 – 5 different cows.

Number of samples analyzed: 216 samples from two experiments conducted (108 each experience) were used in milk study.

Number of repeated analyses: The number of repeated analyses: each study was carried out three times with the number of samples – 216, which amounted to 648 repeated assays.

Number of experiment replication: The study was repeated three times, with the experimental data processed using mathematical statistics methods.

Design of the experiment: The biologically active preparation "Glutam 1M" has been administered to the cows of the experimental groups under the skin behind the shoulder blade in an amount of 20 ml, starting from 270 and 265 days of gestation, once a day for three consecutive days. Cows of control groups have been similarly injected with saline in the same dose.

The biologically active preparation "Glutam 1M" composition includes the following components: monosodium glutamate and isotonic sodium chloride solution 0.9%. The drug is manufactured by the company "Farmak" (Kyiv) following DSTU 4881:2007.

Dairy productivity and quality indicators of cow's milk have been determined by the results of control milkings and by actual milk yields in the accounting journals. They calculated the milk productivity of cows for one, two, three first months of lactation after calving milk yield for 305 days per lactation. Consequently, the qualitative parameters have been determined: the content of fat (acid method) and protein (refractometric method) in milk for the lactation period.

Statistical Analysis

Statistical analysis data have been performed using Xlstat 2022.1 version (Addinsoft). The accuracy of the obtained experimental data has been determined using Student's t-test with a confidence factor of ≤ 0.05 with five parallel determinations.

RESULTS AND DISCUSSION

In our research, "Glutam 1M" was used to increase the reproductive capacity of cows. The biologically active preparation "Glutam 1M" contributes to the intensification of metabolic processes in a cow's body. When using it on the 6 – 8th day of the sexual cycle **[13]**, **[14]**, it contributes to an improvement in the reproductive capacity of cows. "Glutam 1M" is also administered to cows to improve the quality of the obtained embryos by inducing superovulation for their transplantation and subsequent engraftment in the female genital tract. It has been found that the ingredients of the biologically active preparation "Glutam 1M" cause changes in the metabolic processes of cows and have neurotropic properties when administered three times in the postpartum period **[15]** and the last trimester of pregnancy **[16]**, **[17]**, **[18]**, helping to improve the reproductive ability of animals. Therefore, it was necessary to investigate the influence of the "Glutam 1M" on milk productivity and quality of milk for its introduction to dry cows in the last trimester of pregnancy.

The biological action of the "Glutam 1M" in the body of heifers is based on the influence of glutamic acid, which is its main ingredient. Glutamine amino acid is a substituted amino acid, i.e., when it is deficient in the body, it can be synthesized from other amino acids. It is involved in the processes of amino acid reanimation in the body. The nitrogen of most amino acids goes through the stages of inclusion in glutamic, aspartic acid, or alpha-alanine. Glutamic acid is involved in protein and carbohydrate metabolism, stimulates oxidative processes, promotes neutralization and excretion of ammonia, and increases the body's resistance to hypoxia. It enables the synthesis of acetylcholine and ATP, the transfer of potassium ions, which plays an important role in skeletal muscle activity. Glutamic acid belongs to the neurotransmitter amino acids that stimulate the transmission of excitation at the synapses of the central nervous system. This amino acid can be included in energy and plastic metabolic processes

in specific organs or systems of the body, depending on the functional load they perform. As one of the amino acids that are oxidized in brain tissues and serve as an energy source for the activity of neurons, it has a stimulating effect on the hypothalamic-pituitary system **[19]**.

Glutamic acid is a neurotransmitter in many spinal cord and brain parts. This means that groups of nerve cells use glutamic acid to transmit a nerve impulse from one nerve cell to another, mainly excitation pulses. However, glutamic acid also forms inhibitory neurotransmitters, so the excitation pulses are balanced, and the excitatory effect is not observed. Glutamic acid is converted into gamma-aminobutyric acid (GABA) in the brain, which is the main, though not the only inhibitory, neurotransmitter. Glutamic acid synthesizes adenosine monophosphate (AMP), which is subsequently converted into cyclic adenosine monophosphate (cAMP). This intracellular mediator of the hormonal signal increases the sensitivity of cells to sex hormones while stimulating the release of sex hormones into the blood and improving their content in muscle tissue. Glutamic acid is a source of guanidine monophosphatase (GMP). This compound is converted into cyclic guanidine monophosphate (cGMP) in the body. Cyclic GMP is an intracellular mediator of hormonal and mediator signals like a cyclic AMP. For example, cGMP is an intracellular action mediator on muscle and other acetylcholine cells. Acetylcholine is a mediator of nervous excitation in the parasympathetic nervous system **[20]**.

An important criterion on which the different reproduction ability of cows depends is their milk productivity, especially in the first three months of lactation. The duration of the dry period determines the intensity of lactation after calving.

According to Pelekhatyi M.S. **[11]**, the indicators of reproductive capacity and milk productivity of cows are influenced by the age of the first insemination of first-born cows. The optimal period of the first insemination of first-born cows is in the range of 15-19 months. Under the condition of such insemination, high milk yields are obtained, and the reproductive capacity of cows is preserved. The dairy productivity of cows is determined by many factors, in particular, the duration of the dry period. During the dry period, the body of the pregnant cow is preparing for the next lactation. Authors **[12]** also have studied the dependence of milk productivity of cows of the Ukrainian black-spotted dairy breed on their age and live weight during the first insemination and the first calving. It has been found that the highest milk yields were characteristic for cows, which were first inseminated at the age of 16 months with a live weight of 406 – 435 kg, and the age of the first calving did not exceed 25 months with a live weight of 491 – 510 kg. Their live weight more influenced milk yields of cows at the first insemination (23.34 – 34.25%) and the first calving (27.45 – 36.14%) than the age in these periods (12.22 – 18.52 and 12.54 – 17.85%, respectively).

During the dry period, vitamin and biologically active drugs are administered to ensure the physiological course of calving the birth of strong and viable young, to improve the reproductive capacity of cows. However, the introduction of drugs can adversely affect the milk productivity of cows after calving. The improvement of the parameters of the reproductive capacity of cows will lead to milk productivity decreases **[21]**, **[22]**, **[23]**, **[24]**, **[25]**. The use of the biologically active preparation "Glutam 1M" for cows in the last decade of pregnancy causes a reduction in the duration of the recovery and service period of cows. The insemination index decreased. Also, the number of animals bred after the first insemination increased. It means there was an improvement in reproductive performance after calving **[26]**, **[27]**. Therefore, we have decided to test the effect of "Glutam 1M" on milk productivity and milk quality of experimental cows for its use in the last trimester of pregnancy on the 265 – 267th and 270 – 275th days.

A comparative analysis showed that the milk productivity of cows of the experimental and control groups almost did not differ, and some fluctuations in milk yield observed in some months of lactation were within error after the use of the biologically active preparation "Glutam 1M" on the 265 – 267th days. However, it should be noted that in the experimental group of cows, milk yields decreased slightly (by 91.9 kg or 2%), which, in our opinion, is due to a decrease in the duration of lactation, i.e., in the experimental group the service period was shorter compared to the control group.

According to literary sources **[28]**, **[29]**, **[30]**, **[31]**, **[32]**, the increase in the service period causes an increase in the total amount of milk during lactation. It causes a deterioration in the reproductive capacity of cows. Our study showed that the milk yield during the lactation period in the control group of animals increased by 2.9% (141.5 kg). The reproductive performance was worse than the experimental group's animals (Table 1). The parameter 305-days milk yield during the lactation period in the control group of cows was almost the same as in the previous lactation period. In the experimental group of cows, this parameter decreased by 2.9% (136.5 kg).

Table 1 Milk productivity and milk quality of cows after using "Glutam 1M" on the 265th day of pregnancy.

Indicator	Group, n = 27			
	control		experimental	
	lactation			
	prior	after the experiment	prior	after the experiment
Milk yield per lactation, kg	4689.1 ±80.37	4830 ±113.20	4616.9 ±75.97	4525 ±69.78
305-day milk yield, kg	4730.2 ±51.91	4765.6 ±88.88	4748.1 ±59.12	4611.6±93.40
First three months milk yield, kg	2039.3 ±13.12	1943.7 ±38.08	2003.9 ±19.32	1984.3 ±22.83
Mass fraction of fat, %	3.8 ±0.15	3.7 ±0.20	3.8 ±0.17	3.9 ±0.19
Mass fraction of protein, %	2.9 ±0.02	3.0 ±0.02	2.9 ±0.02	3.0 ±0.02

Note: (n = 27, $p \leq 0.05$).

An important indicator of the milk productivity of cows is the milk yield during the first three months of lactation after calving when there is intensive milk production and the peak of the lactation curve increases. The biologically active preparation "Glutam 1M" did not affect the intensity of lactation in the first three months after calving, as the amount of milk in the experimental and control groups was almost the same.

A similar situation was observed during the biologically active preparation "Glutam 1M" on the 270 – 272nd days of pregnancy. In the experimental group of animals, the milk yield of the previous lactation and after administration of drugs remained almost at the same level. The control group of animals decreased by 4.7% (207.1 kg). The parameter of 305-days milk yield in the control group of cows of the previous and after lactation period was almost the same. In the experimental group of animals, there was an increase in this indicator by 2.7% (128.7 kg). For the first three months after calving in the control and experimental groups of cows, milk yield was almost equal. Therefore, the use of "Glutam 1M" did not significantly ($p \leq 0.05$) affect the milk productivity of cows for subsequent lactation. Similarly, as in the previous experiment, the use of "Glutam 1M" did not affect milk quality; the mass fraction of fat and protein remained at a reasonably high level and almost did not differ between groups (Table 2).

Table 2 Milk productivity and milk quality of cows after using "Glutam 1M" on the 270th day of pregnancy.

Indicator	Group, n = 27			
	control		experimental	
	lactation			
	prior	after the experiment	prior	after the experiment
Milk yield per lactation, kg	4416 ±193.38	4208.9 ±114.72	4745.9 ±96.33	4709.6 ±106.3
305-day milk yield, kg	4430.3 ±90.71	4476.7 ±197.80	4672.9 ±94,81	4801.6 ±78.50
First three months milk yield, kg	1977.2 ±22.75	2022.2 ±36.21	2067.9 ±15.06	1986.3 ±36.33
Mass fraction of fat, %	3.9 ±0.15	3.8 ±0.20	3.8 ±0.17	3.9 ±0.19
Mass fraction of protein, %	2.9 ±0.02	3.0 ±0.02	2.9 ±0.02	3.0 ±0.02

Note: (n = 27, $p \leq 0.05$)

It is known that the starting products for the formation of milk get to the breast from the blood. The volumetric rate of blood flow in the udder increases 25 – 30 times, the chemical composition of the blood changes, energy metabolism increases **[33]**.

The primary source for the synthesis of milk proteins – casein, β-lactoglobulin, and α-lactalbumin are free blood amino acids that penetrate the membrane of epithelial cells of the mammary gland of cows both by diffusion (simple or light) and by active transport against the concentration gradient, in which the gamma-glutamyl transferase system is the main one. It is characterized by broad substrate specificity, especially methionine, glutamine, cysteine , and alanine. In synthesizing milk proteins, the gland also absorbs glutathione from red blood cells, an important cysteine, glycine, and glutamate source. Scientists **[34]**, **[35]** suggest that the absorption of some essential amino acids by the mammary gland (glutamic, asparagine, serine, proline, alanine) occurs in smaller quantities than they are excreted with milk proteins.

Among amino acids in quantitative terms, the first place belongs to glutamic acid in the animal body. Thus, the data that characterize the amino acid composition of casein among 15 presented amino acids, glutamic owns 22.4%. It participates in many reactions associated with energy metabolism synthesis of amino acids, protein, carbohydrates, and lipids **[36]**. It is important to emphasize that only glutamic amino acid is oxidized in brain

tissues and is an energy source for the activity of neurons. It has a stimulating effect on the hypothalamic-pituitary system. It activates the centres of regulation of hunger and satiety, which, in turn, leads to better eating of feed by animals **[37]**, **[38]**.

So we can assume that the use of the biologically active preparation "Glutam 1M", which includes the glutamine amino acid, to stimulate the reproductive capacity of cows can affect their productivity and milk quality. Therefore, the study of the chemical composition of milk after administration of the drug to cows is relevant and has significant both scientific and practical interest.

Analysis of the obtained data of our studies shows that a significant difference between the indicators of milk quality of the experimental and control groups is not observed (Tables 1, 2). The use of "Glutam 1M" did not affect its quality indicators, namely fat and protein mass fraction. They remained high and almost did not differ between the groups. Although, there is a slight increase (from 3.8% up to 3.9%) in the mass fraction of fat in the milk of cows of the experimental group during both the first and the second experiments. However, after the statistical processing, the increase in this indicator is unreliable, or in other words – within the error.

Similar results have been obtained by researchers who used a complex of nanocarboxylates Quatronan-Se to stimulate the reproductive capacity of cows on days 1 – 3 of the sexual cycle. Studies of milk productivity have shown that Quatronan-Se and monocarboxylate complexes do not adversely affect the milk productivity of cows. On the second injection day, they increase milk's protein and fat content **[39]**. Other research results have been obtained by colleagues using the drug Nanovulin-VRH to stimulate the reproductive capacity of cows. Fat and protein content in milk increase compared with the control after using Nanovulin-VRH.

CONCLUSION

The use of a biological preparation "Glutam 1M" in the last period of cows' pregnancy stimulates the reproductive ability of cows. It does not significantly ($p \leq 0.05$) affect milk productivity and quality. The fat and protein content were not significantly changed ($p \leq 0.05$). Application of this preparation at 265 – 267th days led to the milk yield decrease by 2% (91.9 kg) in the experimental group and an increase by 2.9% (141.5 kg) in the control group. The parameter 305-days milk yield in the control group of cows was almost the same as in the previous lactation period. In the experimental group of cows, this parameter decreased by 2.9% (136.5 kg). After using the "Glutam 1M" on the 270 – 272th days of pregnancy, the milk yield of animals of the experimental group remained at the same level, and in the control group, it decreased by 4.7% (207.1 kg). The parameter 305-days milk yield remained unchanged in the control group of cows, and in the experimental group we have found an increase in this indicator by 2.7% (128.7 kg). Milk yield of both groups of cows for the first three months after the calving was at the same level. The "Glutam 1M" preparation did not affect milk quality.

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Perspectives for the application of the sous-vide cooking in the development of products for public catering

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ABSTRACT

The effect of different sous-vide cooking temperature-time combinations on beef steak's microbiological, physicochemical, and organoleptic parameters were analysed. The organoleptic quality of souse-vide beef steaks was excellent. The sous-vide cooking had a considerable impact on the physical and chemical parameters of the product. The amino acid composition of the sous-vide cooked meat was similar to the original fresh beef. Souse-vide meat cooking does not denature proteins as much as conventional cooking and frying. In some cases, the microbiological parameters exceeded the expected legislation limit. We recommend additional antimicrobial barriers, such as lower pH and antimicrobial extracts from ginger in a concentration of 0.5 – 1.5% of the weight of fresh meat, combined with garlic powder. The final product had an extended shelf life compared to control samples prepared by boiling and frying.

Keywords: sous-vide, beef, meat, microbiological quality, microstructure, weight loss, product yield, ginger

INTRODUCTION

The sous-vide cooking consists of preparing products closed in a thermally stable vacuum package under strictly controlled temperature and heating duration (Figure 1) **[1]**. The development of this technology responds to the population's need for ready-to-eat, microwave-usable semi-finished products with a high nutritional value, do not contain additives and preservatives, and are affordable **[2]**.

Products prepared by the sous-vide cooking has several advantages. The economic benefits of preparing products using sous-vide cooking are a more efficient use of labour and equipment due to centralised production and an increase in the product's shelf life due to vacuum packaging **[3]**. Sous-vide cooking can be a good solution for reducing food waste in public catering enterprises, where it is impossible to predict the demand for specific menu items **[4]**. In sous-vide products, the growth of aerobic bacteria is limited, and the risk of contamination of the product is reduced. Also, heat transfer to the products inside the vacuum packaging is optimised. Sous-vide cooking can produce food products with constant, reliable, and reproducible organoleptic characteristics. The plastic film prevents the loss of volatile flavours and water during cooking in the sous-vide, which improves the organoleptic quality, promotes juiciness and tenderness of the meat, and increases the product's yield. Sous-vide heat processing preserves the nutritional value of products. It minimises the content of harmful chemicals that are formed during incomplete combustion or pyrolysis of organic substances and are concentrated in meat during frying on coals, grilling, and smoking **[2]**. Thermal denaturation of proteins in cooked meat should be expected. It includes aggregation, binding, oxidation and solvability. These processes affect the release of nutrients and minerals and their bioavailability during digestion, which, in turn, can affect the commercial value of the product. In some studies, the influence of the sous-vide method on the kinetics of protein digestion and the release of minerals such as Cu, Fe, Zn, and Se was studied **[5]**.

Figure 1 Food preparation by the sous-vide method.

The microbiological risks associated with sous-vide cooking is the growth of the spore-forming *Clostridium botulinum* pathogen bacteria and the production of toxins. Non-spore-forming food pathogens in sous-vide products include *Escherichia coli*, *Salmonella*, *Staphylococcus*, *Listeria*, and *Yersinia*. These pathogens must be destroyed during heat processing. However, they can affect consumer health if the raw ingredients are of low microbiological quality when they are secondarily contaminated during production due to improper manufacturing practices **[6]**. Thus, the primary attention in developing the technology for sous-vide production should be directed to ensuring the product's microbiological safety.

The issue remains relevant since no research has been conducted in the Republic of Kazakhstan on using sous-vide technologies for public catering production using regional raw materials. The purpose of this work was to compare the organoleptic, physicochemical, microbiological parameters of beef steak prepared by the sous-vide method and using conventional boiling and frying methods. Ginger was included in the recipe of the sous-vide product, which, according to the literature data **[7]**, has antimicrobial properties.

Scientific Hypothesis

The sous-vide cooking would result in a product with increased shelf life and better organoleptic quality than conventional cooking and frying.

MATERIAL AND METHODOLOGY

Samples

We have used fresh beef meeting the requirements of quality standard GOST 33818-2016 **[8]**. Other ingredients include ginger, salt, garlic powder, butter. Components were obtained from the grocery shop.

Instruments

Beef packaging for the production using the sous-vide method was carried out in a vacuum packaging machine manufactured by Besservacuum of the FAVORIT series with a final pressure of 200 Pa. The heat processing of meat by the sous-vide method was carried out on the Stebra sous-vide SV 2 equipment. During cooking, the meat temperature was controlled by an infrared thermometer with a laser designator and a penetrating food probe Testo 826-T4.

Laboratory methods

Microbiological testing was performed according to technical standard GOST 10444.15-94 **[9]**. The number of mesophilic aerobic and facultative anaerobic microorganisms was analysed. According to legislation, the number of microorganisms in food products should not exceed $1x10^3$ CFU/g.

The content of amino-ammonium nitrogen in meat was determined by standard GOST 55479-2013 **[10]**. The method is based on binding amino groups and ammonia with formaldehyde in a neutral medium, followed by titration with alkali of carboxyl groups, equivalent to the number of free amino groups.

The amino acid composition of the products was determined by standard GOST 55569-2013 **[11]**. The method's principle is to decompose the sample for analysis by acid hydrolysis with the conversion of amino acids into free

forms, the production of FTC-derived amino acids, their further separation, and quantitative determination by capillary electrophoresis.

The acidity of meat was determined by standard GOST R 31470-2012 **[12]**. The method is based on titrating an aqueous extract from the product with an alkali solution. The acidity is expressed in Turner degrees (°T), equal to the number of cubic centimetres of a sodium or potassium hydroxide solution with a molar concentration of 1 mol/dm^3 used to neutralise the acids contained in 100 g of the product.

The pH of the products was measured according to the standard GOST R 51478-99 **[13]**.

The organoleptic evaluation of the product was carried out according to the standard GOST 9959-2015 **[14]** using the method of quantitative descriptive analysis.

Description of the experiment

Firstly we have prepared the product according to the internal recipe. The recipe includes fresh beef meat, ginger in 0.5 – 1.5%, garlic powder – 1.0%, salt – 1.5%, and butter – 2.0% of the mass of raw meat steak. The meat was marinaded in the mixture of above mentioned ingredients. Consequently the prepared meat products were devided into several gropus to be cooked by conventional heat processing (boiled at 100 °C, fryied at 180 °C), and cooked by sous-vide technology. Sous-vide product was vacuum packed and cooked at temperature of 55, 65, 75°C and during 45, 60, and 90 minutes. Another groups of samples was boiled during 30 – 165 min and sous-vide cooked at 70 °C during 30 – 315 min.

The mass of one steak for packaging in a vacuum film was 105 ±8 g. The bags were made of high-density polyethylene with a thickness of 85 – 105 microns. This material is safe and resistant to aggressive environments withstands temperature conditions from -80 to +110 °C. The meat preparation consisted of rubbing the meat with oil, salt, and spices.

Secondly, the final product were analysed.

The microbiological (Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms QMAFAnM) and physical-chemical (pH, acidity and amino-ammonia nitrogen content) parameters of sous-vide meat steaks cooked in the temperatures mentioned above and times were analysed. Also, the dependence of the product's acidity on the content of amino-ammonia nitrogen in 10 cm^3 of the product extract was investigated.

The weight loss of the product during conventional regular boiling and sous-vide boiling at 70 °C, depending on the ageing time (30 – 165 min) was analysed.

The amino acid composition of raw, boiled, fried, and sous-vide cooked meat steak was analysed.

The QMAFAnM of meat steak prepared by the sous-vide method at 70 °C for 90 minutes and control samples prepared by cooking (100 °C) and frying (180 °C) after 5, 24, 48, 72, and 168 hours of storage at a temperature of 2 – 4 °C was analysed.

Finally, the organoleptic evaluation of meat steak prepared by the sous-vide method, in the mode of 70 °C 90 min, was analysed by a trained expert commission. Qualitative organoleptic parameters of the product were determined by the scoring method.

Statistical Analysis

The mass loss during the heat processing in the cooked meat samples was determined as the ratio of the mass of meat before and after heat processing, expressed as a percentage. Experimental results concerning this study are reported as means ± standard deviation (SD). The Student's t-test was used to test the statistical significance of the results at alpha level 0.05. We have used the Xlstat version 2022.1 (Addinsoft) statistical software.

RESULTS AND DISCUSSION

The results of the first experiment are present in Table 1. The pH of the sous-vide products varied in intervals 5.76 – 6.79. We have not found an increase in the pH in all sous-vide products with the same ginger content, ageing time, and different cooking temperature. The pH decreases slightly at temperatures 55 °C and 65 °C, except for the temperature of 75 °C with ginger in concentrations 1.0 and 1.5%. The sous-vide cooking affected the amino-ammonia nitrogen in the product. It can be assumed that under certain conditions of preheating, pH, and microbiological quality of raw materials, the destruction of protein proceeded rapidly, which led to the accumulation of biogenic amines. The increased content of amino-ammonia nitrogen in the product does not corresponds to a high number of mesophilic aerobic and facultative anaerobic microorganisms in all cases. It is possible to note a tendency to decrease the product's acidity with an increase in the content of amino-ammonia nitrogen in it (Figure 2).

Table 1 Microbiological and physicochemical parameters of the sous-vide beef steaks.

Pasteurisation temperature°C	Duration of ageing, min	QMAFAnM, CFU/g*10^{-3}			pH			Acidity, °T			Amino-ammonia nitrogen, mg/10 cm^3 extracts		
		Part of weight of ginger, %											
		0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
55	45	6	18	1	6.35	6.58	6.59	4.55	3.90	4.80	1.25	1.93	1.10
	60	3	1	3	5.79	5.97	5.81	5.55	3.36	5.28	0.95	2.01	1.05
	90	3	1	4	6.17	5.85	6.03	5.99	3.93	3.96	0.91	1.78	0.91
65	45	2	3	7	6.32	6.79	6.40	5.88	4.85	3.85	1.01	1.33	1.85
	60	8	1	5	6.19	6.07	6.19	3.70	5.64	3.90	1.93	0.90	1.93
	90	6	8	3	5.98	5.91	6.08	5.35	5.16	5.25	0.81	0.89	0.91
75	45	6	4	7	6.02	5.94	5.90	5.81	5.85	3.94	0.75	0.93	1.88
	60	7	6	12	5.77	6.40	6.41	3.96	5.99	5.00	1.92	1.01	1.09
	90	6	3	3	6.02	6.60	5.76	4.80	3.96	5.88	1.27	1.85	0.97

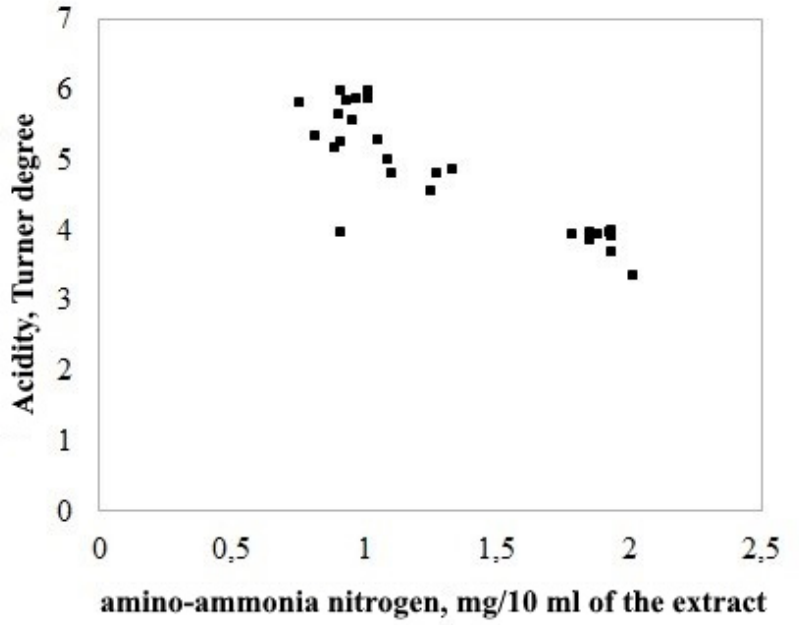

Figure 2 The dependence of the product's acidity on the content of amino-ammonia nitrogen in 10 cm^3 of the product extract.

Table 2 shows the weight loss of meat subjected to conventional boiling and sous-vide cooking at 70 °C, depending on the ageing time. With an increase in the meat ageing from 30 to 165 minutes, the loss of meat mass increased both in the normal and the sous-vide cooking samples, but in the second case, the losses were less. The weight loss decreased with further ageing of sous-vide meat and cooking at 70 °C.

The amino acid composition of the fresh meat cooked by boiling, frying, and sous-vide is presented in Table 3. The amino acid content in the fresh meat and the sous-vide steak is very similar. Therefore the protein denaturation in the sous-vide product is less than in the boiled or fried products. Also, there is no considerable moisture loss from the vacuum-packed steak. The content of all amino acids in the boiled meat increased. It is the consequence of the moisture and fat content decreasing in the sample during the cooking. When frying, it is possible to note a decrease in the content of lysine and alanine in the sample compared to the fresh meat, which may be a consequence of the protein denaturation during frying. There are also small differences in leucine + isoleucine, glycine, histidine, and proline in fried meat compared to fresh meat.

Table 2 Weight loss of the product during conventional boiling and sous-vide boiling at 70 °C, depending on the ageing time.

Ageing, min	Mass loss, %	Ageing, min	Mass loss, %	Ageing, min	Mass loss, %
Regular boiling		The sous-vide method			
30	35	30	35	180	36
45	42	45	38	195	39
60	48	60	39	210	34
75	44	75	32	225	37
90	46	90	42	240	41
105	46	105	40	255	39
120	47	120	42	270	40
135	47	135	40	285	31
150	46	150	44	300	30
165	46	165	41	315	40

Table 3 Amino acid composition of raw, boiled, fried, and cooked using sous-vide method meat steak.

Amino Acid	Mass fraction of amino acid, % in a sample of meat steak prepared by the method:			
	raw	boiled	fried	sous-vide
Arginine	0.95 ±0.38	1.39 ±0.56	1.13 ±0.45	0.89 ±0.35
Lysine	0.85 ±0.29	1.04 ±0.35	0.39 ±0.13	0.78 ±0.27
Tyrosine	0.50 ±0.15	0.64 ±0.19	0.51 ±0.15	0.49 ±0.15
Phenylalanine	0.71 ±0.21	0.93 ±0.28	0.80 ±0.24	0.63 ±0.19
Histidine	0.43 ±0.21	0.48 ±0.24	0.36 ±0.18	0.35 ±0.17
Leucine+	0.79 ±0.20	1.01 ±0.26	0.61 ±0.16	0.72 ±0.119
Methionine	0.40 ±0.14	0.48 ±0.16	0.38 ±0.13	0.38 ±0.13
Valine	0.64 ±0.26	0.83 ±0.33	0.53 ±0.21	0.63 ±0.25
Proline	0.55 ±0.14	0.69 ±0.18	0.71 ±0.19	0.52 ±0.14
Threonine	0.48 ±0.19	0.62 ±0.25	0.47 ±0.19	0.49 ±0.19
Serine	0.28 ±0.07	0.38 ±0.10	0.26 ±0.07	0.29 ±0.08
Alanine	0.63 ±0.16	0.72 ±0.19	0.31 ±0.08	0.54 ±0.14
Glycine	0.43 ±0.14	0.51 ±0.17	0.35 ±0.12	0.38 ±0.13

Notably, on average, there are more acceptable microbiological parameters of the product prepared at a temperature of 55 °C, compared with temperatures of 65 and 75 °C (Table 1). Unfortunately, only in some cases, the microbiological indicator of the product does not exceed the standard value of ($1x10^3$ CFU), for example, at modes 55 °C, 45 min, 1.5%, 55 °C, 60 and 90 min, 1% and 65 °C, 60 min, 1%. These results indicate the antimicrobial properties of ginger are probably maintained only if the temperature does not exceed 55 °C.

The number of mesophilic aerobic and facultative anaerobic microorganisms in meat steaks prepared by the sous-vide cooking (at 70 °C for 90 minutes) and control samples prepared by cooking (100 °C) and frying (180 °C) was analysed after 5, 24, 48, 72, and 168 hours of final product storage at a temperature of 2 – 4 °C (Table 4). Based on this data, the shelf-life of the sous-vide product should not exceed 72 hours.

Table 4 The QMAFAnM (CFU/g) of meat steak prepared by the sous-vide method in the mode of 70 °C for 90 minutes, conventional boiling and frying, after storage at a temperature of 2 – 4 °C.

Time, h	Raw materials	Boiled	Fried	Sous-vide 70 °C 90 min
5	continuous growth	$3.5x10^2$	$2x10^2$	not detected
24	continuous growth	$13x10^2$	$9x10^2$	not detected
48	continuous growth	$22x10^2$	$17x10^2$	$2x10^2$
72	continuous growth	continuous growth	continuous growth	$8x10^2$
168	continuous growth	continuous growth	continuous growth	$36x10^2$

The microstructure of raw, boiled meat prepared using the sous-vide method is shown in Figure 2 and Figure 3. With an increase in the duration of the processing, the bundles of muscle fibres become looser. There are noticeable changes in the meat structure after 130 minutes of cooking, and respectively after 165 min for sous-vide (70 °C, 90 min) cooking.

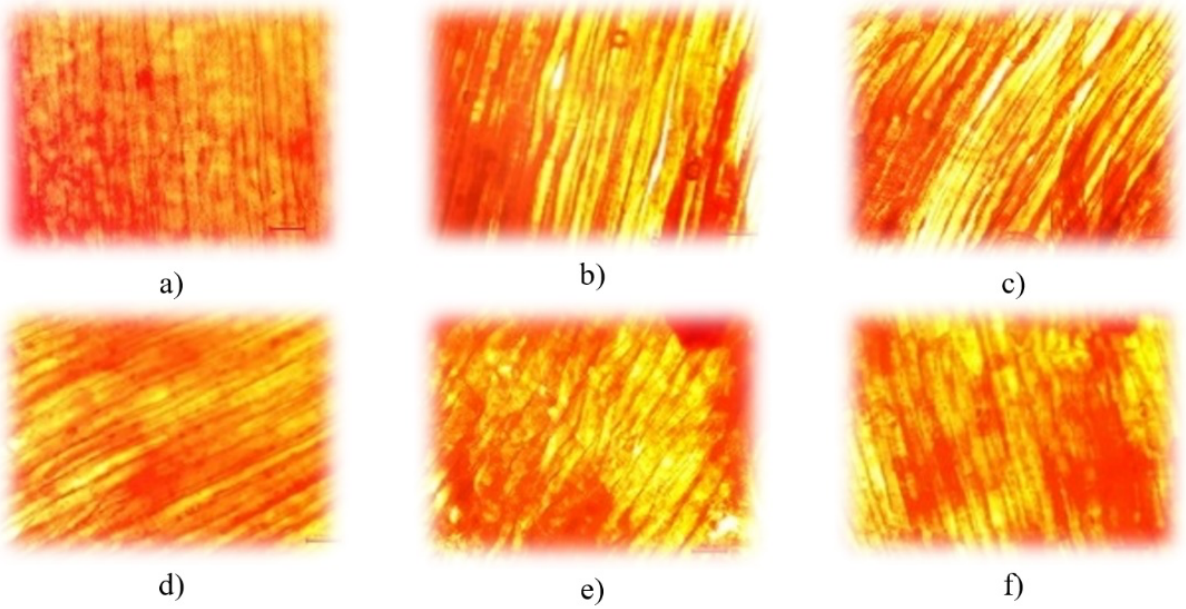

Figure 2 Micrographs of raw and boiled meat.
Note: a) raw meat b) boiled 60 min/70 °C; c) boiled 75 min/70 °C; d) boiled 90 min/70 °C; e) boild 130 min/70 °C; f) boiled 165 min/70 °C (magnified ten times).

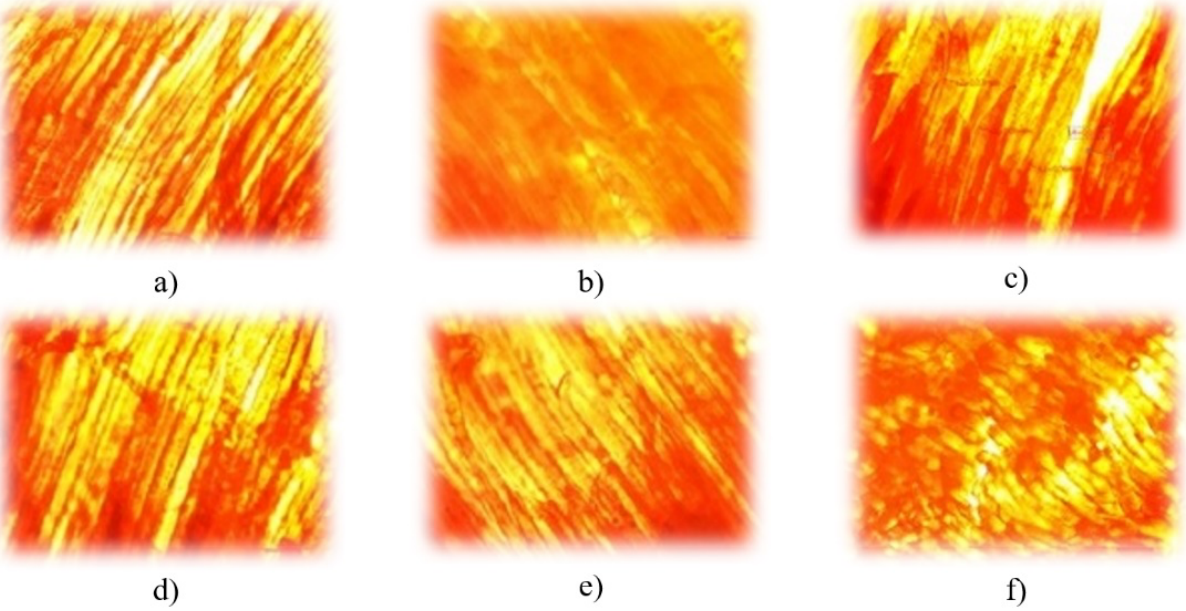

Figure 3 Micrographs of meat prepared by the sous-vide method.
Note: a) 75 min/70 °C; b) 95 min/70 °C; c) 105 min/70 °C; d) 120 min/70 °C; e) 135 min/70 °C; f) 165 min/70 °C (magnified ten times).

The indicators of the organoleptic analysis of the sous-vide product (70 °C, 90 min) are present in Table 5. This cooking mode was the best in comparison with other sous-vide modes tested. The final product reached the best for smell, colour, taste, and consistency characteristics.

Table 5 Indicators of organoleptic evaluation of meat steak prepared by the sous-vide method at 70 °C, 90 min.

Indicator	Evaluation by the commission
The appearance of the base	4.8
Smell	5.0
Taste	4.9
Colour	4.8
Consistency (succulence, tenderness)	4.8
Total	4.9

The most significant technological and organoleptic properties of meat depend on the meat tissue's ability to retain water and form a gel. Myofibrillary proteins retain most of the water inside the muscle. An increase in temperature from 40 °C to 90 °C causes denaturation and compression of these proteins. Also, at 56 – 62 °C, the irreversible change in collagen occurs. Up to 60 °C, the muscle fibres contract in the transverse direction, and the gap between the fibres increases. Above this temperature, the muscle fibres contract in the longitudinal direction, which causes a significant loss of water, the degree of loss increases with the cooking temperature **[15]**. Prolonged meat cooking at a low temperature can lead to protein proteolysis by heat-resistant enzymes, affecting water retention and cooking losses **[16]**. The sous-vide cooking at 70 °C for 30 to 165 minutes was accompanied by a meat steak weight loss of 35 to 44%. After the usual cooking for 30 minutes, the weight loss of the steak was 35%, which is less than with the 3-hour sous-vide cooking at 70 °C. This finding is similar to other research **[17]**. Thus, the duration of cooking in the sous-vide mode has a considerable impact on the yield of the product.

In another study **[18]**, the influence of various combinations of temperature (70 °C and 80 °C) time (60, 90, and 120 minutes) on the yield of the beef sous-vide was studied. The beef meat was marinated in teriyaki sauce or beer. The mass loss ranged from 5.11 to 8.17%. As the temperature increased, there was an increase in weight loss of beef prepared by the sous-vide method. The longer the cooking time, the greater the water loss in the beef samples was **[18]**. The marinating process increases the yield of beef, pork, and poultry; this may be why the loss of beef mass in work **[18]** was less than in the present work. In work **[15]**, the influence of the cooking mode of lamb meat by the sous-vide method on the moisture content and product yield was investigated. Various combinations of temperatures (60, 70, and 80 °C) and cooking time (6, 12, and 24 hours) are considered. Weight losses during the preparation of lamb sous-vide at 60 °C during 6, 12, and 24 hours were 20.77%, 24.72%, and 28.78%; at 70 °C the weight losses were 30.67%, 33.42%, and 32.80% and at 80 °C the weight losses were 35.22%, 35.61%, and 39.41%, respectively. Our results of weight loss are similar to these results. In another work **[4]**, the product yield from chicken breast prepared by the sous-vide method was analysed. The product yield were: 89.4% (1 h, 64°C), 82.4% (80 min, 66°C), and 83.1% (35 minutes, 75 °C). After conventionally cooking steamed chicken breast, the yield was 72.4% and when boiling 69.5%. The product yield from the semitendinous beef muscle prepared by the sous-vide method at 60 °C with 4.5 and 10 hours of ageing was evaluated **[5]**. The weight loss of the product was 20.89 and 28.67%, respectively. The sample size, heating schedule, and raw material history (for example, freeze-thawing cycles) can affect the kinetics of water loss by the product **[16]**.

The formation of amino compounds and ammonia bases during the heat processing of meat products occurs due to the deamination and decarboxylation of both free amino acids and amino acids in proteins and polypeptides. The higher the quality of the heat-processed product, the less ammonia it contains. However, since the initial content of amino-ammonia nitrogen in meat can be different, its absolute content after pasteurisation cannot be used to judge the qualitative changes in the product **[19]**. In this work, the content of amino-ammonia nitrogen in meat samples prepared in different sous-vide modes, depending on the cooking mode, ranged from 0.81 to 2.01.

The content of amino-ammonia nitrogen in vacuum-packed beef processed with a pressure of 400MPa for 3 minutes was 0.10; 0.17, and 0.21 mg/10 cm^3 extracts after 10, 30, and 39 days of storage, with the norm for fresh meat – less than 1.26/10 cm^3 **[20]**. This fact indicates the safety of the protein components of meat. The deterioration of raw meat quality is associated with proteolysis and the growth of microorganisms. The amount of biogenic amines formed under the action of decarboxylase of amino acids of microbial origin depends on several factors, such as raw materials (meat composition, pH, hygienic conditions), additives (salt, sugar, nitrites), storage conditions, meat processing mode **[21]**. In this paper, the increased content of amino-ammonia nitrogen in some sous-vide modes corresponds to samples with a low microbial load (for example, 55 °C, 60 min and 90 min ageing, 1.0% of ginger). However, in most samples with increased content of amino-ammonia nitrogen, the microbiological indicator was higher than average.

Regarding the safety of biogenic amines after the preparation process, the literature data are contradictory. Some authors have concluded that these compounds are heat-resistant, and their level does not considerably decrease during heat processing. According to other authors, heat processing can affect the content of amines **[22]**.

The change in pH is associated with a different degree of increase in the number of free NH_2- and COOH- groups during pasteurisation **[19]**. As a rule, thermal exposure contributes to the rise in the pH of meat, which is explained by a decrease in the available carboxyl groups in boiled meat **[18]**. After marinating and cooking by the sous-vide method (70, 80 °C, 60 to 120 minutes), the pH of beef steak was 5.19 – 6.17, which is lower than in our work 5.76 – 6.79. However, the fresh meat in this work **[18]** had a reduced pH after marinating (4.67 – 5.00). In the research **[5]**, the pH of the semitendinosus beef muscle cooked by the sous-vide method (60 °C for 4.5 and 10 hours) was 5.79 and 5.78. The authors concluded that the beef sous-vide cooking has a negligible effect on the pH of the product. In our work, we have found an increase in the pH of the product was observed with an increase in the temperature and duration of preparation, but not in all cases.

The amino acid composition of poultry meat was studied depending on the cooking method **[23]**. Heat processing led to a certain decrease in the content of all amino acids. Meat samples processed under pressure retained the highest content of total essential, non-essential, and total amino acids. Then, in descending order of the number of amino acids, the following thermal processing methods followed: boiling, microwave processing, and frying. The decrease in the content of amino acids can be associated with both the loss of meat fluid and the denaturation of proteins during heat processing. Several studies have shown a considerable decrease in the amount of sulfur-containing amino acids, i.e., leucine, tyrosine, phenylalanine, and lysine (as essential amino acids), as well as serine, glycine, alanine, histidine, and arginine (as interchangeable amino acids). At the same time, there was a slight decrease in the content of other amino acids after heat processing of chicken breast or thigh meat samples. This work established significant losses of lysine and alanine from meat steak during frying. The loss of amino acids in the preparation of meat steak by the sous-vide method is the smallest compared to other processing methods. Principles of the GMP and HACCP should be applied **[23]**. The HACCP approach is a preventive approach to microbiological quality control and is designed to prevent problems before they occur and not to find them in the finished product **[6]**.

The selected souse-vide mode allowed obtaining a product with a longer shelf life than conventional cooking and frying (Table 4). The microbiological quality of the fresh meat used for cooking is important. The packaging material decreased the risk of the secondary contamination of the product, and the vacuum does not allow the growth of aerobic microorganisms. The microbiological index did not exceed the legislation limit for the sous-vide product within 72 hours after cooking, while conventional boiling and frying – within 12 and 24 hours, respectively. The possibility of extending the shelf-life of sous-vide meat semi-finished products in the refrigerator compared to products prepared using conventional technologies makes the sous-vide method attractive for manufacturers of public catering products. However, the sous-vide method allows increasing the product's shelf life only if the pasteurisation standard is chosen correctly. The data in Table 1 showed that this cooking method can lead to an unsafe product in some cases, which is confirmed by the literature data. For example, in work **[3]**, the safety of sous-vide products against *Clostridium botulinum* was evaluated using control tests with low (2.0 lg CFU/g) and high (5.3 lg CFU/g) contamination. After heat processing, the products were stored at 4 and 8 °C and were checked for botulinum and neurotoxin spores on the end date of the sale period and seven days after the sale date. It was found that most of the thermal processes are unsatisfactory, even with a low level of initial microbial load. Only 2 of the 16 products were negative for botulin and neurotoxin spores in both analysis cases. Based on this study, it was concluded that the safety of sous-vide products requires constant, careful assessment. The time and temperature combinations used in heat processing should be reviewed to improve the processing efficiency and use additional barriers, such as bio preservatives.

The main pathogenic bacteria of concern for sous-vide products are *Salmonella*, *L. monocytogenes,* and pathogenic strains of *Escherichia coli* since they are relatively heat-resistant. The maximum ageing time of the product at a specific temperature will depend on the types and number of microorganisms infecting the product and the heat resistance of each organism. Many pathogenic microorganisms' maximum growth temperature range is 42 – 49 °C, and some can grow at 50 – 55 °C. The temperatures used in the sous-vide cooking modes must be above this range. Slow heating of the product to the cooking temperature can increase the microbial load of the product, so it is recommended to preheat the bain-marie to the cooking temperature **[24]**. The temperature of the fresh meat should be maintained at 2 – 4 °C at all stages before the heat processing. This temperature minimise the growth of pathogens. The correct choice and constant monitoring of the storage modes of raw materials, preparation and storage of the finished product, it can be recommended to use the sous-vide method in the "preparation-serving" option.

A more popular version of the sous-vide "cooking-freezing" processing method, in which products are reheated after several days or weeks of storage in the refrigerator, poses a higher threat to consumers' health. The spore-forming pathogen *Clostridium botulinum* of types A, B, and E can withstand moderate heat processing of sous-vide, and the presence of anaerobic packaging conditions promotes the growth and production of *C. botulinum* toxins in the finished product. *C. botulinum* strains can grow and produce toxins at low temperatures, while *C. botulinum* spores of types A and B can grow at a temperature of 10 – 12°C **[6]**. To reduce *C. botulinum* by six lg, an exposure time of 8 hours 40 min at 75 °C, 75 min at 80 °C, or 25 min at 85°C is required **[1]**. To destroy the spores, it is necessary to apply a pasteurisation temperature above 100 °C. To prevent the reproduction of *C. botulinum* and accumulation of deadly neurotoxin in meat, rapid cooling of the product immediately after pasteurisation is recommended, and then the storage regimes: below 2.5 °C – less than ten days; below 3.3 °C – less than 31 days; below 5.0 °C – less than ten days; below 7.0 °C – less than five days **[1]**. There is a possibility of temperature disturbances during the distribution of sous-vide products, especially in the retail environment. Therefore, additional barriers may be required to ensure the microbiological safety of the final product. These barriers can be combined with pasteurisation to suppress surviving microorganisms in sous-vide products,

including water activity, pH, and preservatives. For example, combinations of water activity and pH reduction have proven effective in controlling the growth of *C. botulinum* type E in caviar stored at room temperature **[6]**. For ready-made chilled products with a shelf life of more than ten days, as additional controlling factors of *C. botulinum*, it is recommended to use:

1) heat processing at 90 °C for 10 minutes or equivalent;
2) pH = 5.0 or less;
3) the minimum salt level is 3.5% in the aqueous phase of the product;
4) water activity 0.97 or less;
5) using a combination of pasteurisation and preservatives **[6]**.

An effective barrier is marinating the meat before cooking. The thermal stability of a mixture of five strains of *Salmonella* and five strains of *L. monocytogenes* in chicken breasts marinated in teriyaki sauce (pH = 4.2) was studied and then prepared by the sous-vide method in the modes 55; 57.5, or 60 °C for an hour **[25]**. The results prove marinades' effectiveness against *L. monocytogenes* and *Salmonella* and other pathogens in meat have been published. The low pH of the marinade reduces the number and increases the sensitivity of pathogenic microorganisms to heat stress. The results of our work suggest that ginger can be used as an additional barrier that reduces the number of microorganisms in combination with a temperature of 55°C and the heating time 45 to 90 min. This antibacterial effect of ginger was also confirmed by **[7]**. Ginger and garlic's antimicrobial effect of aqueous and alcoholic extracts against *Staphylococcus aureus*; *Bacillus spp*., *Escherichia coli*, and *Salmonella spp*. was investigated by **[26]**. Both garlic and ginger's water and alcohol extracts separately did not suppress any test organisms. However, in combination with lime, they suppressed *Bacillus spp*, *Staphylococcus spp*. A combination of alcoholic extracts of ginger and garlic suppressed *S. aureus* and *Bacillus spp. Salmonella* was resistant to almost all extracts. There were no literature sources on the use of ginger as an additional antimicrobial barrier in the preparation of meat products by the sous-vide method. The effect of ginger extract in combination with citric acid on the tenderness of duck breast was studied by **[27]**, since ginger has enzymatic activity and promotes the degradation of myofibrillar proteins during long marinating. Additional studies are needed to confirm the possibility of using ginger as an additional antimicrobial barrier for meat steak prepared by the sous-vide method.

An increase in the gaps between the fibres at 60 °C, and a more compact arrangement of the fibres at 70 – 80 °C was noted by **[15]**. There was no noticeable effect of the cooking time on the microstructure of the fibres. In the works of some authors, a decrease in the diameter of the fibres at 60 °C was observed at 60 °C, followed by an increase in the diameter at 70 – 80 °C. They attributed this shrinkage to the thermal denaturation of intramuscular collagen at temperatures from 60.7 to 61.7 °C. At the same time, further heating leads to the formation of a denatured collagen gel around each muscle fibre. In another research **[28]**, authors found fibre compression and the intercellular space increased in beef meat cooked by the sous-vide method (60 °C for 12 hours). In our work, the change in the fibre structure in micrographs became noticeable after 165 minutes of sous-vide cooking at temperature 70 °C.

The colour stability of the sous-vide product depends on the cooking mode. The darker appearance of the meat may be explained by the higher moisture content of the meat cooked at a lower temperature, which leads to deeper penetration of light into the tissues. The intensity of redness in cooked meat is inversely proportional to the degree of denatured myoglobin. The denaturation process occurs at a temperature from 55°C to 65°C but continues up to 75 – 80 °C **[18]**. Beef cooked at 80 °C with a long ageing time has a more tender consistency than cooked at 125°C in a short time, which emphasises the importance of slowly increasing the temperature to obtain a more acceptable organoleptic quality product. Prolonged ageing affects the tenderness and juiciness of the meat in the opposite way. Sometimes more tender meat was described by experts as less succulent and crumblier.

Given that succulence and tenderness are the most important sensory characteristics of cooked meat, any sous-vide process should be designed in such a way as to obtain optimal values of both characteristics **[16]**. We agree with these authors. The sous-vide temperature and time are important for the final product organoleptic properties. In our study, we produced beef steak with excellent organoleptic properties.

CONCLUSION

The microbiological, physicochemical, and organoleptic parameters of sous-vide beef meat product were analysed. The meat ageing leads to the product's weight loss in conventional (100 °C) and sous-vide (70 °C) cooking. The souse-vide product's weight loss ranged from 36 – 41%. In comparison with traditional cooking, the weight loss was lower. The sous-vide cooking method affected the content of amino-ammonia nitrogen, which varied in the range of 0.81 – 2.01 mg/10 cm^3 of the product extract. The increased values of this indicator in the product could result from increased content of proteases in raw materials with insufficient microbiological quality of fresh meat. The pH of the finished product was in the range of 5.76 – 6.79. The microbiological parameters of

the finished products were not satisfactory in almost all sous-vide modes and exceeded the legislation limit. The antimicrobial properties of ginger are probably maintained only if the temperature does not exceed 55 °C. The addition of ginger and garlic extract with a pasteurisation temperature of 55 °C can be considered as a barrier to microbial growth. However, additional experiments are necessary to confirm this assumption. The use of a more severe pasteurisation regime brings the properties of the finished sous-vide product, such as the yield and the degree of protein denaturation, similar to conventional cooking methods. The sous-vide product shelf-life was max. 72 hours.

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The inhibitory effect of Ukrainian honey on probiotic bacteria

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ABSTRACT

Honey is used in the food industry as a natural sweetener and has therapeutic effects on the human body. Obtaining quality honey involves using organic preventive and treatment agents in beekeeping. The most common of these agents are probiotic supplements. This research aimed to study honey's interaction with an inhibitory effect on the growth of microorganisms from the probiotic supplement Immunobacterin-D under laboratory and experimental field conditions. At the first stage of the research, we assessed the effects of ten honey varieties (buckwheat, sunflower, meadow and forest plants, linden) on *B. subtilis* and *B. licheniformis* from the dry probiotic supplement. The honey-containing nutrient media had an inhibitory effect on the growth of *B. subtilis* colonies. After 24 hours of cultivation under aerobic conditions, the concentration of *B. subtilis* decreased, on average, from 5×10^{12} colony-forming units in 1 g to 3.2×10^{4} and 2.1×10^{5} CFU/g in samples with monofloral and polyfloral honey, respectively. These results emphasize the need for further research on the symbiotic role of microflora in the stability of the microbiota of the hive and bee colony ecosystem. The next stage of the study investigated the probiotic effect on bee colonies in the field. Observations were made on the sanitary conditions of the hives and the behaviour of bees at the Petrodolyna demo apiary. No differences were found at the macro hive-bee colony ecosystem level between control bee colonies (n = 5) and the experimental ones (n = 5) that had received carbohydrate feeding with added probiotics. This confirms the inhibitory effect of honey on the development of bacteria, which eliminates the risk of uncontrolled growth of *B. subtilis* and *B. licheniformis* strain colonies inside the hive and the bacteria getting into bee products. The probiotic had positive effects, increasing the live weight of worker bees by 9.15% by the end of the apiary season compared to the control. This can improve the viability of the bees during wintering. At the last stage of the research, the honey obtained from the experimental colonies was checked for the spores of *B. subtilis* and *B. licheniformis* using melissopalynology.

Keywords: bee family, microbiota, safety, *Bacillus subtilis, Bacillus licheniformis*

INTRODUCTION

Honey production in Ukraine is export-oriented. More than 70% of the honey produced is exported. Up to 86% of it is delivered to the markets of European countries. Safety, quality, and honey botanical identification research methods are a hot topic in the food industry **[1]**. Ukrainian market operators compete fiercely with other producers with proven safety, naturalness, and product quality. The safety of honey and its value for the buyer are the biggest concerns. Considering the international requirements, the need for alternatives to antibiotics in beekeeping is also increasingly pressing **[2]**. Most of the research is concerned with the use of probiotics. There are positive results for using *B. subtilis* in shrimp and fish aquaculture production systems **[3]**. This bacterium is used in various living organism interaction mechanisms, such as synergistic, antagonistic, competitive exclusion, and immune-stimulating effect systems. Various applications of this bacterium include its use as a probiotic, bioremediating agent, bioflocing agent, and, potentially, live vaccine vehicle in aquaculture **[4]**.

It is known that some strains of *Bacillus*, particularly the *B. subtilis* strain DSM32324 or the *B. subtilis* strain DSM32325, are used as direct-fed microbial (DFM), premix, animal feed additive, and animal feed **[5]**.

Other researchers **[6]** have shown that *Bacillus licheniformis* can reduce the incidence of diarrhoea and modify cecal microbiota composition in weaning piglets. This suggests that *Bacillus licheniformis*-fermented feed additive has good potential as a suitable alternative to antibiotics in the swine industry. Scientists have developed a symbiotic bacterium for bee intestines, *Snodgrassella alvi*, to induce eukaryotic RNA interference (RNAi) immune responses **[7]**. They have shown that engineered *S. alvi* can kill parasitic *Varroa* mites by triggering the

mite RNAi response. While the normal flora composition of the main productive animals has been studied extensively, the microflora of hives is currently insufficiently understood. At the same time, a shift of the microflora toward pathogenic or conditionally pathogenic agents with virulence against macroorganisms leads to a significant increase in the bee incidence rate **[8]**.

In recent years, the use of probiotic supplements has also become widespread in beekeeping. It was found that the use of a *Lactobacilli*-containing hive supplement may reduce enzootic-pathogen-related hive losses **[9]**. Samples of honey stomachs, honey, bee bread, bee pollen, and royal jelly from different species of honey bees (*Apis ceranaindica* Fabricius*, Apis melliefra* Linnaeus*, Apis florea* Fabricius*, Apis dorsata* Fabricius*, Tetragonula iridipennis* Smith) were examined for the presence of probiotic lactic acid bacteria **[10]**. The results confirmed that *Enterococcus*, *Micrococcus*, *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Lactococcus*, and *Leuconostoc* typically live in the bee habitat and beekeeping supplies. However, considering the complexity of the individual bee body's biological functions and the whole bee colony as an integrated biological and technological production unit, the use of probiotics as a preventive measure is still little understood.

There are recorded cases of colonies of *Bacillus* strains growing excessively under certain cultivation conditions **[11]**. Given the great variety of modern probiotics and the microorganisms in them, more research is needed on the impact of different species and strains on the quality of the final product, honey **[12]**. New methods for isolating bacteria from honey are also being investigated. For example, it was found that HiCrome *Bacillus* agar combined with simple microbiological tests was beneficial for rapid and reliable identification of most *Bacillus*, *Brevibacillus*, *Lysinibacillus*, and *Paenibacillus* species commonly found in honey samples, facilitating their isolation from polymicrobial honey **[13]**. In the probiotic supplement formula, *B. subtilis* is mixed with *B. licheniformis*. Due to their ability to form spores, these bacteria are resistant to acids, alkalis, sudden temperature changes, and some antibiotics. Numerous studies have shown that they are harmless to animals, even in high concentrations; they have antagonistic activity against a wide range of pathogenic and conditionally pathogenic microorganisms; they have high enzymatic activity, which can have significant regulatory and stimulating effects on digestion; they can carry out antiallergenic and antitoxic actions; they are technological in production, and stable during storage **[14]**. We were interested in the product called Immunobacterin-D, which contains, per 1 kg, bacteria of the species *B. subtilis* and *B. licheniformis* at not less than 6×10^{12} CFU/kg (6×10^{9} CFU/g); xylanase (300.000 U/kg); protease (5000 U/kg); amylase (1000 U/kg); and a filler. This product is widely studied and used in Ukrainian animal husbandry. It was found that in calves, the components of Immunobacterin-D accelerate the population of microflora and the development of ruminal digestion by a factor of 2.5 compared with calves in the control group **[15]**. The use of Immunobacterin-D in cows increases their milk yield by 0.7 – 2.5 L per day **[16]**.

Our research aimed to study the interaction and inhibitory effect of honey on the growth of microorganisms from the probiotic product Immunobacterin-D under both laboratory and experimental field conditions.

Scientific Hypothesis

The main hypothesis of this research is that the presence of aerobic transient spore-forming probiotic bacteria would improve various stages of honey production from the bees *A. mellifera*. There is a possibility of excessive growth of *B. subtilis* inside hives during the vegetation season. This probiotic product is so far untested for use in beekeeping, and it is possible that unanticipated action of the probiotic bacteria would compromise the quality of the honey or interfere with the functioning of the bee colonies. Also, if the bacteria are found in excess in the honey, its naturalness could be compromised.

MATERIAL AND METHODOLOGY

Samples

Sampling of honey was performed according to DSTU 4497 : 2005 **[17]** and GOST 20264.4-89 **[18]**.

Honey samples for group 1 (n = 10) for the research on inhibitory effects were obtained from beekeepers in Odesa Oblast, Ukraine.

Honey samples for group 2 (n = 10) were taken at the end of the beekeeping season from experimental and control colonies on the Petrodolyna demo apiary (Odesa Oblast), where the field experiments were performed to create the average samples for *B. subtilis* and *B. licheniformis* residue testing.

Immunobacterin-D.

Chemicals

The following chemicals were used: sodium chloride (NaCl), c.p.; nutrient agar (composition: enzymatic peptone, meat extract, sodium chloride, agar), brand HMH-Agar Dry Culture Medium; standard set for melissopalynology.

All chemicals were obtained from Khimtest Ukraine TOV, Ukraine.

Animals and Biological Material

The water-soluble probiotic product Immunobacterin-D (dry) was used for the researche. It contains the bacteria *Bacillus subtilis* AX20 and *Bacillus licheniformis* EA22 at a concentration of 5×10^{12} CFU/g, as well as a water-soluble filler (xylanase, amylase, and protease enzymes and nutrient-rich substances) to stimulate the rapid growth of vegetative forms of the bacteria after metabolism restoration.

Immunobacterin-D was manufactured in accordance with TSU 24.4-32430604-001: 2009 (manufacturer KronosAhro TOV, Ukraine). The bacterial strains of *Bacillus subtilis* AX20 and *Bacillus licheniformis* EA22 were initially deposited in the Depository of Institute of Microbiology and Virology of Ukraine with a conclusive confirmation of non-pathogenicity.

10 bee colonies were used (Petrodolyna educational demo apiary, Odesa Oblast, Ukraine).

Instruments

Petri dishes (diameter 60 mm, sterile) and other laboratory glassware (Standard-Lab TOV, Ukraine).

150-mL container for biological samples (plastic) (Khimlaborreaktyv TOV, Ukraine).

Water thermostat Elmi TW-2.03 (Latvia, supplier MEDTECHNIKA TOV, Ukraine).

Water-soluble filler manufacturer VNP (Ukrzoovetprompostach PRAT, Ukraine).

Sigeta Biogenic Led Trino Infinity microscope (China).

Rotator Multi Bio RS-24 (Latvia, distributor BioLabTech Ltd., Ukraine).

Laboratory centrifuge SM-3M.01 (Torhivelnyi Dim Mikromed TOV, Ukraine).

KERN ABJ 220-4NM (220 g/0.1 mg) analytical balance (Kern & Sohn, Germany).

Qc PASS (100 g/0.01 g) lab balance (Biomed LTD TOV, Ukraine).

Laboratory Methods

Method for determining the quantity of aerobic microorganisms present

The research was conducted in a certified veterinary laboratory (Ukrmetrteststandard SE № PT-446/19 certificate of measurement capabilities). We measured the amount of *Bacillus subtilis* bacteria in 1 g of culture medium with an added honey sample. Honey and probiotic sampling were performed in accordance with the requirements of GOST 20264.4-89 **[18]**. The number of viable cells the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) was determined by the test method GOST 10444.15-94 **[19]**.

The method consists of diluting 1.0 g of Immunobacterin-D aseptically in 100 cm^3 of sterile 0.85% sodium chloride solution, followed by suspension. Two series of ten-fold dilutions were prepared from this dilution so that the predicted number of microorganisms in 1.0 g of product could be determined.

The culture media were prepared according to the manufacturer's instructions and aseptically poured into Petri dishes. 1 g of each honey test sample was added to a culture medium. The ten-fold dilutions were then applied on the surface of the solidified nutrient agar, and the Petri dishes were incubated in a water thermostat aerobically for 24 h at +37 ±1 °C.

During the growth phase, bacteria formed individual characteristic colonies, counted selectively. Colony-forming units (CFU) were calculated for the Petri dishes, each held 20 to 300 colonies, considering their morphology. 10 Petri dishes with colonies were taken for counting, based on the number of honey samples. The procedure was repeated two times.

Botanical origin and bacterial residue in the honey after probiotic feeding

Botanical origin was determined according to the adapted harmonized methods of melissopalynology **[1]** and **[21]** using a Sigeta Biogenic Led Trino Infinity microscope (China) with 400× and 2000× magnification, based in the laboratories of the Department of Certification and Standardization of Agricultural Products, NULES of Ukraine, following DSTU 4497:2005 **[17]**.

The method for measuring the bacterial residue in the honey after feeding with Immunobacterin-D involved looking for the presence of an excessive amount of endospores that could generate *B. licheniformis* and *B. subtilis* in a high-sugar medium (i. e., in honey). Melissopalynological analysis shows that Ukrainian honey usually contains no more than 0.3% fungal spores, 0.1% yeast, and 0.3% of other spores, microalgae, and other biological inclusions of natural origin **[1]**. During the melisopalynological analysis, we assumed that bacterial spores, depending on their species, can be up to 2 μm long **[22]**.

Description of the Experiment

Sample preparation: Preparation of honey sample was performed according to DSTU 4497 2005 **[17]** and GOST 20264.4-89 **[18]**.

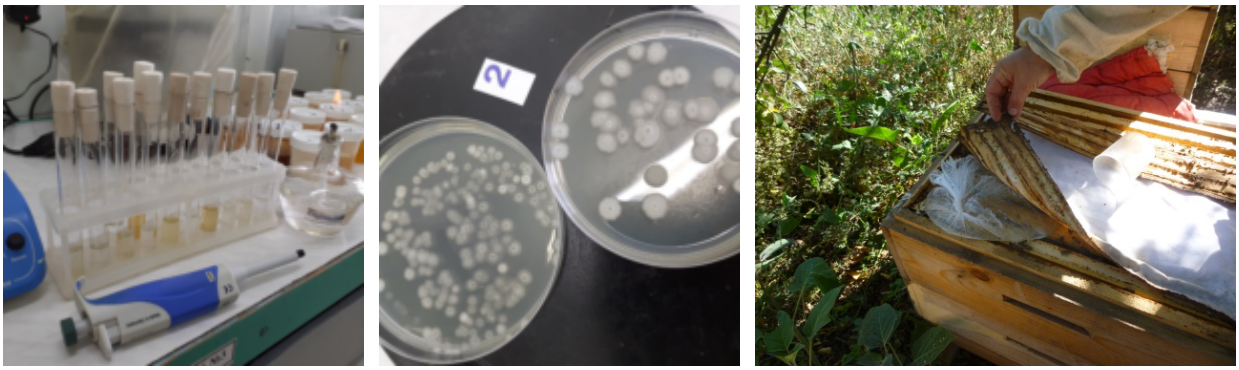
Number of samples analyzed: 20

Number of repeated analyses: 2

Number of experiment replication: 2

Design of the experiment: At the first stage of the research, we assessed the effect of ten different varieties of honey on *B. subtilis* and *B. licheniformis* from the dry probiotic Immunobacterin-D. The second stage of the study

assessed the probiotic effect on bee colonies in the field. Observations were made of the sanitary condition of hives and the behavior of bees at the Petrodolyna demo apiary (Odesa Oblast). At the third stage of the research, the honey obtained from the experimental colonies was checked for the presence of spores of *B. subtilis* and *B. licheniformis* using melissopalynology (Figure 1).



a b c

Figure 1 Selected stages of the research. Note: a – sample preparation; b – growth of *B. subtilis* on the culture medium with honey after 24 hours of cultivation; c – feeding sugar syrup with added probiotic.

Bee feeding arrangement with probiotic

Ten bee colonies were used (5 experimental and 5 control). One feeding was done daily for 5 days, starting on August 22, 2020. Twenty-five doses with Immunobacterin-D were used.

The following feeding procedure was used. Before mixing, the dry Immunobacterin-D was weighed on a KERN ABJ 220-4NM analytical balance. 25 5-g doses were weighed out and aseptically packed. Each dose was diluted in a clean vessel with 5 mL of filtered drinking water (t +20 ±1 °C. The mixture was mixed with syrup (250 mL) and added to a bee feeder. The syrup residue was measured in 12 h (the following day).

Field research procedure

The field (second) research stage was conducted over the summer of 2020 on the Petrodolyna educational demo apiary (Odesa Oblast). The weather was arid, and fodder was low due to poor flowering and insufficient nectar secretion from plants. Five control and five experimental colonies were identified, in which bee colonies were given carbohydrate feed (sugar syrup diluted 1:1 with purified drinking water). 250 mL of feed was given per colony for 5 days. For the experimental colonies, pre-diluted Immunobacterin-D was added to the syrup before feeding, 5 g for each colony. In both the control and experimental colonies, the bees consumed all the feed, as nature had insufficient feed resources within range of a productive summer of bees (r = 2.5 km).

Visual observations

From the beginning of the experiment (August 22, 2020) until October 1, 2020, the experimental and control colonies' condition was observed to determine possible residual effects. The sanitary conditions of the hive were recorded according to the generally accepted rules in terms of the inner surface of the walls and honeycombs, the appearance of the bees, their behavior, flight activity, and aggression/peacefulness during the examination.

Live weight of worker bees

The worker bees were weighed before the first feeding (on August 22, 2020) and again in 21 days, on September 14, 2020. The weighing was carried out at the Petrodolyna demo apiary (Odesa Oblast), in warm, dry weather without wind, between 12:00 noon and 13:30. Two samples of 10 worker bees from each colony were weighed. The sample of worker bees was taken from the middle bee space of the nest. The bees were placed into a container for biological samples, in which ventilation holes had previously been made. The bees did not show aggressive behavior or other atypical reactions during weighing.

Statistical Analysis

Fundamental statistical analysis was carried out with the help of the software package Statistica-6.1 (Sentinel System 7.5.7, V6.1). Student's t-test determined the probability of similarity between the group averages.

RESULTS AND DISCUSSION

After melissopalynological analysis, the honey samples were divided into monofloral (buckwheat and sunflower) and polyfloral (meadow flowers, forest plants, a mixture of linden and clover, and a mixture of linden and maple). During the counting of *B. subtilis* colonies, different numbers of viable cells were found in samples grown in culture media with different varieties of honey (Table 1).

Table 1 The growth of *B. subtilis* colonies on culture media containing honey of different botanical origins.

Monofloral honey (n = 5)		Polyfloral honey (n = 5)	
Botanical origin	**Results**	**Botanical origin**	**Results**
Buckweat[a]	2.7×10^4	Meadow plants[a]	2.0×10^5
Sunflower[a]	6.5×10^4	Meadow plants[b]	6.6×10^4
Buckweat[b]	5.7×10^3	Forest plants	1.5×10^5
Sunflower[b]	2.8×10^4	Linden+melilot	3.1×10^5
Sunflower[c]	3.6×10^4	Linden+maple	3.4×10^5
$\bar{x} \pm s_{\bar{x}}$, CFU.g	$3.234 \pm 0.958 \times 10^4$**	$\bar{x} \pm s_{\bar{x}}$, CFU/g	$2.132 \pm 5.064 \times 10^5$
σ	2.14	σ	11.32
Cv, %	66.3	*Cv*, %	53.1

Note: a, b, c – honey samples belonging to the same botanical variety but having different geographical origins within Odesa Oblast, Ukraine; ** $p < 0.01$ – probable difference from polyfloral honey; $\bar{x}$ – arithmetic mean; $s_{\bar{x}}$ – arithmetic mean error; σ – standard deviation; *Cv* – coefficient of variation.

The average results from our research indicate that monofloral honey has a high inhibitory effect on bacterial growth. The strongest bactericidal properties were observed in buckwheat honey obtained from the Shyriaieve Raion of Odesa Oblast. Other authors **[23]** found that Ziziphus honey had bactericidal effects against *B. cereus* ATCC 10876 and other gram-positive and gram-negative bacteria. In addition, some authors have reported contamination of honey by spore-forming anaerobes, including *B. cereus* and *C. botulinum* **[24]**. However, the research on bactericidal and bacteriostatic properties of honey has focused on pathogenic microorganisms, mainly *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* **[25]**. Ukrainian honey has also been analyzed for the pathogenic microorganisms *Staphylococcus aureus* CCM 4223, *Listeria monocytogenes* ATCC 7644, *Salmonella enterica serovar Typhimurium* CCM 3807, and *Escherichia coli* ATCC 25922 29 **[26]**.

Our results (Table 1) suggest that introducing honey of any type into the culture medium has an inhibitory effect on the development of the bacteria we studied. Immunobacterin-D contained an initial concentration of 5×10^{12} CFU/g *B. subtilis*; after daily contact with media with honey, their average concentration decreased to 3.2×10^4 in monofloral honey and 2.1×10^5 in polyfloral honey. Monofloral honey had significantly higher inhibitory activity compared to polyfloral one ($td = 3.509$; $p < 0.01$). Our results confirm results from the previous studies on the bactericidal properties of honey **[27]**, **[28]**, **[29]**. There are also significant variations in the extent of the inhibitory effect of honey, which correspond to different botanical and regional origins.

The next research stage was to test bees' reaction to consuming the Immunobacterin-D preparation by observing the bee colonies. The results are shown in Table 2. No difference was observed between the behavior and condition of the control and experimental bee colonies. As of February 1, 2021, all colonies were normally wintering (the winter dormancy period for bees in Ukraine lasts from mid-November till the end of February).

During the examination of the hives, their sanitary condition was found satisfactory. There were no microorganism colonies on the hive walls, honeycombs, or brood frames. There were no visible symptoms of disease or parasitism. There were no observable differences in propolis production between bee colonies, except for normal individual fluctuation.

Similar positive results had been obtained by researchers who studied the effect of feeding *B. licheniformis*, *B. subtilis*, and other bacteria on the survival of bees during their infection with *P. larvae* spores (American foulbrood) **[29]**. It has also been shown that after bees infected with *Nosema* spp. were fed the commercial probiotic EM® Probiotic for Bees, the number of spores in the colonies decreased significantly, and the bees' strength increased **[30]**.

Immunobacterin-D normalized the digestive functions of the experimental bee colonies. An analysis of the weight of the worker bees shows that there is no significant difference between the average live weight values of individuals from the experimental and control groups. But there is a positive trend of the average live weight of bees remaining constant in the experimental group, which received Immunobacterin-D, in comparison with the control group (Table 3).

It should be noted that these results were obtained in the summer of 2020 under unfavorable environmental conditions (Odesa Oblast, Ukraine). The conditions included insufficient feed resources for bees; an extremely dry summer (June–August) during the bee-keeping season; and low nectar secretion by honey plants due to a lack of precipitation and low humidity. But even with chronic nutritional stress, the bees retained their body mass, indicating better preparation for winter.

Table 2 Hive sanitary conditions, signs of disease, and behavior of bees after the use of carbohydrate feed with the addition of Immunobacterin-D.

Indicator*	Bee colony groups	
	Experimental (n = 5)	Control (n = 5)
Behavior of bees during honey flow and examinations	no change	no change
Increase in the aggressiveness of bees	n/a	n/a
Increase in dead bees	none	none
Consumption of the daily portion of feed	full	full
Condition of the inner surfaces of the beehive walls	no change	no change
Signs of disordered digestive systems in bee colonies	none	none
Signs of bee brood disease	none	none
Growth of microorganism colonies on the beehive walls, honeycombs	none	none
Change in bee propolis activity	no change	no change
Varroa mite infestation	no change	no change

Note: * – observations of changes in the condition of experimental and control bee colonies were conducted from August 22 till October 1, 2020.

Table 3 The weighing results of worker bees before and after feeding with Immunobacterin-D.

Weighing date	Indicator	Bee colony groups	
		Experimental (n = 5)	Control (n = 5)
August 22, 2020	$\bar{x} \pm s_{\bar{x}}$, mg	114.80 ±4.97	113.80 ±5.46
	σ	11.12	12.21
	Cv, %	9.68	10.73
September 14, 2020	$\bar{x} \pm s_{\bar{x}}$, mg	115.31 ±5.38	103.50 ±3.86
	σ	11.84	8.63
	Cv, %	10.3	8.3

Note: $\bar{x}$ – arithmetic mean; $s_{\bar{x}}$ – arithmetic mean error; σ – standard deviation; *Cv* – coefficient of variation.

Similar results with experimental hives indicate that *B. subtilis subsp. subtilis* Mori2 improved the performance of bees. The micro-organisms stimulated egg-laying by the queen bee, which led to an increase in the number of bees and, consequently, the amount of honey. They also reduced the prevalence of two important bee diseases found worldwide (nosemosis and varroosis) **[31]**.

The changes in the microbiota of the bee digestive system after feeding the probiotic require further research. Similar studies have been performed with the autochthonous strain *Lactobacillus brevis* B50 Biocenol™ (CCM 8618), which had been isolated from the digestive tracts of healthy bees **[32]**. Some strains of *Lactobacillus* have been shown to have a positive effect on bee health **[9]**. Positive results were also obtained using the strain *Bacillus subtilis subsp. subtilis* Mori2 **[33]**. This strain has been shown to cause probiotic effects in bee colonies, including a constant increase in egg laying by the queen bee; high yields of honey; and a decrease in the incidence of nosema and varroosis diseases.

The results may indicate the different modes of action of the strains *Bacillus subtilis* AX20 and *Bacillus licheniformis* EA22, which are part of the probiotic Immunobacterin-D, under different interaction conditions with a functioning bee colony and with honey.

Because of reports of bacterial contamination in honey, including contamination by some species of *Bacillus* spp. (e.g., *B. cereus*) **[24]**, we conducted a microscopic study of the honey, looking for the excessive presence of *Bacillus* spp. spores. The spores could get into commercial honey due to feeding Immunobacterin-D to the bees. It is important to know whether that is the case, given that the supplement would be used in the spring or summer to produce commercial honey.

Honey samples were taken from experimental and control bee colonies and combined to make an average sample, used to compare the microscopic spectrum. The results are shown in Table 4.

The results of the melissopalynological analysis of the honey confirm that Immunobacterin-D is safe to use. No bacterial spores were detected in either sample (control or experimental). The pollen spectrum of the honey was similar in both samples, with a predominance of *Helianthus* spp., but in terms of botanical origin, it was defined as floral polyfloral.

Table 4 The microscopic analysis of honey in percentages.

Parameter	Control	Experimental
Predominant pollen	none	none
Secondary pollen (%) (16 – 49%)	35 (k 40) *Helianthus* spp. (Asteraceae) 16 (k 19) *Onopordum acanthium* (Asteraceae)	23 (k 26) *Helianthus* spp. (Asteraceae)
Minor pollen (%) (≤15%)	6 (k 7) *Bunias orientalis* (Brassicaceae) 4 (k 5) *Erigeron* spp. (Asteraceae) 4 (k 4) *Draba nemorosa* (Brassicaceae) 4 *Artemisia* spp. (Anthemideae) 3 (k 4) *Silene* spp. (Caryophyllaceae) 3 (k 3) *Cirsium arvense* (Asteraceae) 3 (k 3) *Rosa* spp. (Rosaceae) 2 (k 2) *Tripolium pannonicum* (Asteraceae) 2 (k 2) *Helichrysum* spp. (Asteraceae) 2 (k 2) *Inula britannica* (Asteraceae) 2 *Atriplex* spp. (Atripliceae) 2 (k 2) *Marrubium vulgare* (Lamiaceae) 2 *Secale cereale* (Poaceae)	14 (k 15) *Onopordum acanthium* (Asteraceae) 9 (k 10) *Bunias orientalis* (Brassicaceae) 7 (k 7) *Brassica napus* (Brassicaceae) 5 (k 5) *Erigeron* spp. (Asteraceae) 4 (k 4) *Barbarea vulgaris* (Brassicaceae) 4 (k 4) *Helichrysum* spp. (Asteraceae) 3 (k 3) *Cirsium arvense* (Asteraceae) 3 (k 3) *Draba nemorosa* (Brassicaceae) 3 (k 3) Fabaceae 2 *Artemisia* spp. (Anthemideae) 2 (k 2) *Inula britannica* (Asteraceae) 2 *Atriplex* spp. (Atripliceae) 2 *Vinca major* (Apocynaceae) 2 (k 2) *Tripolium pannonicum* (Asteraceae) 2 (k 2) *Urtica* spp. (Urticaceae) 2 (k 2) *Carduus* spp. (Asteraceae)
Trace pollen (≤1%)	*Urtica* spp. (Urticaceae); *Origanum* spp. (Lamiaceae); *Vicia cracca* (Fabaceae); *Senecio vulgaris* (Asteraceae); *Cichorium intybus* (Asteraceae); *Carduus* spp. (Asteraceae); *Centaurea cyanus* (Asteraceae); *Berteroa incana* (Brassicaceae); *Cruciata* spp. (Rubiaceae); *Geranium sylvaticum* (Geraniaceae); *Salvia tesquicola* (Lamiaceae); *Genista tinctoria* (Fabaceae); *Vinca major* (Apocynaceae); *Iris pseudacorus* (Iridaceae)	*Impatiens spp.* (Balsaminaceae); *Silene* spp. (Caryophyllaceae); *Marrubium vulgare* (Lamiaceae); *Centaurea cyanus* (Asteraceae); *Berteroa incana* (Brassicaceae); *Geranium sylvaticum* (Geraniaceae); *Eupatorium cannabinum* (Asteraceae); *Rosa* spp. (Rosaceae); *Senecio vulgaris* (Asteraceae); *Cichorium intybus* (Asteraceae); *Iris pseudacorus* (Iridaceae); *Taraxacum officinale* (Asteraceae); *Centaurea jacea* (Asteraceae); *Echium vulgare* (Boraginaceae)
Damaged pollen, not identified (%)	3	2
HD-Elements, fungal spores	few (0.3)	none
HD-Elements, other spores	none	none
HD-Elements, algae	few (0.3)	none
Yeast content	few (0.1)	none
Starch grains	none	none
Other solid constituents	none	none

Note: k = counts without nectarless plants; HD = honeydew in per 300 pollen; spore, algae, starch graine, yearst content and other solid constituents in per 300 pollen.

Our previous research on the botanical composition of honey **[1], [20]** showed that most Ukrainian honeys contain pollen grains of *Helianthus* spp. in various quantities. Other scientists also mention that sunflower honey is common in Ukraine **[24]**, **[34]**. This is because Ukraine currently ranks first in the world in sunflower production, with a share of 29.3% (40.57 million tons) of total world sunflower production **[35]** and **[36]**.

Furthermore, the proportion of sunflower hybrids is increasing **[37]**. The practicing beekeepers who participated in our research state that sunflowers, especially the drought-resistant varieties, have stopped secreting nectar, and produce only a large amount of pollen, contaminating the honey of other botanical origins.

HD-elements were detected in the control samples of honey from bee colonies that were not fed probiotics; this requires additional research. It may be due to the antagonistic action of spore-forming bacteria. After entering the anterior digestive system of bees (mouth, pharynx, oesophagus, honey sac), the bacteria probably entered a vegetative state. They actively reduced the number of fungal spores on the inner surface of the organs. This is probably the reason for the improved microbiological purity of the honey. However, the research results are not conclusive so the experiment will be continued.

A gamma irradiator is used to sterilise honey in contamination with *Clostridium botulinum* and *Bacillus subtilis* spores **[38]**. Short-wave ultraviolet light (UV-C) has been studied for inactivating vegetative cells of *Escherichia coli* (CECT 405) and spores of *Bacillus subtilis* (CECT 12) and *Clostridium sporogenes* (CECT 553) in honey, inoculated at 104 – 105 CFU/g **[39]**. In addition, it is known that *Bacillus subtilis* and other bacteria normally exist in the digestive systems of honey bees **[40]** and Malaysian stingless bees *(Heterotrigona itama)* **[41]**, **[42]**.

In our opinion, the microbiological purity of honey should be reconsidered, and individual strains of bacteria should be included in the list of permitted bacteria for use in natural honey as a raw material. Some microorganisms are also used for the geographical identification of honey **[43]**.

CONCLUSION

The nature of the microbiota, both in individual bees and in the whole bee colony, remains insufficiently studied. The negative impact of pathogenic or excessive microflora is a threat to the safety and quality of honey, which requires further research.

The objective of the research was to investigate the effects of *Bacillus subtilis* AX20 and *Bacillus licheniformis* EA22 strains, contained in the probiotic supplement Immunobacterin-D, on both the microlevel (honey) and the macrolevel (the sanitary conditions of the hive interior, the bees' behavior, their health, and the products obtained). We confirmed that these strains have different effects on honey and on the bee colony. Cultivation of *B. subtilis* on culture media with added honey showed that honey had an inhibitory effect on colony growth. The initial concentration of 5×10^{12} CFU/g in the probiotic preparation decreased over 24 h, with final values between 2.1×10^{5} and 3.2×10^{4} CFU/g. Immunobacterin-D had no ill effects on colony health or the sanitary conditions of the hive. On the other hand, the probiotic had significant positive effects on most of the bees despite an extremely unfavourable (dry) apiary season. Furthermore, a comprehensive analysis showed that Immunobacterin-D is safe to use during commercial honey production.

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

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Use of laboratory equipment for analysis of external quality of food maize kernel

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ABSTRACT

The purpose of this study was to investigate the influence of the parameters of the grain air-sieve cleaner in laboratory conditions on the external quality of food maize (*Zea mays* L.) kernel in terms of the design for the selection of a suitable sieve mesh for cleaning procedures. The object of the research was maize kernel, variety Pionier P0216, year of cultivation 2019. The available laboratory equipment was used in the study. To evaluate the external quality of food maize kernel, indicators were determined, which were investigated before and after cleaning. An Asus notebook computer with software from Microsoft Windows XP and Ofice 2010 was used to evaluate the measurement results. These results were achieved: an average bulk density of 846.77 kg.m^{-3} was found in the input sample of food maize kernel after harvest, admixtures before cleaning reached an average of 19.1% and impurities of 2.76%, cleanliness of kernels before cleaning averaged 76.9%, the output after cleaning expressed in terms of bulk density reached an average value of 851.15 kg.m^{-3}, admixtures after cleaning reached 0.07% and impurities 4.21%, clean kernels after cleaning reached 94.86% and damage kernels after cleaning decreased slightly by separation of fragments and chipped kernels. In conclusion, it was stated that the laboratory technique for post-harvest treatment of grain is at a high level worldwide. Currently, the issue of post-harvest processing of grain in Slovakia is addressed at an average level. Post-harvest processing and storage of grain in terms of enginery and technological and economic aspects is little researched in the Slovak Republic, so these issues are open to further research.

Keywords: maize kernel quality, laboratory equipment, cleaning, sorting, sieve maize

INTRODUCTION

Prompt and effective identification of harmful factors is important for food safety and protection to prevent foodborne illness. This view emphasises various analytical techniques used to analyse food raw materials, foods, structure, and quality **[1]**. Thus, continuous assessment of food quality and its safety is essential to deal with public health **[2]**. The issue of food safety is currently gaining in importance and is a difficult task for the food industry. The world's population is constantly expanding, which increases the demands on quantity, food quality, and safety. Foods contaminated with biological or chemical agents cause more than 200 diseases **[3]**. Food safety is crucial for public health, from the food industry to distributors, retailers, and consumers. Every year, about 48 million people become ill, about 1.28 million people are hospitalized, and about 3 thousand people die due to consuming harmful foods **[4]**. The main objective of food risk assessment is to maintain their safety concerning public health. Food laboratories exchange their conventional technologies for innovative and modern analytical methods to promote new visions for which they are challenged **[5]**, **[6]**. The nature of the predominant analytical results in food safety is to display accurate information on the relevant official guidelines as accurately as possible without compromising the characteristics of the procedures, such as accuracy, sensitivity, and repeatability **[7]**. Foods consist of nutritionally essential ingredients, including carbohydrates, proteins, fats, vitamins, water and minerals, and fiber, which are necessary for the consumer in terms of nutritional quality. But from consumer health, their health safety is important **[8]**.

It is important to prepare the conditions for maintaining quantity and quality to store grain in warehouses.

It is the vision of every cereal producer to ensure enough food for the population. Farmers need financial investment to cultivate grain. After collection and storage of grain in the warehouse reduces farmers' fears of

quality loss. Still, it can also lead to failures due to reduced quality from damaged grain and unsuitable storage conditions **[9]**. Among grain storage losses, maize kernel is estimated to be susceptible to insect damage of up to 3.5% **[10]**. This is generally due to inadequate post-harvest management practices and an imperfectly designed warehousing structure **[11]**. Grain quality is a set of indicators that qualitatively express the effective parameters of a given crop type according to the purpose of use. There is internal and external quality. Internal quality cannot be increased by post-harvest treatment, only maintained. Internal quality can be affected by improper handling, drying, and storage. It can be low-performance aeration, resulting in the so-called "sweat layer", the alternative method of cooling the products or high temperature during hot air drying. Grain handling affects their external quality, carried out on harvesting lines, transport routes, or storage and retrieval from bins **[12]**. Understanding the impact of post-harvest adjustment procedure on grain quality within farming environments should guide farmers on the choice of better intervention steps, if necessary, to decrease spoilage and post-harvest losses and ultimately contribute to food safety **[13]**. Maize agro-growing environments are changing as a result of climate change. As a result, the conditions under which maize kernel is harvested, handled, and stored continue to vary widely, affecting not only the incidence and severity of loss factors **[14]**, **[15]** but also how farmers respond to post-harvest challenges **[16]**. Traditionally, most crop losses have been associated with insect pests. However, a significant part of the total loss, often not quantified, comes from fungal contamination, rot and disease, rodent damage, mechanical injury, and other defects.

Oil damage is associated with the agricultural environment and harvesting and handling practices. For this reason, the collection and handling procedures are crucial for the safety and health of the grain that is ultimately available for household and market consumption **[17]**.

The purpose of this study is to investigate the influence of the parameters of the air-sieve cleaner in laboratory conditions on the external quality of food maize kernel in terms of the design for selecting a suitable sieve mesh for cleaning procedures.

Scientific Hypothesis

1. The correct cleaning and sorting procedure shall obtain the objective result of clean food maize kernel in post-harvest treatment.
2. The shape and dimensions have a decisive influence on the external quality of the cleaning and grading of the maize kernel.

MATERIAL AND METHODOLOGY

Samples

The subject of the research was the food maize kernel.

Animals and Biological Material

The maize kernel was a variety of Pionier P0216, the year of cultivation 2019, which were removed from the cereal combine harvester after harvest.

Instruments

Laboratory technology was used in the research: Wintersteiger LD 350 laboratory grain thresher, OS-01 grain volume measuring device, SLN-3 laboratory sieve sorter, Ohaus Adventures laboratory digital scale, Numerix laboratory grain counter, Pfeuffer HE 50 grain moisture meter, Testovent 4000 anemometer, and Sonet deviation meter.

Laboratory Methods

The influence of the air sieve cleaner parameters on the external quality grain of food corn was investigated by laboratory technique.

To evaluate the external quality of food maize grain, the following indicators were examined before and after cleaning: dimensional and mass characteristics, critical grain flotation rate in the air stream, grain moisture, impurity content, grain purity, thousand kernels weight (TKW), bulk density, and cleaning efficiency.

Bulk density was determined using an M2/1/t instrument (type OS-1) in $g.dm^{-3}$ and subsequently converted to $kg.m^{-3}$.

Thousand kernel weight was determined based on STN 46 0610 from the proportion of pure grains. Five hundred maize kernels were counted twice (two concurrent repetitions) and then weighed. The weight of the test sample is given in grams. The specified number of 500 maize kernels was determined using a certified NUMIREX grain counter.

Impurities were determined according to STN ISO 950 and STN 46 1011-34 from the test sample. A certified SORTIMAT screening device was used. The individual impurity fractions were sieved on sieves with longitudinal rounded holes 1 mm wide and 20 mm long, with circular holes 4.5 mm in diameter (damaged grain) and manually from the test sample. The weight of the individual impurity fractions and the total weight were determined.

Grain moisture was determined with a certified moisture meter Pfeuffer HE 50 repeated three times based on STN ISO 6540.

The cleaning effect is an indicator for determining the quality of cleaning and calculates based on the formula (1):

$$\eta = \frac{Cv-Cp}{100-Cp}.100\ ,[\%] \quad (1)$$

Where:

CV – cleanliness of the cleaned sample; Cp – sample cleanliness before cleaning.

Description of the Experiment

Sample preparation: Samples were taken after harvesting by grain harvester.

Number of samples analyzed: 3 x 1000 g

Number of repeated analyses: 3x

Number of experiment replication: 3x

Design of the experiment: sample processing, analysis of food maize grain before cleaning, cleaning procedure, analysis of food maize grain after cleaning, calculation of cleaning effect.

Statistical Analysis

The SAS package, version 8.2 was used for statistical evaluation of the results. The basic statistical characteristic ($\bar{x}$ – arithmetic mean and *SD* – standard deviation) was used for mathematical-statistical evaluation of measured data. The statistical comparison of the differences in the observed values between the groups depended on the normality of the data distribution. Analysis of variance (ANOVA) was used to determine normality. The base set in each statistical set compared contained 13 statistical units (n <30). The Schefft's with a *p*-value of 0.05 was used to compare the difference between the groups.

Pearson's correlation coefficient (r) was used to test the relationship between the two variables in each group. The value of the coefficient is between -1 and +1. When evaluating the results, Cohen´s **[18]** procedure was chosen: under 0.1 trivial (simple, easy), 0.1 – 0.3 weak, 0.3 – 0.5 medium, over 0.5 strong (0.7 – 0.9 is often reported as very strong and 0.9 – 1 as almost perfect). Microsoft Windows XP and Office 2010 were used for graphical representation of the results.

RESULTS AND DISCUSSION

Evaluation of dimensional and weight characteristics of food maize kernel food maize kernel

Previous studies **[19], [20]** and **[21]** show that hermetic storage technologies are effective at limiting maize damage in storage. Therefore, one might reasonably expect access to an improved storage method to influence storage decisions.

In terms of cleaning and sorting grain, the most important properties of kernels (there are large considerably, width, thickness) as their size directly affects the choice of shape and sieve mesh. Furthermore, the variability of dimensions, i.e., a considerable interval of the respective size, affects the lower yield and the increase of kernels in class 2, in class 3, respectively, and in the waste. The kernels' dimensional characteristics document the crop's dimensions and the options for choosing the shape and the sieve mesh size, which must be selected for quality cleaning and sorting. The observed crop in our case was food maize kernels. To properly investigate and ensure the input parameters of the treated material on the harvesting line, the evaluated material was sampled directly from the threshing floor. Three hundred grains, the dimensions of which were measured, were randomly selected. When determining the dimensions of the maize kernel, variation curves of the chosen distribution feature were constructed from the measured values. Subsequently, the mass characteristic and the critical speed characteristic were performed. The measured maize kernel values are shown in Figures 1, 2, 3, 4 and 5.

The maize kernel dimensions were measured to distribute their relative and cumulative abundance.

Furthermore, the dimensions of the maize kernel were the basis for the correct choice of shape and sieve mesh in the cleaner.

The dimensional variability of the food maize kernels varies. The kernel width ranged from 5.7 to 10.2 mm, with the most dimensional concentration in the range from 7 to 9 mm. The most significant number of food maize kernels in sample in terms of its length ranged from 9.8 to 14.6 mm and in terms of kernel thickness in the range of 3.5 to 6.5 mm.

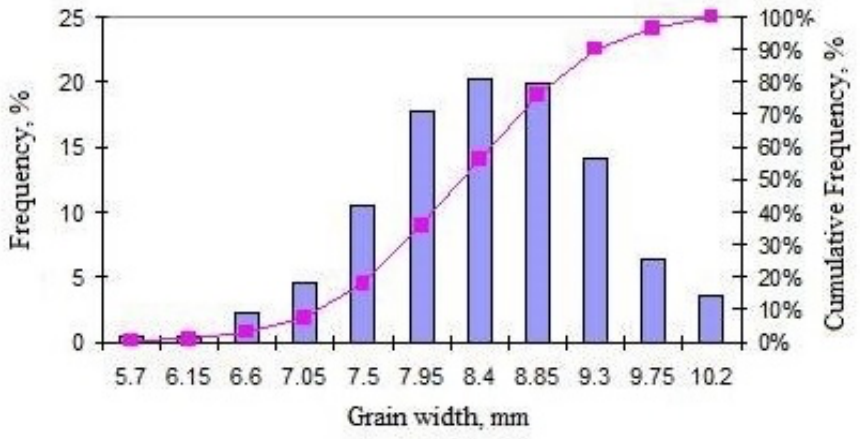

Figure 1 Dimensional characteristics of food maize kernel and cumulative abundance.

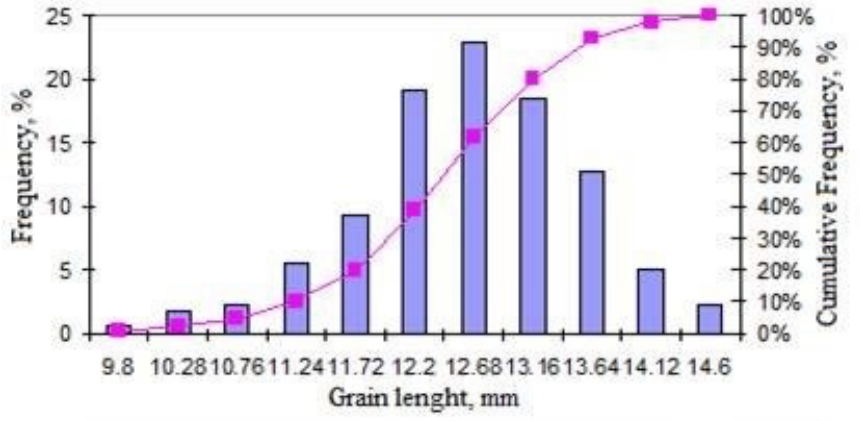

Figure 2 Dimensional characteristics of food maize kernel and cumulative abundance.

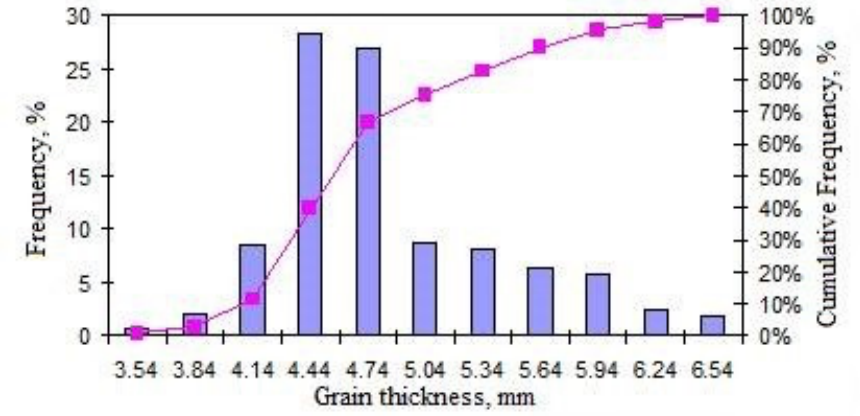

Figure 3 Dimensional characteristics of food maize kernel and cumulative abundance by thickness.

The weight characteristics of the food maize kernel and the cumulative abundance are shown in Figure 4. The weight of the food maize kernels ranged from 0.23 to 0.45 g with an average of 0.31 g. It should be noted that the maize was harvested at a moisture content of 23% to 35%. As a result, the grain weight fluctuated immediately after harvesting.

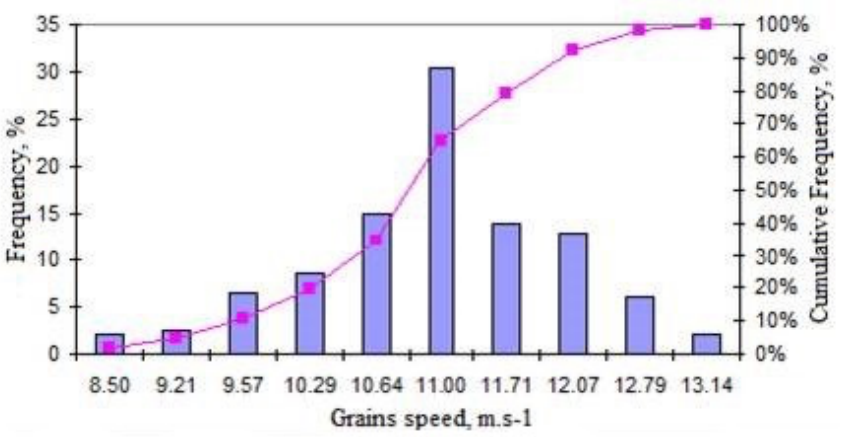

Figure 5 Characteristics of critical speed of food maize kernels and cumulative abundance.

From the point of view of kernel cleaning and sorting, the airflow rate at which the kernel floats and remains in equilibrium is important. This speed is called the lift speed or critical speed. To set the airflow speed in the cleaning machines, laboratory measurements of the given quantities were performed. The results of the measurements are shown in Figure 5. The stated speed was performed on a 4 x 100 kernels sample based on measurements in a modified laboratory grain grader K-293.

Table 1 Statistics on dimensional and weight characteristics of food maize kernels.

	Length, mm	Width, mm	Thickness, mm	Weight, g	Critical speed, $m.s^{-1}$
n	300	300	300	300	400
Mean value	2.41058	8.248598	4.725837	0.34454	10.9775
Median	12.45	8.29	4.54	0.343	11
Modus	12.01	8.21	4.51	0.351	11
SD	0.928611	0.842691	0.645089	0.05493	0.948812
Variance of selection	0.862318	0.710128	0.41614	0.003017	0.900245
Minimum	7.94	2.32	0.89		8.5
Maximum	16.43	11.84	8.7	0.669	13.5

Note: n – multiplicity, *SD* – standard deviation.

Variation distribution curves were processed for individual kernel samples. Next, a cumulative frequency distribution curve was constructed. In this way, the interval of critical speeds and the number of entrained kernels of the monitored crop were determined. Based on the measured values, the critical rate of buoyancy of the maize kernels in the airstream was determined. For maize kernel samples, a value in the range of 8.6 to 13.6 $m.s^{-1}$ was measured with an average of 10.9 $m.s^{-1}$.

Subsequently, from the variation curves of the dimensional characteristics of the maize kernel, sieves were designed for the grain cleaner SLN 3 in order to clean.

Table 2 Designed sieves for the kernel of food maize.

Crop	1. Upper sieve (straw)	2. Medium sieve (sandy)	3. Bottom sieve (sorting)	Air speed
Corn	Ø 11.0 mm/12.0 mm	Ø 45.0 mm	Ø 6.0 mm	13.5 $m.s^{-1}$

Investigation of the influence of air-sieve grain cleaner parameters on external quality grain

The SLN sample grain cleaner is intended for cleaning and sorting seeds, grain of wheat, malting barley, but also other crops such as maize, oilseeds, legumes, as well as more difficult cleanable products such as sunflower, flower and grass seeds, etc. The sieves are fully automatically cleaned using rubber balls. Exchange the sieves are a matter of minutes. The operation is quiet and without shocks (Figure 6).

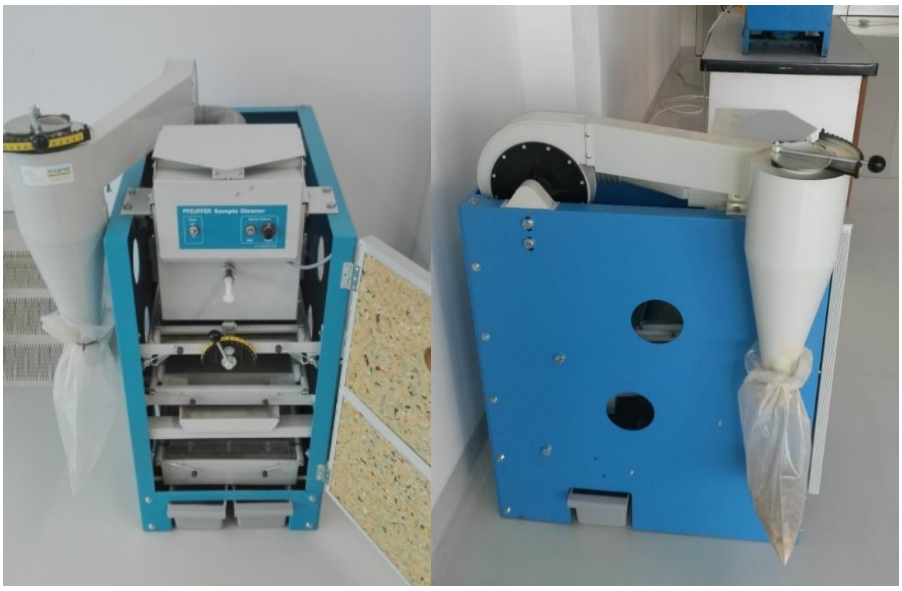

Figure 6 Laboratory air cleaner SLN 3. Note: Source.

Table 3 Technical parameters of laboratory air grain cleaner SLN 3.

Indicator	
Height with cyclone	970 mm
Device heihgt	780 mm
Width with cyclone	580 mm
Width without cyclone	420 mm
Lenght	680 mm
Weight	85 kg
Sample weight	1 kg
Voltage/input power	230 V/0.37 kW
Noise	82/78 dB/A

Evaluation of external quality of food maize kernel, variety Pionier P0216, year of cultivation 2019 in the process of cleaning

Maize intended for use in the food industry must comply with legislative requirementsc**[22]**, **[49]**. It must not contain foreign objects that would pose a problem with its security **[50]**. Maize processors must also implement the HACCP system. In terms of Hazard Analysis and Critical Control Points (HACCP) and food safety, it was necessary to investigate maize kernel's external and internal quality, Pionier variety P0216, year of cultivation, and harvest 2019. System of critical control points, risk analysis, and other quality assurance systems such as the ISO 9000 system can ensure product quality. The ISO 9000 includes standards such as ISO 9001: 2015, ISO 9000: 2015, ISO 9004: 2009, and ISO 19011: 2011 for food hygiene monitoring **[23]**. Table 4 shows the summary results of the investigated quantities of external quality of maize kernel before and after the cleaning process.

Table 4 Evaluation of external quality of food maize kernel before and after cleaning at grain cleaner SLN 3.

Indicator	**Before cleaning** $\bar{x}$ ±*SD*	**After cleaning** $\bar{x}$ ±*SD*	**Analysis of variance**	**Scheffe test**
Bulk density, kg.m^{-3}	846.77 ±32.10	851.15 ±42.71	0.09^{-}	p >0.05
TKW, g	324.47 ±45.85	311.19 ±59.62	0.41^{-}	p >0.05
Impurities, %	9.99 ±2.96	5.14 ±1.64	26.73^{+++}	p ≤0.05
Admixtures, %	0.73 ±0.54	0.36 ±0.40	3.78^{-}	p >0.05
Damaged grain, %	9.26 ±2.83	4.77 ±1.42	26.08^{+++}	p ≤0.05
Clean grain, %	90.01 ±2.96	94.86 ±1.64	26.73^{+++}	p ≤0.05
Grain moisture, %	27.97 ±3.45	28.71 ±4.15	0.24	p >0.05

Note: n = 13 before and the same after cleaning, $\bar{x}$ – mean, *SD* – standard deviation, TKW – thousand kernel weight, p >0.05 – statistically insignificant difference, p ≤0.05 – a statistically significant difference.

Identifying the physical and engineering characteristics of cereal crop grains is very important to optimize the design parameters of agricultural equipment used in their production, handling, and storage processes. So, it is essential to determine and recognize the database of physical and engineering (aerodynamic and mechanical) properties of these agricultural products because these properties play an important role in designing and developing specific machines and their operations such as sorting, separating, and cleaning **[24]**.

Kernel weight is usually represented by 1000-kernel weight. Thousand kernel weight (TKW) is not only directly related to the grain yield and milling quality of grain but also has an impact on the seed vitality and growth, indirectly affecting the yield **[25]**.

Thousand kernel weight was also found to be closely associated with kernel size traits as well, such as kernel length (KL), kernel width (KW), kernel thickness (KT), and the kernel length/width ratio (L/W) **[26]**. As a result, thousand kernel weight is frequently used in grain research as a measurement indicator **[27**].

In the statistical evaluation of the dependent variable maize kernel bulk before and after cleaning, the assumption of a normal distribution of indicator data in groups was observed. The deviations were the same. The variances in these groups were the same, not statistically significant (F 0.09^{-}, $p > 0.05$), which confirmed the result of the analysis of variance, which verified the assumption of similarity of variance. The null H0 hypothesis that there was no difference between the groups was rejected.

The maize kernel bulk density reached 846.77 kg.m^{-3} before cleaning and 851.15 kg.m^{-3} after cleaning. The maize kernel bulk density difference before and after treatment was not statistically significant ($p > 0.05$). The statistical evaluation showed that the measured maize kernel bulk density values fluctuated more in the group after treatment compared to the measured values before treatment (*SD* 42.71 vs. *SD* 32.10). The bulk density is considered to determine the capacity for cleaning and grading equipment **[24]**. The average value of bulk density was found **[28]** at 790 kg.m^{-3} for maize.

In the statistical evaluation of the dependent variable thousand kernel weight of maize grain before and after purification, the assumption of a normal distribution of indicator data in groups was observed. The deviations were the same. The variances in these groups were the same, not statistically significant (F 0.41^{-}, $p > 0.05$), which confirmed the result of the analysis of variance, which verified the assumption of similarity of variance. The null H0 hypothesis that there was no difference between the groups was rejected. Thousand kernel weights of maize grains reached a value before cleaning of 324.47.77 g and after cleaning of 311.19 g. The difference in the thousand kernel weight of maize grain before and after treatment was not statistically significant ($p > 0.05$). The statistical evaluation showed that the measured values of thousand kernel weight fluctuated more in the group after cleaning compared to the measured values before cleaning (*SD* 59.62 vs. *SD* 45.85). Grain number and weight are the main components of corn grain yield. If the corn grain yield is reduced, e.g., under the influence of stress drought, you reduce the number and weight of injuries in maturity **[29]**, **[30]**, **[31]**, **[32]**. Water stress during the vegetative growth stage of maize limits the potential yield (grain number) **[33]**, while stress in the reproductive phase mainly affects the increase in grain weight **[30]**.

In the statistical evaluation of the dependent variable maize kernel impurities before and after purification, the assumption of a normal distribution of indicator data in groups was observed. The deviations were the same. The variances in these groups were the same, statistically significant (F 26.73^{+++}, $p \leq 0.001$), which confirmed the result of the analysis of variance, which verified the assumption of similarity of variance. The null H0 hypothesis that there was no difference between the groups was rejected. Impurities in corn grains reached a pre-cleaning value of 9.99% and a post-cleaning value of 5.14%. The difference in impurities before and after treatment was statistically significant ($p \leq 0.05$). The statistical evaluation showed that the measured values of impurities fluctuated more in the group before treatment compared to the measured values after treatment (*SD* 2.96 vs. *SD* 1.64).

In the statistical evaluation of the dependent variable maize kernel admixtures before and after cleaning, the assumption of a normal distribution of indicator data in groups was observed. The deviations were the same. The variances in these groups were the same, not statistically significant (F 3.78^{-}, $p > 0.05$), which confirmed the result of the analysis of variance, which verified the assumption of similarity of variance. The null H0 hypothesis that there was no difference between the groups was rejected. Maize kernel admixtures reached a pre-cleaning value of 0.73% and a post-cleaning value of 0.36%. The difference in pre-treatment and post-treatment admixtures was not statistically significant ($p > 0.05$). The statistical evaluation showed that the measured values of admixtures fluctuated more in the group before treatment compared to the measured values after treatment (*SD* 0.54 vs. *SD* 0.40).

In the statistical evaluation of the dependent variable damaged maize kernel before and after purification, the assumption of a normal distribution of indicator data in groups was observed. The deviations were the same. The variances in these groups were the same, statistically significant (F 26.08^{+++}, $p \leq 0.001$), which confirmed the result of the analysis of variance, which verified the assumption of similarity of variance. The null H0 hypothesis that

there was no difference between the groups was rejected. Damaged maize kernel reached 9.26% before cleaning and 4.77% after cleaning. The difference in damaged grain before and after the cleaning was statistically significant ($p \leq 0.05$). The statistical evaluation showed that the measured values of damaged grain fluctuated more in the group before cleaning compared to the measured values after cleaning (*SD* 2.83 vs. *SD* 1.42). There can be significant differences in the quality of pre-stored grain with regard to insect populations, the amounts of insect-damaged grain, moldy/diseased/discolored grain, shriveled grain, and non-consumable grain **[34]**. Temperature and humidity affect the incidence and multiplication of insects **[35]**, molds **[36]**, and rodents **[37]**. Large quantities of the broken grains promote the spread of insects and microorganisms, and they are therefore not desirable on lots of grain intended for long-term storage **[34]**.

In the statistical evaluation of the dependent variable maize kernel, which should meet the quality requirements of the pure grain, before and after cleaning, the assumption of a normal distribution of indicator data in groups was observed. The variances were the same. The variances in these groups were the same, statistically significant (F 26.73^{+++}, $p \leq 0.001$), which confirmed the result of the analysis of variance, which verified the assumption of similarity of variance. The null H0 hypothesis that there was no difference between the groups was rejected. The pure maize kernel reached a value of 90.01% before cleaning and 94.86% after cleaning. The difference in maize kernel moisture before and after purification was not statistically significant ($p > 0.05$). The statistical evaluation showed that the measured values of pure maize kernel fluctuated more in the group before purification compared to the measured values after purification (*SD* 2.96 vs. *SD* 1.64).

In the statistical evaluation of the dependent variable maize kernel moisture before and after the purification, the assumption of a normal distribution of the indicator data in the groups was observed. The variances were the same. The variances in these groups were the same, not statistically significant (F 0.24^{-}, $p > 0.05$), which confirmed the result of the analysis of variance, which verified the assumption of similarity of variance. The null H0 hypothesis that there was no difference between the groups was rejected. Maize kernel moisture reached a value of 27.97% before cleaning and 28.71% after cleaning. The difference in maize kernel moisture before and after purification was not statistically significant ($p > 0.05$). The statistical evaluation showed that the measured values of maize kernel moisture fluctuated more in the group after purification compared to the measured values before purification (*SD* 4.14 vs. *SD* 3.45). The moisture content of 22 – 25% is considered ideal for efficient harvesting **[38]**, although field drying to ~18% m. c. is sometimes considered sensible to reduce drying costs. Early harvesting was done within the proper timing in the cooler agro-location but was somewhat late in, the warmer agro-location because of rapid dry-down. Maize kernels are considered physiologically mature when the m. c. is 30 – 35% **[39]**. Compared to the drier grain, the wet grain continued to lose moisture, apparently at a faster rate, eventually attaining a lower moisture level **[34]**.

The correlation between the two variables of the examined food maize grain indicators before purification is shown in Table 5 and Table 6.

Table 5 Correlation relation between the two variables of the examined food maize kernel indicators before purification.

Indicator	TKW	Impurities	Admixtures	Damaged grain	Clean grain	Grain moisture
Bulk density	-054^{-}	0.14^{-}	-0.40^{-}	0.22^{-}	-0.14^{-}	-0.40^{-}
TKW		0.06^{-}	0.60^{+}	-0.06^{-}	-0.06^{-}	0.67^{+}
Impurities			0.31^{-}	0.98^{+++}	-1.00^{+++}	0.42^{-}
Admixtures				0.13^{-}	-0.31^{-}	0.73^{++}
Damaged grain					-0.98^{+++}	0.30^{-}
Clean grain						-0.42^{-}

Note: TKW – thousand *kernel* weight, the number in each row of the column – the result of the correlation coefficient (r); +, ++ and +++ superscript for the number – statistically significant relationship between two variables $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$.

The evaluated results of the correlation between the two variables in the food maize kernel before purification statistically confirmed the existence of a strong positive or negative linear dependence or did not statistically confirm a low, medium, and strong dependence. A strong positive linear relation, statistically significant, was recorded between thousand kernel weight and admixtures ($p < 0.05$), between thousand kernel weight and grain moisture ($p \leq 0.05$), between impurities and damaged grain ($p \leq 0.001$), and between admixtures and grain moisture ($p \leq 0.05$), and a strong negative relation statistically significant between impurities and clean grain (0.001) and damaged grain and clean grain

($p \leq 0.001$). The linear relation between the other monitored indicators was not statistically significant ($p > 0.05$).

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Table 6 Correlation relation between two variables of the examined indicators of food maize kernel after purification.

Indicator TK		Impurities	Admixtures	Damaged grain	Clean grain	Grain moisture
Bulk density	-018^{-}	0.15^{-}	-0.06^{-}	-0.16^{-}	0.15^{-}	-0.01^{-}
TKW		0.33^{-}	0.48^{-}	0.25^{-}	-0.33^{-}	0.51^{-}
Impurities			0.65^{+}	0.97^{+++}	-1.00^{+++}	0.38^{-}
Admixtures				0.46^{-}	-0.65^{-}	0.09^{-}
Damaged grain					-0.97^{+++}	0.43^{-}
Clean grain						-0.39^{-}

Note: the number in each row of the column – the result of the correlation coefficient (r),
+, ++ and +++ superscript for the number – statistically significant relationship between two variables $p \leq 0.05$, $p \leq 0.01$, $\underline{p} \leq 0.001$.

The evaluated results of the correlation between the two variables in the food maize kernel after purification statistically confirmed the existence of a strong positive or negative linear or did not statistically confirm a low, medium, or strong dependence. A strong positive linear relation, statistically significant, was recorded between impurities and admixtures ($p \leq 0.05$), impurities and damaged grain ($p \leq 0.001$), and a strong negative linear relation was statistically significant between impurities and clean grain ($p \leq 0.001$) and between damaged grain and clean grain. The linear relation between the other monitored indicators was not statistically significant ($p > 0.05$).

Thus, the relations between post-harvest management practices, storability concerns, and the adoption of improved maize kernel varieties remain poorly understood. Understanding these relationships is important for future maize productivity and food security **[40]** and **[41]**.

Samples of food maize kernel before and after cleaning are shown in Figure 7 and Figure 8.

Figure 7 Maize kernels before cleaning.
Note: Source **[22]**.

Figure 8 Maize kernels after cleaning.
Note: Source **[22]**.

Cleaning efficiency

The cleaning effect (cleaning efficiency) on the investigated maize kernel cleaning operation was good.
The cleaning effect when cleaning the food maize kernel with grain cleaner SLN 3 laboratory technique was, on average, 47.50%, with a fluctuation of measured values of 10.43 (SD). Cleaning of the food maize kernel increased the purity after cleaning with SLN 3 by 4.49%.

Agricultural grain production is seasonal, while demand for agricultural commodities is more evenly distributed throughout the year **[42]**, **[43]**.

In these circumstances, it is necessary to meet the average demand by storing the surplus supply during the harvest period for gradual release to the market during the off-season. For the regular availability of grain or the stabilization of any country's economy, quality cereals must be supplied to consumers for the production of various products and marketing, as well as to farmers for sowing and growing healthy kernels **[44]**.

Understanding the impact of post-harvest operations on grain quality within the contexts of farming environments should guide farmers on the choice of better intervention steps, if necessary, to decrease spoilage and post-harvest losses and ultimately contribute to food security and safety **[45]**. These agro-environments are also becoming increasingly variable due to climate change. As a result, the conditions under which maize is harvested, handled, and stored continue to vary widely, affecting not only the incidence and severity of loss agents **[35]**, **[36]** but also the way farmers respond to post-harvest challenges **[46]**.

Walls et al. **[47]** state in their study that food safety and food quality are, therefore, key **c**omponents of food systems that have a major impact on consumer welfare. Food safety is closely linked to food-borne diseases and involves food handling. Foodborne diseases harm the health of individuals, sometimes entire families, and have a negative impact on societies and, ultimately, nations. Such diseases disrupt people's livelihoods by having a significant impact on healthcare and business networks. The World Health Organization (WHO) has identified health care, which protects people from imminent potential danger, as one of the five key areas of WHO work in the 12th General Work Program **[48]**.

CONCLUSION

The importance of post-harvest grain treatment lies in securing and maintaining the expected state of quality of grain growing. Cereal grain quality is ensured through cleaning, sorting, and drying.
Our research was focused on investigating and evaluating the cleanliness of the food maize kernel and proposing the selection of suitable sieve pans for the laboratory air-sieve cleaner.
Based on the achieved results, we can state that:
- an average bulk density of 846.77 kg.m^{-3} was found in the input sample of food maize kernel after harvest,
- admixtures before cleaning reached an average of 19.1% and impurities of 2.76%,
- cleanliness of kernels before cleaning averaged 76.9%,
- the output after cleaning expressed in terms of bulk density reached an average value of 851.15 kg.m^{-3},
- admixtures after cleaning reached 0.07% and impurities 4.21%,
- clean kernels after cleaning reached 94.86%,
- damaged kernels after cleaning decreased slightly by separating fragments and chipped kernels. From the obtained results, we found that after cleaning, the bulk density and cleanliness of the kernels were higher, but therefore the admixtures and impurities were lower. The grain-cleaning machine meets the required ISO and STN 461100-8 for food maize kernels, where the impurities together in quality class A (standard) are the most 7% and in class B (minimum) to 12%.

Laboratory technology for post-harvest treatment of grain is at a high level worldwide. Currently, the issue of post-harvest processing of grain in Slovakia is addressed at an average level. This work is recommended as a basis for evaluating the inputs of raw materials from primary production for food production. The research results in laboratory conditions can be used to compile technology in large post-harvest lines. The issue of post-harvest processing and storage of grain in terms of enginery and technological and economic aspects is little researched in the Slovak Republic, so these issues are open to further research.

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Quality changes of sous-vide cooked and blue light sterilized Argentine squid (*Illex argentinus*)

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ABSTRACT

The present work was carried out to investigate the quality changes and shelf life of blue light (Blu-ray) irradiated sous-vide cooked (SVC) Argentine squid (Illex argentinus) during storage at 0, 5, and 10 °C. Sensory evaluation, color, shear force, lipid oxidation levels, total viable counts (TVC), and psychrophilic bacterial count were used to study the changes in storage quality of SVC squid at different temperatures. Results showed that the high-quality endpoints of Blu-ray irradiated Argentine squid were 360, 144, and 72 h, and the shelf-life endpoints were 504, 240, and 120 h during storage at 0, 5, and 10 °C, respectively. The redness values of irradiated squid did not differ significantly (p >0.05) during the storage, the brightness and yellowness values of irradiated squid showed an increasing trend, and the sheer force initially increased and then decreased. The thiobarbituric acid reactive substance of each squid stored at low temperature increased with the extension of the storage period, indicating that they exhibited fat oxidation with the extension of the storage period. The TVC and the number of Psychrobacter species increased with the storage period. The correlation analysis suggested that TVC and Psychrobacter count as indicators of quality changes in Argentine squid during low-temperature storage were in good agreement with sensory scores (R^2 >0.9). Additionally, our results showed that Blu-ray sterilization played a positive role by inducing photosensitive oxidation and decreasing TVC and the total number of Psychrobacter than the control group during storage of SVC squid after Blu-ray irradiation. This study provides a theoretical basis for applying Blu-ray sterilization in aquatic product processing.

Keywords: blue light sterilization, sous-vide cooking, squid, storage period, quality changes

INTRODUCTION

The squid belongs to the family of molluscs and is a member of class Cephalopoda. At present, the main species of squid processed are Japanese common squid (*Todarodes Pacificus*), Argentinean squid (*Illex argentinus*), Peruvian squid (*Dosidious gigas*), and New Zealand squid (*Nototodaras Sloane*) **[1]**, **[2]**. Squid has high economic value; its edible part is 80%, nearly 20% higher than ordinary fish, protein content is 15 – 20%, and fat content is 1 – 2%. Additionally, it is rich in essential amino acids and contains high taurine levels, which can relieve fatigue, restore vision, and improve liver function. It is also abundant in calcium, phosphorus, iron, selenium, and other trace elements **[3]**, **[4]**. In addition, squid is rich in vitamins; every 100 g of fresh squid contains 35, 20, 60, 1600, and 600 μg of vitamin A, thiamine, riboflavin, niacin, and vitamin E1, respectively **[5]**. Therefore, squid is consumed as an essential source of protein.

For easier and better consumption of squid, it can be made into ready-to-eat squid using sous-vide cooking, which is a heat treatment method. The raw materials are placed in a vacuum bag and heated at temperature and time **[6]**. This method reduces water loss from the food during the cooking process, maintains tenderness and juiciness, preserves the color of raw materials, prevents oxidation during the heating process, hampers secondary contamination during storage, and extends the shelf-life **[5]**. Gonnella et al. **[7]** addressed that compared to traditional cooking methods (boiling, steaming, and microwaving), ready-to-eat asparagus prepared using sous-vide microwave cooking increased green color and reduced chlorophyll content, resulting in better consumer

satisfaction, and created a positive impact on the ready-to-eat vegetable industry. Renna et al. **[8]** indicated that sous-vide combined with microwave cooking could control naturally occurring thermophilic aerobic bacteria, yeasts, and moulds in vacuum-packed vegetables for up to 30 days and reduced *Escherichia coli* and *Listeria monocytogenes* levels on chicory by more than 5 LG CFU/g. Furthermore, Cui et al. **[5]** showed that SVC squid's appearance, texture, and preference were better than those prepared using traditional cooking methods (boiling and steaming), along with good quality feasibility for industries processing squid.

Blue light (Blu-ray) sterilization is a non-pharmacological technology and has been widely studied as an alternative to traditional antibiotics **[9]**. This sterilization technology uses Blu-ray for light sterilization at a wavelength of 4.05×10^{-7} – 4.70×10^{-7} m. Blu-ray receptors induce gram-positive and Gram-negative bacteria and fungi to cause physiological reactions **[10]**. Since Blu-ray sterilization does not exhibit any thermal effect and is effective in sterilization, researchers in the food field observed that after Blu-ray irradiation (4.13×10^{-7} m, <2 h, 7.20×10^{5} J/m^2), all *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella Typhimurium*, and *Mycobacterium fortuitum* presented a 5-log inactivation in milk **[11]**. Moreover, Blu-ray irradiation (4.60×10^{-7} m) inhibited Salmonella inoculated on the surface of fresh-cut pineapples without any food additives **[12]**. A previous study found that SVC squid irradiated with Blu-ray was superior to the non-Blu-ray irradiated control group in terms of sensory attributes, shear force (SF), and fat oxidation.

In this study, the sensory evaluation, color, shear force, total viable count (TVC), and the number of *Psychrobacter* were used as indicators to quantify the sensory, physicochemical, and microbiological characteristics of SVC Argentine squid to explore and analyze their quality changes and shelf-life during storage at 0, 5, and 10 ℃ using Blu-ray sterilization. Furthermore, the consistency of each indicator was investigated to provide essential information for better research on the feasibility of making ready-to-eat squid using SVC and optimizing the squid cold chain.

Scientific Hypothesis

Blu-ray is an anti-sterilization technology. This study assumed that Blu-ray exposure would affect the quality of SVC squid and play a positive role during the storage process. According to the results obtained, the SF of the SVC squid was not affected by SVC. The SVC squid obtained after Blu-ray was different from the control group in brightness and yellowness. In addition, the TVC and *Psychrobacter* count in the SVC squid after Blu-ray exposure were lower than those in the control group.

MATERIAL AND METHODOLOGY

Samples

Argentinian squid (about net weight 0.4 kg) was chosen the same size and purchased from an aquatic products market in Qingdao city, China's Shandong Province.

Chemicals

The chemical reagents are of analytical grade and purchased in Sinopharm Chemical Reagent Beijing Co., LTD, China. Ethanol, magnesium acetate solution, glucose solution, anthrone reagent, sulfuric acid solution, boric acid solution, sodium hydroxide solution, phosphate buffer solution, trichloroacetic acid, 2-thiobarbituric acid, casein solution (Sinopharm Chemical Reagent Beijing Co., LTD, China).

Animals and Biological Material

Argentinian squid (*Illex argentinus),* China's Shandong Province.

Instruments

Vacuum sealer (DZ-260, Dajiang Holding Group Electric Co., LTD, China).
Sous Vide machine (A3.2-120V, Anova Culinary, United States of American).
Spectrophotometer (7200, Unico Shanghai Instruments Co., LTD, China).
Digital light meter (Lutron-LX-101A, Lutron electronic enterprise Co., LTD, Taiwan, China).
Colorimeter (CR-400, Konica Minolta Holding Company, Japan).
Texture meter (TA.XTC, Shanghai Baosheng Industrial Development Co., LTD, China)

Laboratory Methods

According to the International Standards and Chinese National Food Standards, the study was carried out.

The sensory evaluation was determined according to ISO 11136:2014 **[13]**. The sensory evaluation group consisted of 15 trained panellists aged 18 and 25. The color, odor, body mucus, and muscle elasticity of squid were evaluated and scored, with 20 being the best quality, 12 being the high-quality period endpoint, and 4 being the endpoint quality.

The squid samples‘ colour (control and irradiated) was measured using a colorimeter. The color was described with the CIELAB color space scale: lightness (L*), green to red hue (a*), and blue to yellow hue (b*).

The thiobarbituric acid reactive substances were determined according to GB/T 35252-2017 **[14]**. For extraction, the sample (0.02 kg) was first mixed with 25 ml of aqueous 20% trichloroacetic acid (TCA) solution and 15 ml of distilled water. The mixture was homogenized and allowed to stand at room temperature (25 °C) for one h. After centrifugation at 3000 rpm for 10 min, the filtrate was diluted with distilled water to 50 ml. Next, 2 ml of the fresh filtrate was mixed with 2 ml of 0.02 M aqueous 2-thiobarbituric acid (TBA) solution, placed in a water bath in a cuvette containing a stopper at 95 °C for 30 min, and then cooled under running water. The spectrophotometer was calibrated at 5.32×10^{-7} m with distilled water, and then the sample absorbance was measured. The colorimetric absorbance obtained from the spectrophotometer was converted to mg malonaldehyde/kg meat to represent TBA content.

The determination of shear force was carried out referring to the method of Baublits et al. **[15]**. The cooked samples were divided into 0.02 × 0.02 × 0.005 m cubes, and samples were sheared along the muscle fibers vertically using a texture meter at 20 °C. The force required to shear the samples were recorded in Newton (N).

The total viable count was determined according to ISO 4833-1:2013 **[16]**. The samples were placed in 10 ml of phosphate-buffered saline (PBS) (10 mM, pH = 7.4, NaCl 8 g, KH_2PO_4 0.00024 kg, $Na_2HPO_4 \cdot 12H_2O$ 0.00363 kg, KCL 0.0002 kg, distilled water 1 L) in a centrifuge tube and sonicated for 5 min, centrifuged (5000 rpm for 5 min), the supernatant removed, and resuspended in 200 µl PBS. The bacterial solution was then diluted in 96-well plates in a gradient ($10^0 - 10^7$) and incubated in Luria-Bertani medium (pH = 7.4 − 7.6, tryptone 10, yeast extract 0.005 kg, NaCl 0.01 kg, agar .0.015 kg, distilled water 1 L) (37 °C, 7 – 9 h) and counted to calculate the TVC.

The samples were placed in 10 ml of phosphate buffer saline (10 mM, pH=7.4, NaCl 0.008 kg, KH_2PO_4 0.0024 kg, $Na_2HPO_4 \cdot 12H_2O$ 0.00363 kg, KCL 0.0002 kg, distilled water 1 L) in a centrifuge tube and sonicated for 5 min. They were then centrifuged at 5000 rpm. The supernatant after gradient dilution ($10^0 - 10^7$) was taken for 5 min and incubated (37 °C, 7 − 9 h) in Luria-Bertani medium (pH = 7.4 − 7.6, tryptone 10, yeast extract 0.005 kg, NaCl 10 g, agar 0.015 kg, distilled water 1 L) and counted. The number of *Psychrobacter* was determined after incubating the plates at 5 °C for 72 h.

Description of the Experiment

Sample preparation: The squid specimens were kept refrigerated with flake ice inside polystyrene boxes provided with a lid and holes for drainage and transported to the laboratory at -18 °C.

Number of samples analyzed: 402.

Number of repeated analyses: 5.

Number of experiment replication: 3.

Design of the experiment: Just before cooking, squid specimens separated into the head, foot (wrist), and ketone body with scissors after thawing and washing. The average weight of the ketone body of squid was 0.02 ±0.004 kg (n = 16), respectively. Each squid specimen's length, width, and thickness of the ketone body of squid were 0.04 ±0.01 m, 0.04 ±0.01 m, and 0.001 ±0.0004 m. The samples were in a plastic vacuum bag (nylon/polyethylene, 0.03 mm, 121 °C/249.8 °F) and sealed using a vacuum sealer and using SV machine heated in water baths, timed experiment time. Next, they were placed in water maintained at a temperature of 60 °C and heated for 30 min until further use **[5]**. After heating, the samples were cooled to 4 °C in cold water and placed in the refrigerator for testing. All samples were randomly divided into the control group and the Blu-ray irradiated group. All the samples were irradiated by Blu-ray at the dose of 2.16×10^5 J/m^2. Then the irradiated and control samples were sent for storage. The storage temperatures were controlled at 0 ±0.1 °C, 5 ±0.1 °C, and 10 ±0.1 °C. According to the pre-experiment results, the samples were randomly taken at appropriate intervals (adjusted based on the spoilage rate at different storage temperatures, and the frequency was increased at a later stage according to the spoilage rate) for physicochemical and microbiological tests.

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

Changes in sensory attributes of squid during storage

The time required to reach the end of the high-quality period (control group and irradiated group) was 360, 144, and 72 h. The sensory score was 12, while those taken to reach the end of shelf-life (control group and irradiated group) was 504, 240, and 120 h, and the sensory score was 4 during storage at 0, 5 and 10 °C, respectively. The quality of squid at different temperatures varied considerably (Figure 1).

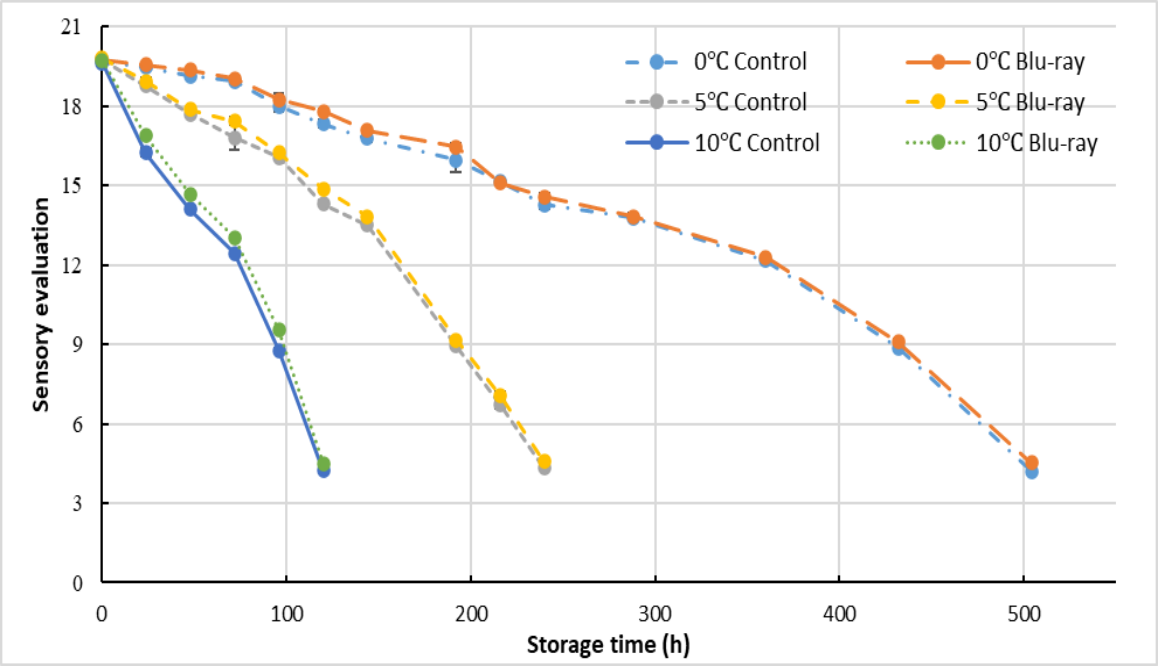

Figure 1 Sensory evaluation of squid during storage.

Other than this, there was no significant difference in sensory scores between the Blu-ray treated and control groups. However, the sensory scores of the Blu-ray group were higher than those of the control group.

Changes in the color of squid in storage

The brightness (L^*), redness (a^*), and yellowness (b^*) values of squid were measured using a LAB colorimeter as recommended by the International Commission on Illumination. The *squid's L* and b* values* at different storage temperatures showed an increasing trend. The trend of a^* was not noticeable; except for a more significant change at 10 °C, the other values were more stable. With an increase in storage time, the color of squid displayed increased brightening and yellowing (Table 1).

Table 1 The value of L^*, a^*, b^* of squid during storage.

Storage temperature	Time	L^*	a^*	b^*
0 °C Control	Initial time	75.61 ±0.24^{b}	-1.58 ±0.43^{a}	2.32 ±0.35^{a}
	The end of high-quality period	78.37 ±0.75^{a}	-2.04 ±0.57^{ab}	2.40 ±0.43^{a}
	The end of shelf period	79.69 ±0.57^{a}	-2.62 ±0.12^{b}	2.50 ±0.47^{a}
0 °C Blu-ray	Initial time	78.79 ±0.72^{b}	-1.24 ±0.09^{a}	1.32 ±0.46^{a}
	The end of high-quality period	79.39 ±0.47^{ab}	-1.69 ±0.36^{a}	2.30 ±0.34^{a}
	The end of shelf period	80.32 ±1.19^{a}	-2.42 ±0.26^{b}	2.33 ±1.28^{a}
5 °C Control	Initial time	79.55 ±1.32^{a}	-1.47 ±0.28^{a}	1.54 ±0.11^{c}
	The end of high-quality period	81.05 ±0.36^{a}	-1.61 ±0.06^{a}	4.02 ±0.42^{b}
	The end of shelf period	81.57 ±0.17^{a}	-1.62 ±0.43^{a}	6.42 ±1.08^{a}
5 °C Blu-ray	Initial time	78.03 ±0.67^{b}	-0.65 ±0.13^{a}	0.11 ±0.26^{b}
	The end of high-quality period	78.42 ±0.23^{b}	-0.74 ±0.02^{a}	3.56 ±0.16^{a}
	The end of shelf period	80.46 ±0.25^{a}	-0.78 ±0.16^{a}	3.68 ±0.20^{a}

Table 1 Continue.

Storage temperature	Time	*L**	*a**	*b**
10 °C Control	Initial time	75.49 ±0.24^{c}	-2.39 ±0.12^{b}	0.76 ±0.53^{c}
	The end of high-quality period	78.49 ±0.72^{b}	-2.11 ±0.10^{ab}	5.73 ±0.24^{b}
	The end of shelf period	80.84 ±0.46^{a}	-1.91 ±0.04^{a}	9.57 ±1.08^{a}
10 °C Blu-ray	Initial time	77.48 ±0.26^{b}	-4.10 ±0.37^{c}	1.47 ±0.08^{c}
	The end of high-quality period	77.75 ±0.17^{b}	-1.84 ±0.30^{b}	4.34 ±0.09^{b}
	The end of shelf period	83.25 ±0.53^{a}	0.35 ±0.02^{a}	5.69±0.14^{a}

Note: Results are mean ± standard deviation (n = 3), values within a column with different superscript letters are significantly different (p <0.05).

Although the skin of the squid samples was peeled, the surface was still protected by a film, and their oxidation rate was low. Therefore, the change in redness was small. Moreover, the mucus moisture produced during the storage period covered the surface layer, resulting in progressive production of specular reflection by the film and thus increasing its brightness (Figure 2).

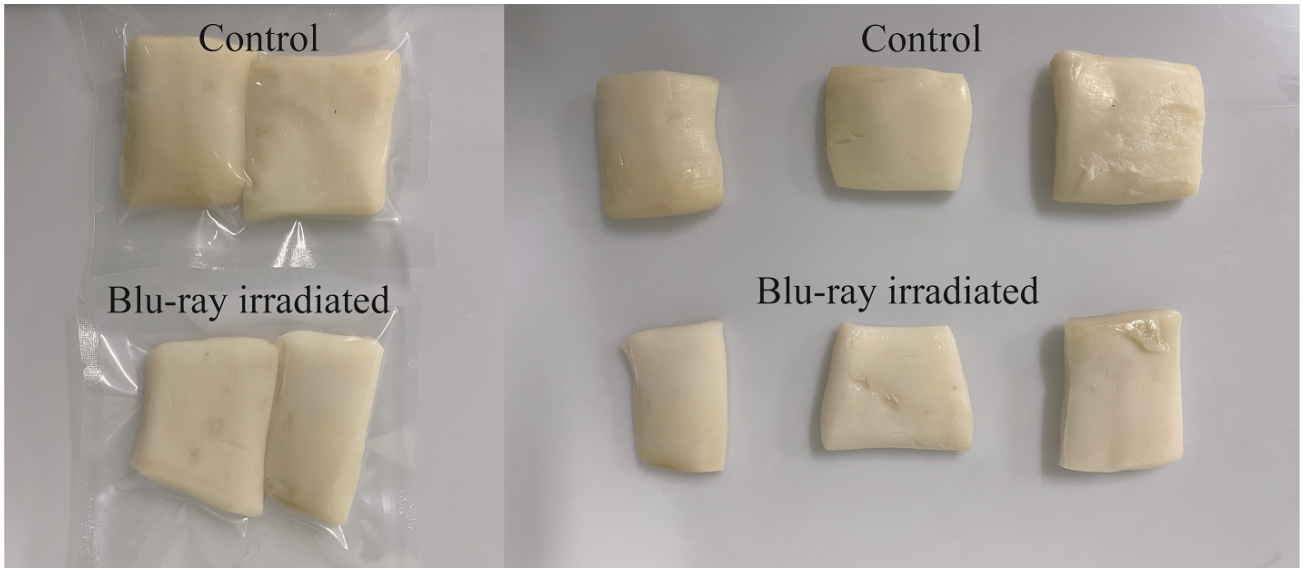

Figure 2 Appearance of squid samples.

Ramirez-Suarez et al. **[17]** found a similar gradual yellowing of color during storage (0 °C, for 15 days) of squid (*Dosidicus gigas*). Our results, to some extent, indicated that with the prolongation of time, the squid surface mucus increased, and the quality decreased, which was consistent with the sensory evaluation **[18]**, **[19]**.

Furthermore, in our study, the color of the samples treated with Blu-ray irradiation had some differences from the control group. Moreover, the samples after Blu-ray irradiation were whitish compared to the control group from the sensory observation. Thus, these changes might be caused by the loss of riboflavin in the squid samples after Blu-ray irradiation **[11]**, **[20]**. Besides, Ghate et al. **[21]** showed that the color of orange juice changed after exposure to Blu-ray (4.60×10^{-7} m). However, the samples irradiated using Blu-ray did not reduce the sensory characteristics of the squid.

SF changes in squid during storage

SF value is widely used to measure the tenderness of aquatic food products, and it reflects the internal structure of the meat; the structural properties of various proteins in the muscle determine the tenderness of the meat and average meat shear value **[22]**. The SF at all three temperatures increased and then decreased, indicating that the flesh quality of squid initially decreased due to muscle tenderness caused by stiffness and then because of storage time, wherein the squid flesh softened due to decomposition (Figure 3).

The storage of squid at 10 °C exhibited the fastest rise and fall in SF value, suggesting that storage in ice could maintain the meat quality. However, the SF at 0 °C was the highest, with a value larger than the initial SF, probably because the meat was slightly harder than fresh squid after storage at 0 °C. Squid is rich in protein and has high elasticity, and as stiffness occurs, it can increase various indicators such as elasticity, hardness, and cohesion. In this study, the temperature of heat treatment was only 60 °C. Therefore, the endogenous enzymes in the squid might not have been wholly inactivated and still functioned in the subsequent low-temperature storage. With the prolongation in storage time, the squid cell structure, muscle tissue, and protein stereo-structure were gradually destroyed by microorganisms, resulting in less SF **[17]**, **[23]**, **[24].** Our results showed that the Blu-ray irradiation treatment had little effect on the SF of the squid compared to each control group during storage.

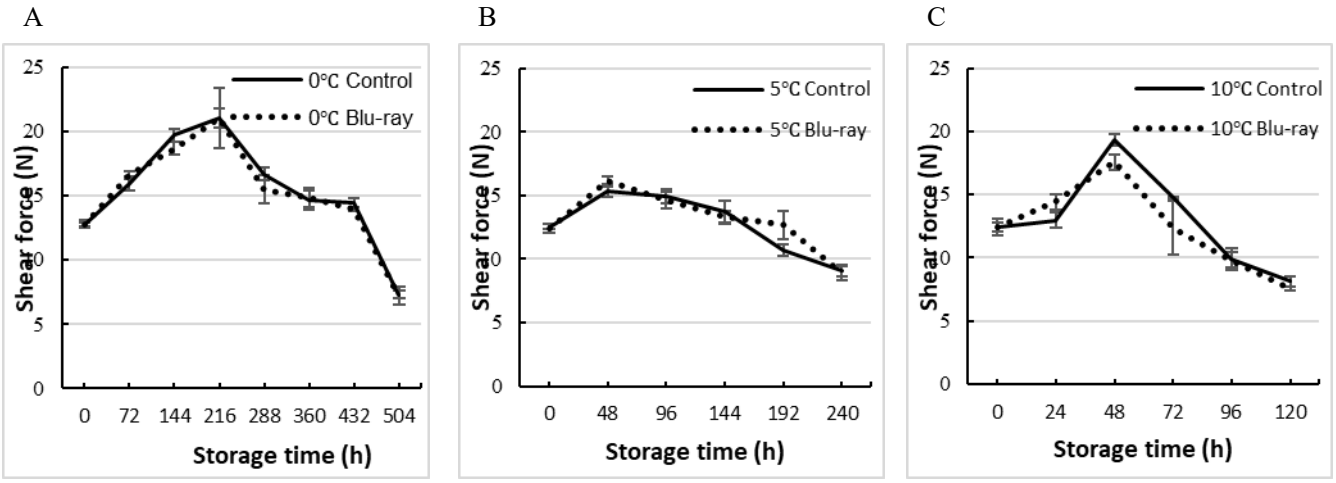

Figure 3 Shear force of squid changes during storage at (A) 0 °C, (B) 5 °C, and (C) 10 °C.

Changes in TBARS in squid storage

The lower the TBARS value, the lower the degree of fat oxidation and the better the quality of the product **[25]**, **[26]**. Lipid degradation products can cause off-flavours in fresh fish during storage **[27]**, **[28]**, **[29]**. As shown in Figure 4, the values of TBARS in squid in both the control and treatment groups showed an increasing trend as the storage time increased.

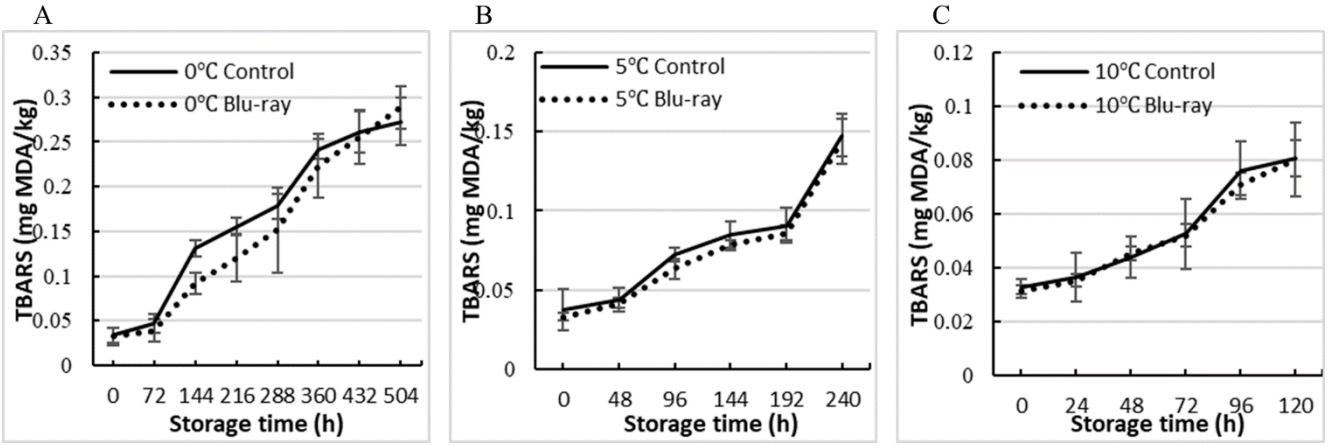

Figure 4 The TBARS changes in squid during the storage at (A) 0 °C, (B) 5 °C, and (C) 10 °C. Note: The increasing trend of TBARS of stored squid at 0, 5, and 10 °C was consistent.

Furthermore, Blu-ray treatment did not induce photosensitive oxidation in squid. It was noteworthy that TBARS increased rapidly after 144 h in control and treated groups at 0 °C. The reason for this is to be investigated in future studies. However, compared with the control group, Blu-ray irradiation reduced the fat oxidation of the squid stored at low temperature, enhanced the anti-bacterial effect, and resulted in a longer shelf-life.

Changes in TVC in squid during storage

The main factors that cause spoilage of aquatic products are microorganisms, as well as enzymatic and chemical changes, and the degree of spoilage of marine products through the growth of spoilage microorganisms **[30]**, **[31]**, **[32]**; therefore, TVC is known as a conventional indicator of aquatic product quality **[33]**, **[34]**, **[35]**. The total number of bacterial colonies in all samples showed an increasing trend with increased storage time. The growth rate of microorganisms was higher under storage at 10 °C, almost directly entering the logarithmic phase, while there were obvious delay periods during storage at 0 and 5 °C. Since microbial metabolism requires enzyme catalysis and the catalytic rate of enzymes depends on temperature, low temperature causes microbial growth to be delayed **[36]**. There was a good correlation between TVC and sensory scores in control and Blu-ray irradiated groups at 0, 5, and 10 °C. In Figure 5, TVC at the end of the high-quality period was 5.92, 6.05, and 5.61 LG CFU/g in the control group and 5.58, 5.74, and 5.50 LG CFU/g in the Blu-ray irradiated group at 0, 5, and 10 °C, respectively.

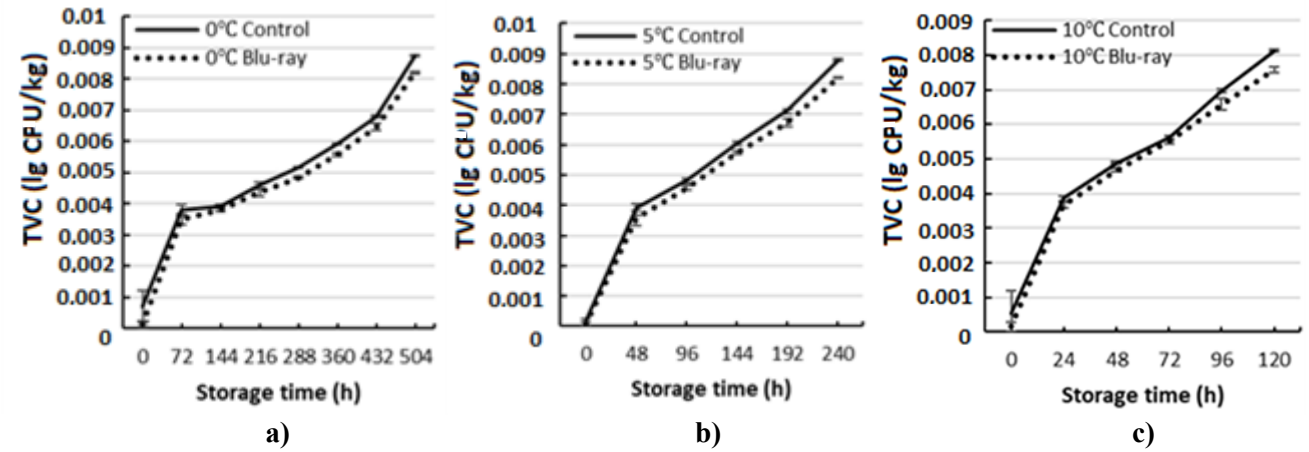

Figure 5 Changes in TVC during the storage at (a) 0 °C, (b) 5 °C, and (c) 10 °C.

At the end of shelf life, the total number of colonies was 0.00879, 0.00879, and 0.00813 LG CFU/kg in the control group and 0.00821, 0.00822, and 0.00757 LG CFU/kg in the Blu-ray irradiated group, respectively. The microbial counts reached 0.0055 LG CFU/kg or more at the end of the high-quality period in both Control and Blu-ray groups. In microbiological quality guides for ready-to-eat foods in Australia and New Zealand, the microbial limit for Class A food (after heat treatment) is proposed to be LG 0.005 CFU/kg **[37]**. Therefore, in terms of the high-quality sensory period of squid, the lower the temperature, the more likely it causes the illusion of food safety, good sensorial characteristics, or high quality; however, its total bacterial count is high, with the total number of colonies at the end of high-quality periods at 0 and 5 °C more than that at 10 °C. In addition, there was a good correlation ($R^2 > 9$) between TVC and sensory scores at 0, 5, and 10 °C (Table 2).

Table 2 Correlation between the total viable counts and sensory evaluation.

Storage temperature	Correlation between the total viable counts and sensory evaluation (R^2)
0 °C Control	0.95
0 °C Blu-ray	0.95
5 °C Control	0.93
5 °C Blu-ray	0.92
10 °C Control	0.92
10 °C Blu-ray	0.99

Changes in the number of *Psychrobacter* during storage of squid

Typical bacteria grow at 25 to 40 °C, while *Psychrobacter* generally grows best between -15 to 20 °C. The most common species of cold-loving bacteria are *Yersinia pestis*, *Listeria monocytogenes,* and *Pseudomonas spp*. Aquatic products and meat are more vulnerable to contamination of food by these species **[38]**, **[39]**, **[40]**. The squid growing in the ocean depths is more likely to become infected with this type of *Psychrobacter*. From Figure 6, we could see that the growth trend of *Psychrobacter* was similar to that of TVC, and the growth of the number

of *Psychrobacter* and TVC was the same in all temperature conditions throughout the storage process, and the correlation between the two was good ($R^2$0.9). During low-temperature storage, the growth of non-*Psychrobacter* might have been inhibited, while the *Psychrobacter* had an advantage over that of non-*Psychrobacter*. Our results suggested that at the initial time and during storage, the Blu-ray treatment group had a lower number of TVC and colonies of *Psychrobacter* than the control group, indicating that Blu-ray sterilization was effective and performed well during the whole storage period.

CONCLUSION

In conclusion, our results demonstrated the positive effect of Blu-ray treatment during the storage of SVC squid by inhibiting microbial growth and reducing fat oxidation. And the end of shelf-life (control group and irradiated group) was 504, 240, and 120 h. Although Blu-ray irradiation affected the color of the squid, especially yellowing, it did not affect the sensory characteristics of the squid. Moreover, our results showed that the quality of squid decreases as the storage time increases. However, storage at lower temperatures could extend the storage time of SVC squid. Our study notes that the evaluation of food quality cannot be done only by sensory evaluation but also requires a comprehensive evaluation concerning other physical and chemical indicators. Future research should focus on the mechanism of Blu-ray for the destruction of specific food-borne micro-organisms and the application of Blu-ray for sterilization of aquatic products.

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The authors have no conflicts of interest.

Ethical Statement:
This article does not contain any research that would require ethical statements.

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The aromachology and possibilities of its application in a selected business entity

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ABSTRACT

Aromachology studies the influence of odours on human behaviour and examines the relationship between feelings and emotions. The submitted paper deals with implementing a new marketing communication tool into practice and the possibilities of its use. Our research deals with the use of the human senses in marketing, where we take a closer look at the sense of smell because it has an important position in the human mind and life. It can awaken our memories and create emotions and improve the mood and often unconsciously influence consumer behaviour. The paper is divided into two parts. The first one aims at the theoretical introduction devoted to basic concepts such as aromachology and aroma marketing. This knowledge was subsequently applied directly to the surveyed company in the results part. The research subject is the influence of aromas on the emotional side of consumer behaviour which is influenced by all stimuli around us. Based on the research, we identified the most suitable aroma category that the company could use in the future. We have developed proposals and recommendations that could help increase awareness of a new product in the company's portfolio through unconscious communication at the point of sale, which will bring the gradual implementation of this marketing tool into practice. Two questionnaires were used – the first one aimed to gain basic information about consumers and their consumer behaviour. The second one was realized as a blind review of selected aromas using facial biometrics. Based on both surveys, we can conclude that introducing a specific aroma in the company certainly could influence the sale of a new product, a chocolate cake.

Keywords: aromachology, aroma marketing, consumer behaviour, smell, marketing tool

INTRODUCTION

The sense organs are used to get to know the external environment we live in. These organs can mediate information between the external environment and the internal organs of the human body and its nervous centre. Nowadays, our sensory organs are also becoming more and more involved in marketing.

The submitted paper takes a closer look at the olfactory sensory organ. First of all, we consider smell to be a sense that we use all the time, and it allows us to perceive different smells and odours. Of course, the smell can evoke different feelings and emotions in us. The relationship that can be created between memory and feelings can influence individuals in their shopping behaviour. When the smell is used correctly in marketing, it can also mean a competitive advantage for a given company because nowadays, shops or businesses are based on enormous competitiveness.

New directions of communicating with consumers in a way that has not been used before have become increasingly common. Here, for the first time, we encounter terms such as aroma marketing or aroma archaeology, also called smell marketing. It is marketing that influences the customer through smells. The first knowledge about the use of aroma in marketing dates back to France, where they were the first traders to use fragrances to promote the sale of their products, such as various types of tea, cosmetics or alcohol. According to **[1]**, the marketing aroma uses knowledge about odours and smells. Aroma marketing can encourage the customer to buy goods and services and thus influences his behaviour and purchasing decisions. According to **[2]**, customers who come to an environment exposed to pleasant smells can improve their mood by forty percent. Not only their mood will improve, but this effect can extend the time spent in the store by at least fifteen minutes. As for the impact of smells, they have a positive impact on customers and employees who are exposed to these smells throughout the

time they spend their work. Therefore, it is very important to think also about employees when choosing a smell so that it can positively influence their performance and, of course, their mood throughout working hours. **[3]** claims that there are about 10,000 smells that humans can recognize and remember in their unique way. Two people cannot feel the same, even if it is the same chemical with the same composition. It is also proven that more than half of what we perceive as taste passes through our olfactory senses. The benefits that aromas in marketing can bring: by effects of smells on a customer's emotional side, they can also contribute to sales growth; the smell can make the customer prefer products that are in the smelled rooms and look for them more and more often; fragrances can make a brand easy to remember, and this contributes to the company's reputation; the smell can support the feeling of quality, customers will get a feeling of a better product or service, and this can trigger the incentive that customers are willing to pay an even higher amount for a given product **[4]**. Even **[5]** argues that smell can be considered our strongest sense. When using a marketing strategy, a suitable smell can connect a brand with customers, especially at their emotional level, leading to the situation that customers even like the brand or product. The smell is also a good tool to attract new customers, support increased sales, as well as to expand brand knowledge. It can cause consumer satisfaction or evoke the memories associated with the smell. It can even establish a long-term connection between the brand and the customer. The aroma can directly affect our limbic system, which controls the memory segments in the brain as well as feelings. **[6]** claims that almost three quarters of the feelings and moods that we have during the day can be influenced by smells. Smell can influence consumer behaviour because it is tied to the brain's emotional centre. It can cause drooling in a person, change the heart rate, evoke attraction or perhaps even resistance to the person, or direct his thoughts to pleasant or even unpleasant moments in his life. Smells and odours are all around us, whether we are at home, on a visit, in a store, a store or a business, and have been shown to impact consumer behaviour at the time **[7]** significantly.

[8] states that more than 70% of decisions are made directly at the point of sale. An important aspect that influences this decision is air quality. When it comes to shopping, the environment has a significant impact on consumer behaviour, as consumers will not spend time somewhere where there is exhaled air or inappropriate temperatures. According to **[9]**, marketers are increasingly using the surrounding smell as a tool to differentiate themselves from their competitors and thus attract more and more new customers and influence their moods and create pleasant experiences from shopping. The excellent aroma can cause a lot of effects concerning customers: the time spent in a store can be extended by a pleasant smell by 10 minutes; products or services can be perceived as better quality; the store is etched in their memory as a place where they felt good, evoked good memories in them and they will likely return there and repeat their purchase **[10]**.

According to **[11]**, the aromas themselves and their use can be divided into several categories:

- an *aroma that can influence the choice of customers* – a positive aroma can very quickly affect the activity of the brain. It is mainly the part of the brain responsible for the external environment, and so gradually begins to change the person's attitude. This can cause a fragrance that has a pleasant effect can attract more and more customers who want to stay in the store longer and thus spend more money;
- an *aroma that affects customers' emotions* – the smell is one of the most emotional senses. A pleasant smell will immediately improve the mood, but must also be paid attention to the other side of the smell because it can have the opposite effect on emotions. An important factor here is the intensity of the smell. A positive smell affects the brain by focusing only on the positive things and omitting the negative ones altogether. An ordinary clothing store, by using a positive smell, can turn into a stylish store that uses better services or prices;
- an *aroma that affects customers' memory* – smells work as a glue that can combine ideas and experiences with a brand. Marketers know how to take advantage of this fact by applying the smell to a commercial or directly to an advertising leaflet or brochure that creates a given sensory experience, thus creating a positive memory of the brand, and customers can remember it more easily;
- an *aroma you can imagine* – with advertisements on television, on the Internet or directly in the catalogue, it is challenging to combine the ad with the aroma. However, research has confirmed that if we tell someone just to imagine a given aroma, the brain can evoke the situation as if it happened. When a customer suspects a given aroma of food, it can lead to greater saliva production, desire, and, consequently, to consuming a certain type of food. The customer can immediately find a service that can satisfy his ideas and needs.
- *aroma and its knowledge of use do not discourage customers from shopping* – but deep care must be taken here because the knowledge that someone subconsciously wants to influence the senses of customers for a reason to spend more money can already discourage them. Attention needs to be paid to which aroma will be used, so the customers do not close themselves and leave the store.

According to **[12]**, when using the aroma of marketing, it is good to follow these bits of advice: it is necessary to test the smell and get feedback from customers; the smell of the brand should be original and mainly associated with the company's brand; to advise on the choice of smell from experts, because an inappropriate smell can discourage customers; aroma marketing works best with a simple smell, complex smells should be avoided –

especially heavy smells, as this can cause health problems; The smell should be easy to remember for your customers. Neuromarketing is used to get feedback in aroma marketing. Customers often consider their purchasing decisions rational because they shop with "the heart". Therefore, it is important to focus on their emotions, which they use when deciding what to buy. Customers are often unaware of their emotions or do not want to reveal them for various reasons. Therefore, marketers decided to use the objective measurement of their physiological manifestations such as heart rate, pressure, dilated pupils or respiratory rate because they can identify and measure their intensity using neuromarketing **[13]**. Neuromarketing is a relatively new area of research. The term was first used in 2002 to refer to the intersection of neuroscience and marketing. Neuromarketing combines knowledge of neurology, psychology, sociology, and marketing. This concept has contributed to a significantly better understanding of human behaviour in recent years because a person can receive far more information than he can consciously process **[14]**. **[15]** states that neuromarketing combines three disciplines: traditional marketing, brain research, and medical technology. The first discipline of marketing is used mainly by the largest global companies. Brain research as a second discipline includes behavioural, primarily economics, neuroscience and psychology, gaining more and more prominence. The third discipline is medical technology, which can determine precisely what is happening in our brain. Various technologies such as EEG (electroencephalography) or fMRI (functional magnetic resonance imaging) are used. The primary use of these technologies is to make diagnoses, but these technologies can provide large amounts of data to help us determine exactly how a person's mind works. We can see exactly where the blood is flowing which parts are in charge of emotions such as anger, fear, joy, sadness, happiness or desire. Neuromarketing would not exist without these three disciplines.

Scientific Hypothesis

We established the following hypotheses, which were statistically tested:

1. To find out if there are differences in individual preferences by gender or if there are no statistically significant differences.
2. What percentage of employed respondents prefer to eat cake over other sweets.
3. Whether there exists a statistically significant difference between the frequency of cake consumption by women and by men.

The above-stated hypotheses were created hypotheses H_0 and H_1, which were verified by statistical tests. Hypothesis H_0 states that there is no difference between the indicators, hypothesis H_1 states that there is a difference between the indicators. The results of the hypotheses are given in the results of the work.

MATERIAL AND METHODOLOGY

Before implementing the second questionnaire survey, we divided the individual aromas into test tubes then marked them as sample No. 1, sample No. 2, sample No. 3 and sample No. 4. Each sample was placed in an envelope. It was necessary to make 100 pieces of samples from each aroma together we needed to create 400 pieces of samples, which we sent to 100 respondents for implementation (Figures 1 – 3). The second questionnaire was compiled based on a blind test. We wanted to verify the smell of respondents and their feelings when smelling aromas, what the aromas are associated with, and especially whether they consider them suitable when selling cakes. We used biometric testing. Before conducting the questionnaire survey, the respondents did not know which aromas were under the given sample number.

Figure 1 Preparation, portioning and storage of aroma samples in test tubes. Note: Own processing 2021.

Figure 2 Portion of sample No. 1 into test tubes. Note: Own processing 2021.

Figure 3 Placing samples in envelopes and then sending them to the respondents to carry out a questionnaire survey. Note: Own processing 2021.

Subsequently, when the respondents received an envelope with aromas, their task was to open a link sent to them by email. After its opening, a questionnaire appeared via the Samolab.online platform. The tests were performed using a special platform, Samolab.online, which allows the whole spectrum to create specialized questions (e.g., association tests, A / B testing). Respondents can perform such testing using their home computers, tablets or even mobile devices outside the laboratory (Figures 4 and 5). Visible manifestations of mimic muscles are recorded through video recording, and these are then processed using analytical tools. The survey was conducted from March 15, 2021, to April 15, 2021.

Emotional feedback was analysed using the somatic biometric method FaceReader 7 from the Dutch company Noldus, which identifies the emotional feedback (valence, excitement) of respondents with maximum accuracy based on observable changes in mimic muscles and recognizes basic micro emotions (happy, sad, angry, disgusted, surprised, neutral) (Noldus Information Technology, 2021).

The validity of the recorded data is mainly influenced by the scanning angle, the brightness of the environment and the resolution of the recording device **[16]**.

The course of the second questionnaire survey:

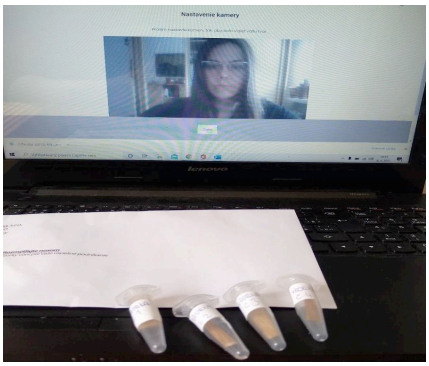

Figure 4 Opening the second questionnaire and setting up the camera. Note: Own processing 2021.

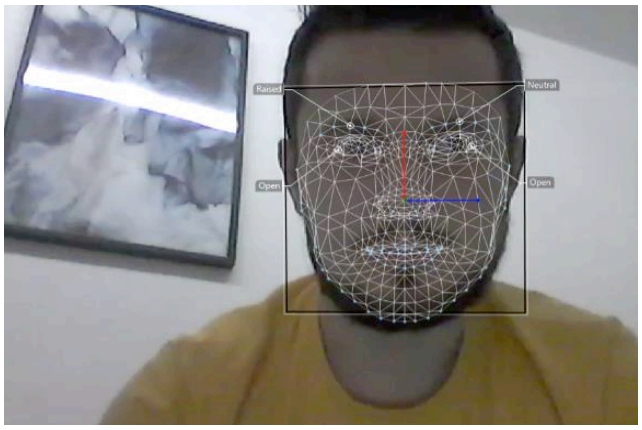

Figure 5 Analysis of the program during the implementation of the second questionnaire. Note: Own processing 2021.

Preparation and implementation of the first questionnaire survey

The first questionnaire survey aimed to obtain basic information about the preferred category of aroma during the purchase decision on the purchase of chocolate cake on primary sample respondents. It consisted of three parts: the questionnaire's introductory information and the inquirer, the classification questions and the research questions. The questionnaire was disseminated mainly through the social network Facebook, sharing in various Facebook groups and sending it to potential respondents directly in personal messages and via e-mails and the social network Instagram. The partial objectives of the first questionnaire were as follows:

1. To find out whether the respondent prefers cakes over other sweets in individual age categories and in what periodicity of consumption.
2. To find out which place respondent prefers when eating cake.
3. To find out what smell first comes to the respondent´s mind when looking at the picture of the chocolate cake.
4. To find out what smell the respondents would prefer when entering the store selling cakes, and whether this smell would influence them when buying a cake or not at all.
5. To find out where, according to the respondent, the given smell should be placed so that the customer can feel it as best as possible.
6. To find out if there are differences in individual preferences by gender or if there are no statistically significant differences.

Together 153 respondents attended the first questionnaire survey. The first questionnaire survey resulted in 4 samples of smells that respondents most associated with the smell of cake: the smell of chocolate, coffee, vanilla, and citrus or fruit- and exactly these aromas were used in our second questionnaire survey.

Preparation and implementation of the second questionnaire survey

The second questionnaire survey was conducted three weeks after the first one. This survey consisted of monitoring the facial biometrics of the respondents. The software was borrowed from Samo Europe Ltd. The second questionnaire survey was used to verify the unconscious influence of aromas on human emotions. The implementation took place through the platform Samolab.online.

We cooperated with the company Aroma marketing, which offered us a choice of aromas for the marketing research implementation. Based on our requirements, the company Aroma marketing sent us 4 aromas that we chose based on the results of our first survey: Vanilla Orange - a balanced aroma of vanilla and citrus; Coffee and Cake - a pleasant aroma of coffee with a hint of sweet cake taste; Wildberry - the sensual and fresh smell of forest fruits; Chocolate - classic chocolate smell.

A total of 100 respondents took part in the second questionnaire survey, but we evaluated the data only for 51 respondents. The questionnaire itself consisted of three parts. The first was focused on the purpose of the questionnaire survey and introduced the interviewer. The second part consisted of questions that were the research subject, and the third part consisted of classification questions. The partial objectives of the second questionnaire were as follows:

1. The ability of respondents to recognize selected aromas based on a primary questionnaire survey.

2. Determining the sample that would subconsciously most positively affect the respondent when buying a cake using facial biometrics.
3. Comparison of conscious and unconscious perception.

Both questionnaire surveys were conducted online, as the pandemic situation did not allow testing in laboratory conditions.

The material used in this paper can be divided into several categories - characteristics of the business entity; preparation and implementation of the first questionnaire survey; preparation and implementation of the second questionnaire survey.

The business entity Sport Pub is focused on hospitality activities, located in the town of Brezno, specifically in the Mazorníkovo district and was registered in the Business Register in 2006. In 2017, the company expanded its activities to include restaurant activities when it opened a new section called Sport Pub Restaurant and enriched its classic menu with a food menu.

We used the following software:

- MS Excel 365,
- Software from Samo Europe Ltd – Samolab.online,
- Analysis using Wordle software,
- FaceReader 7 from the Dutch company Noldus.

Statistical Analysis

For the analysis of the answers to the first questionnaire, we used the right-hand test of the agreement of the proportion with the known constant with the significance level alpha 0.05 (*p*-value 0.05), where we calculated three methods of calculation and also used the second test, namely the Chi-square test for two independent variables.

Right-hand test, resp. right-hand alternative hypothesis:

H_1: $Q > Q_0$; which defines the range of values of the parameter Q to the right of the value Q_0.

The test of the null hypothesis H_0 against the alternative

H_1 is a procedure which, based on a random selection from a given distribution and at the chosen level of significance α (i.e., with the chosen reliability $1 - \alpha$) leads either to the rejection of the null hypothesis (i.e., to the acceptance of the alternative) or to the non-rejection. null hypothesis (i.e., to reject the alternative).

Chi-square test

The test consists in comparing empirical and theoretical frequencies, i.e. what would be empirical frequencies if the variables were independent.

Calculation of theoretical frequencies (1):

$$E_{ij} = \frac{(a_i)*(b_j)}{n} \qquad (1)$$

Where:

(a_i), (b_j) – single-stage frequencies; a_i – represents the number of statistical units with the i-th variant of the variable A; b_j – expresses the number of statistical units acquiring the j-th variant of the variable B; n – range of the sample.

The following hypotheses verify dependency testing:

H_0 – there is no dependence between A and B's qualitative variables, resp. there is no association with the alternative hypothesis.

H_1 – there is a dependence resp. association between characters A and B.

The test criterion can be calculated by using the formula (2):

$$\sum_{i=1}^{m} \sum_{j=1}^{k} \frac{((a_i b_j) - (a_i b_j)_0)^2}{(a_i b_j)_0} \qquad (2)$$

Where:

m – number of categories of the first character; k – number of categories of the second character.

If calculated χ^2 is $\geq \chi^2_{1-\alpha}$ for the significance level α and the degrees of freedom (m-1)*(k-1), we reject hypothesis H_0, i.e. the characters A and B are dependent.

RESULTS AND DISCUSSION

Results of the first questionnaire survey

The first questionnaire survey, which was compiled to initially identify a suitable category of aroma that would be used in Sportsport Pub to introduce the sale of chocolate cake and increase its sales, was attended by 153 respondents of different ages. We used categorization by gender (Table 1), age (Table 2), economic activity, and place of residence to categorise respondents.

Table 1 Share of respondents by gender.

Gender	Absolute frequency	Relative frequency
Female	91	59.5%
Male	62	40.5%
Total	153	100.0%

Note: Own questionnaire survey 2021.

Table 2 Share of respondents by age.

Age	Absolute frequency	Relative frequency
17 – 24 years	61	39.9%
25 – 33 years	42	27.4%
34 – 41 years	14	9.1%
42 – 59 years	24	15.7%
60 years and over	12	7.9%
Total	153	100.0%

Note: Own questionnaire survey 2021.

The economic category of respondents was the following: the largest group consisted of employed persons (37%), the second group were students (29%), the third group were self-employed people (14%), and the smallest groups were women on maternity leave, pensioners and the unemployed.

The share of respondents by residence was as follows: 53.6% live in the city, and 46.4% of respondents come from the countryside.

Subsequently, it was necessary to determine how many of the respondents consume sweets (e.g., cakes). One hundred fifty respondents (98%) answered that they consume sweets, and only three respondents (2%) do not.

The second question of the research part of the questionnaire was intended to specify the respondents who, in the case of choosing a sweet, would prefer a cake. The question was asked as follows: *"Do you consider yourself a type of person who would prefer cake over other sweets?"* With the possibility of answering yes/no. The aim was to identify the target group that prefers cake consumption over other sweets in the situation there is a choice or to confirm the assumption that more than 55% of respondents who have economic activity as employees prefer cake consumption over other sweets. To confirm the given assumption, we used a right-hand test of the agreement of the proportion with a known constant with a significance level of alpha = 0.05. From the questionnaire survey, we obtained 57 answers corresponding to the selection, of which 32 answers were marked “yes” and 25 answers “no”. Other responses were from respondents with an economic activity other than employed (Table 3).

Table 3 Structure of responses corresponding to the survey selection.

Answers	Absolute frequency	Relative frequency
Yes	32	56.0%
Not	25	44.0%
Total	57	100.0%

Note: Own questionnaire survey 2021.

We implemented the method in three ways, namely by the value of *p*-value, interval detection and also by the standard way through TCH.

H_0: At least 55% of employed people prefer to eat cake over other sweets ($\pi = 0.55$).

H_1: More than 55% of employed people prefer to eat cake over other sweets ($\pi > 0.55$).

1. Results of detection by using P value
u = 3.90707; *p* value = 0.00841344746; $\alpha = 0.05$; it means that *p* value $<\alpha$ and thus we reject H_0.

2. Results of the determination by using the confidence interval and the tabular value
centre of the interval $\pi = 0.55$; $\Delta = 0.061$; HH = 0.421; *p* = 0.5614; *p* > HH and it means that we reject H_0.

3. Test results by using the test characteristic
u = 3.90707; u tab = 1.644853 it means that u tab <u and therefore we reject H_0.

Using the three methods of testing, we found that H_0 was rejected, and thus more than 55% of respondents in the selected target group prefer cake over other sweets.

It was important for the research to find out the periodicity of cake consumption by the respondents, while only 1.3% stated that they do not consume cakes at all. In this question, it is interesting to find out the difference between the behaviour of women and men. We performed a Chi-square test for two independent variables, in which we compared the behaviour of women and men.

H_0: There is no statistically significant difference between the frequency of cake consumption by women and men.

H_1: There is a statistically significant difference between the frequency of cake consumption by women and men.

Based on the calculations can be concluded that X2 > the degree of freedom, and we do not reject H0. The differences are not large in terms of statistics. Sport Pub does not have to target a range of cakes to just one gender of customers. The company should create a range of cakes in a design for both men and women. The results of the statistical testing are shown in Table 4.

Table 4 Chi-square test of goodness – the difference between cake consumption of women and men.

How often do you consume cakes?	Empirical frequencies			Theoretical frequencies		Calculation x ^ 2	
	Men	Women	Total	Men2	Women2	(EM-Tm)^2/Tm	(EW-Tw)^2/Tw
Every day	3	6	9	3.57615894	5.423841	0.092825607	0.0612037
At least once a week	15	47	62	24.6357616	37.36424	3.768826106	2.48494029
At least once every two weeks	10	20	30	11.9205298	18.07947	0.30941869	0.20401232
Less often	30	18	48	19.0728477	28.92715	6.260347682	4.12770177
I don't eat cakes	2	0	2	0.79470199	1.205298	1.82803532	1.20529801
Total	60	91	151	60	91		

Note: Own processing 2021.

The following question was perhaps a little unusual for the respondents: *" When you look at the picture of the chocolate cake, what smell will come to your mind first?"* The cake that the company wants to add to its offer and the answers should help us choose the aroma that will be used in the second questionnaire survey.

Increasingly more companies are dealing with an idea of what products and where to place them in the store to impact customers **[17]**.

Figure 6 Picture of chocolate cake. Note: Own processing 2021.

A total of 151 respondents answered this question. The question was opened, and the respondents had to write one word that would come to mind first when looking at the picture of chocolate cake (Figure 6). These answers helped us carry out the second research, which focused on shape biometrics and our sense of smell. To evaluate this question, we used Wordle analysis, which can store words according to the size of the meaning. In other words, it is a visual representation of words, where the size of each word is directly proportional to the number of cases it appears in answers. In Figure 7, we can see that the most common word the respondents imagined when looking at the picture of the chocolate cake was the smell of chocolate, and therefore, this word is also the biggest. Ninety respondents wrote the smell of chocolate (we also included the smell of cocoa). The second most common word was the cinnamon smell, where up to twenty respondents stated they associate this smell with chocolate cake. The third most common word was the smell of coffee with 12 answers and in the fourth place was the smell of vanilla with ten answers. The other smells mentioned by the respondents were, for example, the smell of lemon, honey, nuts or the smell of orange.

According to **[18]**, the use of aromas can also be included in the basic communication functions from the seller to the customer.

There exist products that represent a characteristic feature of a particular product, and the main reason for buying such product is primarily its smell **[19]**.

According to **[20]**, the customers´ ability to smell creates sales opportunities and bring them into the shopping mood.

Figure 7 The most common smells associated with chocolate cake. Note: Based on own processing using Wordle analysis.

The next question was crucial in our questionnaire research, as the main purpose was to narrow down the selection of aromas suitable for use in the premises of Sport Pub. The question was similar to the previous one, but the difference was that the answer was no longer open, but the respondents had a choice of 7 options to choose from. The question was: "What smell would you prefer when entering a store where they sell cakes? ". Again, this question was to help us choose the right aromas to help us with the second research. There were these smells to choose from: chocolate, coconut, vanilla, cinnamon, fruit, citrus, coffee smell and others, where respondents could write their opinion and a smell that still occurs to them. 23% of respondents said they would prefer a fruity smell when entering stores where cakes are sold, and 22% said they would welcome the smell of coffee. We think that the reason for choosing the coffee aroma is that most respondents consume sweets with coffee, and in many cases, it may be a cake. This was followed by the vanilla smell, which 16% of respondents chose, followed immediately by the smell of chocolate by 15% of respondents. Other respondents chose the option of preferring the smell of cinnamon, coconut and gingerbread.

According to **[21]**, aromatic marketing is a series of events in which smell can encourage customers to buy goods and services and increase employee activity.

[22] claims that choosing the right aroma is not always easy, e.g., even with cakes, the addition of a chocolate smell may not be enough.

Another question in the questionnaire survey was the following: "How much is your decision when choosing a cake influenced by the aroma of the space in which you are? "This question aimed to find out whether the respondents are aware that their purchasing decisions can influence aroma. The question was compiled using a linear scale. The scale was set up so that respondents had a choice on a scale from 0, which meant that aroma did not affect them at all, to 10, which represented that aroma greatly affected them. 90% of respondents chose numbers higher than five on the scale, indicating that they think aroma can affect them. Only 10% of respondents out of the total number said that aroma does not affect them, so they chose numbers lower than five on the scale. We believe that most respondents marked that aroma influences them because when customers are hungry and feel a pleasant smell somewhere in a store or the city, the subconscious will immediately convince them that they would like to try it and therefore buy it.

The use of aromas is a new generation of communication tools that measure the impact on the consumer that is created with interactivity in business **[23]**, **[24]**.

The penultimate question in the first questionnaire survey focused on the location of the appropriate aroma release. More than 53% of respondents said that the aroma should be released throughout the space. The reason may be that when the customer smells a pleasant smell in the whole space and all the time, he can unconsciously spend more time in the store because he feels comfortable there. 44% of respondents said that the smell should be released upon entering the store, which we can assume would make a good first impression for customers. Only 3% of respondents said that the smell should be released directly when choosing a cake at the check desk.

Also, **[25]** argues that for setting a good mood, promoting products or brand positions itself can be used the expression "smell marketing".

The last question of the first questionnaire survey was of an informative nature. It concerned the factors that can influence the pleasant atmosphere of the store, with the respondents having a choice of factors and could choose just three of them. Respondents consider the staff, design of the store and its cleanliness to be the strongest factors influencing the pleasant atmosphere of the store. The least influencing factors influencing the pleasant atmosphere of the store are considered: people who are in the store, disposition and where the store is directly located and the air quality in the store.

Results of the second questionnaire survey

One hundred respondents attended the second questionnaire survey, but in reality, it was completed only by 51 respondents. In this survey, it was important for the respondent to be on a device that allows access to the camera, as the research was focused on examining the feelings of facial biometrics.

The first area of interest in the research part of the questionnaire was focused on the quality of the samples used in the research. Respondents had an open question: *"Please take sample No. ..... open it, and smell it for at least 5 seconds. After smelling, write what aroma sample No..... reminds you. "*Respondents had to write a short answer. The question aimed to determine whether the respondents would correctly name the specific aroma they felt. During the smelling of the sample, the camera scanned them and evaluated their emotions and how long they answered the question. It is also possible to determine whether the respondents thought about what they felt or just wrote it. We categorized the respondents' answers according to whether the answers approached the correctness of the sample or at least the things that could remind them of the given sample.

Sample number 2, which represented the smell of coffee and cake, was the most successful. As many as 34 respondents out of 51 determined this aroma. Respondents most often stated that it was the smell of coffee or tiramisu cake. The reason could be that most respondents liked coffee, and therefore the smell was very easily identifiable and recognizable. The last sample used for the research, available to the respondents, was the smell of chocolate, marked as sample number 4. This sample was also easy for respondents to identify, and up to 32 (63%) respondents out of 51 were able to determine that it was chocolate correctly. Again, almost all people like chocolate and know its smell because we consume it and encounter this smell from an early age; therefore, it was not such a problem to identify what aroma it is. Sample number 1 also performed quite well, where it was a smell of vanilla and orange. 29 (57%) respondents out of 51 wrote that it was a citrus smell that managed to hit this aroma and even 4 (8%) hit that it was a vanilla smell. The reason may also be that the aroma is associated with the smell of exotic fruits and is often used in various air fresheners, whether they are room or car air fresheners.

The most difficult was determining sample number 3, the smell of forest fruits. Twenty-three respondents (45%) approached that it was the smell of fruit. The other answers were of the smell of liquorice, chewing gum, baby syrup or candies. The smell was completely different from the previous ones, and we think it was much harder to

recognize. This may also be because people encounter this scent little, as it is mostly used in dairy products, whether it is various yoghurts, milk or kefir, which many people cannot consume due to milk intolerance. Therefore this aroma is more difficult to recognize and less known.

According to **[3]**, it is proven that two people will never feel the same, even though it is chemically the same substance. It is also remarkable that 80% of what we perceive as taste passes through the olfactory sense.

Using a webcam, we evaluated the emotions in the examined samples, so-called unconscious perception. The system captured via a webcam seven emotions that may have occurred to an individual respondent. These were: neutral emotions, happiness, sadness, anger, surprise, fright and disgust.

Figure 8 shows us on the horizontal axis what emotions are involved and the percentages of how the respondents reacted on the vertical axis. Regarding aroma sample number one, Vanilla Orange is dominated by neutral emotions in 70% of respondents, followed by the emotion of sadness, and the third was the emotion of happiness. In sample number 2, Coffee and Cake again dominated the neutral emotion (60%), then the emotion of happiness and emotion of sadness. The third aroma sample Wildberry also had the highest values in neutral emotion as well as the first aroma sample, followed by the emotion of sadness and emotion of happiness and anger that, were both on the same level. In the fourth sample of Chocolate aroma, we can see that there is a slightly smaller neutral emotion (only 55%) than in the other ones where it was higher and compared to the other three samples of aromas the emotion of happiness rose to 20% which may cause people to know this aroma and when smelling it, the idea of something sweet immediately arises inside them, and this can evoke an emotion of happiness in them at a given moment. All these emotions represent the micro emotions of the respondents – partial emotions.

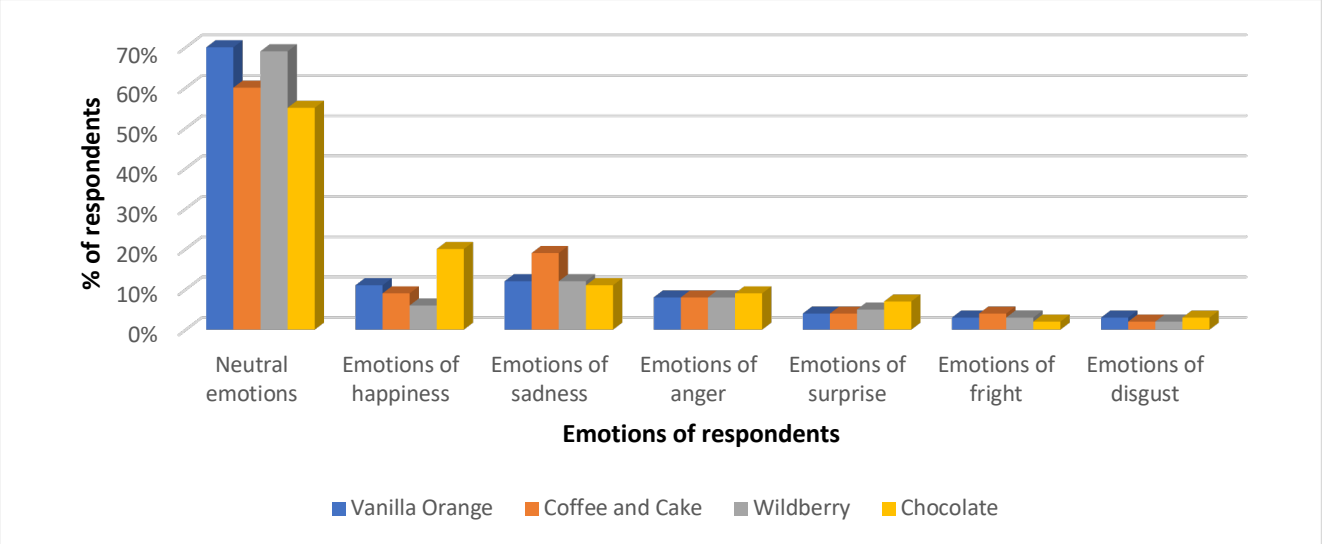

Figure 8 Unconscious perceptions of respondents' emotions when evaluating four samples. Note: Source: Own processing 2021.

The second area we addressed in the questionnaire focused on the aroma sample's suitability. The question was: *" Please indicate on the scale from 0 – 10 the suitability of this aroma sample No. .... associated with the sale of cakes."* In Figure 9, we can see that in conscious perception, respondents had to choose suitability on a scale from 0 – to 10, where 0 represented that the aroma sample is inappropriate and ten that the aroma sample is suitable in connection with the sale of the cake. Respondents chose aroma number 2, with a value of 7.8, as the most suitable one. In the second place, in terms of the suitability of the aroma in connection with the sale of cakes, sample number 4 with a value of 7.16 was placed, and we also consider this sample as a sample easily identifiable for the respondents. Sample number 1 and number 3 had similar ratings from respondents, and the value was around 6.50. All four aroma samples were evaluated as suitable and could be used in connection with the sale of cakes.

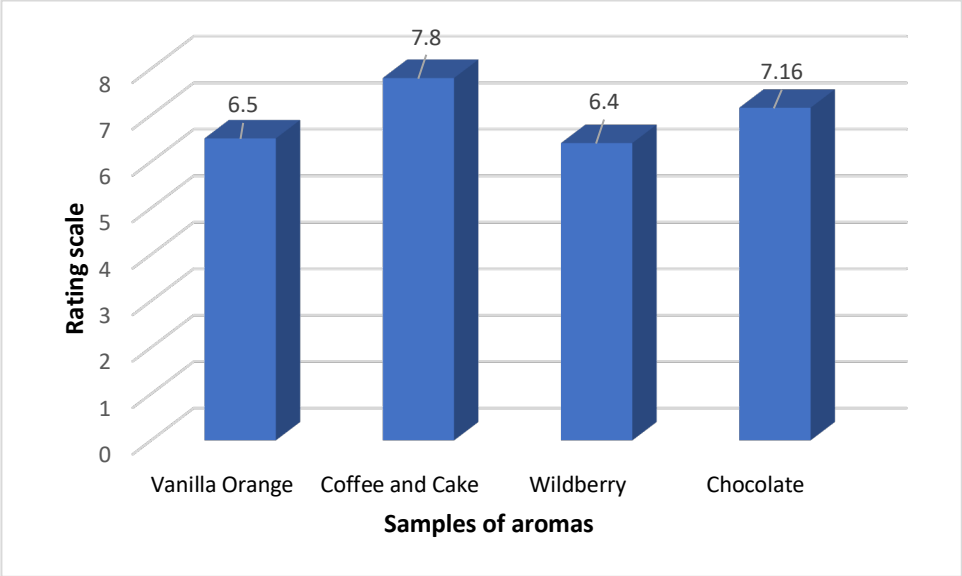

Figure 9 The suitability of a given aroma in connection with the sale of cakes – conscious perception. Note: Own processing 2021.

We also surveyed how the respondents unconsciously reacted to the suitability of the four aroma samples used in the research (Figure 10). From the data evaluated by the software from biometric perception, we focused on excitement, respectively, on the degree of concentration in the verification of aroma samples. The level of excitement already belongs to the group of main emotions and the polarity of emotions. The value of excitement, in other words, the value of concentration, is evaluated on a scale from 0 to 1, where the higher the value from 0, the higher the concentration level. According to the respondents' emotions, aroma sample number 4, i.e., the smell of chocolate, had the highest concentration value, up to 0.42. The reason may also be that most people like chocolate, and this smell is familiar to them, so they pay enough attention to it. Sample number 2, i.e., Coffee and Cake, and sample number 3, i.e., Wildberry, had a slightly lower concentration level, 0.37, based on measurements by facial biometrics. The smallest concentration value, namely 0.34, had sample number 1, namely Vanilla Orange, where we can say that the respondents were the least concentrated in determining this aroma sample. The reason may also be that the smell was uninteresting for them and could remind the smell of cleaning products.

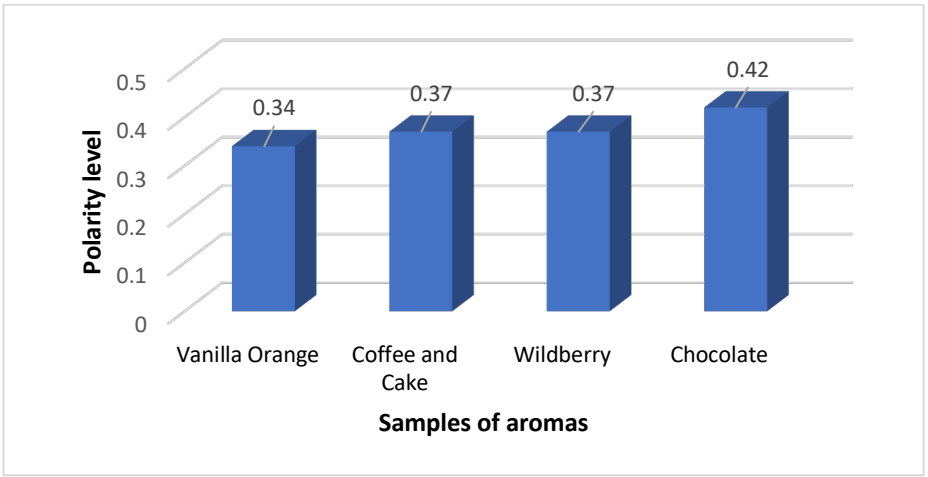

Figure 10 Concentration (excitement) of respondents in the evaluation of aromas. Note: Own processing 2021.

When comparing the conscious and unconscious perceptions, these are a little different. Respondents chose Coffee and Cake as the most suitable aroma in connection with the sale of cake number 2, namely Coffee and Cake, but in unconscious perception won Chocolate aroma sample number 4, which may be due to respondents concentrating best when smelling this aroma sample because they were able to identify it immediately and it made them feel happy.

According to **[26]**, the smell of a product creates a characteristic feature and can be easily identified by the customer. If the product smells pleasant, it acts positively and provides space for a favourable identity.

From the micro emotions we mentioned above, the software recorded the resulting emotions or the main emotions. These emotions include the polarity of the emotions and the excitement of the emotions. The polarity of the emotions determines whether the emotions are negative, positive, or neutral. If the polarity value is in negative numbers, i.e., the numbers are less than 0, then the emotions on the sample are negative. If the values are positive and higher than 0, the emotions in the sample are positive. It can also happen that the value of polarity will be equal to zero, and then we can say that the emotions in a given sample are neutral.

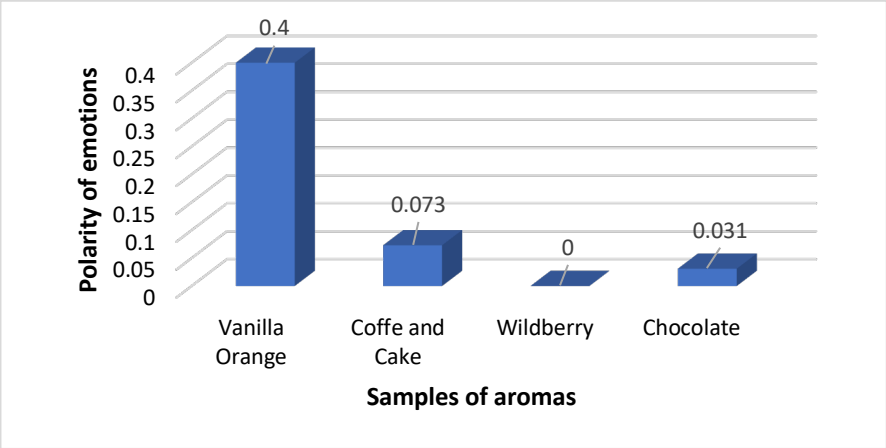

Figure 11 Unconscious perception of aroma samples – polarity of emotions. Note: Own processing 2021.

In Figure 11 can be seen the polarity of emotions of individual aroma samples. The first sample of Vanilla Orange had a polarity of 0.400, and this means that the sample had a positive emotion in the respondents. The second Coffee and Cake sample had lower polarity (0.073), which still represents a positive but low positive emotion. The third sample of Wildberry aroma had zero polarity. It is a neutral emotion. The fourth sample of Chocolate aroma had a very low level of polarity (0.031), which is close to neutral emotions. This result is also interesting because when we found out in the previous part about the excitement (focus) of the respondents, sample number 4, Chocolate, had the highest value and the respondents were able to concentrate on this sample the most and when the value of polarity represented almost neutral emotions, while these mutually do not exclude.

Subsequently, we compared the respondents' answers and their real emotions, which was also served by the question *"How do you feel today?"*. Respondents chose four answers – very good, good, bad and very bad. 66.6% of respondents said they felt good, and even 29.4% of respondents said they felt very well. Only 2% answered that they felt bad, and also 2% chose the option very bad. We think that the answers were certainly influenced by the environment in which they were, as well as the weather as it was on the day we conducted the research.

Thanks to the results evaluated by the system, we were able to look at the micro emotions of the respondents or how they felt from the point of view of biometric measurement.

Comparing the answers and biometric measurements, we found that 66.6% of respondents answered that they felt good, but 63% of respondents had a neutral emotion on this question when we took the results from the software. 29.4% of respondents said they felt very well, but only 11% of respondents had a happy emotion, according to the software. 2% of respondents said they felt bad, and we can confirm from the results of the software that 6% of respondents had angry emotions, and even another 2% were disgusted. As for the other emotions that the software evaluated, as many as 9% of respondents were sad, 6% were even surprised by this question, and 3% were frightened. Here we can see how the human body responds unconsciously to various circumstances.

The following question was: *"Do you think that the aroma of the environment when choosing a cake could influence you?* "This question was aimed at whether respondents are willing to admit the influence of the aroma of the environment on purchasing decisions. We found that 90.2% of respondents said that the aroma of the environment would affect their choice of cake. The reason may be that the human senses suddenly feel something that smells good and immediately wants to buy it. 3.9% of respondents admitted that the aroma of the environment does not affect them when choosing a cake. The other 5.9% said they could not judge.

Olfactory marketing (smell marketing) is increasingly getting into the practice of companies that want to improve their economic situation through research and the use of aromas **[27]**.

The aroma should be selected and applied in a way that corresponds and perfectly adapts to the environment and context (Naščáková and Danková, 2017) **[1]**. Aroma marketing increases commercial results, creates the setting for a pleasant stay in private and public areas, and enhances consumer response, loyalty and trust in the brand (E2 Aroma, 2020) **[28]**.

The last question in the interviewed part of the questionnaire was the same as we used in the first questionnaire, and it was also an open question where the respondents had to express themselves briefly. We asked them which aroma could be combined with the consumption of chocolate cake. As in the first questionnaire, more than 60% of respondents answered in both cases that they were most associated with the consumption of chocolate cake with a chocolate smell. In terms of the answers in this questionnaire, as well as other aromas, the respondents mentioned, for example, the smell of vanilla, cinnamon, rum, coffee or fruit. These answers were also very similar to the answers given by the respondents in the first questionnaire. We also processed this question using Wordle analysis. We can see that the most common aroma associated with chocolate cake is chocolate, coffee, and vanilla, as in the first case (Figure 12).

Figure 12 The most common aromas associated with the consumption of chocolate cake. Note: Based on own processing using Wordle analysis.

Also, in this questionnaire survey, we classified respondents according to gender, age, economic activity and residence. The results are shown in Table 5, Table 6, Table 7 and Table 8.

Table 5 Share of respondents by gender in the second questionnaire survey.

Gender	Absolute frequency	Relative frequency
Women	37	72.60 %
Men	14	27.40 %
Total	51	100 %

Note: Own questionnaire survey 2021.

The low number of 60 years old or older was because not every respondent in this age category can use the device with access to the webcam.

Table 6 Share of respondents by age in the second questionnaire survey.

Age	Absolute frequency	Relative frequency
17 – 24 years	18	35.3 %
25 – 33 years	11	21.6 %
34 – 41 years	5	9.8 %
42 – 59 years	15	29.4 %
60 years and more	2	3.9 %
Total	51	100 %

Note: Own questionnaire survey 2021.

Table 7 Share of respondents by economic activity in the second questionnaire survey.

Economic activity	Absolute frequency	Relative frequency
Student	14	27.5 %
Employed	32	62.8 %
Self-employed	1	1.9 %
Unemployed	1	1.9 %
Maternity leave	1	1.9 %
Retired	2	4 %
Total	51	100 %

Note: Own questionnaire survey 2021.

Table 8 Share of respondents by residence in the second questionnaire survey.

Residence	Absolute frequency	Relative frequency
City	13	25.49 %
Countryside	38	74.51 %
Total	51	100 %

Note: Own questionnaire survey 2021.

Based on the evaluation of two questionnaires, we recommend introducing aroma marketing into its operation over time, i.e., by introducing aroma into the entire company. The questionnaires concluded that the most suitable aroma in connection with the sale of the cake would be the smell of chocolate or the smell of coffee. This is mainly because both aromas are easy for people to remember and have been known to them since early childhood. It was these two smells that the respondents had no problem recognizing, and they are aromas that people immediately remember when imagining a chocolate cake. The questionnaires showed that the influence of aroma could influence respondents to buy a cake, which could economically support the company.

However, the seller must also appeal to other senses by which he can support sales **[29]**.

At Sport Pub, they can choose a separate aroma diffuser unit or add aroma directly into the company's air conditioning. In the case of an aroma diffuser, they must consider the purchase of the device itself and the operating costs. These costs include aroma fillings that need to be changed regularly and staff maintenance or training costs. After communication with the company Aroma Marketing, we can state that the prices of the aroma diffuser range from 55 € to 2,999 €. With the fact that every month it is necessary to fill the device with new fillings, the prices of which range from 50 € to 150 € per month. It all depends on the equipment that the business owner chooses and its parameters. There is also the possibility of renting the device, where the price would represent monthly costs from € 19.90 to € 109.90 for the Sport Pub. Again, the price depends on the type of device and its performance. However, every month the device must be filled with new fillings, where prices range from € 50 to € 150 per month, and as with the purchase of the device, it is also necessary to count on aroma fillings every month service and staff training. A huge advantage when renting is that the business owner can try a modern form of marketing for a few months and decide whether this service is suitable for the company and especially how it affects customers. The owner must realize that with the right aroma, the return on investment is almost certain in the case of purchase and lease. The return on this investment could be reflected in sales, which could increase, given that the satisfied customers who feel good in this environment spend more time here and therefore spend more money.

Sensory marketing helps to understand customer behaviour and purchasing decisions **[30]**.

We recommend Sport Pub to continue focusing on this marketing in the future and also try other aromas over time, such as the smell of fruit or vanilla, because even these samples can be combined with the sale of cakes. However, it is also necessary to try the aromas at different times of the year as we can get the most out of them for the company. For example, in the cold winter, customers would certainly welcome the scent of cinnamon or punch, which could positively influence them to buy the cakes.

Knowing consumers' behaviour, preferences, and reactions provides the company with a better chance of establishing itself in trade **[31]**.

Emotions and memory are affected by the power of smell in very close ties. Find the right smell, and you can bypass the rational ideas **[32]**.

Merchants are increasingly using the surrounding smell as a strategic tool to differentiate themselves from the competition, attract customers, stimulate sales, influence moods, and create an overall enjoyable and memorable shopping experience **[9]**.

Aroma marketing, or so-called Smell marketing, can be used in two areas. The first is ambient smelling, this term means filling the space with a suitable type of smell, and the other area is smell branding, which can be used to create a specific smell. It identifies a brand, product, institution, company, or environment **[33]**.

The COVID-19 pandemic, which is currently here, must also be taken into account. For this reason, antibacterial aromas have also been developed that reduce viruses and fungi in the air. Essential oils have been developed that have antibacterial effects and reduce the risk of infection in wounds. These aromas are mainly used in spaces such as schools, kindergartens, nursing homes or various clinics. Therefore, we can confirm that aroma marketing moves with the times and its use will even benefit society and businesses. Therefore, we also recommend Sport Pub using aroma marketing.

Based on the questionnaires' results, we recommend choosing the aroma by a biometric test for other companies of various specializations. The cost of the survey would, in this case, be more expensive than research using the standard questionnaire, but the costs can be reimbursed in the form of a suitable choice of aroma and higher sales. Because, in case of the wrong choice of aroma, this would cause negative associations and a decrease in sales.

The relationship between the sense of smell and the ability to retrieve memories and evoke emotions is a proven fact and an established marketing tool resulting in increasing sales **[34]**.

By using a suitable aroma, it is possible and proven to force customers to look around the store longer, spend more money, and return more often **[32]**.

As **[35],** consumer behaviour has an increasing role in launching products on the market. Although the brand has a huge impact on the consumer's purchasing decision, it is closely related to the products placed in the stores because the final purchase decision by the consumer is made in the store or point of sale **[36]**.

Also, **[1]** argues that especially smell accompanies the company's image. Companies, businesses or various institutions such as banks, post offices, shops, hotels, waiting rooms, insurance companies, travel agencies or public transport have characteristic smells for their customers that influence them to return there constantly.

[37] also claims that it is important to search for and use new forms of marketing, as we are increasingly saturated with advertising, and this has caused greater immunity to traditional marketing. When selling as well as buying, we always use and need all five senses, which can be used in modern marketing **[38]**.

CONCLUSION

The submitted paper paid attention to introducing the aroma of marketing as a modern tool in practice. Our research was focused on the business entity Sport Pub, located in the town of Brezno, Mazorníkovo district, focusing on hospitality and restaurant services, which has been operating on the market for 15 years.

The use of aroma in marketing is gaining more and more prominence. Many people do not even realize how the aroma can affect their subconscious. Therefore, we decided to focus on how the company Sport Pub could use the aroma of marketing for its operation so that our research would bring benefits to the surveyed company and, of course, higher sales. Introducing the aroma of marketing to the company can cause innovation in its communication with its customers. These facts can contribute not only to the company's competitiveness but also to the development of the company and increase market share.

Two questionnaires were used – the first one was aimed to gain the basic information about consumers and their consumer behaviour and the second one was realized as a blind review of selected aromas by using facial biometrics. Based on both surveys, we can conclude that introducing the specific aroma in the company certainly could influence the sale of the new product- the chocolate cake.

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

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Exploring linkages between food security and economic growth: a Systematic mapping literature review

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ABSTRACT

Food security can be achieved by being carried out simultaneously alongside the economy's growth at the macro level. While many countries worldwide carry out economic growth policies to improve food security, the causal relationship between economic growth and food security is still debated. This study uses a systematic mapping review to analyze the relationship between food security (FS) and economic growth (EG) using a systematic mapping review. There are 26 previous research results from 780 articles obtained. Database from google scholar, ScienceDirect, Elsevier, and JSTOR with a limited date range on published information from 2004-2021. The result shows an empirical gap in the relationship between FS dan EG with 76.92% supporting the correlative relationship between FS and EG, while the other 19.23% claimed that there is no correlation, and 3.85% (one study) explored the relationship between EG and FI (Food Insecurity). Furthermore, 11 studies explained that EG has a positive effect on FS; one study stated that it has a negative impact, and another one hurts Food insecurity. Meanwhile, seven studies revealed that FS has a positive effect on economic growth, one study on the contrary, and two studies explained it has no effect. Availability and GDP per Capita variables were mainly used in describing the relationship between FS and EG.

Keywords: Food Security, Economic Growth, Systematic Literature Review, Empirical Gap

INTRODUCTION

Food security has become a significant focus in today's world's sustainable development. One of the main points in the sustainable development goals (SDGs) discussed the purpose of the action was to end hunger, achieve food security, improve nutrition and promote sustainable agriculture. Food security was indeed a significant focus for countries globally because it could lead to a threat of hunger. FAO **[1]** in 2020 estimates that between 720 and 811 million people faced hungry. Furthermore, nearly 2.37 billion people did not have access to adequate food in 2020. Based on a report by **[2]**, it is stated that around 155 million people in the world experience severe food insecurity. Based on this, it was concluded that food was a basic need that every country must fulfill to achieve prosperity. Several countries such as China, Germany, Australia, and New Zealand, became developed countries due to their agricultural sector progress. The fact proved that food has a strategic role in a country due to its ability to guarantee economic development. According to **[3]**, there was a close correlation between food security and the economic growth of a country. Malthus explained that the lack of food availability has caused the prices to increase due to the imbalance of the increasing population and food availability worldwide. Rapid population growth encouraged a country to maintain its economic growth, particularly in income per capita, so the food prices remained affordable. In addition, to keep food security, the governments must strengthen their sustainability in food production and prevent excessive food consumption. The Food and Agriculture Organization **[4]** formulates the concept of food security as a condition in which everyone at all times, both physically and economically, has access to sufficient, safe, and nutritious food to meet their daily nutritional needs according to their preferences. Furthermore, according to FAO, food security can be seen through four dimensions: availability, access, utilization, and stability. The availability viewpoint viewed food security in terms of supply: the production level, the amount of inventory, and the trade value of food products. The access viewpoint assumed that the government must maintain food supply at the domestic or international level and maintain the food price's affordability for

society. The utilization viewpoint considers the diversity of food that can be utilized biologically for the human body to provide adequate nutrition to determine the individual's nutritional status. The stability viewpoint referred to the continuity of food access regularly and the possibility of the risk which may occur due to poor weather, political instability, and economic factors such as unemployment and increased food prices.

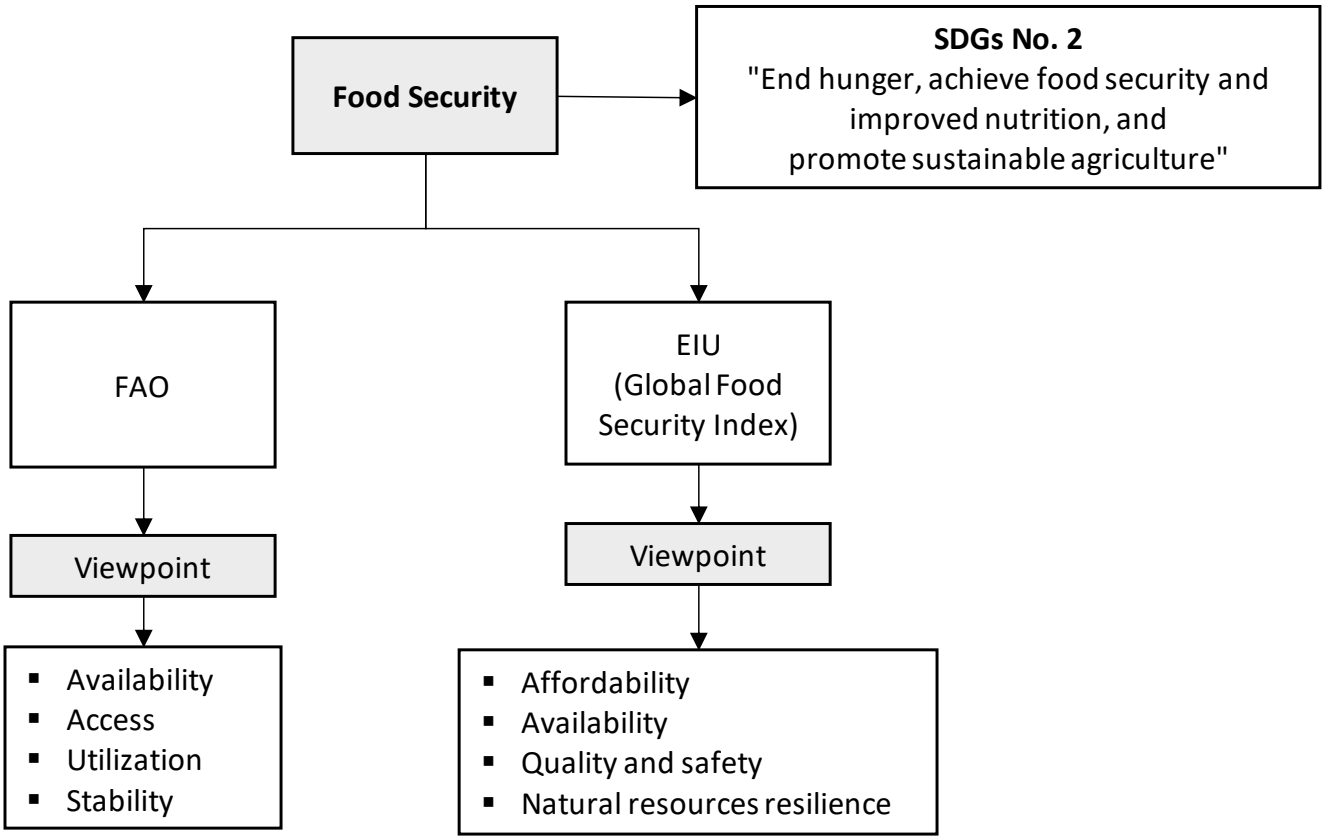

Figure 1 Food Security Concept from Food and Agriculture Organization (FAO) and Economist Intelligence Unit (EIU).

Furthermore, the Economist Intelligence Unit **[5]** described food security as a complex and diverse problem influenced by culture, environment, and geographical area. To identify food security, EIU has formulated a Global Food Security Index, which consists of four aspects, which were: affordability, availability, quality, safety, and natural resources resilience. The affordability viewpoint considered that food security could be achieved with the stability of food prices. No society lived below the poverty line, high GDP per capita, and the availability of a food security net and protection for farmers. The availability viewpoint considered that food security could be achieved with the sufficiency of food supply and agricultural product development. The quality and safety viewpoint described that food security could be achieved with various foods that fulfill the population's nutritional standards. The Natural Resources Resilience viewpoint assumed that food security depended on a country's geographic and demographic factors such as climate and weather, soil and water condition, and population growth and urbanization. In the concept of food security described by FAO and EIU, it was shown that food security required sustainable development, particularly in terms of availability, food prices, and people's incomes which were conditions for achieving stable economic growth. Previous research has attempted to provide empirical facts on the correlation between food security and economic growth. Most of the research was conducted in developing countries **[6]**, **[7]**, **[8]**, **[9]**, **[10]**, **[11]**, **[12]**, **[13]**, **[14]**, **[15]**, **[16]**, **[17]**. That was because the economic growth of the developing countries was highly dependent on food production. In addition, Gross Domestic Product (GDP), or the market value of labour and the products produced by a country in a certain period, were used to measure economic growth. Although many previous studies have attempted to relate food security with economic growth, the causal relationship between the two has not yet been resolved. In other words, whether food security stimulated economic growth, economic growth led to food security, or the economic growth and food security had a two-way causal correlation, the cause has not been found.

This paper will assess previous studies, how the food security linkage with economic growth has been calculated, and recognize shortcomings in future research. A systematic mapping review will investigate the following research question: "Is there a correlation between food security and economic growth?". Therefore, our research

aims to provide an empirical literature review of the relationship between food security based on the results of previous studies. It can be known early identification of the effect of food security on economic growth or vice versa. Therefore, we hope further to identify the connection between food security and economic development to build and enrich the existing literature on food security at the macro level.

Scientific Hypothesis

Based on the theory of Malthus **[3]**, which explains the occurrence of a population trap because the food availability cannot keep up with population growth. Malthus provides an alternative to overcome low food security by increasing per capita income or economic development. However, on the other hand, the population trap can be avoided by strengthening the food supply when population growth is still low so that excess food production can increase per capita income. Based on the two possible relationships between food security and economic development, this study will analyze two alternative hypotheses that contradict using a literature review with the following hypotheses:

H1: Food security affects economic growth.

Otherwise

H2: Economic growth affects food security.

MATERIAL AND METHODOLOGY

This research uses a systematic mapping review to explain the linkage between Food Security (FS) and Economic Growth (EG). Systematic mapping utilizes a straightforward convention to archive each examination interaction step. A systematic mapping review is an experimental investigation that outlines an area to recognize which issues have been thoroughly studied and need extra examination **[18]**. Our review cycle drew vigorously on PRISMA rules for systematic reviews **[19]** and revealed principles for deliberate proof amalgamations in ecological investigation **[20]**, **[21]** to generate the following synthesis ROSES guidelines. The process of systematic mapping review is clarified further in Figure 2. For the information sources and search strategy, the Universitas Indonesia Host was used to access academic and empirical journals to find previous research that discusses the relationship between food security and economic growth. In addition, databases from google scholar, science direct, ScienceDirect, and JSTOR were also included as part of the search process. The date range was limited to published information from 2004-to 2021, and only peer-reviewed and full-text documents were included in the search. The literature search was carried out in August 2021. The following combinations of search terms and keywords were used: the relationship of food security with economic growth, food security and economic growth studies, and empirical evidence of the correlation of food security with economic growth. We found 26 academic and practical journals that discuss the influence or relationship of food security with economic growth from the search results.

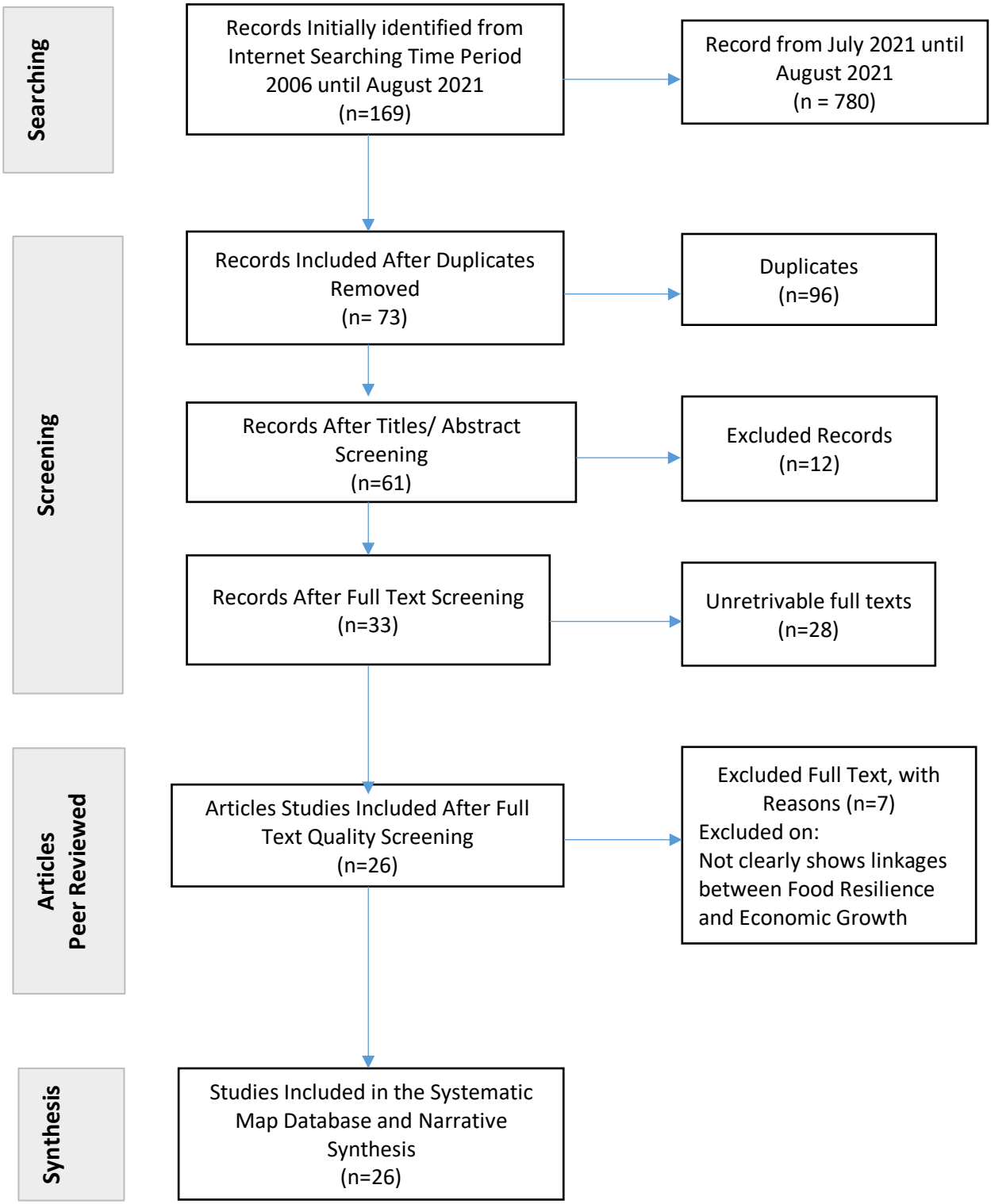

Figure 2 ROSES Flow Diagram for Systematic mapping review process (adopt from **[22]**).

Table 1 Tabulated systematic mapping literature review from selected articles.

No	Reference	Research Scope	Time Horizon	Research Method
1	**Abogahsem, P., et al. (2018) [6]**	Country Level	2014-2015	Qualitative
2	**Aikaterini, K., et al. (2014) [7]**	Selected Countries	2001-2011	Quantitative
3	**Alexander, B.M., et al. (2019) [23]**	Country Level	2018	Qualitative
4	**Andrew, A., et al. (2021) [24]**	Global	2018-2019	Quantitative
5	**Arif, W.W., et al. (2017) [9]**	Country Level	2007-2014	Quantitative
6	**Calestous, J. (2007) [10]**	Selected Countries	Unidentified	Qualitative
7	**Clemens, B., and Olivier, E. (2014) [11]**	Country Level	2009-2013	Quantitative
8	**Desta, A. (2017) [12]**	Country Level	2006-2016	Qualitative
9	**Dikshit, P. and Munisamy, G. (2021) [13]**	Selected Countries	2008-2015	Quantitative
10	**Krystina, S. (2018) [25]**	Selected Countries	2012-2015	Quantitative
11	**Melissa, C.C. (2013) [26]**	Country Level	2008-2012	Qualitative
12	**Michael, C. (2012) [27]**	Global	2005	Mix Method
13	**Singh,Ajay.K (2018) [8]**	Country Level	1961-1990	Quantitative
14	**Olabode, P.O., et al. (2015) [14]**	Region	1990-2014	Quantitative
15	**Paul, T. (2020) [28]**	Country Level	2019	Qualitative
16	**Putra, Y., et al. (2021) [29]**	Region	2000-2018	Quantitative
17	**Shenggen, F., et al. (2021) [30]**	Region	2019-2020	Qualitative
18	**Sujarwo, and Nuhfil, H. (2016) [31]**	Country Level	2010-2014	Qualitative
19	**Supardi, R. and Aries, M. (2017) [32]**	Country Level	2009-2013	Qualitative
20	**Timmer, P.C. (2010) [33]**	Region	1961-2007	Qualitative
21	**Timmer, P.C. and Thomas, R. (2014) [17]**	Region	1990-2012	Qualitative
22	**Torero, M., (2014) [34]**	Global	1996-2007	Qualitative
23	**Susilastuti, D (2018) [35]**	Country Level	2007-2016	Quantitative
24	**Timmer, P (2004) [36]**	Region	2004	Qualititative
25	**Liefert,W (2014) [37]**	Country Level	1990-2000	Qualitative
26	**Manap, and Ismail (2019) [38]**	Selected Countries	1970-2016	Quantitative

Note: Constructed by Author.

Table 2 Empirical results from systematic literature review.

No	Food Security (FS) Dimension	Economic Growth (EG) Indicator	Findings	Empirical Result
1	**Affordability, Utilization**	GDP Growth	FS has an impact on EG	+
2	**Availability**	GDP Growth	EG has no impact on FS	×
3	**Availability**	GDP Growth	EG has an impact on FS	+
4	**Availability, Affordability, Quality and Safety, Natural Resources Resilience**	GDP Per Capita	EG has an impact on FS	+
5	**Availability, Natural Resources Resilience**	GDP Growth, GDP Per Capita, Unemployment, Inflation	FS has an impact on EG	+
6	**Availability**	GDP Growth	FS has an impact on EG	+
7	**Affordability, Availability, Quality and Safety, Natural Resources Resilience**	GDP Growth, GDP Per Capita	EG has an impact on FS	+
8	**Availability**	GDP Per Capita, Inflation	EG has no impact on FS	×
9	**Availability, Affordability, Natural Resources Resilience**	GDP Per Capita	EG has no impact on FS	×
10	**Affordability, Availability, Quality and Safety, Natural Resources Resilience**	GDP Per Capita	EG has an impact on FS	+
11	**Availability**	GDP Per Capita	FS has an impact on EG	+

Table 2 Cont.

No	Food Security (FS) Dimension	Economic Growth (EG) Indicator	Findings	Empirical Result
12	**Quality and Safety, Utilization**	GDP Growth	EG has an impact on FS	-
13	**Affordability, Availability, Quality and Safety, Natural Resources Resilience**	GDP Per Capita, Inflation	EG has an impact on FS	+
14	**Availability**	GDP Growth, GDP Per Capita	EG has an impact on FS	+
15	**Availability, Affordability, Quality and Safety**	GDP Growth, GDP Per Capita	EG has an impact on FS	+
16	**Availability, Affordability**	GDP Per Capita	EG has an impact on FS	+
17	**Availability**	GDP Growth	EG has an impact on FS	+
18	**Availability, Affordability**	GDP Per Capita, Unemployment	FS has an impact on EG	+
19	**Availability**	GDP Per Capita	EG has an impact on FS	+
20	**Availability**	GDP Growth, GDP Per Capita	FS has an impact on EG	+
21	**Availability, Quality and Safety**	GDP Growth, GDP Per Capita	EG has an impact on FS	+
22	**Quality and Safety**	GDP Growth	FS has no impact on EG	×
23	**Availability**	GRP (Gross Regional Production)	FS has no impact on EG	×
24	**Availability, Access, Utilization**	GDP Per capita (Growth)	FS has an impact on EG	-
25	**Availability, Access, Quality, and Safety (Nutrition)**	GDP Growth	EG has an impact on FI	-
26	**Quality and Safety (Dietary Energy Supply)**	GDP Growth	FS has an impact on EG	+

Note: FS (Food Security), EG (Economic Growth), FI (Food Insecure); +means positive impact between variables; - means negative impact between variables; x means no correlation between variables.

RESULTS AND DISCUSSION

About 169 previous studies searched for the connection between FS and EG. However, only 33 previous studies met the criteria that provide empirical evidence of the association between FS and EG. Furthermore, an in-depth analysis of 26 studies was conducted to find the linkage between FS and EG. Concepts and perspectives based on the dimensions of global FS and EG indicators were used to determine the relationship between variables. The literature found that previous researchers used the FS dimension from FAO or other sources such as the Global Food Security Index (GFSI). **[39]** divides FS's dimension into four pillars: availability, access, utilization, and stability. **[40]** also divides FS into four dimensions: affordability, availability, quality and safety, and natural resources and resilience. However, not all sizes of FS from FAO or GFSI were used by previous researchers to clarify the connection between FS and EG. For example, FAO's FS dimensions were not used, such as access and stability.

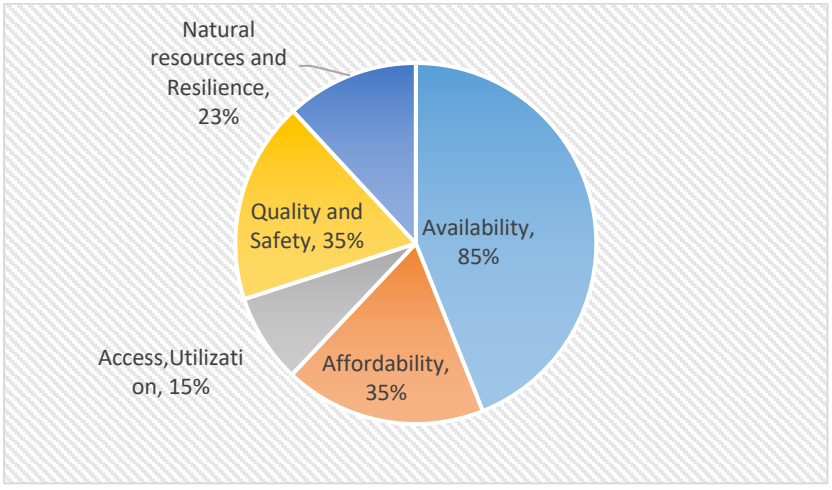

Figure 3 Percentage of Types of Food Security Dimensions Frequently Used.

Furthermore, out of the 26 previous studies (Figure 3), 22 studies (85%) used the availability dimension to determine the linkage between FS and EG. Nine studies explored FS (35%) through the affordability dimension, and two studies used the Access dimension (8%). Nine studies (35%) specifically focused on quality and safety by examining how malnutrition impacts FS and EG. Natural resources and resilience were reviewed by six studies (23%). Finally, two studies (8%) focused on food utilization at a national and global level **[6]**, **[27]**. The results show that availability is the most widely used dimension, which means that the economic approach is still the mainstream in measuring food security. The relationship between FS or FI and EG can be mapped based on their function (independent or dependent) for empirical results (Figure 4). Eleven studies (50%) were found to explain that EG has significantly and positively impacted FS **[8]**, **[23]**, **[24]**, **[11]**, **[25]**, **[27]**, **[14]**, **[28]**, **[29]**, **[30]**, **[32]**, **[17]**. However, One study states that EG hurts FS **[27]**. On the other hand, seven studies (27%) explained that FS has a significant positive impact on EG **[6]**, **[9]**, **[10]**, **[26]**, **[31]**, **[33]**, **[38]**, while four studies (15%) explained that EG has no impact on FS **[7]**, **[12]**, **[13]**, **[34]**. Another interesting finding is that food security has a significant but negative impact on economic growth in the long term. These results refer to the findings of **[36]** in cases in Asian countries. The final finding by **[37]** explains that EG reduces food insecurity.

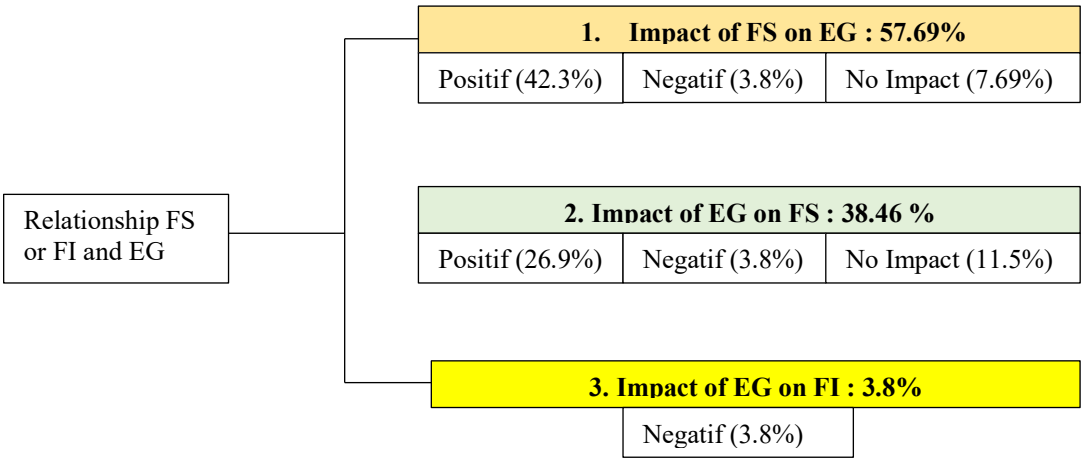

Figure 4 Comparison of Relationship Proportions between FS or FI and EG.

Results from 26 previous studies showed that most of them proved the effect of FS on EG empirically. On the other hand, an important fact (38.46%) of earlier studies explains that EG's effect on FS cannot be ignored and indicates an empirical gap.

Availability Viewpoint

Our review revealed that previous researchers used the availability dimension to show the relationship between FS and EG. Twenty-two previous studies (85%) indicated that FS affects EG regarding the availability dimension. In addition, most previous research explained the connection between food availability and EG through indicators such as GDP growth per capita. From the review, it is found that there were three empirical results:

First, 11 of 26 studies revealed that EG had a significant effect on FS **[28]**, **[23]**, **[24]**, **[11]**, **[25]**, **[14]**, **[28]**, **[29]**, **[30]**, **[31]**, **[17]**. It provided evidence that the increase in EG increases domestic food availability. Furthermore, EG, in terms of GDP per capita, shows economic equilibrium between low-income and high-income citizens **[23]**, **[14]**. It will impact the development of agribusiness infrastructure, which in turn will increase agricultural production. Research on broader-based EG in Yemen proved that massive infrastructure development is needed to return to FS levels [27] quickly. Besides that, the increase in EG also showed an increasing trade in food products, proving an increase in FS in terms of availability **[29]**, **[17]**. Increasing food availability by diversifying imports through trade in food products is a strength for higher-income countries such as Singapore to increase FS in the global sphere **[28]**. In addition, to support imports, Singapore applies a zero-tariff policy for foodstuffs. Furthermore, the government should focus more on maintaining EG to maintain national food availability to control FS **[30]**. It can be done by developing FS by (a) Increasing the availability of food through increasing productivity and production, diversifying the production of food crops, both fresh and value-added processed food, and (b) Increasing community food access to reduce food insecurity through empowering economic and institutional capacities **[31]**.

Second, 6 of 26 studies believe that FS influences EG **[6]**, **[9]**, **[10]**, **[26]**, **[33]**. It means that FS can be achieved

by increasing the quantity and quality of consumption and increasing food availability, economic access, and stability **[9]**. Furthermore, improvement and innovation of agricultural production can help to increase GDP. It is present in a study on agricultural improvement and economic development in Sub-Saharan Africa, which grew 6% per year during 2002 to 2007. It is equivalent to doubling the GDP in sub-Saharan Africa **[10]**. Besides that, increasing food availability is a substantial effort to improve FS through agricultural technology development. It increases domestic food availability to reduce dependence on imports **[26]**. In theory, reducing dependence on imports will significantly affect GDP because it will increase net exports. The total demand for food products such as rice is also essential to ensure GDP growth because rice is still the largest source of calories for most consumers worldwide. The literature review results show that FS in Asia has generally centred around rice creation, advertising, and utilization. However, the region's rapid EG and the structural transformation redefine Asia's needs. The importance of agriculture to the Asian economy is 3.7 times greater than that of the world. This ratio increased to 5.2 times in 2007 **[33]**. The quick change of Asian economies demonstrates that farming remains vital. It is critical because the Asian economy is still inferior. After all, even with rapid economic growth, many small farmers in Asia cannot move into modern and managerial professions in metropolises in just a few decades. In addition, several studies have stated in **[41]** that the vulnerability of climate change has impacted decreasing the income of farmers, most of whom are small and marginal farmers.

Third, 3 of 26 studies assessed no relationship between food availability and EG **[7]**, **[12]**, **[13]**. The empirical results show that the faster EG model has helped developing countries improve their trade balance but does not incentivize overcoming domestic FS by increasing domestic consumption **[7]**. Further empirical evidence of the relationship between FS and EG in Ethiopia proved that food insecurity is caused by inflationary pressures, resulting from the excess money supply, population growth, and budget deficits, not EG **[12]**. It showed that other factors affect FS, with the conditions of each country being different. This is supported by the Food Insecurity Experience Scale (FIES) results for 2015–2017, showing that GDP and capital significantly reduce food insecurity, while most other coefficients are not statistically significant **[13]**. The relapse examination consequences of the percent expansion in GDP per capita (or financial development), food frailty diminished by 0.77%. In the interim, the rate expansion in the portion of arable land declined food uncertainty by 0.27% **[13]**. The impact of capital development on reducing food instability is slightly smaller (0.11%), but it varies with per capita GDP and arable land.

Affordability Viewpoint

The Global Food Security Index (GFSI) defines affordability as a dimension that describes buyers' capacity to purchase food, weakness to value vacillations, and the existence of programs/policies to support consumers when shocks occur. Nine studies (41%) discuss the relationship between the affordability dimension of FS and EG. Most previous studies attempted to explain this relationship through a quantitative approach (6 of 9 studies). Most studies used indicators of GDP growth and GDP per Capita to see the connection between EG and FS. Moreover, few studies used other indicators such as inflation and unemployment rate. The empirical results classify into three:

First, 6 of 9 studies explained that EG has a significant effect on the affordability dimension of FS **[8]**, **[24]**, **[11]**, **[25]**, **[28]**, **[29]**. The result showed that increasing EG and GDP per capita can increase purchasing power, and government support to ensure affordable food prices can increase FS. Further findings are that inflation and population growth rates are the most responsible for food insecurity in most developing countries such as India **[8]**. It supported a causal mechanism by which increased income increases FS without extraordinary local factors. However, based on a review study, the strength of this effect varies depending on the country's condition, either negatively or positively **[24]**, **[11]**. The consequences of an itemized investigation of FS in various areas and nations affirm that the vast regional separation is because of contrasts in financial turn of events and GDP per capita. It tracked down that the geological differences in GDP per capita in 2012-2015 are under regional FS. Compared to countries with the highest GDP per capita, the lowest domestic-income countries are food insecure most **[25]**. For example, Singapore has provided some lessons that can use economic growth and systemic food reserves to find durable solutions to ensure the security of financial services in response to temporary supply shortages. Improving citizens' economic conditions as measured by per capita GDP is essential to ensure economic access to food **[28]**, **[29]**. It is also supported by intraregional trade and trade openness to support EG. It has a positive and significant effect on increasing FS through increased food production.

Second, 2 of 9 studies assess FS from the affordability dimension that affects EG **[6]**, **[32]**. The empirical results show that the economic cost of micronutrient deficiency has reduced the GDP of most developing countries by 0.72%. Due to macronutrient and micronutrient losses, global losses in economic productivity account for more than 2-3% of GDP. Without a state-claimed FS system, there will be a supported unfavourable impact on human resources, adversely impacting government spending. In addition, it will prompt stale financial development over the long haul. This way, the proper FS methodology is fundamental for all nations to ensure affordable food prices for citizens. In addition, the affordability dimension in FS aims to reduce inequality so that EG can be evenly distributed to the community. It can be supported by increasing community food access to reduce food insecurity by empowering economic capacity, encouraging farmer cooperatives, and expanding market information availability **[31]**.

Third, one study does not support the correlation between the affordability of FS and EG **[13]**. This study believes that the FIES measures from 2015 to 2017 show that per capita GDP and FS are not statistically significant. This relationship is the weakest in developing countries, and it is generally estimated that hunger in low-wage countries has been reduced by 0.29%. For low-wage countries that use agribusiness as their primary source of GDP, genetic modification and education seem to provide accessible food for the entire population. So at this point, land assets, capital, and urbanization are the most useful for realizing FS. The FS network access commitment provides an additional way to regularly use economic development to achieve food security.

Quality and Safety Viewpoint

Quality and safety are indicators used in the Global Food Security Index (GFSI), which measures changes in the average diet, food security, and nutritional quality. 8 of 22 studies analyzed the relationship between quality and safety aspects of FS with EG. Most studies tried to see the correlation or influence with economic indicators such as GDP growth, GDP per capita, and inflation. There are two groups based on empirical results from the reviewed literature as follows:

First, 6 of 8 studies support EG's impact on quality and safety dimensions **[8]**, **[24]**, **[11]**, **[25]**, **[28]**, **[17]**. The study identified that EG could push FS and nutrition **[11]**, **[25]**. In addition, broad-based EG will benefit the poor because it will positively impact how nutrition programs are implemented, such as integrated childcare programs and awareness campaigns related to family planning, women's education, and consumption. In addition, previous studies have also identified that countries with good EG, such as Singapore, can improve quality and safety in FS. It works well when EG aligns with increasing GDP per Capita **[28]**, **[8]**. It means increasing people's purchasing power to buy nutritious food to achieve FS. In addition, the government's role in increasing the subsidy budget for the poor to get nutritious food can maintain sustainable FS in developing countries **[8]**, **[17]**.

Second, 2 of 8 studies do not support the correlation between EG and FS or vice versa **[27]**, **[34]**. **[34]** explained that EG does not contribute to FS. Furthermore, Torero argued that economic development is feasible whenever created nations attempt to accomplish FS as a reason for their residents. His observation is still up in the air that expanding 10% in financial development just diminished the persistent lack of healthy sustenance by 6%. Therefore, it determined no direct relationship between EG and FS; Torero stated that economic development without help from others would not take care of the ongoing shortage of healthy food. In other words, economic growth is not strong enough to create food security without equitable development. However, it should be regarded as one of the fundamental factors of any FS strategy. **[27]** supported this view, and he believes that EG cannot be trusted enough to feed the world. He also persuades EG critics to pay more attention to food-related issues in development evaluation. The result comes from empirical testing of the Food Human Security Index (FHSI), which shows no relationship between FS and EG. FHSI enhances conventional thinking as it relates not exclusively to FS yet additionally to development and thriving. Likewise, it will be helpful for future examination to understand better why inequality impacts FHSI indicators, especially among high-income countries.

Natural Resources Resilience Viewpoint

Global Food Security Index (GFSI) defines Natural Resources Resilience as a dimension to measure a country's vulnerability to climate change, natural resource risks, and the efforts the country to adapt to the risks. In addition, 6 of 22 previous studies explained the relationship between EG and aspects of Natural Resources and Resilience. Three empirical results can be grouped based on the review literature as follows:

First, 4 of 6 studies support that EG significantly impacts the natural resources resilience aspect on FS **[8]**, **[24]**, **[11]**, **[25]**. The Global Food Security Index (GFSI) study proves that EG impacts climate factors (i.e., average temperature, a statistically significant negative, and rainfall). Furthermore, empirical results based on the OLS

model show that GFSI can reduce 1.70% with an average temperature increase of 1% **[8]**. In addition, the rise in income in EG also has a critical effect on farmers in improving land management to improve FS. The result discovered blended proof that cereal creation per capita, grain yield per hectare, total administration measurements, coordination execution, and paid business levels are public FS indicators. In addition, the outcome of a deep analysis of FS confirms that differences in economic development cause enormous territorial differentiation and, what is more, along these lines in, GDP and pay per capita. It tracked down the geological contrasts in GDP per capita in 2012-2015 under regional FS. Compared to countries with the highest GDP per capita, nations with minimal homegrown salaries are miniature food insecure **[25]**.

Second, 1 out of 6 studies explains that FS from the aspect of Natural Resource Security impacts EG **[9]**. This study argues that land area, commodity production, CPI (Customer Price Index) of a collection of several commodities, and FIMI (Multidimensional Food Insecurity Index) determine the achievement of FS fairly and equitably. It implies that FS can expand the amount and nature of food utilization and availability, economic access, and stability to influence EG. In addition, this study also explains that the availability of land and climatic factors as endowment factors could increase the number of jobs. It impacts increasing people's income which leads to EG.

Third, one study does not support a relationship between FS and EG from the aspect of Natural Resources Resilience **[13]**. Empirical results were obtained from a meta-regression analysis of the relationship between EG and the Food Insecurity Experience Scale (FIES). Statistical results showed that the natural resources resilience factor has no statistically significant coefficient.

Utilization Viewpoint

[39] explained that utilization is generally understood in how the body uses different supplements in food. Adequate personal intake of energy and supplements results from careful consideration and care of practices, food preparation, dietary diversification, and food flow within the family. Joined with the tremendous natural use of food consumed, this determines people's health. Based on these, 2 of 22 previous studies looked at the connection between utilization dimensions on FS and EG. There are two empirical results where one researcher supports a correlation between utilization and EG, and the other explained that there is no relationship. A study from **[6]** proved a connection between utilization and EG. His research proved that high malnutrition rates could cause a gross domestic product (GDP) loss by 4 to 5 percent. Furthermore, micronutrient's financial expenses also diminish GDP by 0.7-2% in most non-industrial nations. Therefore macronutrients and micronutrients cause global losses in economic productivity that account for more than 2-3% of GDP. It will prompt stale monetary development over the long haul. Hence, a suitable FS system is fundamental for all nations. While the research results from **[27]** refuted this, his empirical study proved that utilization has a significant negative impact on EG. The reason is that EG in developing countries creates inequality. According to this study, the FHSI score is plotted against the degree of national inequality (measured by the Gini coefficient). One hundred twenty-six people found a subtle negative correlation between the variables (correlation coefficient 0.071). However, when low-income countries are excluded, the strength of the negative correlation increases significantly. For example, the correlation coefficient is 0.285 in nations with a per capita GDP more significant than the US $ 20,000 and 0.426 In countries with a per capita GDP of more than the US $ 25,000. Finally, among the highest-income countries, countries with a per capita GDP of at least US $ 35,000, the correlation coefficient is an abnormally stable negative value of 0.97. The differences seem to weaken the evident ability of citizens to make food safe.

Research Priorities and Gaps

Our review revealed the shortcomings of research on the connection between FS and EG. In searching the relevant literature, it is clear that there is a gap in studies of the relationship between FS and EG at the global level. For example, our search revealed only three review-related articles **[24]**, **[27]**, **[34]** that focused on the worldwide level. Therefore, it is not easy to generalize a correlation between FS and EG. Furthermore, most studies have looked at this relationship only at the country level. So that in the future, it is necessary to increase the population of countries at the global level to prove this relationship empirically. Furthermore, we identified that previous studies have mainly used qualitative methods to show the linkages between FS and EG. It was recorded that 12 of the 22 previous studies used qualitative methods to explain this relationship **[6]**, **[10]**, **[12]**, **[26]**, **[28]**, **[30]**, **[31]**, **[33]**, **[17]**, **[34]**. Moreover, nine studies used quantitative methods **[7]**, **[8]**, **[24]**, **[9]**, **[11]**, **[13]**, **[25]**, **[14]**, **[29]** and the only one uses mixed methods **[27]**. So there is an opportunity to expand quantitative or mixed-based research methods in the future. Finally, most studies only focused on the correlation between FS

and EG based on one or a few dimensions of FS. Very rarely does a holistic research approach using global FS indicators such as the Global FS Index (GFSI) and Global Hunger Index (GHI), or other global FS indices. From the review results, only four studies used global FS indicators **[8]**, **[24]**, **[28]**, **[30]**. So for further research, it can be expanded to use the global FS index to see a causal relationship between FS and EG.

CONCLUSION

The results of the literature review show an empirical gap between economic growth (EG) and food security (FS). Although as many as 76.92% of the investigators supported an association between EG and FS, we cannot rule out the 19.23% of the results of other investigators who did not support this result. There are research gaps to be developed in the future, one of which is that most of the previous researchers only used the availability variable as a proxy for food security (FS). In contrast, two more variables determine FS (affordability and utilization). In addition, the object of research is still limited to the national level; there are still few that raise cases at the global level. Therefore, empirical evidence is needed to address this gap. Next, we recommend adding a poverty variable to examine the relationship between EG and FS. It is essential because low food security increases hunger levels (poverty).

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Social responsibility in reducing food losses and waste in the Slovak Republic: the role of policies – the responsibility of all

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ABSTRACT

The study aimed to point out the calls of the European Commission to the social responsibility of the solution of food losses and waste, to evaluate the current state of the researched issues in the Slovak Republic, to point out the trends, and propose measures to improve the situation of the food losses and waste on the poultry meat market in the Slovak Republic. The scientific hypotheses were established. A questionnaire survey was used to obtain primary data. The research object was households and agricultural enterprises of broiler chicken farming (poultry farms) in the Slovak Republic. Data from questionnaires completed by households and poultry farms were examined and processed by the sorting method. Cumulative totals, intervals, and percentage ranges were calculated in each response class. The obtained data for individual objects of research were processed by sorting using Microsoft Word tables – Excel, Office 2016. The chi-square test ($\chi 2$ test) with a contingency table according to the procedure of Social Science Statistics was chosen for hypothesis testing. The SAS program was used for statistical evaluation of the results and answers of the respondents from the questionnaires. The research shows that food losses in Slovak households were up to 40% and on poultry farms at 6.8%. Mould and rot were the most common causes of food degradation. Mortality during breed has been recorded as a cause of food waste in poultry farms. A statistically significant difference ($p \leq 0.001$) was found in the quantity of food losses between gross household income per family member and month. Statistically, no significant difference ($p > 0.05$) was found between the numbers of family members. The proposals were recommended to improve the solution of reducing food losses and food waste in households and poultry farms. Based on the application of a practical approach of households and poultry farms to reduce food losses and support innovative solutions, it is possible to achieve gentle practices in ensuring the security of nutrition, food production, social and economic sustainability as well as environmental protection in the Slovak Republic.

Keywords: food loss and waste, cause of origin, household behaviour, poultry farm, innovative approach

INTRODUCTION

Society's approach to food losses and food waste

Food waste is an unsustainable system of food production and consumption. Food and Agriculture Organization of the United Nations drew attention in 2013 to food waste, which is currently facing an environmental, economic, and social problem **[1]**. Approximately 1.3 billion tonnes of foods are globally degraded or end up as waste, representing a significant share of total food production **[2]**. The United Nations has also included food waste in one of its 17 Sustainable Development Goals (SDGs). Objective 12.3 focuses explicitly on reducing them and the whole production and supply chain. The aim is to halve global food waste per capita by 2030 **[3]**. The European Commission has recognized food waste as a priority area of the European Union's circular economy action plan **[4]**, which aims at a common approach to measuring food waste through appropriate indicators, promoting commitment, knowledge acquisition, and taking over best practices as well as improving legislative measures **[5]**. The European Union's announced strategy for an integrated farm-to-table system means focusing on all stages of the food chain, including food waste **[6]**. The adopted strategy aims to demonstrate how food waste can be transformed into valuable resources and how to create innovations and incentives to reduce it by 50% of its total

weight by 2030 and contribute to the transition to a circular economy **[7]**. The value of food waste, which can be avoided, is estimated based on research in several European countries and ranges between 3.2 and 6.1 €.kg^{-1}. In addition, the European Commission's Joint Research Center (JRC) has proposed a quantification procedure to prevent food waste and reduce environmental impact and economic savings **[8]**. Constructive discussions in academia on food losses from an economic and environmental perspective are taking place in academia. Researchers are also working on macroeconomic strategies that are useful in addressing this issue **[9], [10]**. Researchers are far from reaching a consensus on some topics in discussions on nutrition strategies. Most solutions to food losses are in studies focused on quantity and its impact on the environment **[11]**. Input-output analyses **[12]** are often used for these studies, especially a life cycle assessment **[13]**. These interdisciplinary efforts increasingly point to the complexity of dealing with food waste **[14]**. Households are identified as the sector with the characteristics that contribute most to food waste. Many studies indicate that the food waste of households can be avoided. Estimates suggest that 50 – 60% of losses and waste in the food supply chain across the European Union are generated by households and retails **[15]**, **[16]**. The European Fusions project states that about 60% of the waste generated by consumers (corresponding to 32% of all food waste) is an avoidable waste **[17]**. Estimates of unnecessary food waste from total household food waste vary from country to country **[18]**. The generation of food waste in households cannot be viewed in isolation from the other stages of the food chain, i.e. from the production phase to the consumption phase. Household food waste can also result from measures taken further down the food chain, such as misunderstood date labels, sealable packaging, and marketing strategies such as bulk packaging and special offers **[19]**.

In connection with the above, our research aimed to point out the European Commission's calls for social responsibility to address food losses and waste, evaluate the current state of researched issues, point out trends and propose measures to improve the situation of food losses and waste in the chicken meat market in the Slovak republic.

Scientific Hypothesis

Established scientific hypotheses – households and food waste

The number of members in the household affects the generation of food waste.

The age of household members affects food waste generation.

The income amount of household members affects the generation of food waste.

The amount of household expenditures on the purchase of chicken meat contributes to food waste.

Established scientific hypotheses – poultry farms and food losses

The poultry farm size affects the generation of food losses.

MATERIAL AND METHODOLOGY

Samples

A questionnaire survey was used to obtain primary data. The research object was households, and primary poultry farms focused on breeding broiler chickens in the Slovak Republic.

Instruments

Questionnaire survey.

Laboratory Methods

A questionnaire survey, the method of questioning, was used to solve research tasks. Our evaluation material was questionnaires and the respondents' answers to the questionnaire. The questionnaire contained 14 questions, to which the respondents answered numerically, verbally, or by supplementing the answers. The questions were open to respondents.

Description of the Experiment

Sample preparation: The sorting method examined and processed data from questionnaires completed by households and poultry farms. Cumulative totals, interval, and percentage range in the individual response classes.

Number of samples analyzed: Respondents from the addressed households returned 255 completed questionnaires, representing a 56% return of the questionnaires. The Slovak households and poultry farms survey took place from April 2021 to January 2022. Two companies completed the questionnaire intended for poultry farms for 15 fattening periods, which have a capacity of 500 thousand pcs of broiler chickens and 20 thousand pcs of broiler chickens for the fattening period.

Design of the experiment: Household research was focused on finding access to food losses and identifying foods in their households that are subject to losses. 2 household research factors are important for household research objects, namely the number of household members, while for the answer more members or fewer members the average number of household members was a clue to the answer 2.94 according to the Statistical

Office of the Slovak Republic from 2019 **[20]**. The second factor is income, while for the answer more income or less income was a clue to the answer the average income of € 577.50 per person and month, which results from the income of € 6,930 per person and year according to the Statistical Office of the Slovak Republic from 2019 **[20]**. Research in the poultry farms was focused on identifying the root causes of food losses with the possibility of addressing effective measures to reduce them.

Statistical Analysis

The data obtained from the questionnaires for individual objects were examined and processed by sorting using Microsoft Word tables – Excel, Office 2016. Cumulative totals, interval a percentage range. The SAS package, version 8.2, was used to statistically evaluate the results between household members and food waste and between total household income and food waste. Statistical evaluation of the results was performed based on descriptive characteristics by groups according to the values of a certain quantity ($\bar{x}$ – arithmetic mean, SD – standard deviation) and t-test for statistical significance of the difference between the groups.

The Chi-square test ($\chi 2$ test) with a contingency table was chosen to test the hypotheses. This test is suitable for comparing quantitative quantities. It is used to determine whether the abundances in the individual categories are distributed randomly, naturally, or whether a certain stimulus influenced the distribution of abundances in the individual categories. Based on this test, the frequency of occurrence is tested, and the dependence between the variables is determined. The Chi-square value of the test is compared with the theoretical Chi-square distribution to determine the probability of obtaining a random value. This probability represents the value of significance. The frequencies are significantly different if the significance value is lower than the significance level. In the calculation, we set *p*-value $\alpha = 0.05$. We tested the hypotheses in the same way: we determined a dependent and an independent variable. We have formulated hypotheses (H_0 - there is no statistically significant connection, H_A - there is a statistically significant connection). Reporting the Chi-square test results included the result of the statistical evaluation with Yates correction and the achieved *p*-value of statistical significance. We performed the calculations according to a freely accessible procedure published on the Social Science Statistics website.

We established the conclusions of the findings:

a) if the result of $\chi 2$ is $p \leq 0.05$, hypothesis H_0 was rejected, and hypothesis H_A was accepted,

b) if the $\chi 2$table >0.05, hypothesis H_0 was accepted, and hypothesis H_a was rejected.

By processing and evaluating the obtained research results, food losses and the main causes of their generation in the investigated objects were identified and characterized.

RESULTS AND DISCUSSION

Food losses and waste in households and poultry farms, causes of their generation

We used the Chi-square test to test the established hypothesis that the number of members in the household affects the generation of food waste. Contingency Table 1 provides information on the observed cell totals (expected cell totals) and the statistical evaluation according to the Chi-square test for each cell.

Table 1 Contingency table testing the hypothesis of the influence of the number of household members on the creation of food waste.

Number of members in the household	The number affects members	The number of members does not affect	Boundary row totals
More than 2.94	143 (127.5) [1.88]	112 (127.5) [1.88]	255
Less than 2.94	112 (127.5) [1.88]	143 (127.5) [1.88]	255
Boundary sums of columns	255	255	510 (total sum)

Source: Own research.

According to the chi-square test with Yates correction, the result of the statistical evaluation is 7.0588, and the *p*-value is 0.007888. A statistically significant dependence of $p \leq 0.05$ exists between the number of household members and food waste, i.e. food waste arises from a dependent variable.

Machate's [24] study shows a positive but weak correlation between family size and the amount of food waste generation per household. The results confirm the previous findings **[21]**, **[22]**, **[23]**.

We used the Chi-square test to test the established hypothesis that the age of household members affects food waste generation. Contingency Table 2 provides information on the observed cell counts (expected cell counts) and statistical evaluation according to the Chi-square test for each cell.

Table 2 Contingency table testing the hypothesis of the effect of age of household members on food waste.

Age in years	Age affects	Age does not affect	Boundary row totals
Up to 20	4 (51.00) [43.31]	251 (204.00) [10.83]	255
20 - 35	80 (51.00) [16.49]	175 (204.00) [4.12]	255
36 - 49	80 (51.00) [16.49]	175 (204.00) [4.12]	255
50 - 65	63 (51.00) [2.82]	192 (204.00) [0.71]	255
Over 65	28 (51.00) [10.37]	227 (204.00) [2.59]	255
Boundary sums of columns	255	1020	1275 (total sum)

Source: Own research.

The statistical evaluation results according to the Chi-square test are 111.8627. Value $p \leq 0.00001$. There is a statistically significant dependence between age and food waste at $p \leq 0.05$, i.e. food waste arises from a dependent variable.

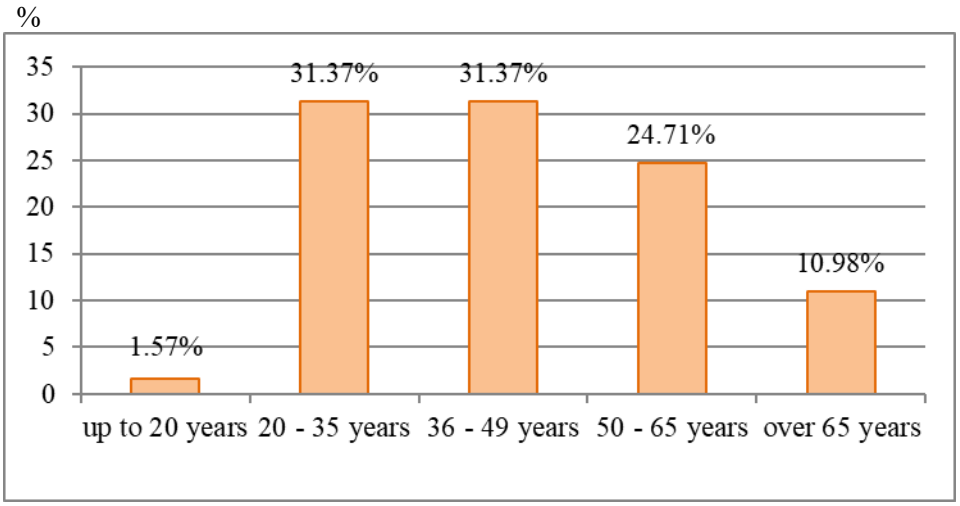

Figure 1 Involvement of household respondents to research by age. Source: Own research.

The total number of involved household respondents by age category in the survey was 255 (Figure 1). The most significant proportion were household respondents of the specified age category 20 - 35 years, i.e. 80 (31.37%) and 80 (31.37%) in the age group 36 - 49. This was followed by the age category of respondents of the household 50 - 65 years in 63 (24.71%). Respondents over 65 accounted for 10.98%, i.e. 28, and the least respondents participated in the research of the age group up to 20 years, only 4 (1.57%). It is also known from other research carried out in this area that respondents over the age of 20 to 35 are the most involved in similar research. An important category in assessing food waste is young people who, unlike seniors, have several characteristics that affect their food waste behaviour, such as education, lifestyle, shopping habits, and eating and storing food (especially when studying or working away from home). The results confirm an appositive and strong correlation between age and amount of food waste generation at a regression coefficient of 0.7 **[24]**.

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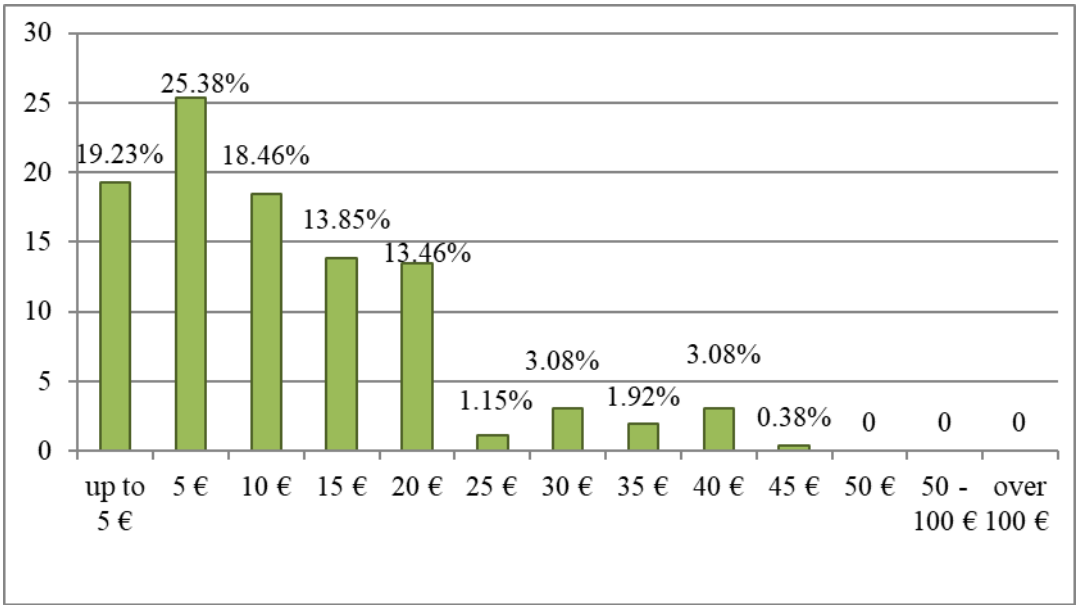

Figure 2 Household budget expenditure related to food waste per member and month in €.
Source: Own research.

Two hundred fifty-five household respondents answered the amount of budget expenditure per household member and month (Figure 2). When asked about household budget expenditure related to food waste and throwing food into the trashcan. Respondents indicated budget expenditures related to food losses in their household per member and month at most 66 (25.88%) at € 5, followed by food waste up to € 5 marked by 50 respondents (19.61%) and 48 household respondents (18.82%) food waste of € 10. Respondents reported food losses at almost the same level as € 15 and € 20, i.e. 36 (14.12%) and 34 (13.33%). Other respondents described food waste as higher but lower. For € 30 and € 40 of budget expenditures from the budget related to food waste per 1 member and month in €, respondents also indicated 7 (2.75%) and an even lower number of respondents, 4 (1.57%) indicated expenditures related to food waste of € 35 and 2 respondents (0.78%) of € 25 per member and month. The highest amount of household expenditures from the budget related to food waste per 1 member and month was given by one respondent (0.39%). Other amounts mentioned above of household expenditures from the budget pertaining to food waste per 1 member and month were not indicated by any respondents in the questionnaire. If we conducted research to address household budget expenditures related to food waste per member and month on these days after January 2022, the results would be different, higher as food prices have risen.

Tables 3 and Table 4 show a statistical evaluation of the impact of the number of members and the amount of income on household budget expenditures related to food waste per 1 household member and month. We found (Table 3) the average value of expenditures per 1 member and month in € from the household budget related to food losses € 11.37 with the number of household members more than 2.94 and € 12.78 with the number of household members less than 2.94. The difference in budget expenditures related to food waste per member and month between households with more than 2.94 members and households with less than 2.94 members was not statistically significant (p >0.05).

Table 3 Statistical evaluation of expenditure per member and month in **€** from the household budget related to food waste among the number of members in the household.

Number of members in the household	n	$\bar{x} \pm$ SD	t-test
More than 2.94	143	11.37 ±9.08	
Less than 2.94	112	12.78 ±9.55	0.2366

Note: n - multiciplity, $\bar{x}$ - mean, SD - standard deviation, 0.2366 - p value of the t-test (p >0.05), 1.1865^{-}) - a statistically significant difference. Source: Own research.

However, evidence from a 2019 survey showed that households the one adult and minor members could not afford lunch to satiety every other day. Up to one-third of the respondents involved in the research indicated this situation. On the other hand, households with two adults and one or two children are the least affected by this situation, which means that only about 6% of households with several members cannot afford lunch the next day **[25]**.

We used the Chi-square test to test the established hypothesis that the income amount of household members affects the generation of food waste. Contingency Table 4 provides information on the observed cell counts (expected cell counts) and the statistical evaluation according to the Chi-square test for each cell.

Table 4 Contingency table testing the hypothesis of the impact of members' income amount on food waste generation.

Income of members in the household	The number affects members	The number of members does not affect	Boundary row totals
More than 577.5 €	189 (127.5) [29.66]	66 (127.5) [29.66]	255
Less than 577.5 €	66 (127.5) [29.66]	189 (127.5) [29.66]	255
Boundary sums of columns	255	255	510 (total sum)

Source: Own research.

According to the Chi-square test, the statistical evaluation results are 118,6588 and $p \leq 0.00001$. There is a statistically significant dependence between household income and food losses at $p \leq 0.05$, i.e. food waste arises from a dependent variable.

We found (Table 5) the average value of household expenditures per 1 member and month of € 13.08 from the household budget related to food waste at the income of more than € 577.50 per member and month. If the household income was less than € 577.50 per 1 member and month, the average value of expenditures in such a household per 1 member and month from the household budget related to food waste was lower, € 6.89. The difference in budget expenditures related to food waste per member and month between households with more than € 577.50 and households with less than € 577.50 per member and month was statistically significant ($p < 0.001$).

Table 5 Statistical evaluation of expenditure per member and month in € from the household budget related to food waste between income per member and month in the household.

Household income	n	$\bar{x} \pm SD$	t-test
More than 577.50 €	189	13.08 ± 8.79	
Less than 577.50 €	66	6.89 ± 4.73	0.001

Note: n - multiciplity, $\bar{x}$ - mean, SD - standard deviation, 0.001 - p-value of t-testu ($p \leq 0.001$, 4.5177^{+++}) - a statistical significant difference.

Within the European Union, households in Slovakia generate less food waste than households in the other Member States. According to statistics, most per capita food ends in the Netherlands' trashcan. Food waste is the least generated in Greece, Malta, but also in the Czech Republic. The value of avoidable food waste is estimated based on research carried out in several European countries in 2020 and ranges between 3.2 and 6.1 €.kg^{-1} **[25]**. The complexities of food waste generation in its entirety are a subject of the social and economic profile of the generator. Evidence from Gustavsson et al. **[26]**, one of the leading global authors in food loss management, shows that food waste generation increases proportionally with the levels of development. As a result, developed countries generate more food waste than their developing counterparts **[24]**.

Respondents mentioned the foods that represent the biggest waste in their household, which are the most critical, and at the same time stated the percentage of their waste. This means which foods are most critical in their household. Respondents identified fruits, vegetables, dairy products, meat products, prepared food, pastries, bread, milk, and meat as the most critical foods. The enormous waste reported by household respondents with a maximum percentage of 40% of all food waste is fruit, vegetables, pastries, meat products for temporary storage, dairy products, especially cheese, yoghurt, cream, and prepared food. Household foodstuffs were most often degraded and damaged by fibrous microscopic fungi (mould), rot, fermentation, drying, and hardening (change in sensory properties), expiration date, large volume or quantity of prepared food - uneaten residue discarded or inedible, long storage time and ageing in stocks. Respondents in the questionnaire stated deterioration and damage of several types of foodstuffs.

Studies **[26]** and **[27]** argue that consumers in developed countries buy more food than they need. They support that high household income is proportional to increased food waste production. In contrast, consumers in

developing countries buy smaller quantities of food with each purchase. This process affects the way food is prepared or cooked and, consequently, the amount of food that is disposed of as waste. Households prepare and serve more significant portions of food than they can consume, leading to more residues **[28]**. Household income affects not only food waste generation but also waste generation in its broadest sense **[29]**, **[30]**, **[31]**, **[32]**.

Machate [24] in the study, presents a strong, negative correlation between income in the household and the quantity of food loss generated by the households in the five selected suburbs in the city of Tshwane (South Africa). These results imply that the household's monthly income, the lesser the quantity of food waste generated. A significant number of possibilities can be attributed to these findings: the educational levels, employment status, ages, and other demographic factors of individual household members. The findings of this study are contrary to most previous studies. However, these results are consistent with the findings of **[33]**, who found no correlation between income levels and the amount of food wasted. Machate **[24]** states in his study that looking at the income level can reveal the living standards of the members of the households, which in his research meant that more than 50% of the sampled households lived below the poverty line. The author also states that the employment status of individual household members influences the monthly household income, which ultimately has proven to influence food waste generation directly.

We used the Chi-square test to test the established hypothesis that the amount of household expenditure on the purchase of chicken meat contributes to food waste. Contingency Table 6 provides information on the observed cell counts (expected cell counts) and the statistical evaluation according to the Chi-square test for each cell.

Table 6 Contingency table for testing the hypothesis of the impact of the household expenditure on the purchase of chicken meat and chicken products on the generation of food waste.

Expenditure on the purchase of chicken meat (€)	Expenditures affect	Expenditures do not affect	Boundary row totals
0	17 (56.30) [27.43]	272 (232.70) [6.64]	289
up to 10	38 (49.68) [2.74]	217 (205.32) [0.66]	255
10 - 15	68 (49.68) [6.76]	187 (205.32) [1.64]	255
15 - 20	59 (49.68) [1.75]	196 (205.32) [0.42]	255
Over 20	73 (49.68) [10.95]	182 (205.32) [2.65]	255
Boundary sums of columns	255	1054	1309 (total sum)

Source: Own research.

According to the Chi-square test, the result of the statistical evaluation is 61.6474 and a p-value $p \leq 0.00001$. There is a statistically significant dependence between the expenditure incurred for buying chicken meat and food waste at $p \leq 0.05$, i.e. food waste arises from a dependent variable.

A total of 255 respondents answered the question of the set household expenditure from the budget for purchasing chicken meat and chicken products per member and month in € (Figure 3). Of most respondents involved in the research, 73 (28.63%) reported expenditures on chicken meat and chicken products of over € 20 per member and month. Other respondents, numbering 68 (26.67%), indicated costs of chicken meat and chicken products per month per member for € 10 to € 15, but also € 15 to € 20 (59 respondents, 23.14%). Respondents followed this with lower expenditures below € 10 (38 respondents, 14.90%) and € 0 (17 respondents, 6.67%). In the case of expenses on chicken meat and chicken products in the amount of € 0, the respondents also stated the reason that they are vegetarians (4 respondents) or have their broiler chickens (6 respondents), or the respondents eat in the common dining room during working days where chicken meat is often prepared food, so they do not buy it (5 respondents), respectively. 2 respondents do not purchase chicken meat because its smell hinders it.

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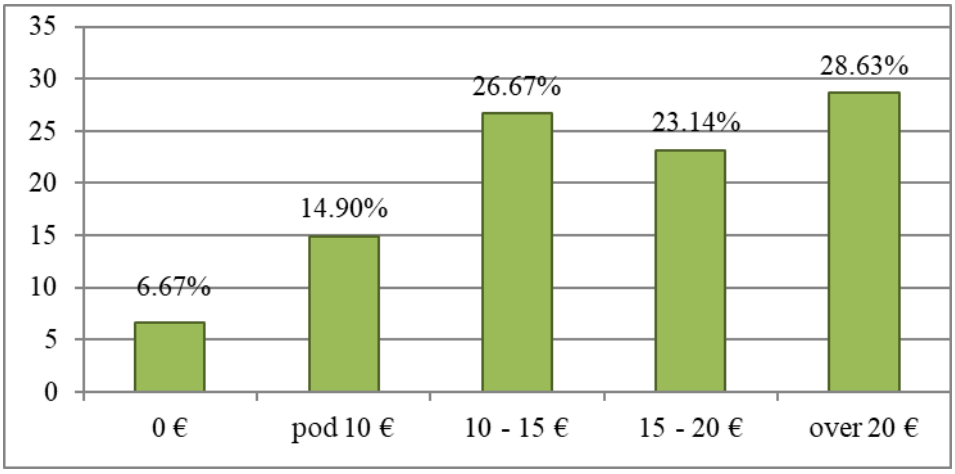

Figure 3 Household expenditure from the budget for buying chicken meat and chicken products. Source: Own research.

The increased demand for proteins from animal sources in consumer diets is related to urbanization growth, living standards, diet, livestock production growth, and consumer prices. The affordability contributed to making poultry meat of choice for consumers worldwide, especially in developing countries **[34], [35]**. Chicken meat and chicken products are globally popular, which can be explained by the fact that quality chicken products are available at affordable prices, although their production costs may vary [**36],** [**37], [38]**. Chicken meat is a popular type of meat; any religion does not limit it in comparison, e.g. to pork. It is characterized by relatively high nutritional value and dietary properties. It is a good source of protein and is low in fat and cholesterol. It is affordable for the consumer and is easy to cook. Respondents of households involved in the research also took a stand on the amount of losses of chicken meat from prepared food on a plate, i.e. uneaten chicken meat and skinless chicken per 1 household member per month, shown in Table 7.

For some respondents, 44 (17.25%) out of all household respondents 255, it was a problem to answer a numerical value in terms of the amount of chicken meat lost as part of the prepared dish on a plate without skin and bones. The individual questionnaires of the household respondents stated that they like chicken meat, so they also buy it and prepare it culinary, or they do not like chicken meat, but eat in the staff canteen, where there is very often chicken meat with side dishes.

Respondents also reported that children ate at home during Covid-19 online learning. The dishes did not like the prepared meals very much, including the meal with chicken meat. This may be related to a different cooking process or different food additives in the preparation of meals. This group of respondents stated in the questionnaire either a comment or an answer: I can't estimate, and I don't know.

Table 7 Amount of chicken meat waste from prepared food on a plate.

The loss of chicken meat from the prepared food on a plate	Percentage of respondents to the answer	The loss of chicken meat from the prepared food on a plate	Percentage of respondents to the answer
Various comments	17.25%	50 g	3.92%
0	59.61%	70 g	0.39%
Minimum	3.14%	100 g	3.92%
10 g	1.57%	150 g	2.35%
15 g	1.18%	200 g	0.78%
20 g	2.35%	250 g	1.18%
30 g	0.78%	300 g	0.39%
		500 g	1.18%

Source: Own research.

The overwhelming majority of 152 household respondents (59.61%) out of all involved respondents stated that the value of chicken meat waste from prepared food on a plate was zero. In this group, some respondents from the city and the municipality indicated that they keep a cat or dog at home, so they have no waste. The minimum losses of chicken meat (stated by the respondents) from the prepared food on a plate with chicken meat without a numerical value were expressed by eight respondents (3.14%). Other respondents reported waste of culinary chicken designed on a plate from 10 g (4 respondents, 1.57%) to 500 g (3 respondents (1.18%). Of the

44 respondents (17.25%) who expressed losses of chicken meat prepared and presented on a plate in numerical value, the most (10, 3.92%, and the same) stated 50 or 100 g.

The current worldwide production of broiler chickens is approaching 60 billion pcs per year **[39]**. Broiler chickens are transported for slaughter from their geographically dispersed farms. On-farm harvesting uses either manual or mechanical harvesting, placing them in crates, then loaded onto vehicles and transported to slaughterhouses **[40]**. The chickens are unloaded with crates from the cars upon arrival at the slaughterhouse and kept in temporary housing set up for various lengths of time or killed immediately **[41]**. Handling broiler chickens before killing them causes varying degrees of stress that threaten their welfare **[42]**. Collection and broiler chickens in poultry farms and handling are considered the most frequent injuries. The animals then suffer during transport to the slaughterhouse **[43]**.

We used the Chi-square test to test the established hypothesis that the size of a poultry farm affects the generation of food losses. Contingency Table 8 provides the following information on the observed cell counts (expected cell counts) and statistical evaluation according to the Chi-square test for each cell.

According to the chi-square test with Yates correction, the result of the statistical evaluation is 0.5333, and the *p*-value is 0.465209. There is no statistically significant dependence of $p > 0.05$ between poultry farms and food losses by the chicken mortality, i.e. food losses arise from an independent variable.

The respondents of the poultry farms were to comment on the losses of chickens caused by death during each fattening period from the housed dormitory and report the losses of chickens to deaths during fattening, harvesting, and poor health (Figure 4).

Table 8 Contingency table for testing the hypothesis of the impact of the size of poultry farms on generating food losses.

Poultry farms	Losses generate	Losses do not generate	Boundary row totals
Large enterprise	9 (7.50) [0.03]	6 (7.50) [0.03]	15
Smaller enterprise	6 (7.50) [0.03]	9 (7.50) [0.03]	15
Boundary sums of columns	15	15	30 (total sum)

Source: Own research.

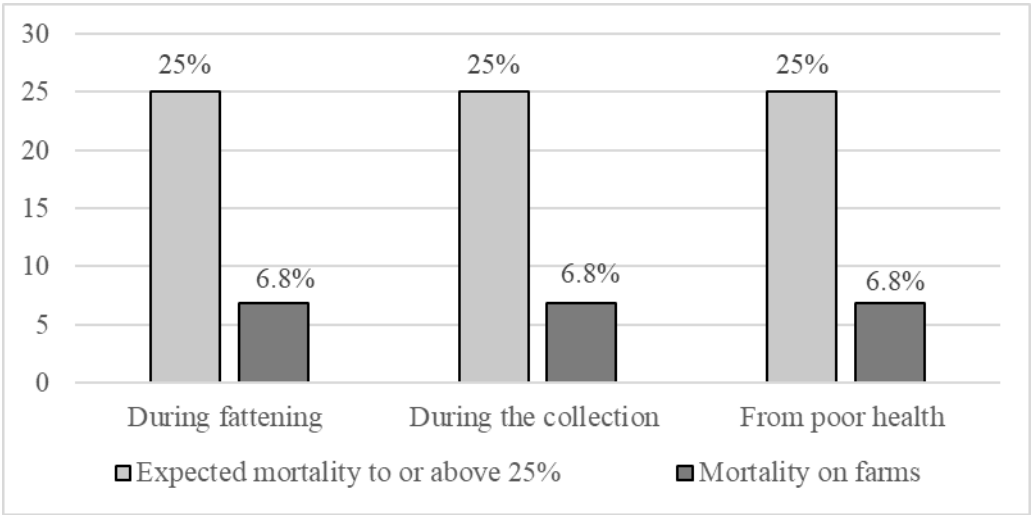

Figure 4 Respondents of poultry farms are aware of the causes of food losses - mortality. Own research.

We determined expected losses of up to 25% and over 25% as a tool for respondents. The poultry farms were also asked to comment on food losses during the transport of broiler chickens in the questionnaire. The addressed poultry farms order an animal transport service when they fill the hall with day-old chicks or harvest broiler chickens in the hall at the end of the fattening period. They are not monitoring the mortality of broiler chickens during transport.

Of the total number of broiler chickens that were filled with halls in poultry farms during fattening periods, the mortality losses of chickens averaged 6.8%. These losses evaluated for individual fattening periods ranged from 6 to 8%. Interestingly, lower mortality losses of broiler chickens were in a larger large-scale broiler chicken farm (6.44%, i.e. 30,600 to 42,375 pcs per fattening period). This agricultural company has been operating for many years and is managed by experts with university degrees in agriculture and many years of experience.
In a smaller poultry farm, larger losses were reported by respondents, with 6 to 8% (1,228 to 1,652 pcs per fattening period). In a smaller poultry farm, these losses represent an average of 7.33% (1,500 pcs per fattening period).

All respondents reported a generation of losses due to broiler chicken mortality altogether during the fattening periods. Respondents reported the same values of broiler chicken mortality during fattening periods in the hall, harvest, and ill-health. It is known from the literature that broiler chickens during harvest experience intense stress, similar to transport. This issue in poultry farms requires more attention in research.

Poultry farms must comply with the legislative measures for the protection of this type of livestock and apply the principles of welfare under the five fundamental freedoms and the legislative measures of the hygiene packages, and despite strict compliance, mortality occurs. Three interrelated factors are important for the breeding of broiler chickens: genetics, breeding conditions, and nutrition. Their effect in small deviations may have a different effect on the viability of the placed animals in individual fattening periods if they are not dangerous infectious diseases. Some manipulations with broiler chickens create a stressful behavioural pressure that results in a deviation from their natural behaviour. One of these manipulations is the collection of broiler chickens in the hall, described as a critical stage in breeding. In large-scale farms, it is probably challenging to divide the mortality of broiler chickens on the farm according to the cause, as we identified in the questionnaire. This issue is an open question for future research. Respondents reported an average loss of 54,623.67 kg caused by broiler chicken mortality on farms from the planned live weight when collecting chickens for one fattening period. The stated average amount of losses of broiler chicken mortality arises in poultry farms with a capacity of 510 to 565 thousand and a capacity of 20.21 to 20.66 thousand pieces. In a poultry farm with a higher broiler chicken breeding capacity, the losses of chicken mortality per fattening period ranged from 76,500 to 105,937.5 kg, and with a lower broiler chicken breeding capacity ranged from 3,070 to 4,130 kg. These losses of broiler chickenst the farm's economy, increase its costs and reduce profits. In line with the measures taken to reduce food losses, this issue is suitable for solution in future research from a social, economic, and environmental point of view.

A published study **[44]** recommends an appropriate procedure for handling broiler chickens at harvest in the hall to reduce mortality and suffering from bleeding, bruising, and fractures. Mortality is observed throughout the breeding period and of varying intensity in poultry farms **[45]**. The procedure is developed to calculate the weekly mortality, taking the number of dead broilers per week. Food losses (meat) from broiler chickens are related to the lack of technical equipment at the slaughterhouse level for the recovery of the edible part of chicken meat **[46]**. The yield of chicken meat may also be related to processing costs that are too high to allow commercialization or feed costs. There are currently major concerns about welfare, hygiene, and disease control, resulting from tremendous genetic pressure to increase meat production. Genetic pressure to improve the productive performance of animals adversely affects their well-being and innate immunity, and thus tolerance to disease. Genetic selection achieves improved breeding, disease control, and nutrition handling practices **[47]**. The transport of broiler chickens is considered a critical point in the chicken meat production chain **[48]**, which is explained concerning the possible consequences for the welfare of broiler chickens **[49]**.

It is recommended to address the process of reducing food losses in households:

- to buy food in retail in smaller volumes and more often without creating large stocks,
- to establish food sales closer to the consumer,
- reduce the amount of the prepared meals and submitted portions of the food,
- strictly control food labelling during purchase and storage,
- show more respect for the produced food (bread),
- strengthen legislative measures to change people's approach to reducing food waste,
- stimulate consumer education.

It is recommended to address the process of reducing food losses by chicken mortality in poultry farms:

- application of current knowledge based on science and research in the protection of broiler chickens (welfare),
- compliance with good poultry farm practices, including nutrition and safe feed,
- addressing more environmentally friendly practices through the handling of broiler chickens.

Our research on food losses and waste in Slovak households and poultry farms supports the definition of food waste reduction strategies according to the European hierarchy for waste prevention and management, which sets waste prevention as the preferred option. Strategies and targets for the prevention and recovery of food waste are

important, including waste management and food safety from an economic, social, and environmental point of view, to optimize the efficiency and effectiveness of data collection, evaluation, and enforcement.

The evaluated research results processed in the presented study are beneficial for the further development of science and have their use in practical conditions in the field of measures taken to prevent and reduce food losses.

CONCLUSION

The research shows that in Slovak households, the food waste is up to 40% and in poultry farms the average of the food loss is 6.8%, which is primarily caused by the mortality of broiler chickens. Household budget expenditures related to food waste per member and month were most often reported at € 5 (25.88%), with a small difference of up to € 5 (19.61) or € 10 (18.82%), € 15 and € 20 (14.12% and 13.32%), respectively. A statistically significant difference ($p \leq 0.001$) was found in the amount of food waste between the amount of household income per family member and the month and no statistically significant difference ($p > 0.05$) between the number of family members. Foods that generate the most household waste include fruit, vegetables, pastries, meat and dairy products, and prepared meals. Mold and rot were the most common causes of food spoilage. Broiler chicken mortality losses during breeding have been recorded as the cause of food losses in poultry farms. Suggestions for improvement were recommended to address the process of reducing food losses and waste in households and farms. By applying a practical approach of households and poultry farms to reduce food losses snd waste and supporting innovative solutions, it is possible to achieve gentle practices in ensuring the security of nutrition, food production, social and economic sustainability, and environmental protection in the Slovak Republic. Not only policy makers, food producers, and retailers, but above all households must realize that with the current economic, environmental, and geopolitical changes, it is not possible to generate as many food losses and waste as they have done so far.

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The effect of storage conditions on the microstructure of sterilized canned meat

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ABSTRACT

The article presents the results of studies of changes in the microstructure of the meat system as a whole and its protein component during freezing, subsequent defrosting, and storage of canned meat. Microstructural analysis of the prototypes showed the presence of several types of destruction of muscle fibers, loosening of collagen fiber bundles, and the formation of multiple cavities due to the action of ice crystals. The main components of sterilized canned meat had new characteristics after thawing, such as decreased transverse striations of muscle tissue fibers, loosening of myofibrils, changes in the size and shape of sarcomeres, violation of sarcolemma integrity, and multiple fiber fragmentation with the formation of a fine-grained protein mass. Freezing did not lead to a decrease in the content of the high-molecular-weight protein fraction of the nitrogen system, the ratio of the peptide fraction content to the residual nitrogen remained equal to 5.2. However, the ratio of non-protein nitrogen to total nitrogen decreased by 1.8 times due to the destruction of low-molecular-weight nitrogen under the action of ice crystals. The dynamics of the eh values of control and experimental canned food samples during storage indicated the loss of oxidative stability of the protein system of the samples subjected to freezing. Based on the results, we would like to recommend that logistic organizations sort and confirm canned meat safety and quality requirements after thawing in the case of unforeseen circumstances.

Keywords: canned meat, model, freezing, storage, microstructure, physicochemical processes

INTRODUCTION

The life cycle of sterilized canned meat includes a storage phase. Storage of such products is a long and complicated process, requiring conditions to maintain quality and safety. According to the regulatory documents of Russia, sterilized canned meat is stored at a temperature between 0 and 20 °C and not higher than 75% humidity. Such conditions ensure the stability of quality indicators for 1 – 5 years, depending on the technological features of obtaining canned meat. Sterilized canned meat is a closed system where external access of oxygen to the meat system is excluded. Therefore, the system exchanges only thermal energy with the environment; an important role in this exchange is assigned to the transportation temperature and storage temperature.

Histology and sensory analysis methods can effectively resolve an issue relating to the quality of raw materials and finished products. While studying the sensory characteristics of the finished product makes it possible to determine its potential consumer acceptability, histological studies make it possible to identify changes in the morphological components at the cellular level when exposed to various technological factors. Also, histological studies enable the purposeful improvement of meat processing technology and quality monitoring of finished products, including during storage. So, using microstructure analysis, **[1]** established that a modified gas atmosphere influences the formation of fissures and pores in ground beef during storage. Disruption of the structure of muscle fiber bundles and loosening of the endomysium were detected in beef steaks under the influence of electrohydraulic shockwaves. These microstructure changes have been detected using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) **[2]**. In a study of porcine longissimus muscle, the results of circular dichroism (CD) spectral analysis and fluorescence spectroscopy indirectly proved that thawing can cause protein cross-linking and degradation, destruction of secondary structure, exposure of non-hydrophilic domains, and conformational changes of samples **[3]**.

Scientific data on the histological and physicochemical changes that occur in the main components of canned meat in case of violation of the normal temperature and humidity of storage conditions and their impact on quality indicators are not systematized. Such violations include freezing canned meat during its transportation to consumers in the Arctic zone. It is known that recrystallization of ice due to fluctuations in storage temperature leads to tissue cell restructuring and a change in the morphological characteristics of muscle tissue. These changes, in turn, negatively affect the quality of frozen meat **[4]**. Fluorescence optical microscopy and SEM have also been used to confirm the effect of the freezing temperature on the size of ice crystals in frozen samples of minced beef **[5]**. As for sterilized canned meat, we obtained the first results of studies on changes in the morphological components of such products under the influence of model low temperatures **[6]**. The microstructural analysis is one of the traditional and effective tools for analyzing past transformations through visualization of the microstructure.

This study aimed to study the destructive changes in canned meat after sterilization, model freezing, and subsequent storage.

Scientific Hypothesis

We hypothesized that negative storage temperatures are the root cause of destructive changes in the meat sterilized system.

MATERIAL AND METHODOLOGY

Samples

Sterilized canned meat was selected as the object of this study. Canned food was made from chilled beef.

Chemicals

Chemicals are necessary for fixing samples during histological analysis, such as a neutral aqueous solution of formalin and gelatin, as well as substances necessary for staining samples: Ehrlich's hematoxylin and 1% aqueous-alcoholic solution of eosin were purchased from AppliChem GmbH (Germany). Other reagents and chemicals used in this study were purchased for analytical purposes from OOO TH CHIMMED and OOO LABTECH (Moscow, Russia). All chemicals obtained were of analytical grade unless otherwise indicated.

Instruments

AxioImager A1 light microscope with AxioCam MRC 5 video camera and AxioVision 4.7.1.0 computer image analysis system (Carl Zeiss, Germany); potentiometer FE20 FiveEasy instrument (Mettler-Toledo, Switzerland); seaming- machine B4-KZK-79A (Russia); horizontal autoclave AG-1200 (Russia)

Laboratory Methods

According to the methodology of histological analysis (Saprikin et al., 1997) for fixation, the samples were placed in a 10% neutral aqueous solution of formalin at a temperature of 21 – 23 °C for 48 h **[7]**. After that, the samples were washed with cold water for 4 h, after which they were impacted in gelatin. For this, the sample was first impregnated with a 12.5% gelatin solution for 6 h at 37 °C; then the impregnation was carried out in a 25% gelatin solution in a thermostat at 37 °C for 12 h, then the samples were filled with fresh 25% gelatin solution and quickly cooled in the refrigerator. After cooling, blocks with a size of 15 mm x 15 mm x 4 mm were cut out. Sections 16 μm thick were made on a microtome. Sections were stained with Ehrlich hematoxylin and a 1% aqueous-alcoholic solution of eosin and were enclosed in glycerine gelatin under a coverslip. Histological preparations were examined using the light microscope with a video camera and computer image analysis system.

The content of nitrogen fractions in the canned meat was determined by methods based on the ability of protein substances to precipitate under the influence of various reagents. Protein nitrogen was precipitated with trichloroacetic acid, followed by mineralization of the precipitate and determination of the nitrogen in it according to the Kjeldahl method (ISO 937:1978). Peptide nitrogen was determined by the difference between nitrogen precipitated with phosphotungstic acid and nitrogen precipitated with trichloroacetic acid. The amount of residual nitrogen was the difference between the amount of total nitrogen and the amount of protein and peptide.

The pH value was determined from the potential difference between the glass electrode and the reference electrode inserted into the product sample using a method authentic to the international standard ISO 2917: 1999, IDT.

The redox potential was determined on the potentiometer.

Description of the Experiment

Sample preparation: To perform histological analyzes, the cans with canned food opened, pieces of meat removed, and cut across the muscle fibers into thin pieces and they used for research. Physicochemical

indicators were determined in the average sample of canned food. For this, the contents of the jar were taken out and thoroughly crushed to a homogeneous state.

Number of samples analyzed: 115.

Number of repeated analyses: 3.

Number of experiment replication: 3.

Design of the experiment: Samples of canned meat produced in an industrial plant from one batch of chilled beef weighing 1500 kg. After deboning the half carcasses and trimming the meat, the beef was chopped on a meat-cutting machine into pieces weighing 80 – 120 g. The rendered beef fat was heated to a temperature close to

70 °C. The edible salt, ground black pepper, and crushed bay leaf were thoroughly mixed. The ingredients are packaged in metal cans in the sequence: a mixture of salt, pepper and bay leaves, fat, and meat. The cans rolled on the seaming machine (Russia) and were placed in autoclave baskets. Sterilization with a water shower was carried out in the horizontal autoclave at a temperature of 120 °C and a system pressure of 0.18 – 0.22 MPa for 80 minutes. 600 pieces of canned meat were produced.

After sterilization, the entire batch of canned food was placed in a refrigerator with a temperature of minus 12 °C. After 7 days of storage, 300 cans were transferred to the defrosting chamber. Accelerated defrosting was carried out at an air temperature of 16 – 20 °C and its movement speed of 0.2 – 0.5 m/s. After defrosting with canned food, a set of studies was carried out according to the abovementioned methods.

Statistical Analysis

Microsoft Excel and XLSTAT software were used in this study for statistical analysis. All determinations were performed in triplicate. Significant differences between the average values of protein fractions were established using Student's test at $p < 0.05$.

RESULTS AND DISCUSSION

It reported changes in myofibrillar proteins and collagen of the connective tissue during heat treatment of different types of meat at the temperature range from 60 to 100 °C. The obtained results of studies of the microstructure of meat confirm the general trend of destructive changes in muscle tissue **[8], [9]**, **[10], [11], [12], [13].**

Kaur et al. **[14]** noted that boiling beef at a temperature of 100 °C for 30 minutes led to an increase in the diameter of myofibrils by 3 – 4 times about their diameter in raw meat. After boiling, a decrease in the length of beef myofibrils by 186 – 189 nm was noted. The result is shown in Figure 1.

Industrial modes of sterilization of canned meat are in the temperature range 115 – 120 °C. Therefore, destructive changes in muscle proteins will be more significant, confirmed by our results.

The muscle tissue microstructure after sterilization is shown in Figure 2. Figure 2(a) shows that muscle tissue microstructure after sterilization is characterized by straight muscle fibers, the boundaries between them distinguishable. The transverse striation of the fibers was clearly defined; the length of the sarcomeres was 2.7 – 2.9 μm. Destructive changes were detected in the form of micro-fissures or isolated narrow transverse fissures.

The sarcolemma of the fibers was swollen and detached in the main part of the muscle fibers. A slight increase in fine-grained protein mass was visualized under the detached sarcolemma and between the fibers. The reason for its formation was the transition of sarcoplasmic and myofibrillar proteins into the inter-fiber spaces of muscle tissue during heat treatment of the canned meat.

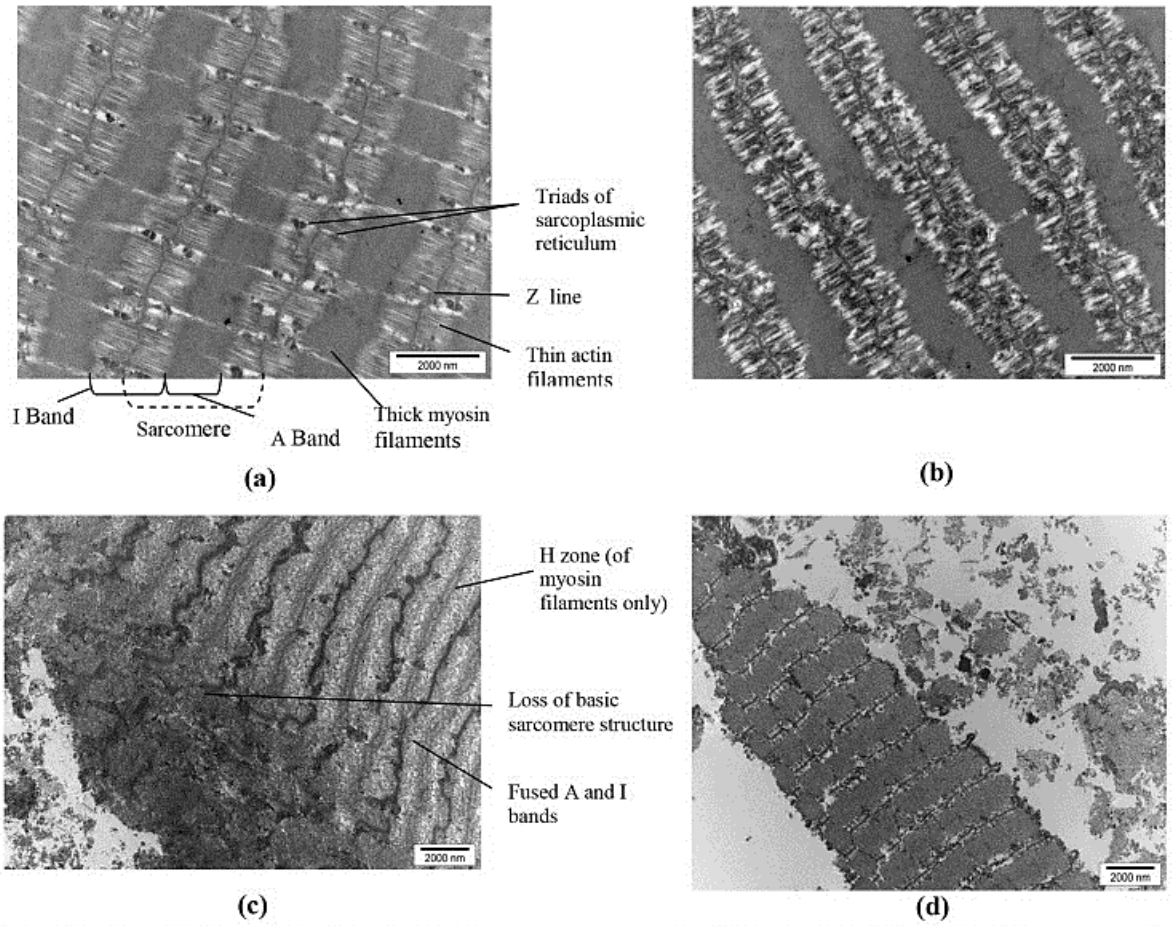


Figure 1 TEM micrographs of (left) raw and (right) cooked (100 °C for 30 min) beef meat myofibril showing sarcomere structural detail (a – b); myofibrils after 30 (c – d) min.

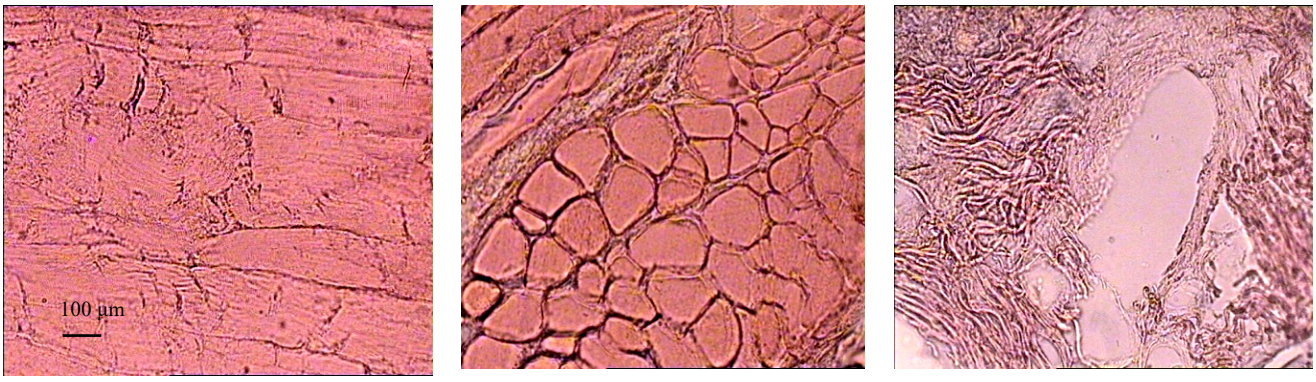
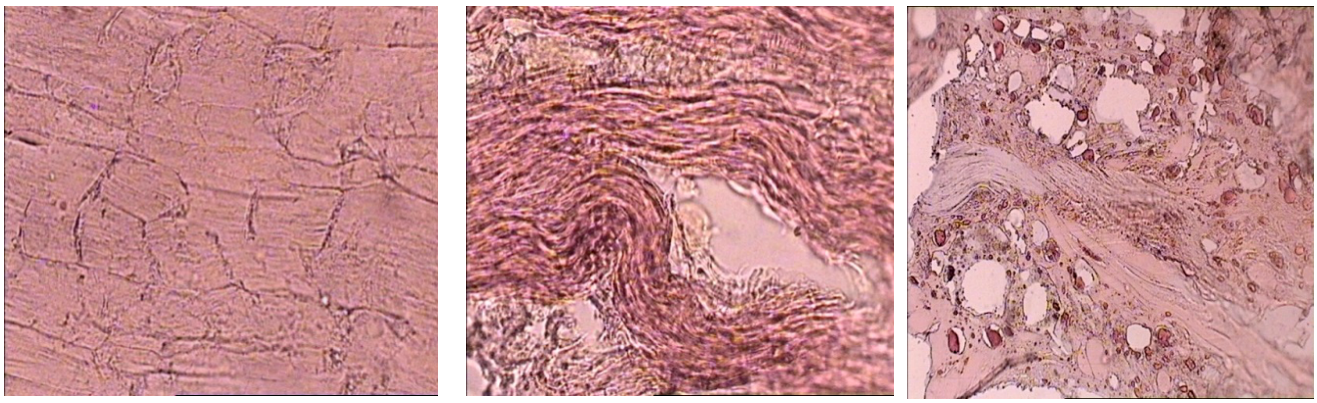


Figure 2 Muscle tissue microstructure after sterilization: (a) longitudinal section: destructive changes in the form of micro-fissures and isolated narrow transverse fissures; (b) cross-section of a muscle fibre; (c) elastic fibres of the connective tissue. Note: Magnification 260×.

In cross-section, the muscle fibers were polygonal; the average fiber diameter was 42.6 µm (Figure 2(b)). The destructive effect of sterilization also affected the connective tissue. Connective tissue layers displayed varying degrees of destruction: large bundles of collagen fibers were swollen, broken into separate collagen fibers; smaller ones were a homogeneous basophilic mass of heat-treated collagen. In the structure of the interlayers, elastic fibers are clearly defined (Figure 2(c)). Heat-treated collagen mass is homogeneous, penetrated by micro-capillaries with clearly defined edges.



(a) (b) (c)

Figure 3 Muscle tissue microstructure after freezing: (a) longitudinal section; (b) loosening of bundles of elastic fibers; (c) multiple cavities – areas of ice crystal localization. Note: Magnification 260×.

The northern territories of our country are huge and differ in climatic conditions. Suppose we assume that during transportation in a zone of high negative temperatures, the equipment of the railway transport that supports the necessary conditions of transportation is out of order. In that case, the canned food will be frozen for, for example, 7 days.

The question was how deep is the effect of negative temperatures on the microstructure of muscle tissue **[15]**, **[16]**. It has been reported that the sarcomeres of raw meat can shrink by 50% after freezing or subsequent thawing **[17]**. Freezing and thawing meat lead to a deterioration in its quality **[18]**, **[19]**. During meat storage in a frozen state, the protein undergoes several changes. With an increase in the freezing time, the heavy chains of myosin and actin degraded to varying degrees **[20]**. We also wanted to see how the destructive processes would affect sterilized canned meat storage capacity in normative storage conditions.

The muscle tissue microstructure after freezing is shown in Figure 3. Straight or slightly wavy fibers characterize muscle tissue microstructure after sterilization; their boundaries are distinguishable. The transverse striation was shallow and close; the length of the sarcomeres was 1.5 – 1.7 μm. Destructive changes in muscle fibers were of varying degrees in different parts of the canned meat.

In some areas, there were multiple changes, in the form of narrow transverse fissures with partial fragmentation of fibers and the violation of sarcolemma integrity. There was less destruction of muscle fibers, and it was defined mainly by micro-fissures and transverse fissures without the violation of sarcolemma integrity.

As shown in Figures 3(b) and 3(c), multiple cavities of indefinite shape – areas of ice crystal localization – were identified; elastic fibers were loosened.

In industrial production or at home, meat products are subjected to various types of processing, most of which cause destructive changes in protein **[21]**. Therefore, at low cooking temperatures, a slow rate of denaturation of myofibrillar proteins was observed **[22]**, **[23]**. During thermal long-term low-temperature treatment, denaturation of the connective tissue of beef was noted at temperatures below 68 °C **[24]**. Destructive changes in pork connective tissue proteins are reported to begin at temperatures between 57 °C to 60 °C **[25]**, **[26]**.

The issues of destruction of sarcoplasmic, myofibrillar proteins **[27]**, **[28]** and proteins of connective tissue **[29]** under traditional heat treatment modes are studied deeply and comprehensively, much attention is paid to prolonged low-temperature heat treatment **[30]**, in particular its effect on the destruction of the protein system, changes in the color of meat **[31]** and tenderness of muscle tissue, due to the activity of cathepsins **[32]**. However, due to the peculiarities of the processes occurring in a closed system – a hermetically sealed can – under the influence of a temperature of 120 °C and a pressure of up to 0.22 MPa, it became difficult to compare the results obtained with the results of other scientists. Therefore, we focused our attention on studying the dynamics of the nitrogen fraction of the meat system. For this reason, as far as the authors know, destructive changes in the protein of canned meat, depending on the duration of their storage in a frozen state, have not yet been studied.

The effect of storing canned food in frozen form on the degree of change in the nitrogen forms of canned food before freezing is shown in Figure 4. After thawing, canned goods were stored for 12 months under normative temperature and humidity conditions. It was noted that freezing and subsequent storage of thawed canned food for 6 months caused an increase in the proportion of non-protein nitrogen fraction by 4.9 times compared with the control sample; after 12 months of storage, this ratio decreased to 1.5 times. The growth of the non-protein

nitrogen fraction is associated with the accumulation of low-molecular-weight compounds, as evidenced by the dynamics of residual nitrogen concerning the non-protein fraction.

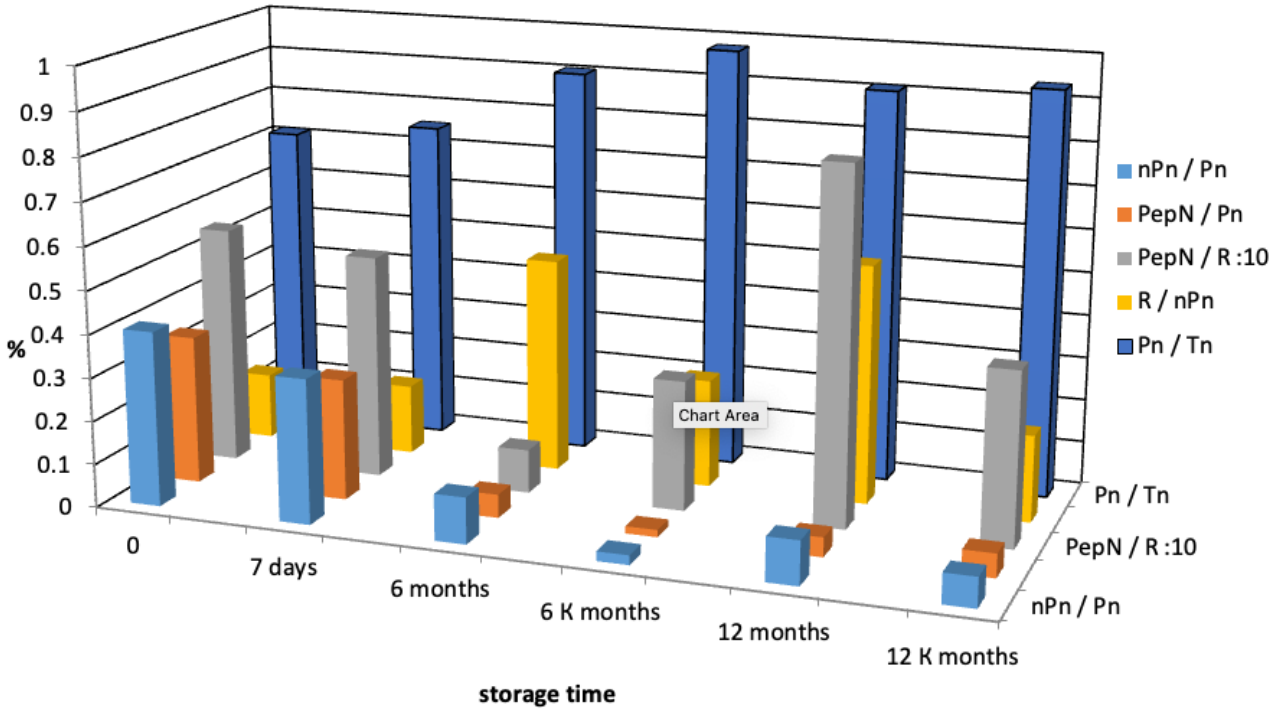
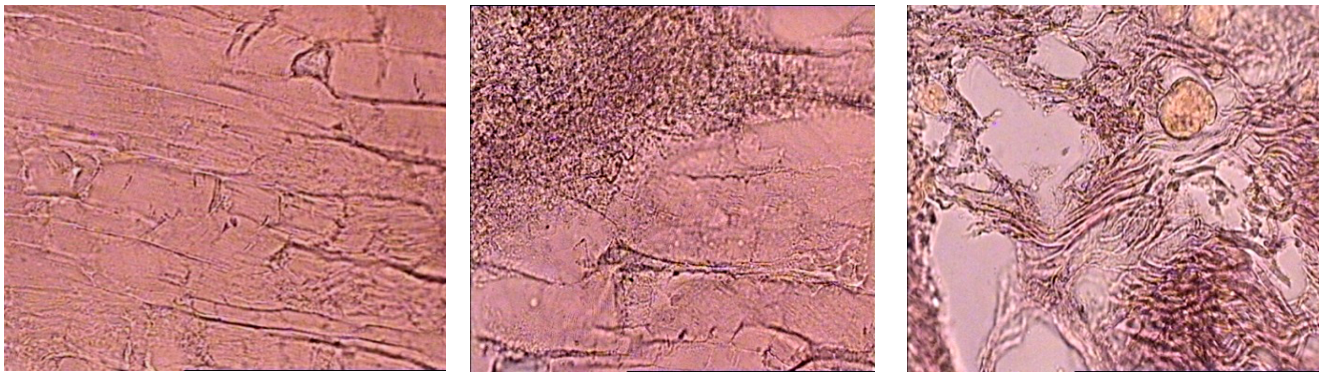


Figure 4 Dynamics of the nitrogen fraction ratios during storage. Note: nPn / Pn –£ non-protein nitrogen / protein nitrogen, PepN / Pn – peptide nitrogen / protein nitrogen, PepN / R – peptide nitrogen / residual nitrogen, R / nPn – residual nitrogen / non-protein nitrogen, Pn / Tn – protein nitrogen / total nitrogen.

Freezing did not lead to a decrease in the content of the high-molecular-weight protein fraction of nitrogen systems. The ratio of protein nitrogen to total nitrogen tended to grow (0.71 to 0.79) and the ratio of non-protein nitrogen to total nitrogen to decrease (0.41 to 0.26), which can be explained by the destruction of low molecular weight nitrogen forms under the action of the ice crystals formed after 7 of storage. Partially relevant data was obtained in the study of pork tenderloin stored at a given negative temperature of -3 ± 0.5 °C for 7 days: excessive protein denaturation was noted in the myofibrillar fraction with an increased storage time **[33].**

The muscle tissue microstructure after 12 months of storage is shown in Figure 5.



(a) (b) (c)

Figure 5 Muscle tissue microstructure after 12 months of storage: (a) longitudinal section; (b) fine-grained protein mass; (c) loosening and destruction of collagen bundles in connective tissue. Note: Magnification 260×.

A longitudinal section (Figure 5(a)) showed that straight muscle fibers with well-defined boundaries characterized muscle tissue. The transverse striation was weak. The length of the sarcomeres was 2.7 – 2.9 μm, the nuclei of the fibers were lysed, myofibrils were loosened, the sarcolemma of the fibers was peeled, and in some places, its integrity was violated. In the inter-fiber space, much more fine-grained protein mass was detected than unfrozen sterilized canned meat (Figure 5(b)). Destructive changes in muscle fibers were detected

in the form of multiple transverse narrow fissures or transverse fissures with fibers broken into fragments. In some areas, the length of the fragments was 100 – 350 μm. The connective tissue layers were loosened, homogeneous, marked areas of collagen bundles decayed to a fine-grained protein mass (Figure 5(c)).

The redox potential (Eh) is reported to be one of the potential barriers to food safety and quality **[34]**. The Eh value depends on several factors, in particular, on the level of dissolved oxygen in the medium, temperature, pH, and the concentration of components capable of oxidation or reduction **[35]**, **[36]**, **[37]**. To date, certain material accumulated on the dynamics of Eh about the storage of chilled meat **[38]**, **[39]** obtained data on the Eh values of cooked, raw smoked, and offal sausages, minced meat with several food additives.

To assess the quality of canned food, Eh is used in a limited number of scientific works. Therefore, the measurement of Eh has not yet entered the practice of research in canned food. We have accumulated a knowledge base on the Eh values of raw materials and canned food made from them **[40]**, **[41]**. The obtained experimental data are consistent with those available in the technical literature; scientific data on the dynamics of Eh of canned food during production and storage are new today.

The dynamics of the redox potential of canned meat after thawing and during storage are shown in Figure 6. The pH value of the investigated canned food underwent minor changes within the limits of the experimental error. However, the dynamics of these changes are different. So, in the control samples, the pH values were in the range of 6.41 – 6.36 units. In experimental samples – in the range of 6.41 – 6.26 units, which correlates with the dynamics of the redox potential values and demonstrates a decrease in the stability of the meat system after freezing during further storage.

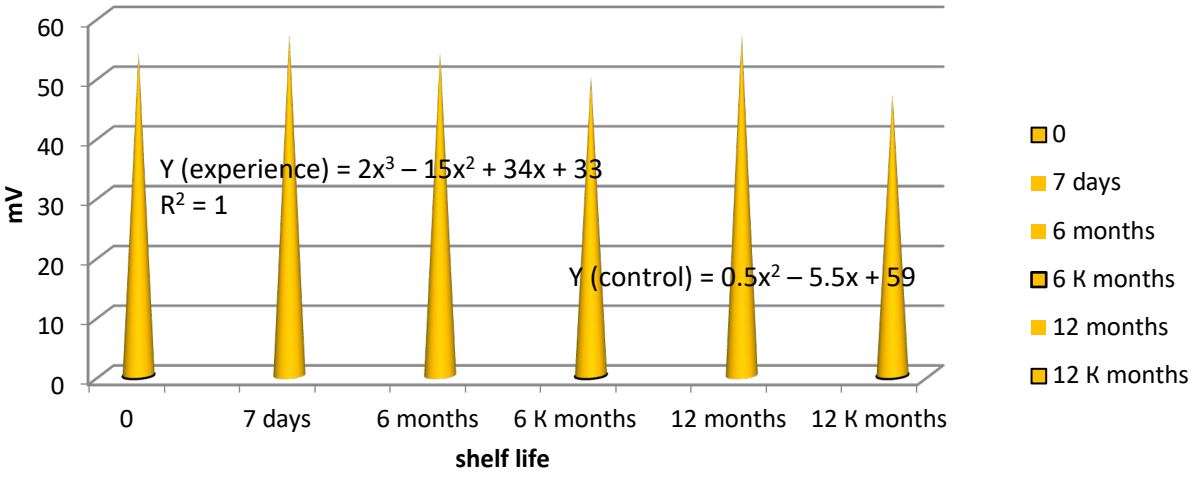


Figure 6 Dynamics of the oxidation-reduction potential of canned meat.

The dynamics of the Eh values of control and experimental canned food samples during storage had the opposite direction. The Eh of the control samples decreased from 52 to 46 mV after 12 months of storage, which indicates the stability of oxidative reactions in the meat system. At the same time, the dynamics of Eh of canned food subjected to model freezing and thawing were unstable: a decrease in the Eh value after 6 months and a subsequent increase by 12 months of storage of canned food. This may indicate the loss of oxidative stability of the protein system of the samples subjected to freezing. The results obtained agree with the data on the dynamics of nitrogen forms in canned food samples.

CONCLUSION

The results obtained showed that short-term freezing of sterilized canned meat led to significant changes in the histological characteristics of the main structural components of muscle and connective tissues. The formation of ice crystals led to a loosening of the structural components of muscle tissue and elastic fibers, deformation of muscle fiber bundles, and the formation of cavities. This increased the degree of destruction of muscle and connective tissues and the release of myofibrillar and sarcoplasmic proteins into the interfiber space with the formation of a fine-grained mass. Freezing did not cause a decrease in the content of the high-molecular-weight protein fraction of nitrogen in the meat system. In contrast, the destructive effect of ice crystals on low-molecular forms of nitrogen was significant. It led to a 1.8-fold decrease in the ratio of non-protein nitrogen to the total. The ratio of the values of peptide nitrogen to residual nitrogen is most indicative. The predominance of peptide nitrogen in canned food after 7 days of freezing demonstrates the preservation of canned food protein: the PepN / R ratio was 5.2.

The dynamics of Eh as a factor of the redox stability of the meat system showed that freezing and thawing of canned food led to a violation of stability. A decrease in the Eh value evidences this after 6 months and a subsequent increase in Eh after 12 months of canned food storage. The results obtained agree with the data on the dynamics of nitrogen forms in canned meat samples.

Destructive changes in the main structural components of muscle tissue became the main reason for the increased degradation of proteins during the further storage of sterilized canned meat under standard conditions. Additional physical, chemical, and organoleptic studies are needed to determine the shelf life of the product after freezing.

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Nutrition of older adults in the Republic of Kazakhstan

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ABSTRACT

This article discusses a study on the nutrition specifics of older adults living in social service institutions in three major cities of the Republic of Kazakhstan: Nur-Sultan, Almaty, and Shymkent. The direction of the research meets the priorities of the World Health Organization to achieve goals on aging and health. The diets of older adults in the Republic of Kazakhstan were studied, food preferences were identified, and needs for basic nutrients were established. This article presents the results of sociological surveys of older adults who answered questions about nutrition, preferred foods, raw materials, and meat products. Based on the survey results, technologies of herodietic meat products aimed at enriching the diet with proteins were developed, along with practical recommendations for a balanced diet. This area of research is relevant due to the lack of products with a herodietic profile on the Kazakhstan market.

Keywords: balanced nutrition, older person, questionnaire, aging, herodietic product

INTRODUCTION

The development of the food industry in Kazakhstan is a strategic task designed to provide high-quality and diverse food products in response to the country's growing population and the significant increase in food consumption **[1, 2]**. Research on the health and longevity of modern man has shown that 10% depend on health care, 20% on genetics, 20% on ecology and the state of the environment, and 60% on lifestyle **[3]**, **[4]**. The life expectancy of citizens in the Republic of Kazakhstan has increased by almost five years over ten years (from 68.41 years in 2010 to 73.15 years in 2018). However, in some Organization for Economic Cooperation and Development countries (Chile, Turkey, and others) with the same level of GDP as in Kazakhstan, the indicator is about 80 years. The first task of the State Program for the Development of Healthcare of the Republic of Kazakhstan for 2020 – 2025 is to form a commitment to a healthy lifestyle among the population **[5]**. One of the main reasons that a person does not reach the upper age limit of life lies in premature aging due to a violation of an optimal lifestyle and, to a large extent, nutritional characteristics. A balanced diet influences the nature of metabolism and the state of human organs and systems, allowing for s activity, and reduce correcting its homeostasis, maintaining activity and reducing aging **[6]**, **[7]**, **[8]**, **[9]**. The median forecast of the total population of Kazakhstan predicts almost 19 million at the end of 2020, more than 21 million in 2030, more than 23 million in 2040, and 25.5 million in 2050.

According to the probabilistic forecast of the sex and age structure of the population of Kazakhstan until 2050, the upper limit for expected life expectancy is 93 years for women and 85 years for men **[10]**. An aging population is inevitable, so governments must adopt and implement relevant policies regarding health, employment, social protection, housing, food security, migration, poverty reduction, and others **[11]**. There is no consensus on the sharp increase in life expectancy. This phenomenon is associated with evolutionary development, improved housing conditions, sufficient food and medical care, and many other factors **[12]**. With age, there is a weakening of the two components of taste – the sense of smell and taste receptors. These changes interact with each other to reduce the pleasure of eating. A slight increase in the threshold values of taste, which occurs with age, suggests a need for richer tasting food for elderly patients. Many complaints about food quality can be explained by changes in the pleasure of eating as age increases. Elderly patients are particularly susceptible to malnutrition if they suffer from a chronic mental or physical illness. In cases of significant malnutrition, there is clear evidence of the benefits

of nutritional support, showing that good nutrition and even the use of vitamin and mineral supplements can play an important preventive role in maintaining the health and quality of life among the elderly **[12]**, **[13]**, **[14]**. Slowing metabolism, lack of appetite, undesirable side effects from taking numerous medications, a tendency to overeat, a high risk of food poisoning, and atherosclerotic changes are the main problems that every older adult will inevitably face. Changes should be made to the usual diet to reduce the negative consequences.

According to statistics, about 75% of elderly people have some kind of nutritional disorder: about 20% overeat, and 60% eat irrationally (more often men), which is expressed in the predominance of meat and flour products with a high content of animal fat, sweets, muffins and insufficient consumption of dairy products, fish, vegetables, and fruits **[15]**. This is a special social group of the population in need of state social protection. There is no unified law on the rights and freedoms of the elderly in Kazakhstan that would regulate the issues of social protection of the aging population, including taking into account access to quality food. With age, certain needs appear that also require attention from the state. Kazakhstan has several regulatory legal acts regulating certain types of legal relations involving the elderly, for example, the Law of the Republic of Kazakhstan On Pension Provision and the Law of the Republic of Kazakhstan On benefits and social protection of participants, invalids of the Great Patriotic War and persons equated to them. However, not all the needs and problems of the elderly are taken into account in these documents.

Following the UN General Assembly initiative of December 14, 1990, the International Day of the Elderly is celebrated in Kazakhstan on October 1. A large-scale social protection system for the elderly has been established. Older adults have various benefits and guarantees, and pensions are regularly increased.

The concept of "quality longevity" plays a unique role and significance in developing an anti-aging model to increase the expected duration of quality life. The model's effectiveness should be determined using assessing the quality of life and forming the scientific foundations of this problem. One of these directions is "assimilation - health improvement through the principles of proper nutrition (health improvement aimed at forming the principles of rational and balanced nutrition)" **[16]**. The demographic situation in Kazakhstan is characterized by a steadily increasing proportion of people over 60 years old, which corresponds to the global aging process of the population. Significant changes are taking place in the public consciousness, and the value orientations, functions, and roles of the elderly in modern society are also changing. International documents point to the awareness of the value of elderly persons, their contribution to the development and functioning of social systems, and the possibility of active participation in society's economic and cultural life. At the same time, it is important to move from simple awareness to building a flexible gerontosocial policy at the national level **[17]**.

The solution for issues related to the aging of the population requires an integrated approach. Therefore, it occupies a worthy place among the directions of state policy.

Qualitative growth of human potential, including older people as one of the fastest-growing groups in Kazakhstan, is an important factor in our country's sustainable economic and social development **[18]**, **[19]**. To maintain health, efficiency, and longevity, it is necessary to observe the basic principles of rational nutrition: energy balance and regular satisfaction of the human body's need for macro- and micronutrients **[20]**.

Kazakhstan is at an early stage of joining the category of countries with a predominantly elderly population. The solution for issues related to the aging of the population requires an integrated approach. This is impossible without developing a unified concept of state policy concerning the elderly. Its strategic goal should be to increase the level and quality of life of older people based on social solidarity, forming a new attitude to the place of old age in the life cycle.

In addition to the general age-related changes, the functional state of each person's organs and systems has unique characteristics. The needs of people for absorption largely depend on the flowing state of health **[21]**. Scientists have shown that the elderly population is the largest demographic group with a disproportionate risk of inadequate nutrition and malnutrition. Aging is associated with a decrease in many physiological functions that can affect the state of nutrition. The muscle mass of the body decreases, the metabolic rate decreases, the gastric secretion of digestive juices decreases, changes occur in the oral cavity, and the sensory functions of the gastrointestinal tract are disrupted. The nutritional status of the elderly is an important factor in determining the quality of life, morbidity, and mortality **[22]**, **[23]**, **[24]**, **[25]**, **[26]**. One of the important goals of the World Assembly on Ageing, adopted in Madrid in 2002, is to promote adequate nutrition throughout life for all older people, preferably based on the consumption of local foods, and in particular by defining national nutrition goals **[27]**. Scientists involved in herodietics believe that a healthy diet and lifestyle can help prevent disease, especially chronic disease. Given the high growth rates of the elderly and senile population around the world, scientists and manufacturers began addressing the problem of maintaining the health status of this population group by changing the nature of its nutrition **[28-40]**.

The results of innovative research within the framework of the cooperation of the European Regional Office of the World Health Organization in 2016 – 2017 demonstrate that to ensure sustainable development, the

popularization of healthy nutrition should become one of the priority areas of work in Kazakhstan. Measures should be developed in effective cooperation with healthcare, agriculture, education, mass media, and culture **[31]**.

Thus, the essence of herodietics is that food should satisfy the body's needs in energy and food substances and contribute to the prevention of the development of chronic non-communicable diseases of modern man, the preservation of health and longevity, and the prevention of premature aging.

The development of formulations that meet the requirements for herodietic products involves using various animal and plant origin components. Animal proteins contained in meat and fish are complete in amino acid composition, vegetable oils and animal fats are sources of polyunsaturated fatty acids, and vegetable raw materials are sources of vitamins and of macro- and microelements, therefore specialized products are always multicomponent **[33]**. The concept of the state policy in the field of healthy nutrition for the population of the Republic of Kazakhstan should rationalize the population's nutrition through the extensive development and introduction of specialized food products enriched with biologically active components. One of the most effective ways to implement this approach is the production and consumption of a new food category – fortified products, which are traditionally consumed products with added essential nutrients and minor components of food. According to the FAO/WHO Food Code, food fortification is defined as adding one or more nutrients to food products to prevent or correct the existing deficiency of one or more nutrients in the population or in a separate group **[34]**. There is not enough information about the development and creation of products for herodietic nutrition in the Republic of Kazakhstan. The domestic food industry does not in practice produce special food products intended for the elderly and older adults. Modern food production technologies do not consider the specifics of nutrition of older age groups. However, the experience of specialists in medicine, dietetics, and gerontology testifies to the need to introduce the technology for the production of herodietic products in our country as a factor in the prevention of pathological conditions with regular physiological aging **[35]**.

Scientific Hypothesis

Based on the study results, food technologies will be developed that take into account the preferences of older adults, which will greatly improve the quality of life.

MATERIALS AND METHODOLOGY

Samples

Our sample consisted of respondents living in nursing homes in three major cities in Kazakhstan: Almaty City Veterans Home (Almaty), the municipal state institution Sharapat Social Service Center (Nur-Sultan), and the state institution Shymkent Boarding House for the Elderly and Disabled of Turkestan region (Shymkent). These institutions are designed to accommodate people who have reached retirement age and disabled people of groups I and II, lonely and requiring constant outside care. In total, 1,000 residents live in these institutions: 400 elderly people in Nur-Sultan, 400 in Almaty, and 200 in Shymkent. The structure of the respondents was as follows:

- Total number of respondents: 500
- Men: 46.4%
- Women: 53.6%

Age categories:

- 60 – 74: 52.4%
- 75 – 80: 23%
- 81 – 90: 22.8%
- 90 and above: 1.8%

Education:

- Basic (primary school): 23.4%
- Secondary (high school): 47%
- Higher education (university): 29.6%

Description of the Experiment

Questionnaire preparation: The authors developed a questionnaire to enable them to study the main health problems of older people in Kazakhstan and understand their food preferences. The questionnaire consists of 12 multiple-choice questions (Table 1) that ask about gender, age, health status, preferences for various foods, knowledge about herodietic products, and factors of human life expectancy. The survey passed a preliminary test before distribution. All questions were mandatory.

Survey: The survey period was from June 2018 to January 2019. It was conducted in Almaty in June 2018, in Nur-Sultan in September 2018, and in Shymkent in January 2019. Official permission to conduct the survey was

obtained from the institutions' administration, and the ethical aspects were agreed upon, including the consent of the participants, voluntary participation, and confidentiality. Respondents had the option to answer the questionnaire in written or oral form.

Number of answers:

The total number of processed answers was 500.

Creation of the dataset:

We processed the raw data. Each verbal answer was examined. Offensive and vulgar responses were deleted. We performed grammatical correction of text answers and prepared the final dataset for further processing in Microsoft Excel (Office 365). The structure of the dataset was adapted to further statistical processing.

Processing of the responses:

We evaluated all questions and visualized the consumers' opinions by figures. We evaluated the individual text answers and formulated the most frequent opinions of the participants. These answers are presented in the discussion section of this article.

Statistical Analysis

Multiple correspondence analysis was used to visualize the data obtained from the questionnaire survey. Statistical significance was determined based on the significance of the *p*-value. The statistical program R studio (vs. 1.3.959) was used for data processing. Multiple correspondence analysis (MCA) is an extension of simple correspondence analysis to summarize and visualize a data table containing more than two categorical variables. It can also be understood as a generalization of the main components' analysis when the analyzed variables are qualitative instead of quantitative **[36]**.

RESULTS AND DISCUSSION

The survey was conducted from June 2018 to January 2019 in Almaty (June 2018), Nur-Sultan (September 2018), and Shymkent (January 2019). The official permission of the administration of the institutions to conduct the survey was obtained, and the ethical aspects were agreed upon, including the consent of the participants, voluntary participation, and confidentiality. Respondents had the opportunity to answer the questionnaire in written or oral form. Respondents noted that the questions were clear and accessible (Table 1).

When developing the research methodology, it was planned to interview 1,000 respondents. Ultimately, 500 respondents were interviewed: 200 in Nur-Sultan, 200 in Almaty, and 100 in Shymkent, which is 50 percent of the residents of each institution.

Table 1 Survey Results of Elderly People Living in Veterans Homes.

No	Questions	Location of the survey (cities)			Total, people
		Nur-Sultan	Almaty	Shymkent	
1	2	3	4	5	6
	Number of respondents	200	200	100	500
1	**Your gender?**				
	Male	96	98	38	232
	Female	104	102	62	268
2	**Your age?**				
	60–74	110	94	58	262
	75–80	24	76	15	115
	81–90	66	26	22	114
	over 90	0	4	5	9
3	**What diseases have you been diagnosed with?**				
	high blood sugar	26	30	11	67
	obesity/overweight	14	6	6	26
	anemia	18	16	0	34
	diseases of the digestive system	36	32	22	90
	high blood pressure	50	44	30	124
	diseases of the musculoskeletal system	42	40	22	104
	high cholesterol	14	12	9	35
	no health complaints		20		20
4	**Do you know what herodietic nutrition is?**				
	I know	2	0	2	4
	I do not know	198	200	98	496
5	**What, in your opinion, affects a person's life expectancy?**				
	heredity	22	18	6	46
	accessibility of healthcare	30	24	8	62
	ecology	32	42	36	110
	proper nutrition	68	70	32	170
	physical activity and active mental activity	48	46	18	112
6	**Do you prefer milk and dairy products?**				
	yes	138	100	64	302
	no	28	88	29	145
	I find it difficult to answer	34	12	7	53
7	**Do you prefer vegetable dishes and fruits?**				
	yes	74	95	41	210
	no	82	55	39	176
	I find it difficult to answer	44	50	20	114
8	**Do you have a need for fish and fish products?**				
	yes	88	116	46	250
	no	112	84	54	250
	I find it difficult to answer				
9	**Do you prefer dishes made from cereals and legumes?**				
	yes	80	74	69	223
	no	80	82	26	188
	I find it difficult to answer	40	44	5	89

Table 1 Cont.

No	Questions	Location of the survey (cities)			Total, people
		Nur-Sultan	Almaty	Shymkent	
1	2	3	4	5	6
	beef	24	62	15	101
	lamb	46	30	24	100
	horse	50	62	25	137
	pork	32	6	2	40
	poultry	42	36	30	108
	camel meat	6	4	4	14
11	**What meat products do you prefer?**				
	sausages	72	106	29	207
	pates	38	32	14	84
	jelly	46	38	36	120
	cutlets	44	24	21	89
12	**What kind of dishes do you prefer according to the type of heat treatment?**				
	boiled	66	100	86	252
	fried	72	68	14	154
	pickled/smoked	62	32	0	94

Note: The distribution of respondents by age categories achieved as a result of the survey is shown in Figure 1. Older adults of different ages took part in the survey: 53.6% of the survey participants were women, and 46.4% were men.

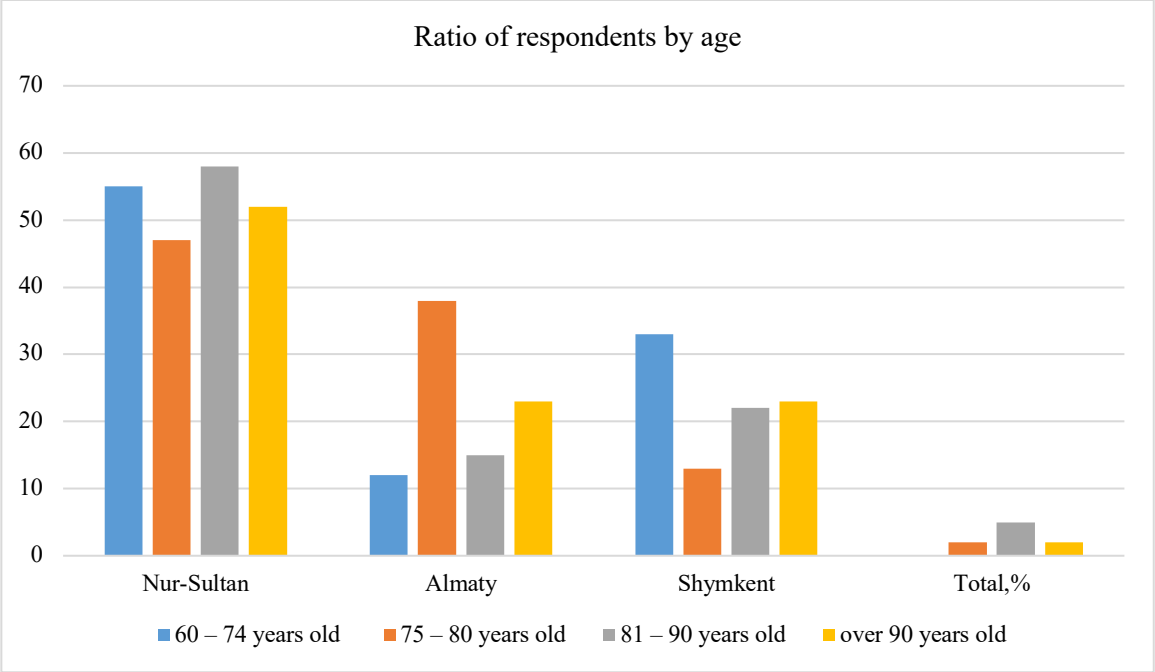

Figure 1 Distribution of Respondents by Age Categories shows that about half of the respondents (52%) are between 60 and 74 years old, 23% of elderly people are aged 75 to 80 years, 23% of respondents are aged 81 to 90 years, and centenarians make up 2%. Note: The survey results on the diseases of older adults living in veterans' homes are shown in Figure 2.

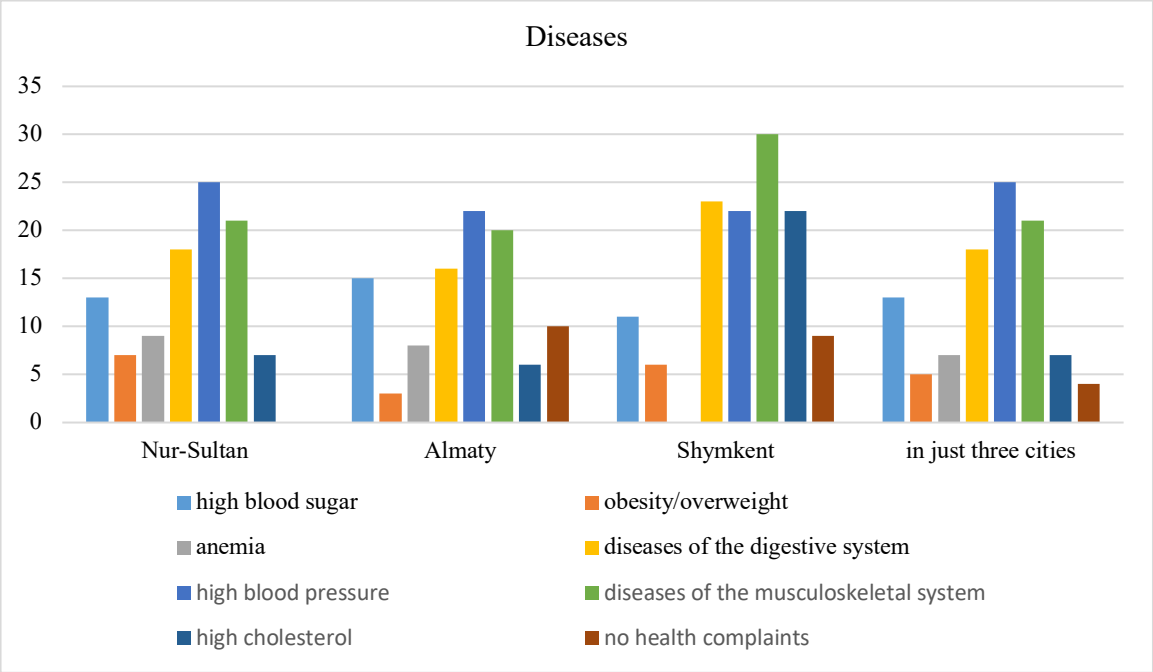

Figure 2 Number of Diseases Noted by Respondents.

The study showed that high blood pressure (25%), diseases of the musculoskeletal system (21%), and diseases of the digestive system (18%) are common among respondents, followed by elevated blood sugar (13%), high blood cholesterol (7%), anemia (6%), and obesity/overweight (5%). Four respondents had no health complaints. It is important to note that most of these diseases can be treated with dietary adjustments.

In general, respondents avoid assessments of their health and instead consider it satisfactory. It is important to note that most of these diseases can result from insufficient and improper nutrition.

It should also be noted that 99% of the survey participants do not know about herodietic products (gerontological nutrition). This shows that there is insufficient information about nutrition and products intended for the elderly category in Kazakhstan. It was revealed that older adults are not aware of the connection between diseases and nutrition.

The respondents also answered questions about factors affecting longevity, as shown in Table 2.

Table 2 Respondents' Opinions on Factors Affecting Human Life Expectancy (survey results).

Factors affecting human life expectancy	Nur-Sultan	Almaty	Shymkent	All three cities combined
	Factors affecting life expectancy (%)			
Heredity	11	9	6	9
Accessibility of healthcare	15	12	8	12
Ecology	16	21	36	22
Proper nutrition	34	35	32	34
Physical activity and active mental activity	24	23	18	22

Among the list of factors affecting human life expectancy, respondents identified proper nutrition (34%), physical activity and active mental activity (22%), and ecology (22%).

A very important factor affecting health and life expectancy is nutrition. The preferences of elderly and senile people regarding food, in general, are presented in Table 3.

Table 3 Preferences of Elderly People Regarding Food (survey results).

Participant responses	Milk and dairy products	Vegetable dishes and fruits	Fish and fish products	Dishes from cereals and legumes
	Factors affecting life expectancy (%)			
Yes	60	42	50	45
No	29	35	50	38
I find it difficult to answer	11	23	-	17

Most respondents in the survey on food preferences prefer milk and dairy products (60%). With age, however, a person's ability to digest lactose decreases. Not all elderly people tolerate milk well, experiencing discomfort in increased gas formation, abdominal pain, and loose stools.

Table 3 shows that respondents prefer vegetable dishes and fruits (42%), fish and fish products (50%), and cereals and legumes (45%).

One of the objectives of this study is to investigate respondents' preferences for types of meat for the development of herodietic meat products since meat is a source of protein.

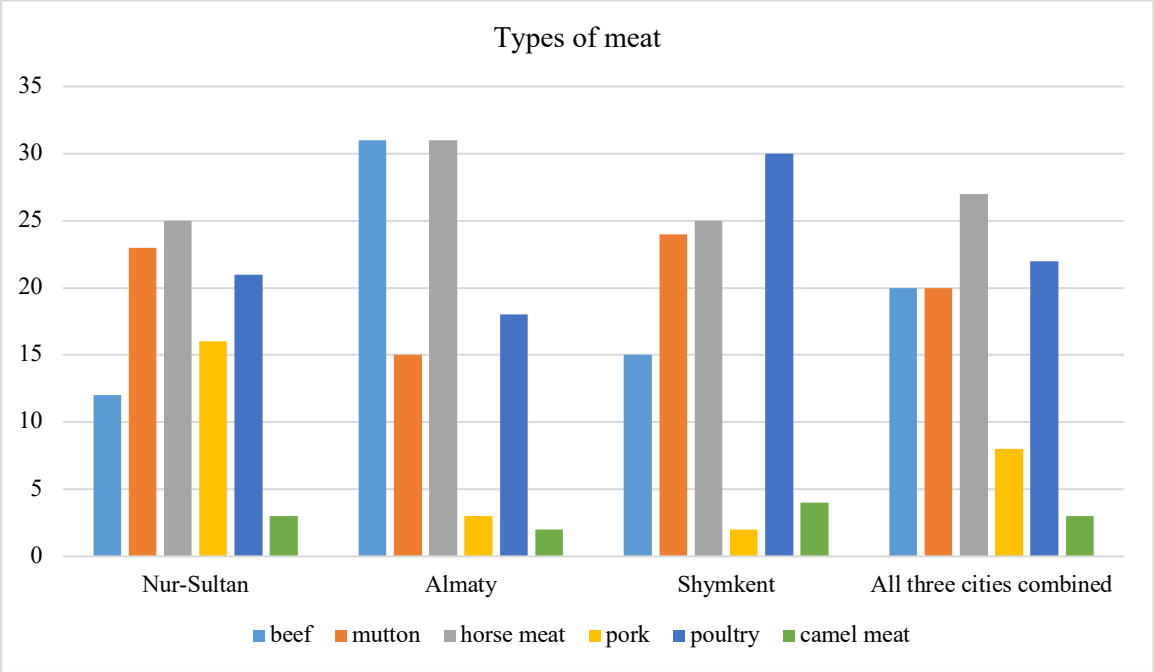

Figure 3 Distribution of Preferences for Types of Meat Products.

The survey revealed that respondents from Nur-Sultan prefer horse meat the most (25%), respondents from Almaty prefer horse meat and beef equally (31%), and respondents from Shymkent prefer poultry (23%) (Fig. 3). The difference between official statistics and the survey results is due to the cost of meat.

In general, Kazakhstanis prefer horse meat (27%), while poultry is in second place (22%), followed by beef and mutton (20% each). The survey participants from Nur-Sultan and Shymkent would like to have more fish and fish products in their diet.

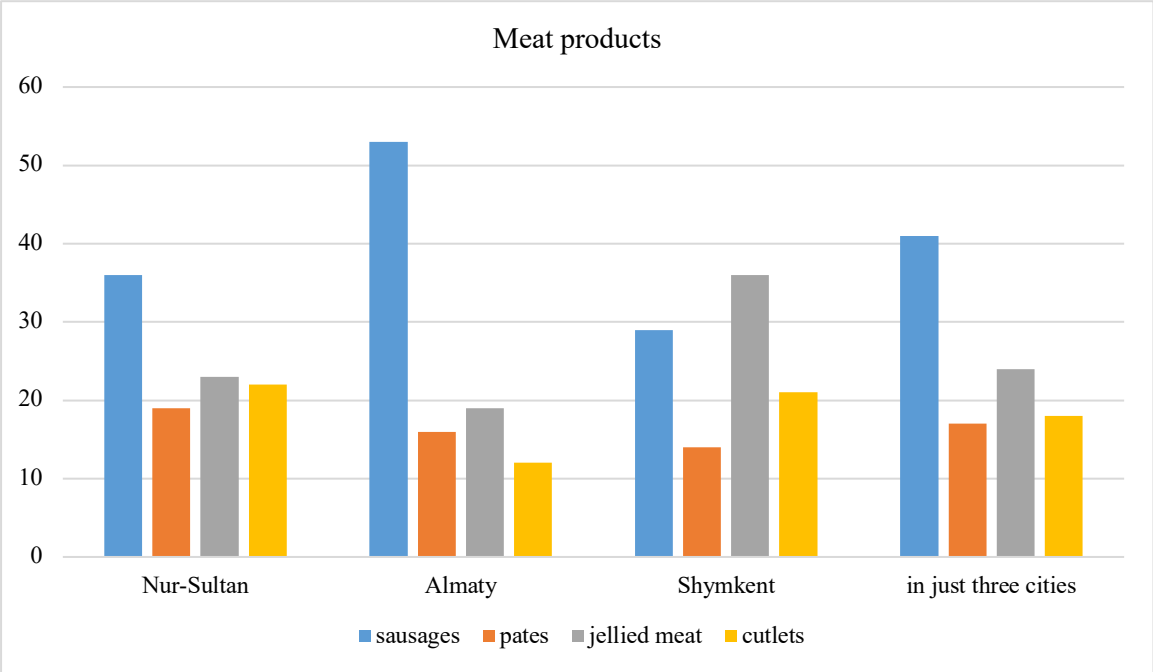

Figure 4 Distribution of Respondents' Preferences for Meat Products.

The survey results from Fig. 4 show that older people prefer sausage products (41%), jellied meat (24%), cutlets (18%), and pates (17%) to meat products.

As for the methods of heat treatment of food, 50% of the survey participants prefer boiled or steamed food, 31% prefer fried food, and 19% prefer pickled and smoked food.

Gruver et al., who has been studying the aging process in Okinawa for several years, believe that life expectancy is determined by five factors: proper nutrition, lack of stress, a caring environment, a high level of physical activity, and spiritual mood. The Japanese island of Okinawa holds the world record for the number of centenarians. Men live for an average of 88 years, and women for 92 years, which is 10 to 15 years longer than in the rest of Japan **[41]**. Swedish scientists Kumar and Manish emphasize that the main negative consequence of aging is immunogenicity, which can be defined as a decrease in the immune system's functionality, which can cause changes in the structure and composition of the intestinal microflora in the elderly. Dieting and the use of probiotics and prebiotics can help prevent and treat age-related physical conditions, support the beneficial intestinal microflora, and thus promote healthy aging **[42]**. The aging process includes changes in a person's physiological, pathological, social, and psychological conditions. Nutrition is an important element of the health of the elderly, and it affects the entire aging process **[43]**. In old age, proper nutrition is necessary to maintain a normal state and efficiency of the body. Proper nutrition throughout life is the key to healthy aging and longevity **[44]**. Demographic aging impacts labor and financial markets, demand for goods and services such as housing, transport, social protection, and the family structure and relationships between people belonging to different generations, significant additional requirements for health and financial services **[45]**. Scientists have shown that the elderly population is the largest demographic group with a disproportionate risk of inadequate nutrition and malnutrition. Aging is associated with a decrease in several physiological functions that can affect the state of nutrition. This results in a declining basic metabolic rate and decreased gastric secretion of digestive juices, disorders of the sensory function of the gastrointestinal tract, and chronic diseases. The nutritional status of the elderly is an important determining factor in the quality of life, morbidity, and mortality **[22]**, **[46]**. The recommended dietary allowance (RDA) in foreign countries for protein is 0.8 g of protein/kg of body weight per day for adults, regardless of age. This is the minimum amount of protein needed to prevent the gradual loss of muscle mass. Studies have shown that protein intake greater than the RDA helps improve muscle mass, strength, and function in the elderly and improves immune status, wound healing, and blood pressure **[47]**. Fats are part of a healthy diet. They are the most energy-intensive nutrient stored in the adipose tissue of the body in the form of triglycerides, a source of essential fatty acids that are not produced in the human body **[48]**. Italian researchers believe that more than half of the elderly suffer from obesity and, at the same time, loss of muscle mass due to an unbalanced diet rich in carbohydrates and lipids but poor in valuable proteins and amino acids **[49]**. American researchers recommend protein supplements for elderly people with sarcopenia, which affects about 45% of men and about 26% of women **[50]**. The nutrition of elderly people is greatly influenced by their socio-psychological and material conditions, which can lead to malnutrition or the consumption of cheap, low-quality products. There

is a shortage of biologically active substances with aging, and those with low incomes cannot afford sufficient amounts of these substances **[51]**. The English scientist Leaker connects the problem of longer wound healing times in the elderly with malnutrition **[52]**. Australian researchers K. Schouten, M.A. Lindeman, and J. Reid speak about the need to develop and introduce a national program on gerontological nutrition, highlighting, among other problems of the elderly indigenous population of Australia, an increased percentage of people with obesity and malnutrition. According to the results of studies by Brazilian scientists on the nutrition of the elderly in the city of Sao Paulo, 33% of people in this category eat inadequately for their needs, and 60% need to alter their diet **[53]**. Various tools have been developed and recognized to assess the nutritional status of older people. The most widely used universal screening tool for malnutrition in the UK is the Malnutrition Universal Screening Tool (MUST), a five-step screening tool to identify elderly people who are malnourished or at risk of malnutrition **[54]**. Many scientists have proven the health impact of altering the nutrition of older people. Japanese scientists found improved well-being in elderly people who expanded the range of their diets and increased the frequency of their meals **[55]**. A study by Australian scientists identified the effectiveness of natural antioxidants in product composition concerning increasing the immunity of older people **[56]**. Scientists from the United Arab Emirates conducted experiments proving a reduction in depressive symptoms in the elderly while ensuring their needs for minerals and vitamins by 100% **[57]**. American scientists used the amino acid β-alanine as a biologically active food supplement, which positively affected the performance of elderly people **[58]**. Studies by Canadian scientists provide data on taurine and L-carnitine in the composition of the NOS Energy Drink and their effect on weight normalization and improvement of cognitive abilities in older people **[59]**. To enrich sausage products of herodietic purpose with calcium, scientists conducted research using various types of mineral raw materials: mussel shells, quail egg shells, bone marrow, alginic acid salt, bone paste **[60]**. Living alone and its substantial impact, along with the associated social isolation and loneliness, were highlighted in many of the discussions. Given the possible implications for nutritional intake, further work is recommended in this area. Likewise, steps should be taken to improve food access, increase opportunities for commensal eating, and, fundamentally, address social isolation and loneliness in the older population **[61]**.

After the survey, a lecture on the unique characteristics of nutrition in older adults was organized and held in each institution, who became interested in this topic, asked questions, and actively participated in the discussion.

Respondents, psychologists, methodologists, and the administration expressed their gratitude for the interest in the health of older people and are open to cooperation with scientists in the field of lectures and seminars, the development and randomization of herodietic products. Thus, the survey revealed that the majority of respondents showed an interest in herodietic products intended for the nutrition of the elderly. The data reflect the well-being of older adults in veterans' homes relatively well and a need for products for this age group. The strengths of this study are the large sample sizes and different regions of Kazakhstan. However, the authors acknowledge that the results are based on only one group of elderly people – residents of nursing homes – and the results may differ for elderly people who live alone or with a family. A disadvantage of the survey is that the quality of the information received depends on respondents' perception of the questions and their accuracy and attentiveness. It is necessary to consider such features when a person cannot or does not want to answer the questions. In general, the questionnaire is not intended to clarify the underlying causes of any phenomenon. The obtained data fix the phenomenological side of socio-political processes and need further conceptual interpretation and theoretical explanation.

CONCLUSION

The survey results serve as material for developing practical recommendations for the preservation and promotion of health through nutrition, particularly the development of herodietic meat products. Thus, the questionnaire method studied the structure of nutrition and food preferences of older adults living in social security institutions in the Republic of Kazakhstan. It was found that most respondents prefer sausage products (41%), It was found that most respondents prefer sausage products (41%) to meat products (insert %). In comparison, 42% prefer vegetable dishes and fruits, 50% prefer fish and fish products, and 45% prefer dishes made with cereals and legumes.With regard to the choice of meat products, Kazakhstanis prefer horse meat (27%), followed by poultry (22%) and by beef and lamb (20% each). It was found that 99% of the survey participants have no idea about herodietic products and the existing connection between diseases and nutrition.

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A multiplicative approach to optimize the consumer properties of quick-frozen semifinished products from cultivated champignons

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ABSTRACT

It is possible to maximise the consumer properties of grown fruit and vegetable products, significantly reduce their losses during the life cycle, and satisfy the public demand for products ready for consumption by using different preservation methods, particularly freezing. It has been found that the freezing of mushrooms without pretreatment does not provide a high-quality finished product. It justifies the expediency of mushroom pretreatment before freezing to stabilize their consumer properties. The inhibition effect of high temperatures on the oxidoreductase activity has been confirmed, ensuring the high preservation of cultivated champignons' natural color and consistency. A quasimetric assessment of the quality of quick-frozen cultivated mushrooms was performed, and the optimal heat treatment parameters were determined. Before freezing, blanched mushrooms' efficiency with polysaccharides has been scientifically proved. Rational concentrations and types of polysaccharides for mushroom processing have been determined. It has been established that blanching champignons in 0.1% citric acid solution followed by xanthan gum (0.2%), guar gum (0.1%), and lamidan (0.1%) processing ensure stability of consumer properties of quick-frozen semifinished products made of cultured champignons. After defrosting, they have an attractive appearance, natural light brown colour, elastic consistency, well-expressed mushroom flavour, and harmonious taste.

Keywords: mushrooms, semifinished product, cultivated champignons, quality models, consumer properties

INTRODUCTION

The production of quick-frozen vegetable raw materials is the first step toward rational nutrition that saves maximum consumer properties, significantly reduces losses of cultured products, and meets consumer demand for products ready for consumption. The works of **[1], [2]**, **[3]**, **[4]**, **[5]**, **[6]**, **[7]**, **[8]**, etc., are devoted to the impact of freezing on the quality of fruit and vegetable products. Scientists studied and analyzed the components of the biological value of broccoli, the variety of Parthenon cultivated in the regions of Ukraine. The research results are reported, the changes in the content of ascorbic acid and isothiocyanates were analyzed, as well as a pigment composition: chlorophyll and β-carotene in the freshly harvested broccoli, as well as after its pretreatment before freezing: by blanching and aging in a solution of food salt **[1]**. In freshly harvested cabbage, pre-frozen in different ways before freezing, the mass fraction of moisture and the form of its connection with dry matter were determined **[2]**. Dubinina A. et al. studied the effect of thermal treatment on the degree of chlorophyll destruction in rhubarb and gooseberry. We established the effect of stabilizing additives on the transformation of chlorophylls and a change in the colour of rhubarb and gooseberry **[3]**. Zamorska I.et al. proposed the technology of production of strawberry jam from strawberries with the replacement of pectin solution with apple puree **[4]**. The problems of the formation of assortment and quality of frozen fruit and vegetable products are investigated in work **[5]**. A complex of organoleptic, commercial, physical, and thermophysical indicators of eggplant, sweet pepper, and tomato fruits was developed to determine their harvesting time **[6]**. Scientists' influence of cryomechanodestruction is examined on the activation and destruction of heteropolysaccharides when developing the nanotechnologies of plant supplements, in particular, frozen nanopuree from carrot, sweet pepper, pumpkin,

tomato, apricot, buckthorn **[7]**. Scientists have proved that there are colour changes and loss of cell sap in plant raw materials after defrosting because of high enzymatic activity, which negatively affects the nutritional value of products. Among cultivated mushrooms, champignons are subjected to freezing. Still, there are almost no data in the scientific literature on the study of their nutritional value after freezing and long-term low-temperature storage. Unfortunately, the traditional technology of mushroom freezing does not provide a high quality of the finished products, so the development of methods of mushroom pretreatment before freezing to stabilize their consumer properties is an important issue.

Scientific Hypothesis

The hypothesis of the scientific work lies in the supposition about the possibility of pretreatment of semifinished products made of cultivated champignons before freezing. Based on the established trends of changes in the quality of quick-frozen semifinished products from cultivated mushrooms, the mechanism of stabilization of their consumer properties by treatment of xanthan gum, guar gum, and lamidan pre-blanched mushrooms in citric acid solution has been scientifically substantiated. In-depth scientific ideas about the patterns of color stabilization and consistency of quick-frozen semi-finished products from cultivated mushrooms.

MATERIAL AND METHODOLOGY

Samples

The object of the study was quick-frozen semifinished products made of cultivated champignons (*Agaricus bisporus*) of the brown race, strain No. 117, with a closed cap of the first collection wave.

Chemicals

Potassiumiodate, KJO_3 (Energostroyinvest Trading House LLC, 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); citric acid, $C_6H_8O_7$ (Ecotechnics LLC, Ukraine) **[9]**; xanthan gum, E 415 (Criamo LLC, Ukraine) **[10]**; guar gum, E 412 (SOSA Ingredients, Spail) **[11]**; lamidan (Lamidan LLC, Ukraine) **[12]**.

Biological Material

The quick-frozen semifinished product made of cultivated champignons (*Agaricus bisporus*) included raw materials that met the requirements of regulatory documents and had the conclusions of the state sanitary and epidemiological expertise: cultivated champignons **[13]**, **[14]**. Cultivated champignons were grown in the commercial firm and purchased to produce semi-finished products.

Instruments

Titration unit (Labor-Technik LLC, Ukraine).

Analytical electronic scales KERN ABS 120-4 (Khimtex SE, Ukraine).

Termia electric cooker EPCH-1.5/200 (Mayak Vinnytsia plant PJSC, Ukraine).

Laboratory Methods

Experimental studies were conducted using modern standard, conventional and special organoleptic, physicochemical, biochemical methods, and mathematical modeling using modern computer programs (Microsoft Excel and Origin 8). The frozen product mass determined mass loss during storage; water holding capacity was determined by the difference in the frozen and unfrozen product mass; oxidoreductase activity-ascorbinatoxidase, polyphenoloxidase was determined by the quantity of ascorbic acid converted to dehydrator 1 g of tissue per unit time. The experimental data were processed by multicriteria optimization.

Description of the Experiment

Sample preparation: Brown race cultivated champignons of strain 117 with the closed cap of the first collection wave were used for the experiments. To produce a test batch of quick-frozen semifinished products, the mushrooms were cleaned of dust, soil, and other foreign impurities and inspected for quality, sorted, washed, and cut into 0.5 cm thick slices. The mushrooms were blanched in hot water at 85 °C ±2°C for 30, 60, and 90 s with the addition of citric acid concentration 0.05; 0.1 and 0.15% in stainless steel boilers, cooled with cold running water (4 – 5 °C), placed on a colander to remove excess moisture. Pre-blanched mushrooms were treated with xanthan gum, guar gum, carrageenan, and lamidan concentrations of 0.1 and 0.2% and their combinations, thoroughly mixed for uniform distribution of polysaccharides, and kept for 1 hour at plus 18 ±2 ºC for polysaccharides swelling. Mushrooms were frozen in a freezer at minus 27 ±2 °C and stored in a freezer at minus 20 ±2 °C.

Number of samples analyzed: test samples were quick-frozen semifinished products of brown race, cultivated mushrooms of strain No. 117 with the closed cap of the first collection wave. Mushrooms were pre-blanched in different concentrations of citric acid for a different time, and treated before freezing with different types and

concentrations of natural polysaccharides. Cultured champignons without pre-blanching and pre-treatment with natural polysaccharides, frozen under similar temperature regimes, served as a control sample.

Number of repeated analyses: average samples were used to determine all quality indicators in five-fold repeatability. The indices were determined, taking a mass loss during freezing and low-temperature storage into account.

Number of experiment replication: each trial was carried out five-fold replication for the test and the control sample.

Design of the experiment:

- mushroom stored in a freezer at minus 20 ±2 °C;
- mushroom preparation: the mushrooms were cleaned of dust, soil, and other foreign impurities and inspected for quality, sorted, washed, and cut into 0.5 cm thick slices;
- mushroom blanching in hot water at 85 °C ±2 °C for 30, 60, and 90 s with the addition of citric acid concentration 0.05; 0.1 and 0.15% in stainless steel boilers;
- treatment with polysaccharides of natural origin: the mushrooms were treated with xanthan gum, guar gum, carrageenan, and lasmiditan concentration of 0.1 and 0.2% and their combination;
- measurement of the organoleptic parameters, mass loss during blanching, weight loss during freezing, moisture-holding capacity, and enzyme activity;
- Microsoft Excel and Origin 8 produced statistical analyses.

Statistical Analysis

Microsoft Excel and Origin 8 produced the statistical analysis data. The accuracy of the obtained experimental data was determined using the Student's test for confident probability ≤0.05 based on the number of parallel determinations of at least 5.

RESULTS AND DISCUSSION

The problem of insufficient protein in the diet of the world's population is currently acute. Fish and fish products play an important role in solving the problem of supplying the world's population with animal protein **[15]**, **[16]**, **[17]**. Cultivated mushrooms are one of the most accessible plants protein sources with high digestibility. However, mushrooms have low storability in fresh form, which is confirmed by the results of scientific research by domestic and foreign scientists and indicates the urgent need to find effective ways of storage, timely processing, and quality control in the distribution process **[18]**, **[19]**, **[20]**, **[21]**, **[22]**. Various processing methods are widely used to reduce the losses of mushrooms and expand the range of mushroom products, such as salting, canning, drying, and freezing. The authors studied the effect of blanching on the stabilization of the consumer properties of cultivated brown race champignons. Citric acid was added to the blanching water to preserve the mushrooms' tissue structure, and natural colourand reduce the enzymatic activity that causes the mushrooms to darken more intensively. The results of the studies were used to calculate (CQI) of the cultivated brown race champignons depending on the blanching time and the amount of citric acid (Table 1).

Table 1 Comprehensive assessment of the quality of fresh-frozen cultivated champignons of the brown strain No.117.

Experiment options		Organoleptic parameters					CQI
Duration of blanching, s	**Citricacidconcentra-tion,%**	**Appearance, ball**	**Color, ball**	**Scent, ball**	**Taste, ball**	**Consistence, ball**	
Control (without blanching)		3.70 ±0.19	3.50 ±0.17	4.50 ±0.22	3.90 ±0.19	3.60 ±0.18	0.40
Experiment							
	0.05	3.80 ±0.19	3.57 ±0.17	4.54 ±0.22	3.96 ±0.19	3.70 ±0.18	0.61
30	**0.10**	3.85 ±0.19	3.59 ±0,17	4.56 ±0.22	3.96 ±0.19	3.72 ±0.18	0.61
	0.15	3.87 ±0.19	3.60 ±0.18	4.57 ±0.22	3.98 ±0.19	3.73 ±0.18	0.62
	0.05	3.90 ±0.19	3.60 ±0.18	4.59 ±0.23	4.00 ±0.2	3.78 ±0.18	0.62
60	**0.10**	3.90 ±0.19	3.60 ±0.18	4.60 ±0.23	4.00 ±0.2	3.80 ±0.19	0.63
	0.15	3.90 ±0.19	3.60 ±0.18	4.60 ±0.23	4.00 ±0.2	3.81 ±0.19	0.62
	0.05	3.87 ±0.19	3.59 ±0.17	4.50 ±0.22	4.10 ±0.2	3.65 ±0.18	0.60
90	**0.10**	3.80 ±0.19	3.60 ±0.18	4.50 ±0.22	4.10 ±0.2	3.67 ±0.18	0.60
	0.15	3.84 ±0.19	3.59 ±0.17	4.50 ±0.22	4.10 ±0.2	3.65 ±0.18	0.60
Coefficient of weight		0.10	0.10	0.10	0.10	0.11	

Table 1 Cont.

		Physico-chemical parameters				
		Mass loss during blanching, %	Moisture holding capacity, %	Enzyme activity, conventional units		
				ascorbate-oxidase	polyphenol-oxidase	
Control (without blanching)		0	63.0 ±3.15	0.30 ±0.01	8.62 ±0.43	0.40
Experiment						
	0.05	3.50 ±0.17	65.88 ±3.29	0.27 ±0.01	6.81 ±0.34	0.61
30	**0.10**	3.80 ±0.19	65.90 ±3.29	0.26 ±0.01	6.80 ±0.34	0.61
	0.15	3.70 ±0.18	66.03 ±3.30	0.25 ±0.01	6.78 ±0.33	0.62
	0.05	6.21 ±0.31	68.25 ±3.41	0.20 ±0.01	5.10 ±0.25	0.62
60	**0.10**	6.12 ±0.31	70.94 ±3.54	0.15 ±0.01	4.92 ±0.24	0.63
	0.15	6.17 ±0.31	69.15 ±3.45	0.13 ±0.01	4.91 ±0.24	0.62
	0.05	7.67 ±0.38	68.25 ±3.41	0.07 ±0.003	4.65 ±0.23	0.60
90	**0.10**	7.50 ±0.37	69.00 ±3.45	0.08 ±0.004	4.63 ±0.23	0.60
	0.15	7.57 ±0.31	69.96 ±3.49	0.09 ±0.004	4.61 ±0.23	0.60
Coefficient of weight		0.15	0.15	0.09	0.10	

The control samples were champignons without blanching. Brown race champignons pre-blanched in a 0.1% citric acid solution for 60 seconds proved to be the best organoleptic and physicochemical properties. After freezing and defrosting, the experimental samples had a slightly less attractive appearance than the fresh ones but significantly better than the control ones. The addition of citric acid helped preserve the natural colour of the mushrooms compared to the control ones. A more elastic consistency was also found in brown race mushrooms pre-blanched in a 0.1% aqueous citric acid solution for 60 seconds. Increasing the blanching time to 90 seconds harms the consistency of the mushrooms, softening the tissues due to their structure changes. The highest mass loss was observed in mushrooms irrespective of race, subjected to heat treatment for 90 seconds. Preliminary heat treatment of mushrooms before freezing positively affected the water holding capacity (compared to control ones). However, champignons were still characterized by a significant loss of cell sap, confirming the need to find additional ways to pre-treat mushrooms before freezing. As a result of these studies, thein inhibitory effect of blanching on the activity of oxidoreductases in experimental samples was determined compared to the control ones (without blanching), characterized by high activity of polyphenoloxidase, which caused the change in colour of mushrooms during defrosting. Experimental studies to determine the optimal blanching time, citric acid concentration, type, and rational concentration of natural polysaccharides require a long time and significant material costs. Therefore, the use of mathematical apparatus and the development of mathematical models of quality allows us to determine the optimal parameters for the effective preservation of product quality. Mathematical processing of the experimental results using Origin 8 software was carried out using the method of multi-criteria optimization. The initial variables (Table 2) were selected as blanching time (s) – x1 and citric acid concentration (%) – x2. The optimization criteria were: organoleptic parameters, mass loss during blanching, water holding capacity, and polyphenoloxidase enzyme activity.

Table 2 Parameters of cultivated champignon pretreatment.

Experiment options	x_1	x_2
1 (control without processing)	0	0
2	30	0.05
3	30	0.1
4	30	1.5
5	60	0.05
6	60	0.1
7	60	1.5
8	90	0.05
9	90	0.1
10	90	1.5

Mathematical models of dependence of consumer properties of cultivated brown champignons of strain No. 117 ($z_1 – z_4$) from the duration of blanching (x_1) and citric acid concentration (x_2):

1) organoleptic quality assessment (z_1):

$$z_1 = – 4.35 \cdot x_1^2 + 1.45 \cdot x_2^2 + 2.83 \cdot x_1 \cdot x_2 – 4.27 \quad (1)$$

2) mass loss during blanching (z_2):

$$z_2 = – 3.13 \cdot x_1^2 + 3.04 \cdot x_2^2 + 1.89 \cdot x_1 \cdot x_2 – 2.0 \quad (2)$$

3) water holding capacity (z_3):

$$z_3 = 2.03 \cdot x_1^2 – 2.36 \cdot x_2^2 – 3.16 \cdot x_1 \cdot x_2 + 1.43 \quad (3)$$

4) polyphenoloxidase activity (z_4):

$$z_4 = 6.47 \cdot x_1^2 + 2.08 \cdot x_2^2 – 5.92 \cdot x_1 \cdot x_2 – 7.59 \quad (4)$$

The mathematical models obtained make it possible to proceed to the nextstage of the study, which is to determine the optimum duration of blanching and concentration of citric acid. The optimal blanching parameters for cultivated brown champignons of strain No. 117 were determined graphically (Figure 1).

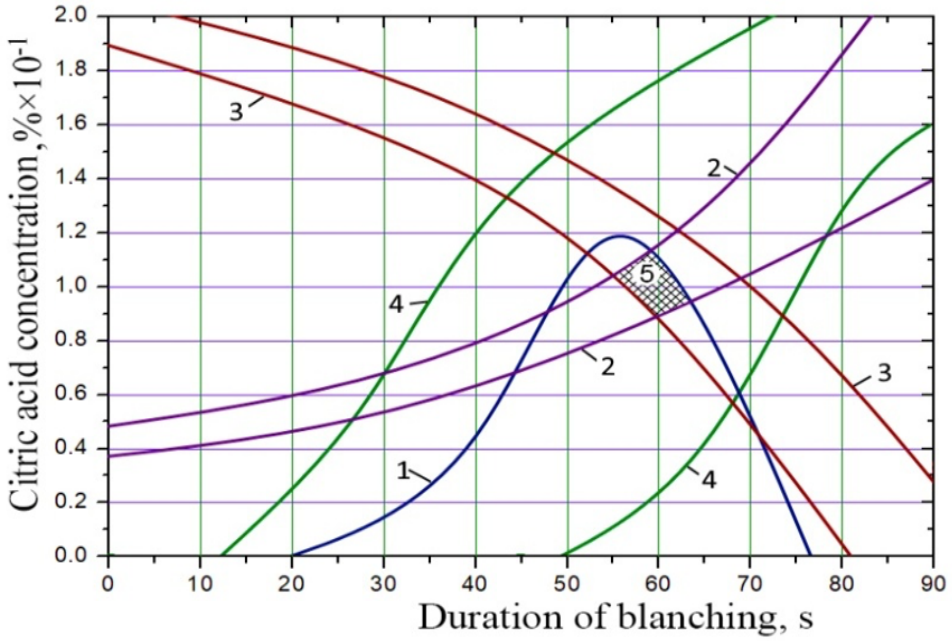

Figure 1 The optimal blanching parameters for cultivated brown champignons of strain No. 117. Note*:* Range of parameters: 1 – organoleptic assessment; 2 – mass loss during blanching; 3 – water holding capacity; 4 – polyphenol oxidase activity; 5 – the range of optimal compromise values of blanching parameters.

The studies and mathematical modeling results confirmed the positive effect of blanching on organoleptic parameters, water holding capacity, and enzymatic activity of cultivated champignons. The developed mathematical models determined the optimal blanching parameters for mushrooms in 0.1% aqueous citric acid solution for 60 seconds (taking into account CQI).

The next step was the pretreatment of cultivated champignons before freezing with natural polysaccharides: xanthan gum, guar gum, carrageenan, and lamidan at 0.1% concentration (Experiment). Samples of champignons without adding natural polysaccharides served as a control one. The main criteria for selecting polysaccharides were the organoleptic properties, mass loss after defrosting, and the water holding capacity of the raw mushroom material. Before freezing, the cultivated brown champignons of both the control and experimental variants were characterized by an attractive appearance, a well-defined natural colour, a clean, pleasant, harmonious, inherent taste without a foreign taste and flavour, and a dense, elastic consistency. The average organoleptic score was 5.0.

The studies showed that the most noticeable deterioration of sensory indicators was observed in the control sample (without treatment) after defrosting, 3.84 points (Table 3). Thus, defrosted mushrooms of control variants had a less attractive appearance (compared with the experimental ones), dark brown colour, deterioration of taste, and significant deterioration of consistency, which, in our opinion, is associated with significant physical, chemical, and biochemical changes of the product. Among the experimental variants of mushrooms, the mushrooms pretreated with xanthan gum, guar gum, and lamidan were the best in organoleptic indicators. After

defrosting, the mushroom samples were characterized by an attractive appearance and uniform, nearly natural colour.

Table 3 Organoleptic assessment of the quality of frozen cultivated brown champignons of strain No. 117 depending on the type of natural polysaccharides.

Organoleptic parameters	Control (without processing)	Experiment (concentration 0.1 %)			
		Xanthan gum	Guar gum	Carrageenan	Lamidan
Appearance	3.7 ±0.18	4.2 ±0.21	4.2 ±0.21	4.0 ±0.20	4.3 ±0.21
Color	3.5 ±0.17	4.0 ±0.20	4.0 ±0.20	3.7 ±0.18	4.0 ±0.20
Taste	3.9 ±0.19	4.3 ±0.21	4.3 ±0.21	4.0 ±0.20	4.5 ±0.22
Scent	4.5 ±0.22	4.8 ±0.23	4.5 ±0.22	4.7 ±0.23	4.6 ±0.23
Consistence	3.6 ±0.18	4.1 ±0.20	4.2 ±0.21	3.8 ±0.19	4.1 ±0.20
Secondary ball	3.84	4.29	4.24	4.04	4.30

The testing samples' taste and flavour of the frozen mushroom semifinished products were pleasant, rich, and normal, without foreign tastes and flavours. However, it should be noted that the flavour of mushrooms, which were pretreated with lamidan, was somewhat different from the other samples and had a pleasant seaweed flavour. A high level of consistent preservation of the experimental mushroom samples compared to the control ones was also noted, which is due to the ability of the natural polysaccharides to bind moisture. Testing samples of champignons treated with carrageenan had slightly lower quality indicators (compared to other types of natural polysaccharides). After defrosting, the mushrooms had significant changes in colour and consistency.

Mass loss during freezing and the water holding capacity of mushrooms had a significant impact on preserving the quantitative and qualitative characteristics of the products. Brown mushrooms (0.179%), pretreated with xanthan gum, had the lowest mass loss during freezing. This indicator was 1.64% higher for champignons of the brown race, which were treated with guar gum. The highest mass loss (0.245%) was observed in the mushrooms of the control samples (with no treatment). The cultivated brown mushrooms of the control samples were characterized by the lowest water holding capacity (63%). The highest moisture-retaining ability was characterized by samples of brown mushrooms, which were pretreated with xanthan gum (81.07%).

This indicator was lightly lower for guar gum (76.49%). This can be explained by the high content of dietary fibers in polysaccharides, which can form colloidal solutions and thus bind free water and participate in the formation of additional intermolecular bonds. Carrageenan-treated mushrooms had the lowest water holding capacity (66.92%).

The research results confirm the practicality of using natural polysaccharides to stabilize the consumer properties of quick-frozen mushroom semifinished products. Preservation of the appearance, taste, and aroma of mushrooms, their elastic consistency, reducing weight loss, and increasing the moisture-retaining capacity of semifinished products is due to the formation of a film on the surface of mushrooms during pre-treatment with natural polysaccharides.

The experiment to determine the optimal types and concentrations of natural polysaccharides is shown in Table 4. Champignons without added polysaccharides – variants No.1 were control samples, and No. 2 – 10 –pretreated with polysaccharides were experimental samples.

Table 4 Types and concentrations of natural polysaccharides.

Experiment options	Carrageenan	Xanthan gum	Guargum	Lamidan
1	0	0	0	0
2	0.1	0	0	0
3	0.2	0	0	0
4	0	0.1	0	0
5	0	0.2	0	0
6	0	0	0.1	0
7	0	0	0	0.1
8	0	0.2	0.1	0.1
9	0	0.2	0	0.1
10	0	0.2	0.1	0

The criteria for choosing the optimal concentration and type of natural polysaccharides were organoleptic properties of quick-frozen cultivated brown champignons of strain No. 117, mass loss during freezing, water holding capacity, and polyphenol oxidase activity (Table 5).

This study indicates that cultivated brown champignons of variant No. 8 (xanthan gum 0.2%, guar gum 0.1%, lamidan 0.1%) had the highest average organoleptic score (4.5 points) compared with the other variants. The control variant of brown champignons (No. 1) had the lowest averages core (3.84).

Table 5 Quality parameters of quick-frozen cultivated champignons depend on natural polysaccharides' type and concentration.

Quality parameters	Experiment variant number									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Organoleptic parameters , ball									
Appearance	3.7 ±0.18	4.0 ±0.20	4.0 ±0.20	4.2 ±0.21	4.2 ±0.21	4.2 ±0.21	4.3 ±0.21	4.5 ±0.22	4.2 ±0.21	4.3 ±0.21
Color	3.5 ±0.17	3.7 ±0.18	3.9 ±0.19	4.0 ±0.20	4.1 ±0.20	3.5 ±0.17	3.7 ±0.18	3.9 ±0.19	4.0 ±0.20	4.1 ±0.20
Taste	3.9 ±0.19	4.0 ±0.20	4.0 ±0.20	4.3 ±0.21	4.3 ±0.21	4.3 ±0.21	4.5 ±0.22	4.5 ±0.22	4.4 ±0.22	4.5 ±0.22
Scent	4.5 ±0.22	4.7 ±0.23	4.7 ±0.23	4.8 ±0.24	4.8 ±0.24	4.9 ±0.24	4.8 ±0.24	4.7 ±0.23	4.9 ±0.24	4.8 ±0.24
Consistence	3.6 ±0.18	3.8 ±0.19	4.0 ±0.20	4.1 ±0.20	4.1 ±0.20	4.2 ±0.21	4.1 ±0.20	4.3 ±0.21	4.2 ±0.21	4.2 ±0.21
Secondary ball	3.84	4.04	4.12	4.29	4.30	4.24	4.30	4.50	4.36	4.38
Quality parameters	**Experiment variant number**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Physical and biochemical parameters									
Weight loss during freezing,%	0.245 ±0.01	0.231 ±0.01	0.216 ±0.01	0.179 ±0.008	0.170 ±0.008	0.182 ±0.009	0.181 ±0.009	0.144 ±0.007	0.185 ±0.009	0.160 ±0.008
Moisture retention capacity,%	63.0 ±3.15	66.92 ±3.34	68.03 ±3.40	81.07 ±4.05	81.45 ±4.07	76.49 ±3.82	75.10 ±3.75	83.60 ±4.18	81.50 ±4.07	81.70 ±4.08
The activity of polyphenol-oxidase, c.u.	8.62 ±0.43	8.63 ±0.43	8.63 ±0.43	8.61 ±0.43	8.61 ±0.43	8.61 ±0.43	8.60 ±0.43	8.59 ±0.43	8.60 ±0.43	8.60 ±0.43

The dependence of organoleptic properties, mass loss, water holding capacity, and polyphenol oxidase activity on the types and concentrations of natural polysaccharides can be described mathematically.

Based on the experimental data, we obtained the following mathematical descriptions:

5) organoleptic quality assessment (z_1):

$$z_1 = -0.04 \cdot x_1 + 4.67 \cdot x_2 - 1.06 \cdot x_3 + 0.16 \cdot x_4 + 0.11 \cdot x_1 \cdot x_3 - 0.38 \cdot x_2 \cdot x_3 + 1.26 \cdot x_2 \cdot x_4 - 0.83 \cdot x_2 \cdot x_4 - 0.47 \quad (5)$$

6) mass loss during freezing (z_2):

$$z_2 = 0.21 \cdot x_1 - 0.12 \cdot x_2 + 3.07 \cdot x_3 - 2.67 \cdot x_4 + 0{,}19 \cdot x_1 \cdot x_3 + 2.08 \cdot x_2 \cdot x_3 - 1.46 \cdot x_2 \cdot x_4 - 1.84 \cdot x_2 \cdot x_4 + 1.22 \quad (6)$$

7) water-holding capacity (z_3):

$$z_3 = -0.76 \cdot x_1 + 2.41 \cdot x_2 - 1.47 \cdot x_3 + 0.26 \cdot x_4 + 2.18 \cdot x_1 \cdot x_3 + 0.27 \cdot x_2 \cdot x_3 + 1.48 \cdot x_2 \cdot x_4 - 1.21 \cdot x_2 \cdot x_4 - 2.04 \quad (7)$$

8) polyphenoloxidase activity (z_4):

$$z_4 = 0.46 \cdot x_1 - 1.25 \cdot x_2 + 2.04 \cdot x_3 - 1.27 \cdot x_4 + 0.66 \cdot x_1 \cdot x_3 + 0.94 \cdot x_2 \cdot x_3 - 0.06 \cdot x_2 \cdot x_4 - 2.73 \cdot x_2 \cdot x_4 + 2.07, : \quad (8)$$

where x_1 is carrageenan content, x_2 is xanthan gum content, x_3 is guar gum content, x_4 is lamidane content, %):

Xanthan gum and lamidan content were the most important for organoleptic assessment, guar gum, and xanthan gum – for mass loss during freezing, guar gum, and xanthan gum – for water-holding capacity, and guar gum – for polyphenol oxidase activity. The effect of carrageenan on organoleptic parameters and water-holding capacity was inversely proportional. Organoleptic parameters and water holding capacity decreased significantly with increasing carrageenan content.

The mathematical models were a prerequisite for the next stage of the study, the task of which was to validate the polysaccharide treatment parameters (Figure 2) mathematically.

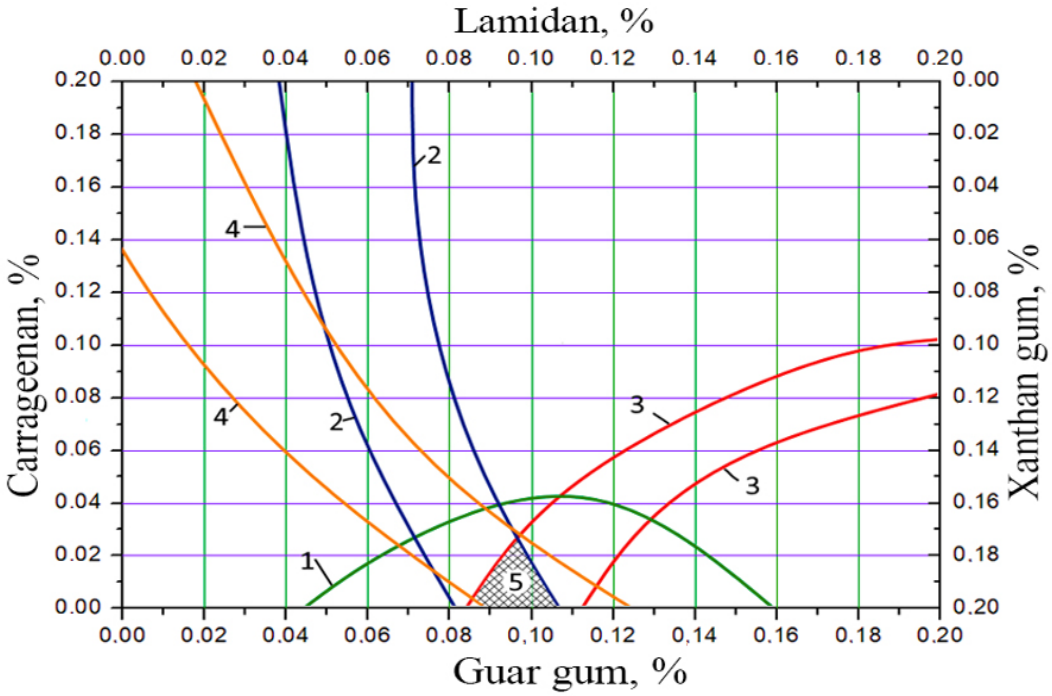

Figure 2 Optimal range of concentrations of natural polysaccharides for cultivated brown champignons of strain No. 117. Note: range of parameters: 1 – organoleptic assessment; 2 – mass loss during frosting; 3 – water holding capacity; 4 – polyphenol oxidase activity; 5 – the range of optimal compromise values of natural polysaccharide concentrations.

Optimal types and concentrations of natural polysaccharides are mathematically proved: 0.2% xanthan gum; 0.1% guar gum; 0.1% lamidan.

Quick-frozen mushroom semi-finished products treated with natural polysaccharides had an attractive appearance, natural light brown colour, elastic consistency, and well-defined taste and smell (Figure 3).

Figure 3 Quick-frozen semifinished product made of cultivated brown champignons of strain No. 117.

Scientists **[23]** proposed a method to control the relative humidity of shiitake mushrooms and found that mushrooms should be dried in an environment with low relative humidity. The use of ultrasound as a method of pretreatment of mushrooms before drying to improve their quality has been studied by foreign scientists. Scientists analyzed the effect of ultrasound and pre-blanching on the drying of mushrooms using a logarithmic model according to given criteria and parameters. It was found that pretreatment of mushrooms reduces the drying time by 9.5% compared to untreated samples **[24]**. Farahnak R. et al. investigated the effect of different ultrasound modes on the defrosting rate and the quality of champignons compared to conventional defrosting methods. They found that using probe-type ultrasound at 250 W could increase the defrosting rate and reduce protein denaturation without significant structural changes **[25]**. The works of Islam et al. **[26]** are devoted to the effect of ultrasound on the quality and rate of mushroom freezing. Aday M. investigated the effectiveness of electrolyzed water on the storage life of mushrooms and found that electrolyzed water at concentrations of 25 mg/l and 50 mg/l preserve the quality of mushrooms better than other treatments **[27]**. The Isfahan University of Technology studied the effect of static electric field on freezing parameters and microstructure of mushrooms (*Agaricus bisporus*). The experimental mushroom samples were frozen at minus 30 °C in an electrostatic field with 0; 4.5; 9.0, and 13.5 kV voltages. It was found that freezing in an electric field increases the generation temperature and reduces the ice crystal size, improving the mushroom microstructure. The smallest ice crystals were formed at voltages of 4.5 and 9.0 KV **[28]**, **[29]**. Scientists **[30]** analyzed the water holding capacity, loss of cell membrane integrity, and changes in the state of structural polymers of the cell wall of *Agaricus bisporus*. Marcal Sarah et al. reflected the effect of canning methods on mushrooms' nutritional and biological value **[31]**. The effect of different combinations of freezing and defrosting on preserving the quality of *Pleurotus eryngii* and the fruit bodies of shiitake mushrooms (*Lentinula edodes*) was studied **[32]** in the works **[33]**. Octavian Baston et al. investigated the quality of *Agaricus* and *Pleurotus* mushrooms during freezing and low-temperature storage **[34]**. Scientists **[35]** investigated the effect of different cooking and canning methods on the *Amanita Zambian* mushroom's nutritional value and phytochemical composition. The work **[36]** analyzed and conducted a comparative assessment of sensory indicators of *Cantharellus cibarius*, *Craterellus tubaeformis*, *Boletus edulis*, and *Lactarius camphoratus* wild mushrooms with *Agaricus bisporus* cultivated mushrooms. Scientists from Ukraine proposed the expansion of the range of sausages with increased biological value by combining meat and mushroom raw materials and found that the mass fraction of fat decreases, the proportion of carbohydrates increases, and the composition of the protein is closer to the “ideal” one in the finished products**[37]**. Egyptian scientists proposed to add fresh or dried mushrooms of the *Pleurotus ostreatus* genus to processed cheese. Organoleptic, physicochemical, and microbiological parameters were studied, and it was found that the finished product is characterized by increased nutritional value and improved sensory properties **[38]**. Scientists **[39]** analyzed cream and Enoki (*Flammulina velutipes*) mushroom extract combinations. They found a slowing of ice crystal growth in whipped cream by adding 0.1% of Enoki mushroom extract, which provides a reduction in quality changes during low-temperature storage. Scientists **[40]** proposed the use of microperforated packaging material to extend the shelf life of fresh-sliced mushrooms. The proposed design of the micro-perforation process, which will be used in passive modified atmosphere packaging, was based on the diameter and number of microholes, and the shelf life of fresh-sliced mushrooms was determined. The empirical equation used in this research can be determined to be applied to microperforated packaging design for fresh-sliced mushrooms. The fresh-sliced mushrooms' shelf life was 8 days, while it was less than 7 days (4, 5, or 6 days) when packaged with non-microperforated packaging material.

CONCLUSION

We determined the positive effect of blanching on organoleptic and physico-chemical parameters. Based on the complex index of quality calculation quality index, the rational parameters of the heat treatment process, namely blanching raw material in 0.1% solution of citric acid for 60 seconds, were determined. Based on the determined changes of consumer properties of quick-frozen semifinished products of cultivated champignons, the rational methods of pretreatment are scientifically proved: blanching of champignons in citric acid solution (0.1%) with the subsequent treatment with xanthan gum (0.2%), guar gum (0.1%) and lamidan (0.1%). It has been scientifically proven and experimentally confirmed that fast-supply semi-finished products with cultivated ovens, treated with certain types and concentrations of polysaccharides of natural origin after thawing, have a small attractive appearance, light brown color, elastic consistency, well-defined mushroom smell, and harmonious nature. Previous moisture-retaining political strength and lower phenol oxidase activity provided less weight loss during freezing.

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Development of a laboratory method for determination of the quality and freshness of frozen poultry meat

Daulet Shalginbayev, Raushangul Uazhanova, Akmaral Mateyeva

ABSTRACT

Traceability of poultry meat quality imported into Kazakhstan is an urgent task. To increase their benefits, some suppliers resort to falsification – they misrepresent thawed meat for chilled raw materials or carry out several cycles of freezing-thawing meat. The objective of these studies is to develop reliable methods for determining the quality and freshness of frozen natural semi-finished poultry meat, including the number of cycles of freezing and thawing meat. Dressed broiler chickens developed by the manufacturer from the Russian Federation using gas and electric stunning of poultry were selected as the research objects. A synchronous analysis device was used for thermal analysis in the heating-cooling process. Histomorphological studies were carried out on a microscope with an eyepiece magnification of x7. Histological examination revealed alterations in the structure of re-frozen and thawing lean tissue. The differential scanning calorimetry (DSC) analysis showed that the specific heat of thawing broiler fillets stunned by electric current during the first thawing is 218.8 J/g, at the second 209.5 J/g, and the third 201.4 J/g, and the specific heat of thawing broiler fillets stunned by gas during the first thawing is 176.5 J/g, at the second 171.9 J/g, and the third 162.6 J/g. Samples of broiler fillets stunned by electric current lost about 2.5% at each thawing stage, and with gas stunning 3% of moisture. The obtained results and research methods can be used to establish the falsification of the thermal state of broiler chicken meat by its undeclared freezing-thawing.

Keywords: poultry stunning, broiler chicken meat, freezing, thawing, morphological changes

INTRODUCTION

Poultry meat production is increasing its volumes in Kazakhstan and abroad due to the availability and continuous improvement of technical support at all stages of the technological process **[1]**, **[4]**. The technological processing of poultry meat includes several interrelated stages that significantly affect the final quality indicators of meat and, consequently, of finished ready-to-serve foods **[2]**. The quality of the food product largely depends on the chemical, physical and structural changes that occur in the muscles during autolytic transformations after poultry slaughter **[3]**. One of the main stages of technological processing of dressed poultry is the stunning process, where the electric current of certain parameters is used in most cases of foreign and domestic practice. An alternative method of stunning is a regulated gas medium used in many European enterprises **[4]**, **[7]**. The advantages of stun technology in a controlled gas environment are to improve the quality of meat: there are no bruises, the colour and taste of meat are significantly improved, due to more intensive bleeding, the carcass and liver have a better appearance **[5]**, **[8]**. Currently, the stunning birds in a gaseous atmosphere are practically not used in Kazakhstan. This technology has found its application in Europe only recently, and in Kazakhstan, it has hardly been studied, and in this regard, it is of great scientific and practical interest **[22]**. Despite the increasing rates of poultry meat production in Kazakhstan, the share of imported meat is still high. Over the past 2020, the main suppliers of poultry meat to Kazakhstan were Russia, Ukraine, the USA, and Belarus (Figure 1) **[1]**. Traceability of the quality of meat raw materials imported into the republic is an urgent task. Some suppliers resort to falsification to increase their benefits – they misrepresent thawed meat for chilled raw materials or carry out several freezing-thawing cycles **[6]**.

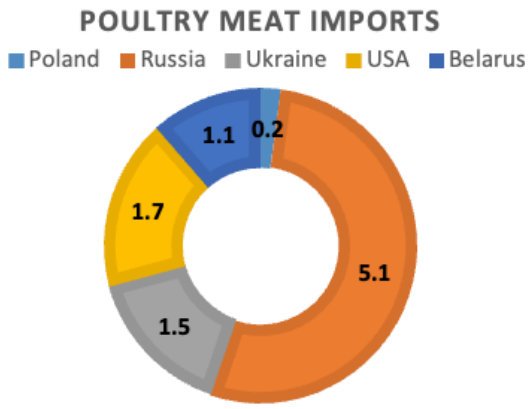


Figure 1 Structure of poultry meat imports to Kazakhstan, million tons.

Scientific Hypothesis

Studied the dependence of storage method on moisture bonding and histomorphological characteristics of chicken meat.

MATERIAL AND METHODOLOGY

Samples

To maintain the experimental integrity, dressed broiler chickens bred in the conditions of the agro-industrial complex of BEPFC of "Belgrankorm" (Belgorod region, Russia) using gas and electric stunning were selected as research objects.

Fresh frozen broiler chicken fillet stored before freezing at a temperature of 0 – 4 °C for a day after slaughter and broiler chicken meat subjected to several freeze-thawing cycles were used for research.

Instruments

A synchronous thermal analysis device STA 449 F3, Jupter by NETZSCH was used for thermal analysis during heating-cooling. The device simultaneously records the curves of differential scanning calorimetry (DSC) and mass loss (ML). Histomorphological research was carried out were studied on a Bio lamP 1 U4 2 microscope under lenses 3,2; 10; 40 with an eyepiece magnification of 7×.

Laboratory Methods

Histomorphology research methods were carried out according to GOST 19496-2013 **[12]**. The thermal analysis method was used to determine the forms of moisture-binding in raw materials **[21]**.

Description of the Experiment

Sample preparation: The thermal analysis method was used to determine the forms of moisture-binding in raw materials. To perform thermal analysis, a piece of cloth was cut out with a blade and placed in a crucible, then covered with an aluminum lid. A sample of lean tissue weighing 24 mg was taken for analysis.

The device simultaneously records the curves of differential scanning calorimetry (DSC) and mass loss (ML). The analysis of the broiler sample was carried out in a copper furnace connected to a Dewar flask in oxidized aluminium crucibles in a helium atmosphere. The accuracy of the temperature measurement was ± 0.3 °C.

The meat sample was continuously cooled – heated at a rate of 5K/min, according to the developed temperature program shown in Table 1.

Table 1 Temperature research program.

No.	Process	Process conditions and modes	
		initial temperature, °C	resultant temperature, °C
1	Freezing of fresh meat	25 in a closed crucible	-30, nitrogen cooling
2	Heating (Thawing)	–30 in a closed crucible	25
3	Freezing	25 in a closed crucible	-30, nitrogen cooling
4	Heating (Thawing)	–30 in a closed crucible	25
5	Freezing	25 in a closed crucible	-30, nitrogen cooling
6	Heating	–30 in an open crucible	250

Measuring and processing the output information in calorimeters is controlled from an IBM-compatible personal computer using a special software package, "NETZSCH-Proteus". Programmatically, calorimeters are

configured, modes are selected, experimental parameters are set, calorimeters are graduated based on measurements of the properties of standard samples, parameters are optimized, operation control, output information processing, printing and storing analysis results.

During the measurement process, the display of the personal computer is displayed in the mode of real-time heat flux values [мW] – (Y axis) as a function of temperature [t, °C or K] or time [τ, min or sec]. Upon completion of the experiment, the desired temperature of phase or structural transformation (T, °C or K), specific heat of phase or structural transformation (ΔH, J/kg), and specific heat (C, J/kg.K) are calculated using a special software section.

For histomorphological research, samples were prepared: scraps of 2 x 3 mm meat were cut off from the dressed poultry fixed in 10% neutral formalin for seven days. The samples were dehydrated in alcohols of ascending concentration starting from 50% and ending with absolutely anhydrous (100% concentration) with an interval of 4 – 6% and the duration of each stage of 24 hours. Dehydrated samples were filled with paraffin. Sections with 7 – 10 microns were made from paraffin blocks on a sledge microtome, stained with hematoxylin-eosin. Boehmer's alum hematoxylin was used as a nuclear dye, and alcohol eosin was used as the main one.

For histomorphological methods of broiler meat studies, samples intended for research were fixed in 10% neutral formalin.

The slices were obtained on the OMT 0228 microtome cooler mounted based on a sledge microtome. The hematoxylin-eosin method was used for staining, Ehrlich's alum hematoxylin was used as a nuclear dye, and eosin was used as the main one.

Number of analyzed: 12
Number of repeated analyses: 5
Number of experiment replication: 5

Statistical Analysis

Fundamental statistical analysis was carried out using the Statistica-6.1 software package (Sentinel System 7.5.7, V6.1). The Student's t-test determined the probability of similarity between the average values of the samples.

The average value of the trait before the experiment is 201.333 ±8.200 (m = ±4.734).
The average value of the trait after the experiment is 209.900 ±8.707 (m = ±5.027).
The number of degrees of freedom (f) is 2.
The Student's paired t-test is equal to 23.079.
The critical value of the Student's t-test for a given number of degrees of freedom is 4.303.
tnabl > tkrit, changes in the trait are statistically significant ($p = 0.002$).

RESULTS AND DISCUSSION

Histological examination of fresh broiler chicken meat showed a clear division of muscle tissue into peculiar slices **[14]**. Myocytes had a polygonal shape with rounded corners. Almost all cells had a uniform colour of the cytoplasm **[20]**. The cores were visualized clearly and had a rich colour. The striation of the fibers was distinguishable (Figure 2).

After a single cycle of freezing and thawing, the studied muscle tissue had a very similar structure to the cooled one; however, it was noticeable that the intercellular space was edematous, which is noticeable why the cells were located less densely and looked dormant. On the longitudinal sections of the fibers, their somewhat uneven colouring was visible, the nuclear apparatus was preserved, and the striation was weakly expressed (Figure 3).

Defects in muscle tissue that appeared due to thawing meat when injured by ice crystals are partially expressed **[16]**, **[19]**. Histological examination revealed alterations in the structure of re-frozen and thawing lean tissue **[7]**, **[20]**. The distinctive features were local layering of myocytes on top of each other, swelling of the cellular space, collapsed and destroyed connective tissue, and dormant myocytes **[8]**, **[10]**. At the same time, the striation and nuclear apparatus of the muscle fibers also changed: the pattern of the fibers was fuzzy, and the nuclei were destroyed. Muscle tissue frozen more than once is represented by a typical microstructure of highly fragmented muscle tissue after low-temperature treatment **[17]**, **[19]**. The pattern of transverse striation of muscle fibers was not visible. The nuclei of muscle cells were absent. Multiple "fractures" of muscle cells specific to low-temperature exposure were detected (Figure 4). Moreover, these signs were specific to these methods of stunning.

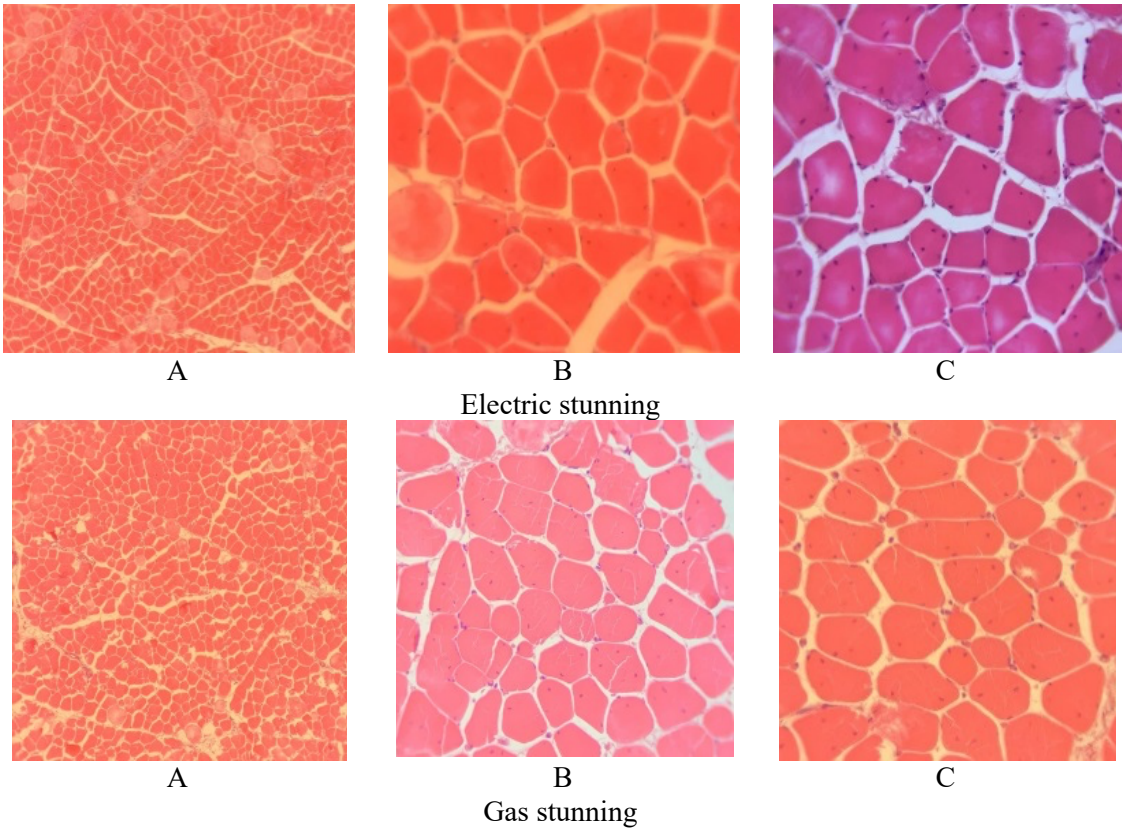


Figure 2 Histological structure of cooled muscle tissue of broiler chicken. A (mag. x100); B, C (mag. x400).

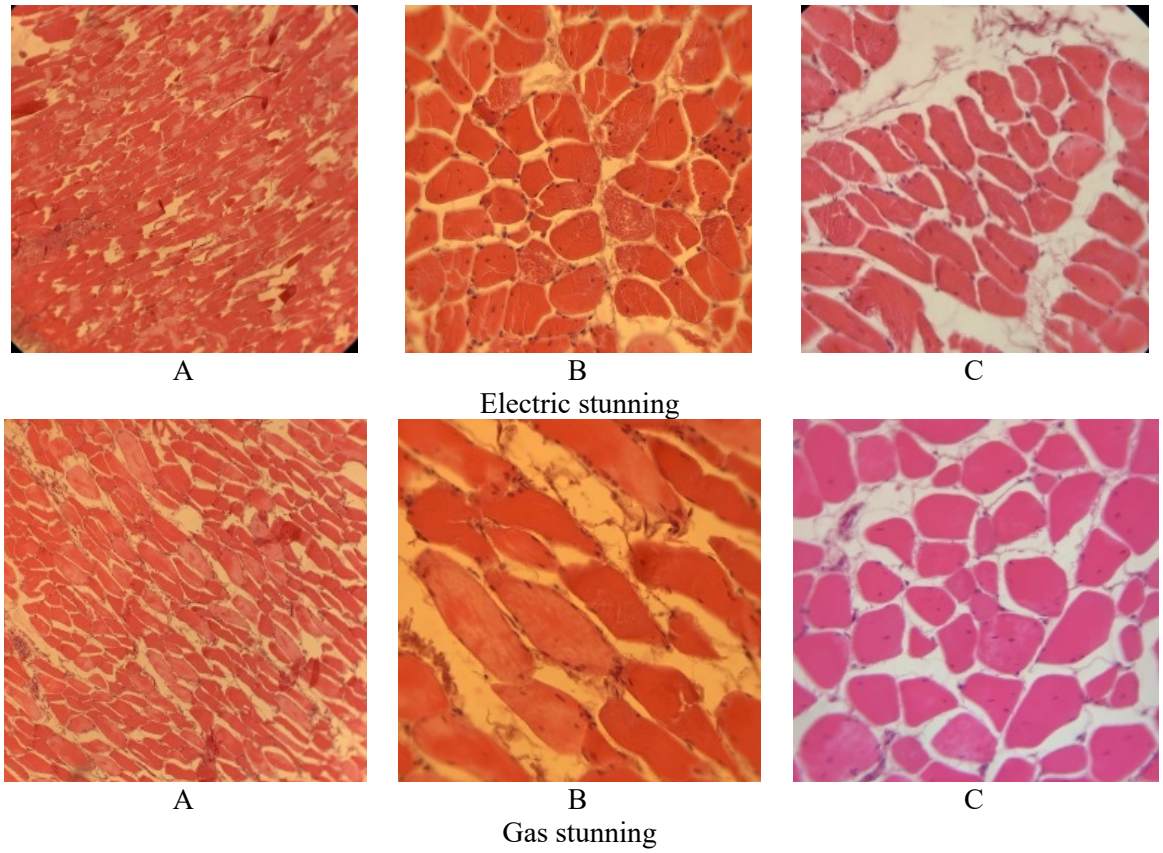
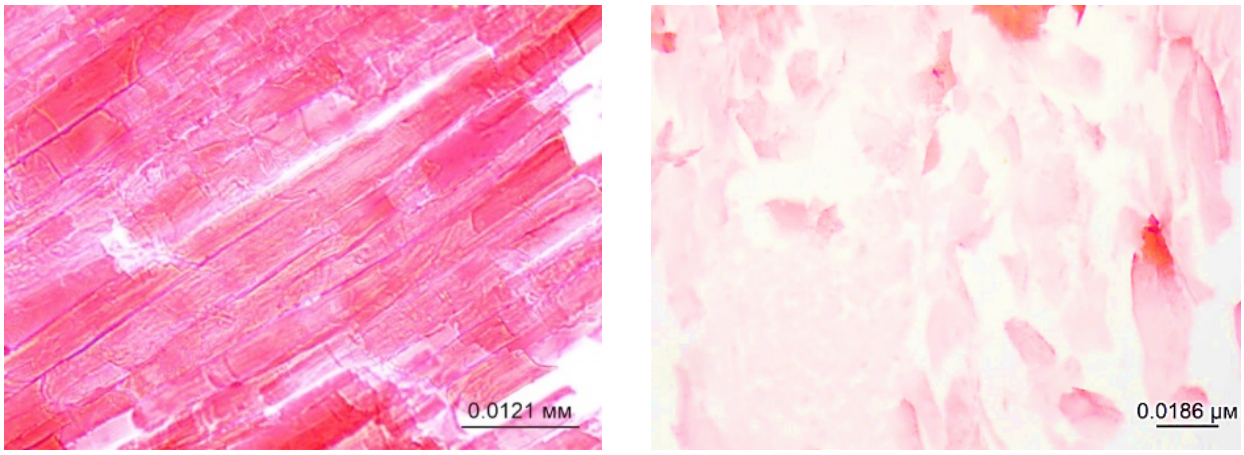


Figure 3 Histological structure of poultry muscle tissue after a single cycle of freezing and thawing A (mag. x100); B, C (mag.x400).



A B

Figure 4 Microstructure of the muscle tissue of a broiler chicken after several freeze-thawing cycles. Mag. x200. A – two cycles, B – three cycles.

Thus, by the type of histological sections of muscle tissue, it can be concluded that the multiplicity of freezing-thawing of broiler chicken meat and the terms and the change in the amount of moisture contained in muscle fibers during freezing **[21]**, **[22]**. And thawing can also be traced using thermal analysis by differential scanning calorimetry **[18]**, **[20]**.

Table 2 presents the results of thermal analysis of raw meat subjected to several cycles of freezing and thawing by differential scanning calorimetry.

Table 2 Results of differential scanning calorimetry of chicken fillet samples of various methods of stunning freezing cycles.

Stun method	Parameters	1 thaw	2 thaws	3 thaws
Electrical stunning	Geometric beginning °C	-3.3	-3.3	-3.4
	Area, J/g	218.8	209.5	201.4
	Shrinkage, %	2.51	2.54	2.52
Gas stunning	Beginning, °C	-3.3	-3.5	-3.6
	Area, J/g	176.5	171.9	162.6
	Shrinkage, %	3.35	3	3.15

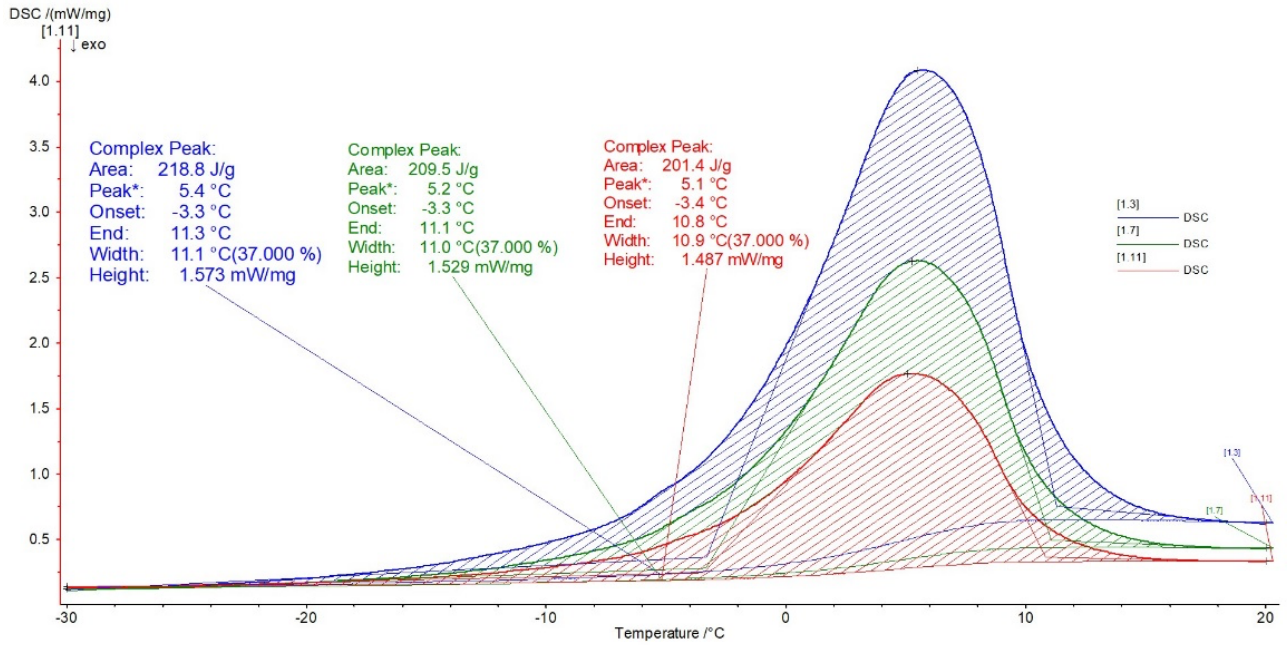


Figure 5 DSC of chicken fillet stunned by electric current (1, 2, 3 thaws).

The geometric beginning of melting moisture in broiler fillet stunned electrically is in the range from -3.3 to -3.4 °C, and in the broiler, fillet stunned by gas from -3.3 to -3.6. The specific heat of thawing of broiler fillets stunned electrically during the first thawing is 218.8 J/g, at the second 209.5 J/g, and at the third 201.4 J/g (Figure 5).

The specific heat of thawing broiler fillets stunned by gas during the first thawing is 176.5 J/g, the second 171.9 J/g, and the third 162.6 J/g (Figure 6).

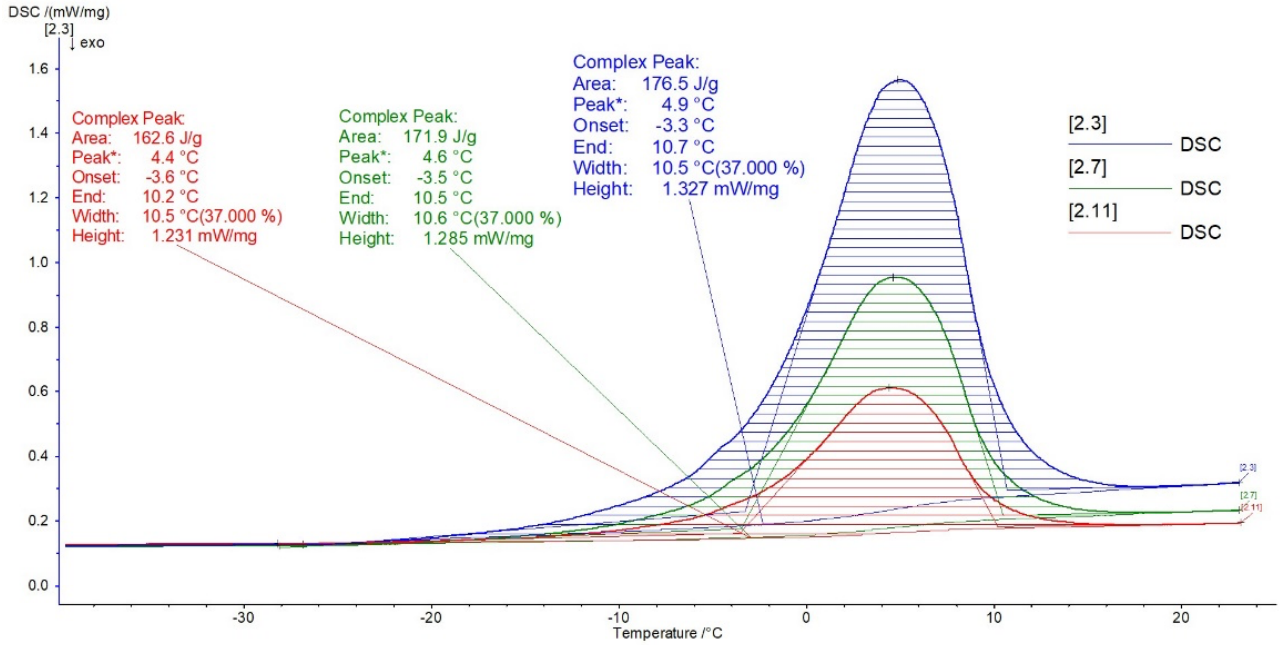


Figure 6 DSC of chicken fillet stunned with a gas mixture (1, 2, 3 thaws).

At each thawing stage, the samples lost some moisture due to evaporation. Samples of broiler fillets stunned by electric current lost about 2.5% at each thaw stage and 3% during gas stunning.

The DSC analysis curves showed that the object of study loses the amount of bound moisture with each freezing-thawing cycle, which is illustrated by a decrease in the specific heat of thawing **[17]**, **[21]**.

Temperature fluctuations cause the most noticeable damage to meat quality and its food safety. Repeated freezing and thawing of meat temperature fluctuations lead to recrystallization of ice. As a result, along with a decrease in the number of crystals, their sizes increase, violating the integrity of muscle fibers, protein denaturation, and large moisture losses **[8]**, **[10]**, **[13]**. Accordingly, the consistency of flesh becomes flabby and dry. In addition, with an increase in temperature, oxidative processes occur more actively, which is 2 – 3 times higher, for example, at a temperature of -9 °C than at a temperature of -18 °C, resulting in a rancid taste and smell **[11]**. Also, at temperatures above -10 to -12 °C moulds, some bacteria assimilate half-life products of proteins and cause the formation of ammonia, and hydrogen sulfide is in a viable state **[12]**. Repeated freeze-thawing cycles aggravate the picture: muscle fibers lose their clearly distinguishable structure and decrease volume. All of the above changes in meat can be observed during histomorphological studies **[9]**, **[15]**. The revealed difference in the stunning methods is most likely explained by the complete bleeding process at the primary processing stage of poultry meat **[7]**, **[8]**. The maximum deviation between the five measurements for each sample was no more than 0.5%.

The changes occurring during the refrigeration process are identical and reliably characterize the loss of moisture during repeated thawing and meat freezing.

CONCLUSION

Histological examination revealed alterations in the structure of thawed broiler meat tissue. The developed temperature program for conducting DSC studies of broiler meat allows using the thermoanalytical curves of differential scanning calorimetry to determine the difference in the quantitative characteristics of the processes of its one- and two-fold freezing in a closed oxidized aluminum crucible in a helium atmosphere in the temperature range from 25 to -30 °C in terms of cryoscopic temperature, peak area numerically equal to the exothermic effect of the freezing process. An urgent problem for poultry processing enterprises and trade enterprises is the objective assessment and classification of poultry meat based on the thermal state and types of

cooling, following the scheme of technological processing and logistics. The expediency of using a synchronous thermal analysis device for monitoring the storage, processing, and transportation of fresh and chilled poultry meat has been proved, and the facts of falsification of poultry meat in a thermal state in analytical laboratories have been revealed. The developed method does not require complex sample preparation, is suitable for operational or operational control of processing, and ensures the objectivity and reliability of measurement results since it allows you to identify changes in poultry meat in the surface layer and in muscle tissue. The obtained results can be used to establish the falsification of the thermal state of broiler chicken meat by its undeclared freezing-thawing.

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The possibility of a halal mix probiotic medium for the cultivation of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae*

Yetti Marlida, Harnentis, Azizah, Yuliaty Shafan Nur, Frederick Adzitey, Norliza Julmohammad, Nurul Huda

ABSTRACT

This study aimed to determine the effects of interaction between media type (halal mix preparation) and culture mixtures of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* (probiotics). A completely randomised factorial design (CRFD) consisting of 2 factors and three replications was used, where factor A was a mixture of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* at a ratio of 1:1 (A1); 1:2 (A2) and 2:1 (A3) and factor B was the type of growth media, that is, control (B1), whey tofu, molasses, and fish waste flour (B2), and coconut water, onggok flour and shrimp waste flour (B3). The variables measured were viability, cell biomass, and pH. The results showed interactions between factors A and B, which were significantly different (p <0.05) in terms of viability, cell biomass, and pH. Based on the results of the study, it can be concluded that the mixture of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* at a ratio of 2:1 (A3), using coconut water, onggok flour, and shrimp waste flour (B3) as medium and incubated at 36 °C for 24 hours was the best medium. It had a 2.37 viability, 42.33 mg/ml biomass cell, and a pH of 2.37.

Keywords: Halal, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, viability, biomass cell

INTRODUCTION

Probiotics are microorganisms that harbour and maintain the digestive system of humans and animals. They are eaten by humans and given to livestock primarily as feed additives. Probiotics are live microorganisms supplied directly (direct-fed microbes) and might be a single culture or a blend. When given in adequate amounts, they provide health benefits to the host **[1]**. The benefits of probiotic bacteria for livestock include increasing the immune system and helping nutrient absorption **[2]**. Farmers use probiotics as feed additives because several countries have banned antibiotics as growth promoters and the tendency for pathogenic bacteria to develop resistance to certain antibiotics **[3]**. Lactic acid bacteria and *Saccharomyces cerevisiae* are two types of probiotics derived from bacteria and yeast that are extensively utilized in livestock. In recent years, lactic acid bacteria (LAB) and yeast have become more popular as probiotics in the industrial sector.

Lactobacillus plantarun N16 isolated from fermented buffalo milk called dadih is a probiotic due to its ability to survive at low pH, resistance to 0.03% bile, and ability to kill pathogenic bacteria such as pathogenic bacteria as *E. coli*, *S. aureus,* and *S. Enteritidis* **[4]**. *Saccharomyces cerevisiae* isolated from fermented fish or budu has also been reported to be a probiotic **[2]**. A combination of these two probiotics need to be considered because many commercial probiotics contain various types of microbes, for example, PoultryStar ME has *Enterococcus faecium*, *Lactobacillus reuteri*, *L. salivarius* and *Pediococcus acidilactici*) **[5]**, PrimaLac has Lactobacillus spp., *E. faecium* and *Bifidobacterium thermophilum* **[6]**, and Microguard contains various species of *Lactobacillus, Bacillus*, *Streptococcus*, *Bifidobacterium*, and *Saccharomyces* **[7]**. Lactic acid bacteria and yeast can be combined as probiotics to produce a symbiotic relationship. This was found in the research of Lara-Hidalgo et al. **[8]**, which reported that yeast could increase the number of lactic acid bacteria as probiotics for digestion and fat absorption in the digestive tract. This was supported by the findings of Paramithiotis et al. **[9]**. They reported that lactic acid bacteria produce lactic acid that can be used by yeast as a food source, and yeast produces catalase which can

eliminate H_2O_2 produced by lactic acid bacteria making yeast stimulate the growth of lactic acid bacteria. Rahman et al. **[7]** added that the number of *Lactobacillus* and *Saccharomyces cerevisiae* cells in a mixed culture growth medium was higher than in separate culture growth media.

Adequate nutrition is needed to ensure the survival of bacteria and yeast. Some of the nutrients required include carbon, nitrogen, and other minerals **[10]**. Commercial growth media such as MRS are specific media for the growth of lactic acid bacteria. However, its use on an industrial scale is still a challenge because it is relatively difficult and expensive to obtain. It is necessary to replace costly media with relatively cheaper media that support microbial growth in some communities - like Muslim and Vegetarian communities, where components in MRS broth/medium is an issue. Beef extract and peptone, nitrogen derived from animal sources used for MRS medium, should be avoided. For Muslims, all components of MRS must be halal (permissible for a follower of Islam)-certified, including its animal-derived parts. The primary media for *Saccharomyces cerevisiae* is YPD (bacto yeast extract, bacto peptone, D-glucose, and bacto agar), which must be changed to incorporate less expensive components and take into account the Muslim and Vegetarian communities.

The potency of waste as a natural growth medium for an economical source of carbon and nitrogen is expected to be an alternative solution to the problem of environmental pollution. This study explored natural growth media made from tofu liquid waste, molasses, fish waste flour, coconut water, onggok flour (tapioca waste flour), and shrimp waste flour to grow *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae*. So far, there have been no studies reporting on alternative media (mixed halal preparation) for the growth of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* as probiotics.

The research aimed to determine the viability, cell biomass, and pH of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* grown as culture mixture (halal mix preparations).

MATERIAL AND METHODOLOGY

Samples

Lactobacillus plantarum N16 and *Saccharomyces cerevisiae* were used as starter cultures. They were obtained from the Laboratory of Feed and Technology, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia. The cultures were stored in a 10% skim milk mixture and 1% sucrose under -20 °C. Alternative materials such as whey tofu, molasses, fish waste flour, coconut water, onggok (tapioca waste flour), and shrimp waste flour were purchased from the local market.

Chemicals

Chemicals used in this study were MRS Broth medium (de Man Rogosa and Sharpe Broth), PDA (Potatoes Dextrose Agar), and PDB (Potatoes Dextrose Broth). All media used were also purchased from Merk, Germany. Tofu liquid waste, molasses, fish waste flour, coconut water, onggok flour, and shrimp waste flour were purchased from the local market.

Animals and Biological Material

Biological materials involved in this study were *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* of our own collection isolated from the previous study.

Description of the experiment

The experiment consisted of 2 factors (A and B), where factor A was a mixture of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* at 1:1 (A1), 1:2 (A2), and 2:1 (A3), and factor B was the type of growth media, thus, control (B1), whey tofu, molasses, and fish waste flour (B2), and coconut water, onggok flour, and shrimp waste flour (B3). The variables measured were viability, cell biomass, and pH.

Laboratory Methods

Viability determination

Cell viability assay measures the number of live/metabolically active cells in a population. Viability was measured according to Pires et al. **[11]**. Viability tests were carried out before and after incorporating *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* on natural media to ensure their growth using the plate count method. A total of 1 ml of the dilution was plated on a sterile petri dish, poured on MRS agar media, and shaken until evenly distributed. It was then incubated at 37 °C for 24 hours. Afterwhich viability was tested by measuring OD (Optical Density) using a spectrophotometer at an absorbance wavelength of 600nm.

Cell biomass determination

Cell biomass was measured based on the weight of the precipitate in the supernatant according to Pires et al. **[11]**. Centrifugation was carried out twice. Firstly, 10 ml of each sample was centrifuged at 1500 rpm for 10 minutes to remove media deposits. Secondly, 2 ml of each sample was centrifuged at 4,000 rpm for 10 minutes to separate bacteria from the media. The discarded supernatants and the remaining precipitates (pellets) were weighed to determine the wet weight. This research was conducted in three replications. The cell weight (X) was calculated using the following formula:

X (mg/ml) = weight of tube containing wet cells (mg) – weight of empty tube (mg) divided by sample volume (ml).

pH determination

pH was measured for each natural medium according to Matouskova et al. **[12]**. The natural medium was placed in a measuring cup and immersed in a calibrated pH meter. The pH value displayed on the pH screen was read when it was stable.

Sample preparation: There were two alternative media: 1) the media based on whey tofu consisted of whey tofu, molasses, and fish waste meal; 2) the media based on coconut water consisted of coconut water, cassava waste, and shrimp shell meal. The alternative media were prepared by a mixture of whey tofu or coconut water (90%), molasses or onggok flour (5%), and shrimp shell or fish waste meal (5%). The combination of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* (probiotic mixed) were based on the TPC (Total Plate Count) results and were divided into three ratios, namely 1:1, 1:2, and 2:1, cultured on MRS-B and incubated at 37 °C for 24 hours. The experiment was triplicated, and the total number of samples analysed was 18.

Statistical Analysis

The data from this research were entered into SPSS 26.0. (SPSS Analytics Partner). And was analysed using a two-way ANOVA (Analysis of Variance) at 0.05 to find the effects of viability, pH, and cell biomass from incorporating *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* in the natural growth media. Tukey's test was applied to determine significant differences.

RESULTS AND DISCUSSION

Effect of culture and media type on the viability

Microbial growth curves are mathematical models that can aid in the study of microbial growth and behavior, as well as the selection of ideal growth circumstances. The turbidimetric method is an excellent alternative to study bacterial growth since optical density (OD) measurement gives real-time values of bacterial population and has practical significance when dealing with bacteria samples in high cell densities **[13]**, **[14]**. Compared to other techniques such as the standard viable count method, estimation of microbial growth characteristics based on absorbance measurement offers the advantages of being quick, non-destructive, affordable, and reasonably straightforward to automate **[14]**.

Table 1 shows the results for optical density (OD) measurements. There was significant interaction (p <0.05) between the cultures and media types, where A3 (culture with a ratio of 2:1 for *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae*) and B3 (containing coconut water, onggok flour, and shrimp waste flour) exhibited the highest viability value of 2.37; this value was not significantly different (p >0.05) from culture ratios A1:B3, A2:B3 and A3:B3, but significantly different (p <0.05) from other tested halal mix probiotic media.

Table 1 Viability of probiotics (*Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae*) on various culture and growth media.

Ratio of probiotics	Type of media			Mean
	B1 (Control)	B2 (Media 1)	B3 (Media 2)	
A1 (1:1)	1.32^{b}	1.94^{d}	2.24^{e}	1.83
A2 (1:2)	0.75^{a}	1.64^{cd}	2.27^{e}	1.55
A3 (2:1)	0.75^{a}	1.61^{bc}	2.37^{e}	1.58
Mean	0.94	1.73	2.29	

The growth of *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae* significantly affected the media because different media will support the growth of bacteria at different rates. This finding is consistent with other researchers **[2]**, **[12]**, **[15]**, **[16]**. The composition of the nutrients in media determines the growth rate, the product type, and the biomass yield. Acu et al. **[16]** reported that enrichment with fruit puree significantly affected *Lactobacillus paracasei* and *Bifidobacterium* spp. in terms of viability, colour, appearance, flavour, taste, and overall sensory scores of ice cream samples.

A medium must contain all the necessary nutrients or elements required to grow the microorganisms of interest. These elements, e.g., C, N, O, S, and P required by *Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae,* must be provided in a suitable form and ratios that are designed to achieve specific effects. The growing cells may require additional complex organic molecules (micronutrients) that they cannot synthesize but are essential for their growth **[17]**. The stability of the viability value of the probiotic mixture in B3 was influenced by its nutrient composition. B3 had abundant carbon due to the combination of coconut water and onggok flour. Meanwhile, B2 could have excess nitrogen (N) from the combination of tofu whey and fish meal waste. Agricultural wastes,

including woody materials, crop residues, and food by-products, are widely available and explored for LAB production because they offer potential environmental and economic benefits **[18]**. Low-cost nitrogen sources can be obtained from slaughtering by-products, fish processing by-products, agricultural waste, dairy industry by-products, and plant products. For example, the by-products of fish processing (chitinous, heads, viscera material, wastewater, etc.) are excellent nutrients for microbial growth **[19]**, **[20]**.

Effect of culture and media type on cell biomass

The highest biomass production was realized in the interaction between A3 (2:1) and B3 (90% coconut waste, 5% onggok flour, and 5% fish waste flour), which was significant (p <0.05) from other treatments (Table 2). The biomass for A3B3 was 42.33 mg/ml, while the lowest biomass production, 16.00 mg/ml, was observed for A2B3 interaction, with the same media but different culture ratios (Table 2). In this study, the higher the number of *Lactobacillus plantarum* N16 in the culture, the higher the biomass produced. Contrarily, the lower the ratio of *Lactobacillus plantarum* N16 in the culture, the lower the biomass produced. Stadie et al. **[21]** reported the symbiosis relationship between *S. cerevisiae* and *Zygotorulaspora florentina*, and *Lactobacillus nagelii* and *Lactobacillus hordei* led to an increased cell yield for all microorganisms. They also discovered that LAB's acidity of the medium helped *Z. florentina* to thrive, while the yeasts' synthesis of amino acids and vitamin B6 boosted Lactobacilli development. Liu et al. **[22]** experimented with improving the stability of *Lactobacillus rhannosus* in fermented milk using *Williopsis saturnus* var. *saturnus*. They found that *Williopsis saturnus* var. *saturnus* improved the stability of the milk for eight days in comparison to the control, which they attributed to the release of nutrients such as amino acids, peptides, and vitamins by the yeast.

Table 2 Biomass of probiotics (*Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae*) for various cultures and growth media (mg/ml).

Ratio of probiotics	Type of media			Mean
	B1 (MRSB)	B2 (Media 1)	B3 (Media 2)	
A1 (1:1)	20.00[a]	24.67[a]	17.33[a]	20.67
A2 (1:2)	22.00[a]	23.00[a]	16.00[a]	20.33
A3 (2:1)	23.00[a]	19.33[a]	42.33[b]	28.22
Mean	21.67	22.33	25.22	

Note: MRSB = de Man, Rogosa & Sharpe Broth.

In this research (Table 2), the novel and halal growth media biomass for *L. plantarum* N16 and *S. cerevisiae* were good quality compared to MRS broth. However, this commercial media has been optimized and used for five decades **[23]**. Nonetheless, coconut water, onggok flour, and shrimp waste flour in appropriate concentrations demonstrated the potency to be used to substitute MRS broth. Different researchers have reported that halal processed-peptone, yeast extract, and whey were preferable to MRS broths **[24]**, **[25]**, **[26]**.

Effect of culture and media type on change in pH

Probiotics (*Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae*) generated pH variations in the halal growing media, as shown in Table 3. Studies describing how changes in pH of the media affected the growth of bacteria or the production of some metabolites are widely available, however, few studies are available on the effects of pH of the medium during the growth of microorganisms. In this study, the initial pH was the same for the three media but differed at final growth.

Table 3 pH reduction caused by probiotics (*Lactobacillus plantarum* N16 and *Saccharomyces cerevisiae*) in the various culture and types of growth media.

Ratio of probiotics	Type of media			Mean
	B1 (MRSB)	B2 (Media 1)	B3 (Media 2)	
A1 (1:1)	0.95[a]	0.89[a]	2.37[e]	1.40
A2 (1:2)	1.21[b]	0.90[a]	2.21[d]	1.44
A3 (2:1)	1.51[c]	0.90[a]	2.38[e]	1.59
Mean	1.22	0.89	2.32	

Note: MRSB = de Man, Rogosa & Sharpe Broth.

Statistical analysis revealed significant differences (p <0.05) between factors A and B concerning the final pH of the medium. Based on the DMRT test, the highest pH reduction was A3B3 (2.38) and was not significantly

different (p >0.05) from treatment A1B3 (2.37) but significantly different (p <0.05) for other treatments. Nahariah et al. **[27]** reported that the decrease in pH is caused by fermentation activity which converts carbohydrates or sugars into acids. According to Maslami et al. **[28]**, the lowered pH was attributable to the formation of acetic and lactic acids by *L. plantarum* and *S. cerevisiae.* Both *L. plantarum* and *S. cerevisiae* ferment produced organic acid (malate acid) **[4]**.

Marlida et al. **[2]** and Younis et al. **[29]** reported that *S. cerevisiae* can inhibit the growth of pathogenic organisms by causing pH changes in the medium as a result of competition for nutrients, organic acid production, growth coupled with ion exchange, secretion of antibacterial compounds, production of high concentrations of ethanol, and release of antimicrobial compounds such as "mycocins" or killer toxins. *L. plantarum* also inhibits the growth of pathogenic bacteria by producing lactic acid and antimicrobial agents like bacteriocin **[4]**. Xie et al. **[30]** worked on improving the stability of *Lactobacillus rhamnosus* in fermented milk using *Williopsis saturnus* var. saturnus. Their work revealed that *Williopsis saturnus* var. *saturnus* enhanced the stability of *Lactobacillus rhamnosus* in the milk compared to the control. The enhanced stability was attributed to the excretion of peptides, amino acids, and vitamins by the yeast **[22]**. In addition, yeast metabolites have an important role in *L. rhamnosus* survival **[31]**.

CONCLUSION

Coconut water, onggok (tapioca waste flour), and shrimp waste flour (B3) were used to make a halal (permissible for a member of the faith of Islam) mixed probiotic medium for *L. plantarum* N16 and *S. cerevisiae* as an alternative media for MRSB, which was cultured for 24 hours at 36 °C. It had a viability of 2.37, a biomass cell concentration of 42.33 mg/ml, and a pH of 2.37.

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

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The effect of the cooking method on rainbow trout (*Oncorhynchus mykiss*) fillets

Ali Aberoumand, Milad Masoudi

ABSTRACT

Fish is nutritious seafood and contains protein with high biological value and essential nutrients for the human body. In Iran, the fish *Oncorhynchus mykiss* is locally known as Ghezelala and is a commercial fish species. Different methods were used to process the fish: boiling, frying in sunflower oil and grilling. This research investigated on effect of various cooking methods on proximate pH and cooking loss of fresh fish (*Oncorhynchus mykiss*). The highest and lowest values for protein were found in fish processed using grilling (18.51%) and frying (16.52%), respectively (p <0.05) compared to the fresh sample (18.20%). The fat content of the fried sample showed significantly highest (5.16%) (p <0.05), while the lowest fat content was found for the boiled sample (20.57%) compared to the fresh fat (24.36%) (p <0.05). Comparing the loss percentage of samples in different cooking methods showed that the boiled sample had the lowest value (25.46%) and the fried sample with the highest value (45.02%) (p <0.05). pH value in the boiled sample was the highest (6.74%), while the grilled sample had the lowest (6.63) compared to the fresh sample (6.54). The highest energy value was found for the fried sample (578.48 kcal/100g), and the lowest was for the boiled sample (269.29 kcal/100g). The results suggest that the boiled and grilled fish found higher nutritional quality due to the relatively high protein content, the most needed nutrients. The results also showed that all cooking methods did not significantly affect in mineral content of the fresh fish.

Keywords: cooking methods, *Oncorhynchus mykiss*, nutrients, cook loss, pH

INTRODUCTION

One of Iran's most important and desirable farmed aquatic animals was the rainbow trout (*Oncorhynchus mykiss*). Its production in Iran was 853 tons in 1992, about a 32% increase. This fish species has high popularity due to its ability to farm in Iran compared to other aquatic animals. The sale of this fish species as fresh and live since 1997 has increased. This fish species is rich in fat. This cold-water fish is farmed in the cold areas of Iran, is readily available for consumption in Iran, and has many consumers. Rainbow trout can be caught fresh from fish ponds.

Iran's three standard fish cooking methods are boiling, frying, and grilling. After cooking in recent decades, people have kept fish in the refrigerator in the refrigerator for consumption. In this article, we investigated and compared the nutritional and energy values of rainbow trout with three methods of cooking, grilling, frying, and boiling, after storage for two days in the refrigerator, then measured the factors of cooking loss, pH, moisture, ash, fat, and protein contents **[2]**.

Fish is a nutritious food, an excellent source of animal protein needed in the human diet, and is also rich in essential vitamins and minerals **[1]**. In Iran, fish is not eaten raw but is processed using various cooking methods such as roasting. Frying and boiling mainly improve the taste **[2]** and kill microorganisms. Pathogenic **[3]**. However, many studies have shown that different cooking methods always affect the nutritional value of fish. In particular, vitamins, flavourings, and polyunsaturated fatty acids **[4]**, **[5]**.

The present study aimed to investigate on proximate composition and physicochemical properties of fresh *Oncorhynchus mykiss* and fried, grilled, and boiled samples.

Figure 1 Rainbow trout (*Oncorhynchus mykiss*).

Scientific Hypothesis

The nutritional quality of processed fish depends on the method and thermal conditions of production and the preservation of fish nutrients in the processed product.

MATERIAL AND METHODOLOGY

Samples

This research work was carried out in 2021 at Behbahan Khatam Alanbia University of Technology, Iran. The fish were caught in Shiraz, Iran. All samples were packaged and stored in a refrigerator at 4 °C for two days. All samples were placed on ice and were transferred to the laboratory.

Chemicals

All chemicals were purchased by Shiraz Company in Iran and were of analytical grade quality.

Animals and Biological Material

The Latin name of the fish species was rainbow trout, and the scientific name was *Oncorhynchus mykiss*. Sunflower oil is the non-volatile oil pressed from sunflower seeds (*Helianthus annuus*).

Instruments

The charcoal, water, meter, scale, oven, electric furnace, Chinese container, Petri dish, distilled water, homogenizer, and pH meter were used in this research.

Laboratory Methods

The three cooking methods (boiling, grilling, and frying) are used in the present research.

Description of the Experiment

Sample preparation:

The fish was filleted with a knife; then, the fillets were weighed by a scale. The weight of the fillets was 1.235 kg. The fillets were divided into four parts A, B, C, and D.

Design of the experiment:

The fish fillets were divided into four groups: Part A, with a weight of 262.4 g, was considered for the control treatment, packaged without processing, and stored in the refrigerator at 4 °C. Part B, with a 425g, was grilled with charcoal heat for 10 min. The sample was cooled for 25 min at an ambient temperature of 24 °C. The weight of sample B was measured after processing (297.8 g). Part C, weighing 265g C, was fried in 200 ml of liquid sunflower oil at 180 °C for 12 min. The sample was cooled at an ambient temperature of 24 °C for 25 min. The weight of sample C was measured after processing, and its weight was 145.7 g. Part D, with a weight of 260 g, was boiled in 500 ml of boiling water at 98 °C for 20 min. The separation of fat drops in boiling water was observed during cooking. The sample was then cooled at an ambient temperature of 24 °C for 25 minutes. The weight of sample D was measured after processing (193.8 g).

The following formula calculated the cooking loss percentage:

The amount of initial raw weight of fillet – sample weight after each cooking process / the amount of raw weight × 100

Biometrics: Fish length: 53 cm. Fish width: 16 cm. Fish weight 2155 g.

Moisture, protein, fat, ash, and pH in the fresh and cooked fish were analysed according to AOAC **[6]**. All analyses were performed in triplicate.

Each sample was placed in the container. Samples were placed in an oven at 105 °C for 90 min. The samples were moved from the oven and weighed with a scale. Measurements continued until the weight remained constant; then, the samples were placed in the oven again for 15 min and weighed. There was no weight change.

The moisture content was calculated as follows according to 10g each sample:

$A1_{moisture} = 10 - 3.9968 = 6.0032$
$A2_{moisture} = 10 - 4.5637 = 5.4363$
$B1_{moisture} = 10 - 4.2365 = 5.7635$
$B2_{moisture} = 10 - 4.6230 = 5.3770$
$C1_{moisture} = 10 - 7.3245 = 2.6755$
$C2_{moisture} = 10 - 6.8367 = 3.1633$
$D1_{moisture} = 10 - 4.1517 = 5.8483$
$D2_{moisture} = 10 - 3.6958 = 6.3042$

PH measurement

The 5 g of each sample was measured, and then the pH value was measured. All samples were crushed separately by a homogenizer and mixed with 45 ml of distilled water. The pH value of each sample was measured by a pH meter model PHS-550 digital device made in China.

Number of samples analysed: 24 samples.
Number of repeated analyses: All chemical analyses were conducted in triplicate.
Number of experiment replication: 2 times.

Statistical Analysis

Data analysis was done in three replications. Duncan's mean comparison test was used at a 5% level. The calculations were performed using SPSS 19 software(IBM, USA). Results were expressed as the mean of triplicate trials. Data were analysed by one-way analysis of variance on the means of values (p <0.05).

RESULTS AND DISCUSSION

The study results are present in Figures 2, 3, 4, 5, 6, 7 and 8.

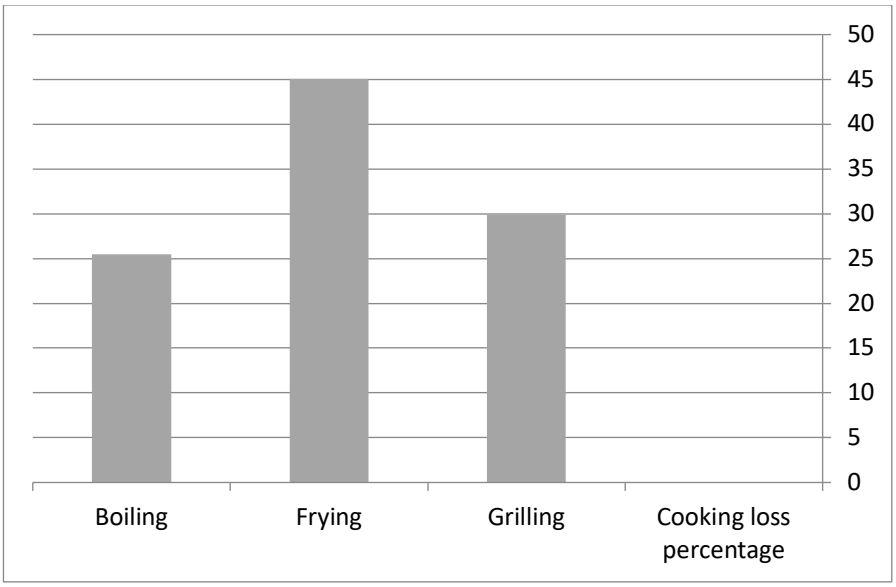

Figure 2 Comparison of cooking loss percentage of samples in different processing methods**.**

The pH of sample A (control) was 6.54. The pH of sample B (grilled) was 6.63. The pH of sample C (fried) was 6.66. The pH of sample D (boiled) was 6.74. The cooking loss percentages in all methods were below respectively.

$B_{cooking\ loss\ percentage} = (425 – 297.8) / 425) \times 100 = 29.93\%$
$C_{cooking\ loss\ percentage} = (265 – 145.7) / 265) \times 100 = 45.02\%$
$D_{Cooking\ loss\ percentage} = (260 – 193.8) / 260) \times 100 = 25.46\%$

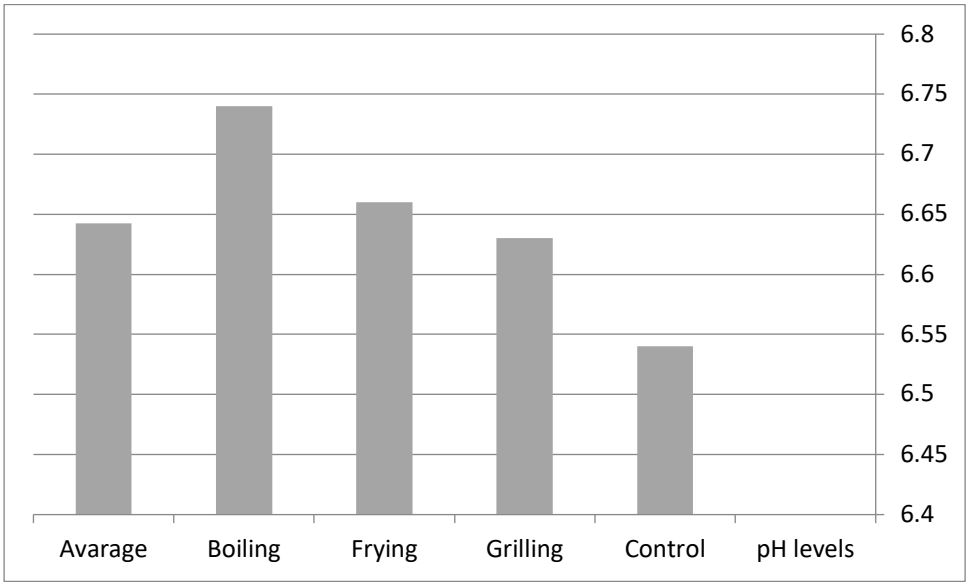

Figure 3 Comparison of pH values of samples in different processing methods.

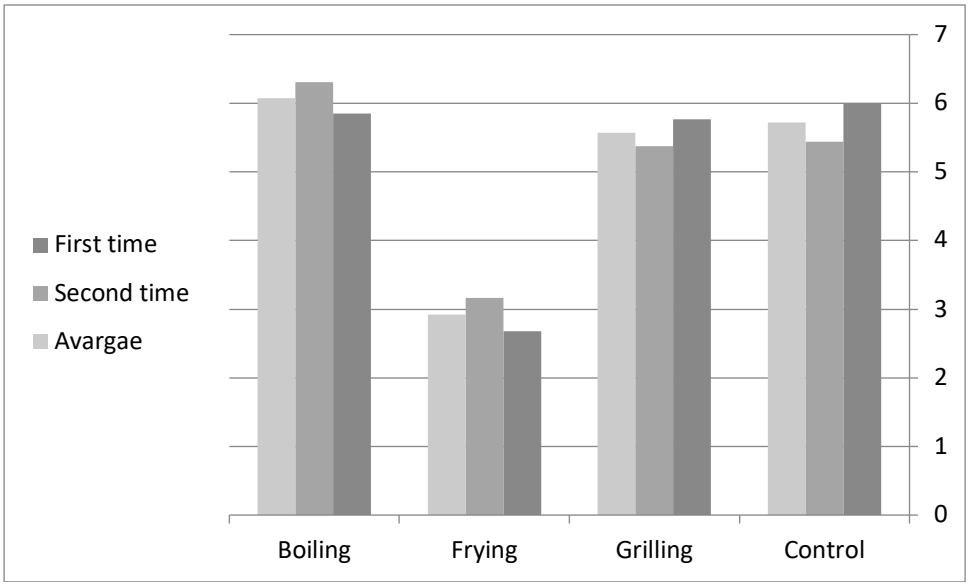

Figure 4 Comparison of moisture content of fish fillets in different processing samples.

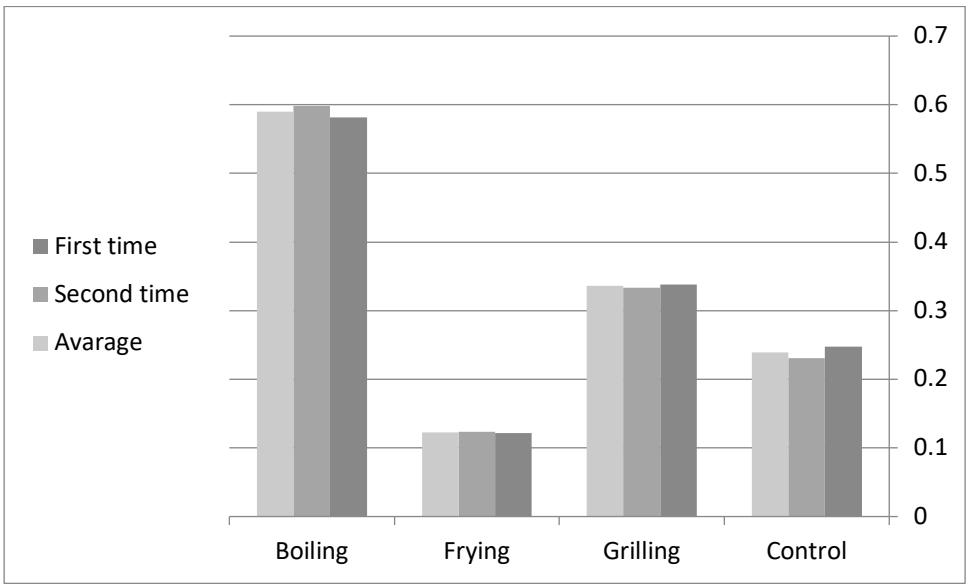

Figure 5 Comparison of ash content in fish fillets in different processing samples.

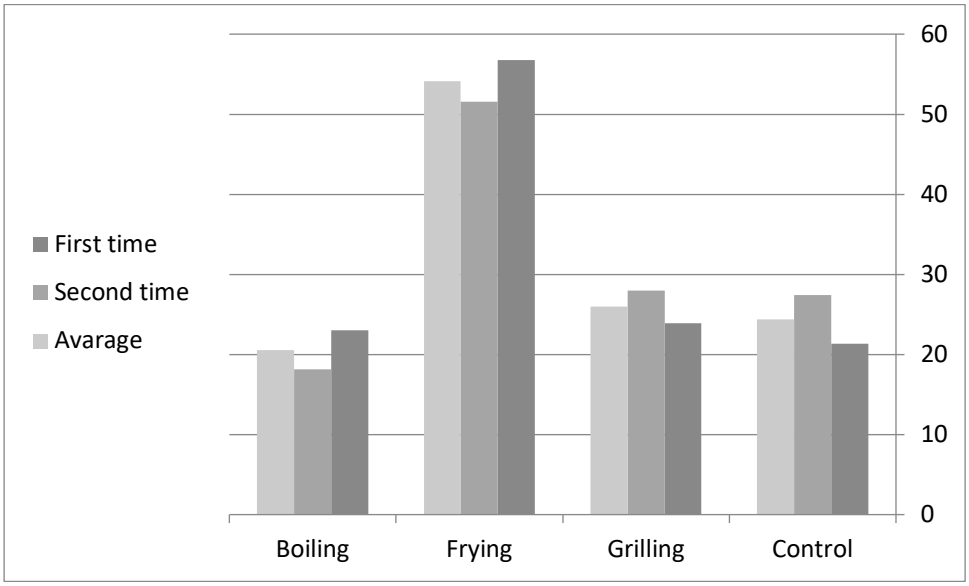

Figure 6 Comparison of fat content in fish fillets in different processing samples.

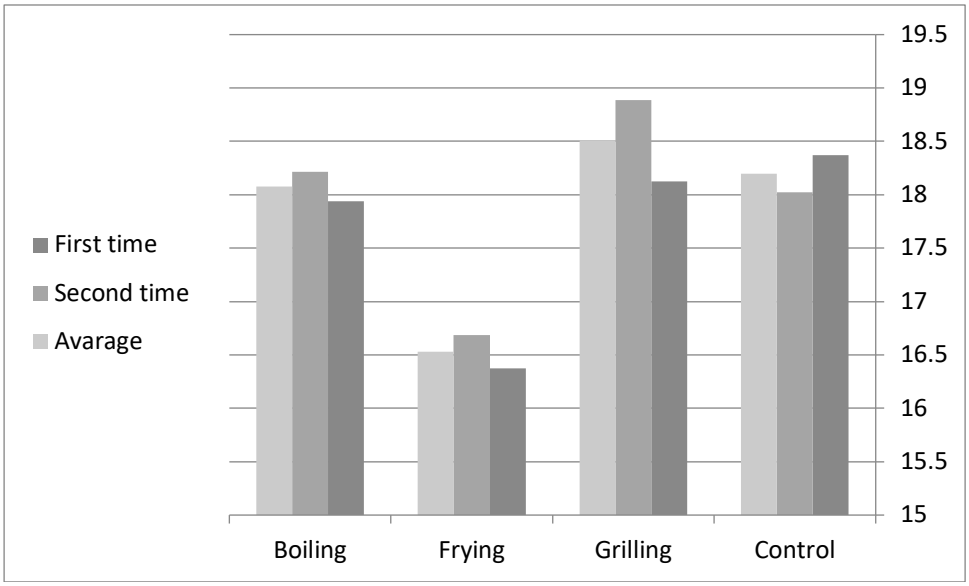

Figure 7 Comparison of protein content in fish fillets in different processing samples.

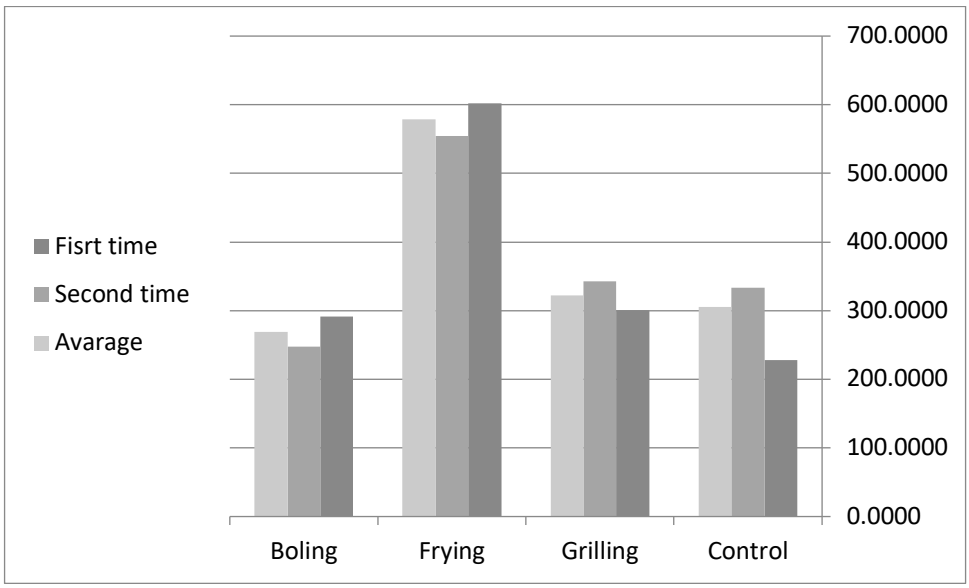

Figure 8 Comparison of energy value (Kcal) in fish fillets in different processing samples.

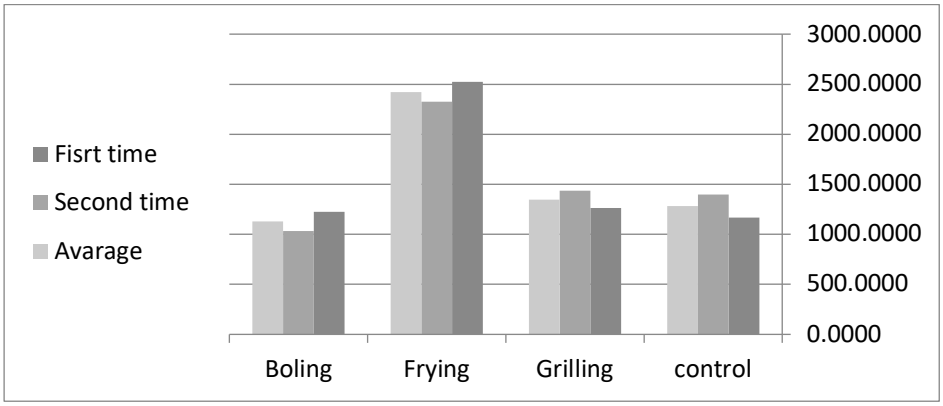

Figure 9 Comparison of energy value (KJ) in fish fillets in different processing samples.

Loss of cooking was found in different methods significant respectively: fried>grilled>boiled, respectively (p <0.05). The boiling process was better from the point of economical compared to the other samples.

The pH value of the samples fillets was found to be significant in different methods, respectively: boiled>fried> grilled>control (p <0.05).

The fat percentage and energy value were significantly different in fried fish than in other methods and control samples. Fat loss during cooking was observed the lowest percentage and energy value of fat in the boiling method. Due to cooking, fat content changed and was observed in the fried sample more than in a boiled sample, while in the boiled sample was more than in a grilled sample. In samples, energy levels and fat percentage were observed, significant respectively, fried>control>grilled>boiled (p <0.05).

There was no significant difference in protein percentage and energy value in different cooking methods compared to the control sample (p <0.05). The percentage and energy value of protein in the grilling method was due to two days of storage in the refrigerator, which was even better than the control sample due to less denatured protein than the control sample. The samples‘ energy level and protein percentage were observed significant: grilled>control>boiled>fried (p <0.05).

The total energy of the fish fillet (total energy values of fat and protein) was observed, respectively: fried> grilled>control>boiled. Fried fish contains a significant amount of fat recommended for children who need more energy (p <0.05). Boiled fish has a lower energy level and fat content, so for people on a weight loss diet or with high blood fats.

The grilled preserved protein and energy levels better against denaturation over time and is recommended for athletes who consume more protein in their diet.

Because of the optimum ratio of fat to protein compared to the control sample and the total energy of the samples, it can be concluded that the grilling method for the studied fish for healthy nutrition after two days of cold storage was the most suitable method processing.

Significantly changes in the fish nutrient contents were reported in various cooking methods (p <0.05). Therefore, these cases reflect many previous reports that the type of processing leads to changes in the food quality of fish **[2]**, **[4]**, **[5]**. Both fish nutrition and quality are related to macronutrient composition **[7]**.

The boiled fish contains high protein significance (p <0.05). because, during cooking, denatured proteins are not mainly lost **[8]**. Al-Jeddah et al. **[9]** also reported that fish muscle is more digestible than other animal proteins due to the smaller amounts of connective tissue. Due to the need for essential amino acids **[10]** – boiled fish is superior to other methods due to its higher protein content.

The frying and boiling methods showed the lowest and highest moisture content, respectively, compared to the fresh fish. Due to the extended contact with boiling steam and water, the moisture content in fish in the boiling method was highest **[11]**.

The ash contents in various cooking methods except frying indicated that this fish species was rich in minerals (p <0.05). Minerals are always not destroyed by cooking heat processes **[12]**. The duo to frying process causes some minerals (from the scales and spine) to enter the fat, and the mineral content in fried fish was low **[13]** and **[14]**.

Physicochemical quality properties of processed fish

One of the good indexes for quality assessment of fish fillets is pH value. It is necessary to determine fish texture quality **[15]**. The changes in pH value of raw and cooked fish in different methods are presented in Figure 2. The pH value showed a slight increase after the cooking process. The recorded pH value of raw, fried, boiled, and grilled fish was 6.54, 6.66, 6.74, and 6.63, respectively.

These results are similar to those of Bett and Dionigi **[16]**. They reported that the decomposition of nitrogen components after also catching bacterial growth **[17]** increased the pH of fish fillets. The acceptance limit of fish pH value was determined between 6.8 and 7, while values above 7 were spoilage indices **[18]**, **[19]**. The duo to breakage of hydrogen bond and electrostatic interactions, pH value in boiled processed *Oncorhynchus mykiss*) fillets increased from 6.54 (raw) to 6.74 **[20]**.

The cooking loss percentage in fish fillets increased with time and temperature, although most cooking loss occurs during primary heating. Similar results were found for Atlantic fish (*Gadus morhua*) **[21]**, pink salmon (*Oncorhynchus gorbuscha*) **[22]**, and blue oyster (*Mytilus edulis*) **[23]**. Loss of cooking and then shrinkage of fish muscle is generally due to denaturation of heat-induced protein and consequent shrinkage of myofibrillar protein. High heat time and degrees lead to more denaturation, resulting in more cooking losses and shrinkage, which agreed with the present study results, which showed the frying temperature of fish in oil (180 °C) was higher than grilling on charcoal and boiling in water (98 °C). As a result, the cooking loss in the fried sample was higher than in other samples.

Here, we suggest that during *post-mortem* proteolysis during cold storage, myofibrils and sarcomeres decompose slowly, resulting in less muscle shrinkage during heating and cooking loss. The effect of fish freshness on quality characteristics such as cooking loss should not be ignored. Loss of cooking and shrinkage of fish fillets is not only affected by the intensity of heat treatment but also by cold storage conditions before reheating, and the mechanism behind this may be due to post-mortem proteolysis, which leads to less shrinkage when heated, which is associated with contraction **[24]**.

Some researchers reported that frying was the only cooking method to change the fatty acid content of the rainbow trout fillet. These results suggest that the cooking methods that optimize n-3 PUFA consumption of rainbow trout are baking, grilling, microwaving, or frying pans in CO, CaO, or PO **[25]**.

In general, protein and ash content increased after cooking in all evaluated methods (p <0.05). According to Ersoy and Ozren, the increase in protein, fat and ash content can be explained by the decrease in moisture **[26]**. These results are similar to the findings of other researchers **[27]**, **[28]**.

CONCLUSION

The cooked fish in the water had the highest content of nutrients, especially protein, which may indicate that food production contains high-quality nutrients. The highest energy value was found for the fried sample (578.48 kcal/100g), and the lowest was for the boiled sample (269.29 kcal/100g). However, boiled fish containing more moisture may lead to low shelf life, because water promotes microbial spoilage. Fish oil is suitable for healthy nutrition. Therefore, The fried fish indicated a longer shelf life due to the highest amount of fat with the lowest moisture, although soft minerals are desirable. There was no difference in mineral content for boiled and grilled fish, except for the grilled sample, which had lower moisture, indicating a cooked product with better shelf life. All cooking methods except frying had minor mineral changes, which may show that the cooking process does not affect the mineral content of the fish, except for the frying. Comparing the cooking loss percentage of samples in different cooking methods showed that the boiled sample had the lowest value (25.46%) and the fried sample with the highest value (45.02%). The cooking methods may decrease the nutrient content, indicating caution in processing fresh fish. Although fish is a nutrient-rich food, the most important component of fish for consumers is protein. The choice of cooking method should be maintained according to protein preservation. Therefore, the boiling process should be best method with protein content 18.25%.

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Thermomechanical processing of components of combined feeds by the expansion method

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ABSTRACT

The availability of high-quality combined feed largely determines the level of economic development in animal husbandry and poultry farming since, in the structure of the cost of livestock products, feed costs reach 65 – 70%. At the same time, the authors of this article aim to compare the current situation around the feed industry in the Republic of Kazakhstan with a similar situation in the Russian Federation, as the formation of this industry in our countries has common roots. Thermomechanical processing methods are proposed to implement deep physicochemical changes in the protein-carbohydrate complex of components of combined feeds. As a result, the quality of the combined feeds improves. Starch gelatinization occurs, and starch grains transition into a more digestible form, contributing to its better assimilation. Bacterial contamination decreases, and coliform bacteria, colon bacillus, mould fungi, and salmonella are destroyed.

Keywords: feed industry, combined feeds, extrusion, expansion, exhibit

INTRODUCTION

On behalf of the President of the Republic of Kazakhstan, a large-scale project, "Development of meat animal husbandry for 2018 – 2027", is being implemented in Kazakhstan, the main task of which is to increase the number of beef cattle for the production of products for export. Subsidies for purchasing livestock, feed, and breeding work are rising annually. Based on the results of this project, existing regional and zonal laboratories of the feed industry will be recreated, meeting modern requirements, the main tasks of which are to analyze veterinary indicators and prevent the receipt of substandard (substandard) raw materials. At the same time, the critical bet in the program is on the development of farms, which should become the leading "players" in the industry. At the same time, the provision of jobs for the rural population will increase from 100 thousand to 500 thousand. Beef and lamb production will grow from 0.6 million tons to 1.6 million tons, while the industry's export revenue will increase to 2.4 billion US dollars.

In recent years, Kazakhstan has significantly reduced the production and consumption of combined feeds, premixes, and various feed additives, without which the productivity, fertility, and safety of young animals decrease significantly, and the quality of livestock products deteriorates. The quality of livestock products profitability of the industry decreases. All these factors are caused not only by a small proportion of highly productive livestock but also by poor feeding and poor quality of the feed produced. The area of sowing of grain crops decreased by 40%, including grain-forage - by 70%. The areas of forage crops were sharply reduced - by more than 4 times. The yield of fodder crops remains low, and the collection of fodder units from 1 ha does not exceed 2.5 – 6.0 kg/ha. The lack of protein in animals' diets leads to overspending of feed by 30 – 40%, increasing its cost. When young animals, during the period of adaptation of the enzymatic system of the food tract, the plant part of the combined feed is poorly absorbed. The use of moisture-thermal and barothermomechanical processing (expansion, extrusion, micronization, granulation, flocking, steaming) of cereals and legumes, as well as vacuum spraying and draining of feed raw materials with thermolabile components (multi-enzyme complexes, vitamins, fat, amino acids, etc.), will allow the production of highly

efficient, environmentally friendly, highly nutritious, easily digestible combined feeds of a new generation (with programmable properties). As a result, a synergistic effect of improving the quality of combined feeds is provided. During baro-thermal processing, under the influence of moisture and heat, starch gelatinization and the transition of starch grains into a more digestible form occur, contributing to its better assimilation. In addition, bacterial contamination decreases, and coliform bacteria, *E. coli*, mold fungi, and salmonella are destroyed **[1]**, **[2]**, **[3]**.

At the same time, the volume of feed products in the Republic of Kazakhstan and the Russian Federation, after a well-known decline in the perestroika years, has grown since 2001. Thus, the production of combined feed in the Russian Federation, according to statistics from 9.8 million tons in 2002, amounted to 30.4 million tons in 2012 and increased more than three times. About half of the demand for protein-vitamin-mineral concentrates (PVMC) and premixes is produced at enterprises of the Russian Federation. The rest is supplied from abroad. But it should be taken into account that in the production of premixes, imported raw materials are almost 100%, and in the production of PVMC – 75% **[4]**, **[5]**, **[6]**.

Before 2008, premixes and PVMC were imported to Kazakhstan from abroad. But recently, the volume of these products in Kazakhstan has been growing annually. In 2010, the production of PVMC increased 2.6 times compared to 2008 – up to 22 thousand tons, and in 2011 – up to 28 thousand tons. The same growth rates are observed in the production of premixes, which in 2011 amounted to 12.2 thousand tons, an increase of 36.6% compared to 2010. However, the achieved level of combined feed production does not meet the needs of animal husbandry both quantitatively and qualitatively.

Therefore, the development of technology for preparing a concentrated protein supplement is based on a mixture of legumes and oilseeds to balance the amino acid composition, providing special heat treatment to increase nutrition and reduce antinutritional factors.

Scientific Hypothesis

The idea of the research is a scientifically based selection of technological methods to process all components of raw materials that make up the combined feed, to maximize their assimilation by the digestive tract of farm animals and birds. To do this, it is planned to use the following technological operations: scientifically based selection of feed mixture components, their steaming with subsequent expansion, cooling of the expansion product expandate (product expansion) with simultaneous evaporation of moisture, and subsequent vacuum spraying with thermolabile components.

The main scientific hypothesis: scientific justification of the choice of the formula composition of the mixture for the production of highly nutritious, easily digestible combined feeds of a new generation for the normalized feeding of farm animals and birds; study of the basic laws of the processes of moisture-thermal and barothermomechanical processing of highly digestible combined feeds of a new generation; selection and justification of rational parameters of the process of obtaining highly digestible combined feeds of a new generation; substantiation of the composition and method of application of thermolabile components (enzymes, vitamins, amino acids, fat) for obtaining easily digestible combined feeds of a new generation for normalized feeding of farm animals and birds; conducting a comprehensive assessment of the quality of highly nutritious, easily digestible combined feeds of a new generation with a designed composition and nutritionally balanced components; determination of the effectiveness of the use of highly digestible combined feeds of a new generation on various groups of farm animals and poultry to identify the dynamics of weight gain growth, reduction of fattening time, reduction of animal and poultry mortality, reduction of feed conversion.

The research strategy is based on a combination of analytical, statistical, empirical, and experimental research methods and a scientifically based choice of approaches to the application's order, sequence, and completeness.

To implement the proposed hypothesis, generally accepted and special methods of mathematical statistics, a systematic approach, and methods of comparative analysis were used. Information processing and analysis methods were also used, including sociological, organoleptic, physicochemical, microbiological and instrumental methods.

MATERIAL AND METHODOLOGY

According to world experience, and research by domestic and foreign scientists, there are two directions for increasing the nutritional value and digestibility of combined feeds: by introducing various enriching and balancing additives and premixes into the combined feed, as well as through such types of moisture-heat treatment of grain components of combined feed as extrusion, expansion, micronization **[4]**, **[5]**, **[6]**.

During expansion, the product is subjected to temperature treatment from 80 to 130 °C and pressure up to 40 MPa, depending on the product type, but for a concise period, since the total duration of the product passes through the expander is no more than six seconds. Processing parameters such as humidity, temperature,

pressure, and flow rate affect the physical characteristics in the expander **[7]**, **[8]**, **[9]**. During expansion, the product is also subjected to short-term (4 – 5 sec) thermal steam exposure, followed by compression in the expander to a pressure of 3.0 MPa. At the expander's exit, the product's melt falls into the low-pressure area. At this moment, the product appears to swell, connections at the cellular level are broken, starch is modified, and the availability of carbohydrates for the action of digestive enzymes increases **[10]**, **[11]**.

The main task of expansion is to obtain homogeneous products with a narrow range of sizes **[12]**, **[13]**. The expansion process ensures the destruction of pathogenic bacteria, prevents the development of pathogenic microflora, mould, etc., partial hydrolysis of starch, and preservation of natural and injected vitamins. This follows based on the analysis of the works of Sharshunov V. A., Shirov Yu. P., Lukhta N. V. and other authors **[14]**, **[15]**, **[16]**, **[17]**.

The product processing process in the expander takes place in four zones. The product is mixed and moved along the screw in the I zone, and its compaction begins. In zone II, there is an increase in pressure, compression, and destruction of particles. In zone III, a further pressure increase and temperature increase, and the product passes into a viscoplastic state (into a melt). And finally, in the IV zone, the melt is forced through the holes of the output head (Figure 1).

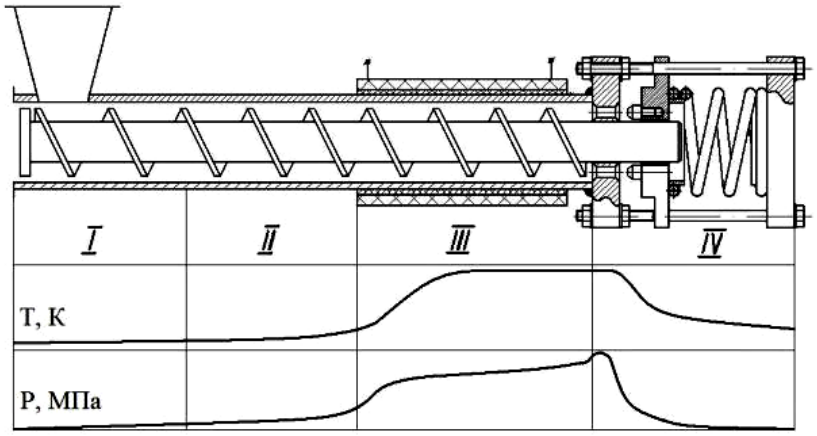


Figure 1 Product processing zones in the expander and combined dependences of temperature and pressure changes of raw materials during movement in the expander working chamber.

Samples

Protein supplement based on a mixture of legumes and oilseeds; amino acid composition of highly digestible combined feeds of a new generation with programmable properties; components of combined feeds to increase nutritional value and reduce antinutritional factors, subject to special heat treatment.

Chemicals

Determination of the chemical composition of the feed (mass fraction of protein, starch and fiber content, and ash content).

Animals and Biological Material

Farm animals were used to implement the study. All animal care procedures were carried out following the Guidelines for the Maintenance and Care of Animals. The animals were provided with food and water. A 12-hour light-dark cycle was maintained in the room. Temperature (22 ±2 °C) and humidity (55 – 60%) were monitored daily.

The measures envisaged in the framework of our study were implemented following humanity towards animals and high ethical standards concerning their well-being.

Instruments

The Chopin Alveograph (France), Farinograph-AT company Brabender (Germany), Case drying SESh-3M (Russian Federation), and The Infrascan-105 "KAN" analyzer (Russian Federation) were used in this research.

Laboratory Methods

The research was carried out at the Kazakh-Japanese Innovation Center (hereafter – KJIC) of the Kazakh National Agrarian Research University. KJIC allows carrying out fundamental, exploratory, and applied scientific research in various branches of the agro-industrial complex. By Order No. 228-OD dated 03/18/2020, the National Accreditation Center LLP KJIC was accredited for compliance with the ST RK ISO/IEC 17025-2018 "General requirements for the competence of testing and calibration laboratories".

Scientific equipment and unique scientific stands and installations of the centre for collective use, "Control and Management of Energy-efficient Projects", located at the Voronezh State University of Engineering Technologies, were also used.

The sequence of research: the implementation of a literary review and patent search, justification of the choice of the sequence of technological operations in the developed technology for the production of highly digestible combined feeds of a new generation with programmable properties, the study of kinetic patterns of the processes under study, justification of the choice of rational parameters, the development of the compounding composition of combined feeds, conducting a comprehensive assessment of the quality of combined feeds for various types of farm animals.

Description of the Experiment

Number of samples analyzed: We analyzed 30 samples.

Number of repeated analyses: Repeated analyses = 3.

Number of experiment replication: Triple.

Design of the experiment: The most important experiments of such basic processes as mixing of the main feed components, their moisture-heat treatment (humidification and steaming with hot steam), extrusion, drying-cooling, and vacuum spraying of liquid components on the surface of extruded granules were carried out at the original installations. In the course of the research, the main kinetic patterns of the processes under study were identified, and the most significant factors affecting the intensity and efficiency of their course were determined. The nature of the influence of the most significant factors on the change of the following main technological parameters is also established: 1) the uniformity of the distribution of the components of the mixture and the influence on the kinematic and design parameters of the mixer for the mixing process; 2) the nature of the temperature and pressure changes along the length of the working chamber of the extruder for the extrusion process; 3) the nature of the change in temperature and humidity of the extruded granules for the drying-cooling process and 4) the nature of the change in pressure (vacuum) in the working chamber of the vacuum sprayer and the intensity of diffusion of liquid components deep into the porous granules for the vacuum spraying process.

The methodological basis of the research includes a complex of general scientific (analysis and synthesis, verification of the truth of theory by referring to practice, interpretation of the results obtained, etc.) and private, scientific (abstract-logical method, modelling, empirical method, statistical-probabilistic method, etc.) methods of cognition.

IR spectroscopy, atomic absorption spectroscopy, capillary electrophoresis, high-efficiency gas chromatography, acid hydrolysis, etc., were used to determine the content of vitamins, amino acids, and other quality indicators of highly digestible combined feeds **[7]**, **[26]**, **[27]**, **[28]**.

Basic information was obtained on the original installations to study the processes of moisture-heat treatment (humidification and steaming), extrusion, drying-cooling, and vacuum spraying to solve the project's tasks.

The obtained information arrays of experimental data were processed using modern software products (Mathcad, Statistica, etc.) to ensure their reliability and reproducibility.

Scientific statements, conclusions, and recommendations are based on fundamental physical laws. They are consistent with the theoretical concepts generally accepted in this field of research. The reliability of the research and the results of the conducted research are based on proven mathematical methods. The calculated ratios obtained are subjected to thorough experimental verification. At the same time, we rely on the experimental data we have obtained and the kinetic regularities of the processes under study (mixing, moisture-heat treatment, extrusion, drying-cooling, vacuum spraying). All scientific statements, conclusions, and recommendations are substantiated and confirmed by experimental studies and fully comply with the data of the experimental protocols.

The degree of reliability of the results of the conducted research is confirmed by a deep study of the literature sources on the topic of research, the formulation of the necessary number of experiments, and the use of modern instrumental methods of analysis. Applied computer programs are used for the mathematical processing of research results.

Statistical Analysis

The ultimate goal of the study is to create a new-generation highly digestible combined feed with controlled properties due to the use of the technology of effective use of thermomechanical treatment of components of combined feed by expandation. To achieve the aim of the study, generally accepted and special methods of mathematical statistics, a systematic campaign, and comparative analysis methods were used. Information processing and analysis methods, such as sociological, organoleptic, physicochemical, microbiological, and instrumental, were also used.

RESULTS AND DISCUSSION

The processing of grain and other components of combined feed in extruders provides a profound transformation of the structure and properties of materials. There is complete gelatinization of starch, the content of dextrins and other low-molecular carbohydrates increases, and the attack of proteins by enzymes increases; all of this contributes to complete assimilation of the nutrients of the combined feed and with less energy consumption for the digestive process. The range of components of combined feeds and ready-made extrudates is expanding significantly.

However, the extrusion process requires high energy consumption: its specific consumption is 120 – 150 kWh/t. Therefore, specialists were actively searching to develop an equally effective but a less energy-intensive variant of thermomechanical processing of products, including feed production. The result of this search was the development of a new expander device. The principle of operation of the expander and its basic design is similar to an extruder. The difference is that the product is pressed out not through a die with dies but through an annular gap, the value of which is regulated by a special hydraulic system. Steam is supplied to the expander casing, which also provides the product's heating.

Due to such changes, the specific energy consumption for expansion is reduced by 2.0 – 2.5 times compared to extruded and is 25 – 60 kWh per 1 ton of raw materials.

An additional advantage of the expander is introducing up to 20% fat and up to 20% molasses into the expander. In contrast, the fat input cannot be higher than 5% during extrusion because the extrudate granules lose connectivity and crumble.

The pressure in the expander reaches 10 MPa. The product is heated to 170 °C. As a result, starch is completely gelatinized and hydrolyzed, proteins are denatured and split, and organic complexes of proteins and fragments of starch molecules are also formed. The nutritional properties of the exhibit are high.

In total, the advantages of the technology that uses the expander are as follows **[1]**, **[2]**, **[3]**, **[4]**:

- the quality of the granule is improved primarily in the case of hard-pressed components;
- less energy is spent on the production of the expandate (product expansion), and the cost of the product is lower than conventional pellets;
- the expandate (product expansion), as a rule, can be used directly instead of granulated combined feed;
- it becomes possible to introduce a large amount of feed fat, molasses, fish hydrolysate, and liquid protein feeds into the combined feed;
- during the expansion process, vitamins and other biologically active additives are not destroyed, and their feed value is preserved in the expected order of magnitude;
- salmonella, bacteria, and fungi are destroyed, and the combined feed is obtained free of microbial contamination;
- starch is completely gelatinized, and its macromolecules are split into low-molecular fragments, as a result of which the nutritional value of the combined feed increases significantly;
- the activity of proteinase inhibitors in those products where they are contained (soy and other legumes) is sharply reduced; this allows the use of legumes in a significant amount as components of combined feeds, providing the necessary protein content in the combined feed;
- due to the high sanitary purity of the combined feed, the use of any preservatives is not required.

In addition, expanders require a small area for their placement, so they can be used in most feed mills or workshops.

Thus, according to domestic and foreign experts, expanders are currently the most economical and efficient way to produce combined feeds, compared to extrusion and double granulation.

The advantage of expanded products is the shape of small or medium grits, which allows you to reduce product losses during transportation and feeding. The sterilization of products achieved during the expansion process is critical in conditions of unstable sanitary quality of raw materials arriving at factories **[18]**, **[19]**. The creation of innovative technology for the production of expanded combined feeds adapted for various animals will increase the requirements for the quality of combined feeds, and broaden the range of raw materials and the product range **[20]**, **[21]**. A new method of moisture–heat treatment of grain crops - expansion or pressure conditioning ("High-Temperature-Short–Time Conditioning") - short-term hydrothermal treatment of grain in an expander, which allows you to get an expanded structured product ready for use, has become increasingly popular in recent years **[7]**, **[8]**, **[9]**. The principle of operation of the expander is similar to that of an extruder, but the product is pressed out not through the holes of the matrices, but into an annular gap. An auger is installed on the expander, with the help of which the product is moved and additionally warmed up due to friction forces up to 85 – 100 °C **[12]**, **[13]**, **[14]**. The compressed product is discharged through a conical diffuser equipped with a locking cone, which regulates the value of the output annular gap and the value of the working pressure on the product. At the exit of the expander, as a result of a sharp pressure drop, the moisture in

the product evaporates, and the product increases slightly in volume. In addition, the expanded combined feed is subjected to coarse grinding on a blade crusher and sent for granulation or cooling **[19]**, **[21]**. The main technological and market trends in the industry under consideration are the following leading Western and Russian firms (Amandus Kahl (Germany), Sprout-Matador (Denmark), Zheng Chang (China), Buhler (Switzerland), Wenger, April, TRONKA-AGROTECH, Arsenal, etc.) **[22]**, **[23]**, **[24]**, **[25]**, **[26]**.

Scientists from many countries of the world have conducted a number of studies on the splitting of starch during grain processing on various types of equipment, proving the preferential properties of expandate (product expansion) as an expansion product. The splitting of starch improves digestion in animals. Therefore, the problem of starch splitting is most relevant to piglets.

A large proportion of the split starch allows piglets to digest starch even before it enters the colon – thereby eliminating the cause of diarrhea, and the stabilization of the gastrointestinal tract is especially important for small animals.

Figure 2 shows the indicators of the degree of starch cleavage during expansion. The splitting of starch helps to improve digestion in animals **[27]**, **[28]**, **[29]**.

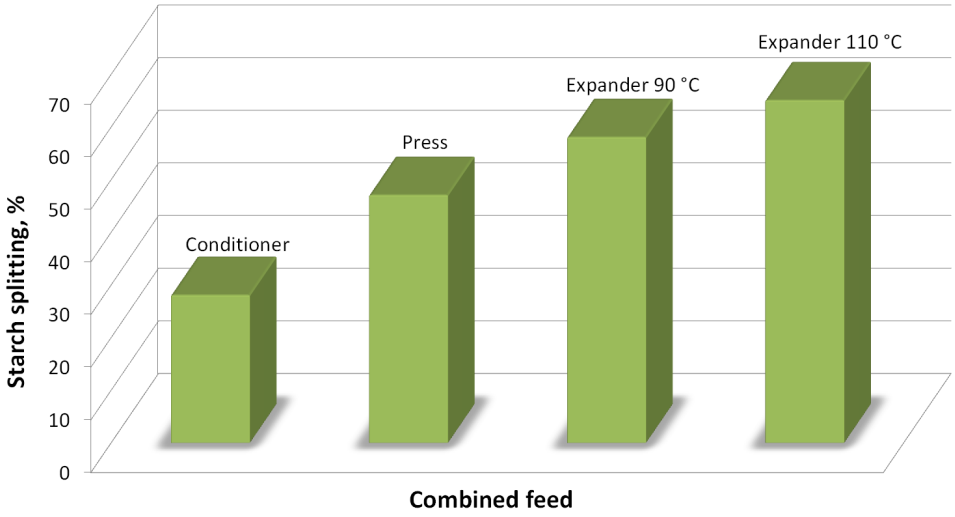
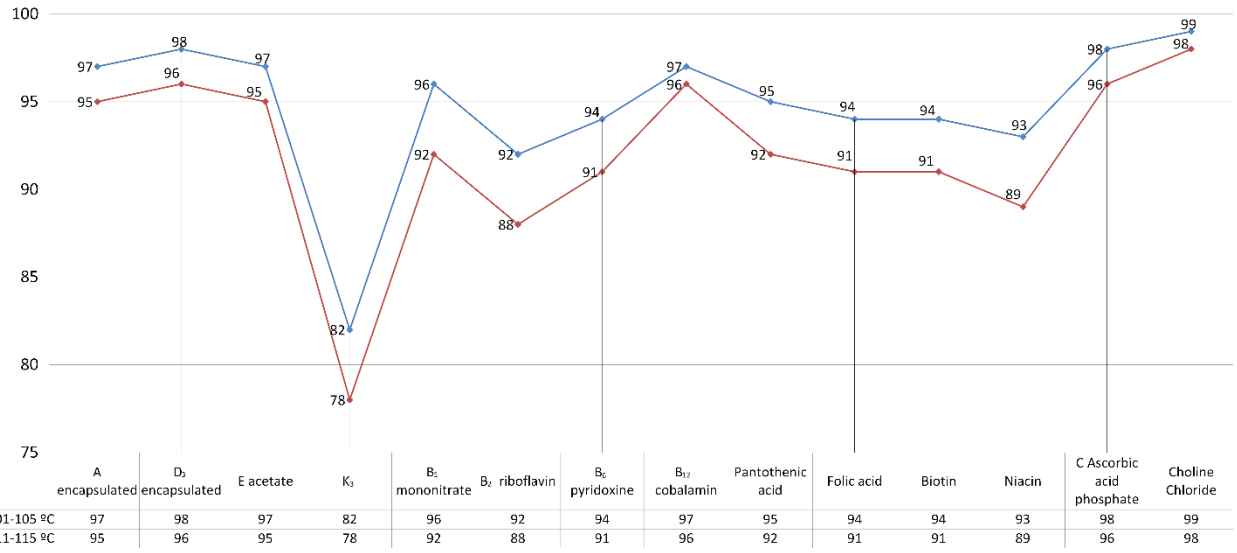


Figure 2 Dependence of starch splitting during processing on various types of technological equipment.

The disinfection principle is based not only on heat treatment but also on the dynamic effect during the passage of the product through the working area of the expander.

The expansion process affects the safety of biologically active substances introduced into combined feed with premix. First of all, this applies to the vitamin complex.

Figures 3-5 show the residual activity of vitamins in the expanded combined feed with various processing methods.



	A encapsulated	D_3 encapsulated	E acetate	K_3	B_2 mononitrate	B_2 riboflavin	B_6 pyridoxine	B_{12} cobalamin	Pantothenic acid	Folic acid	Biotin	Niacin	C Ascorbic acid phosphate	Choline Chloride
Expander 101-105 ºC	97	98	97	82	96	92	94	97	95	94	94	93	98	99
Expander 111-115 ºC	95	96	95	78	92	88	91	96	92	91	91	89	96	98

Figure 3 Residual activity of vitamins after feed processing on the expansion, %.

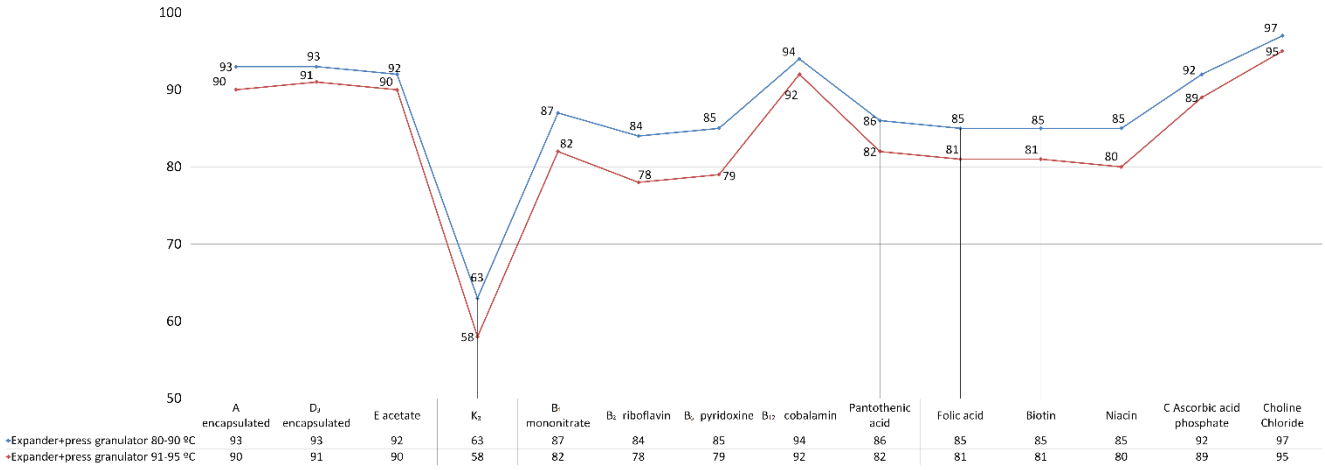


Figure 4 Residual activity of vitamins after feed processing on expansion+ pressing + granulation, %.

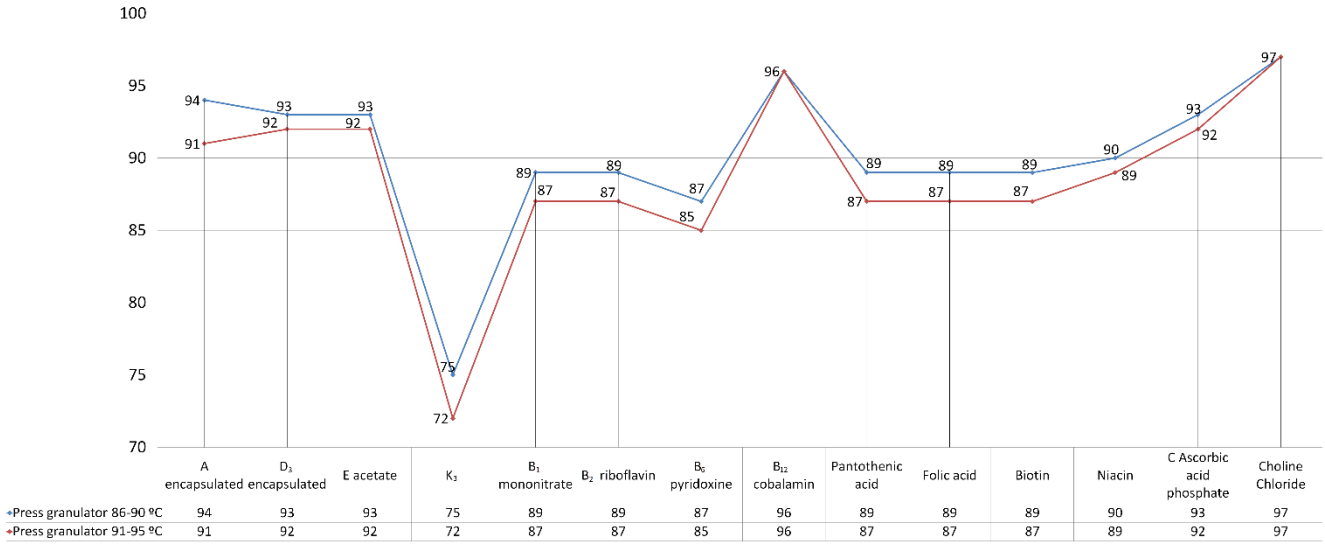


Figure 5 Residual activity of vitamins after feed processing on a granulation + pressing, %.

An analysis of the dependency graphs in Figures 3, 4, and 5 shows the following. The residual activity of vitamins (in %) was established during the expansion of combined feeds in comparison with other methods of feed processing. For example, when expanding (Figure 3) combined feeds at a temperature of 101 – 105 °C, the residual activity of most vitamins ranges from 92 to 99%, except for vitamin K_3, the residual activity of which is 82%. And suppose the expansion process of combined feeds is carried out at a temperature of 111 – 115 °C. In that case, the residual activity of most vitamins ranges from 88 to 98%, except for vitamin K_3, the residual activity of which is 78%.

The above indicators of the residual activity of vitamins during the expansion of the combined feeds differ favorably compared to the processing of feed by expansion-pressing granulation (Figure 4) at appropriate temperatures of 80 – 90 °C and 91 – 95 °C. The same difference is observed compared to feed processing of feed by pressing granulation (Figure 5) at the corresponding temperatures of 86 – 90 °C and 91 – 95 °C.

Another proof of the high efficiency of expansion is the determination of the stability of biologically active components (amino acids) after expansion. Figure 6 shows the content of amino acids before and after expansion under different temperature conditions of feed processing.

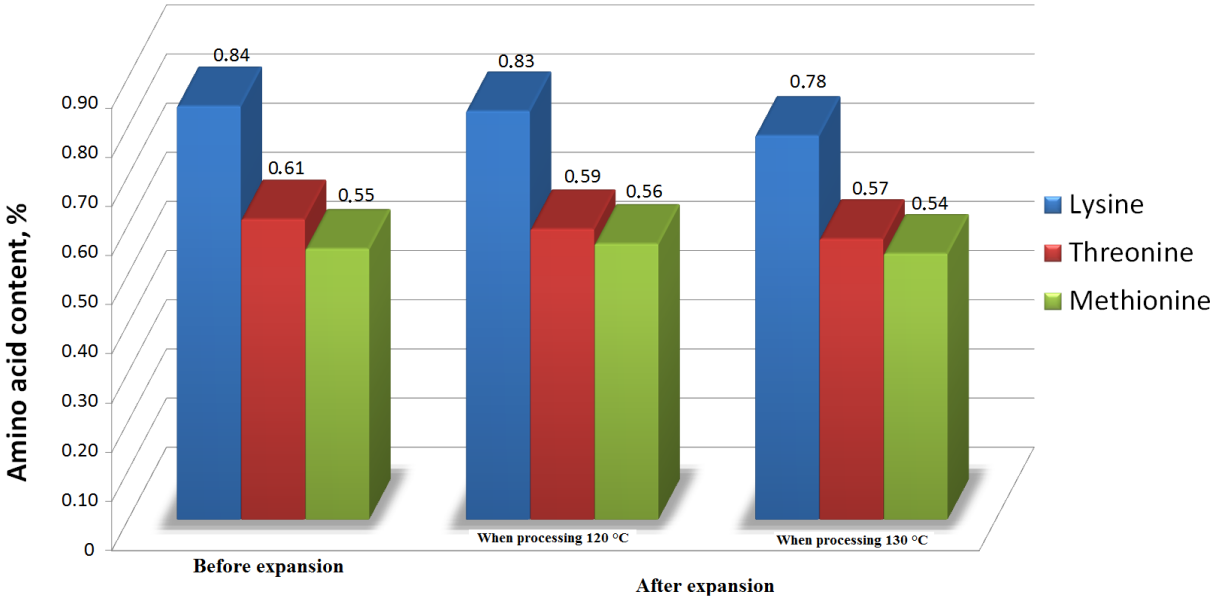


Figure 6 Amino acid content before and after expansion.

The economic efficiency of expanded combined feeds compared to similar loose, and granular combined feeds are confirmed by the following results of our studies on fattening pigs (from 9 to 30 kg) with various combined feeds (Table 1) and feeding laying hens with various combined feeds (Table 2) **[7]**, **[20]**.

Table 1 Indicators of pig fattening (from 9 to 30 kg) with various combined feeds.

Indicators	Loose combined feed	Granular combined feed	Expanded combined feed
Feed consumption, g/day	1007	955	922
Increase in body weight, g/day	473	470	476
Feed costs per 1 kg of weight gain	2.13	2.03	1.94

The economic efficiency when fattening pigs with expanded combined feeds compared to loose and granular combined feeds is as follows (Table 1): feed consumption (g/day) – with expanded combined feed 922 g/day, while with loose and granular combined feed 1007 and 955 g/day, respectively; increase in live weight (g/day) – 476 g/day, 473 and 470 g/day, respectively; the use of feed per 1 kg weight gain is with expanded combined feed 1.94, whereas with loose and granular combined feed 2.13 and 2.03, respectively.

Table 2 Results of feeding laying hens with various combined feeds.

Indicators	Loose combined feed	Expanded combined feed
Number of eggs	289	302
Egg weight, g.	18.11	18.65
Feed consumption, g/day	115.9	108.6

When feeding laying hens with expanded combined feed compared to loose combined feed, the following results were obtained (Table 2): an increase in the number of eggs by 13 more, by weight of eggs by 0.54 g more. At the same time, the savings in feed consumption are 7.3 g/day.

At the same time, there are several misconceptions in the literature about the negative impact of expansion. These misconceptions are explained by the fact that at high temperatures of 150-240 °C and prolonged exposure to them, the protein content in the grain decreases, as well as the availability of lysine decreases **[5]**, **[10]**, **[21]**. At the same time, the operating temperature of the expander is 80-130 °C, and the processing time is no more than 6 seconds **[8]**, **[12]**, **[22]**. Short-term processing at high temperatures means better assimilation of the combined feed, which affects the fattening indicators. This experience was confirmed by research conducted by the University of Göttingen, one of the Saxony feedlots **[11]**, **[12]**.

It is also known that feeding the exhibit leads to a significant decrease in drinking water consumption,

manure, and a decrease in nitrogen content in manure. It is also essential that is possible to obtain a "coarse grinding" structure when expanding.

Thus, the final result of our research is the creation of a new generation of highly digestible combined feeds with adjustable properties through the use of technology for the effective use of thermomechanical processing of components of combined feeds by the expansion method.

CONCLUSION

The use of expandate (product expansion) produced due to thermomechanical processing of components of combined feeds by the expansion method is promising and has significant advantages. The utilization rate of expanded combined feed per 1 kg of weight gain compared to loose combined feeds increases by 9%. The expansion process affects the safety of biologically active substances introduced into the combined feed with the premix. This applies primarily to the vitamin complex. We have established the residual activity of vitamins (in %) during the expansion of combined feeds compared to other feed processing methods. So, for example, when expanding the combined feed at a temperature of 101 – 105 °C, the residual activity of most vitamins ranges from 92 to 99%, except for vitamin K_3, the residual activity of which is 82%. And suppose the expansion process of the combined feed is carried out at a temperature of 111 – 115 °C. In that case, the residual activity of most vitamins ranges from 88 to 98%, except for vitamins K_3, the residual activity of which is 78%. The above indicators of the residual activity of vitamins during the expansion of combined feeds differ favorably in comparison with the processing of feed by expansion + pressing-granulation at appropriate temperatures of 80 – 90 °C and 91 – 95 °C and with the processing of feed by pressing-granulation at appropriate temperatures of 86 – 90 °C and 91 – 95 °C. The economic efficiency of expanded combined feeds compared to similar loose, and granular combined feeds is confirmed by our research results on fattening pigs (from 9 to 30 kg) with various combined feeds and feeding laying hens with various combined feeds. The economic efficiency when fattening pigs with expanded combined feeds compared to loose and granular combined feeds is as follows: feed consumption (g/day) – with expanded combined feed 922 g/day, while with loose and granular combined feed 1007 and 955 g/day, respectively; increase in live weight (g/day) – 476 g/day, 473 and 470 g/day, respectively; the use of feed per 1 kg weight gain is with expanded combined feed 1.94, whereas with loose and granular combined feed 2.13 and 2.03, respectively. When feeding laying hens with expanded combined feed, compared with loose combined feed, the following results were obtained: an increase in the number of eggs by 13 pcs more, by the weight of eggs by 0.54 g more. At the same time, the saving of feed consumption is 7.3 g/day. The final result of our research is the creation of a highly digestible combined feed of a new generation with adjustable properties through the use of technology for the effective use of thermomechanical processing of components of combined feeds by the expansion method.

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The yield of adipose tissue and by-products in the course of the slaughter of inbred and outbred bulls of the Ukrainian beef breed

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ABSTRACT

The research focuses on analysing and generalising the distribution of internal adipose tissue and organs that are not part of the carcasses of inbred and outbred bulls of *the Ukrainian beef breed*. Animal stock inbreeding was determined based on five breeding records according to Wright's method modified by Kyslovskyi. Two experimental groups of 5 bulls were formed. The average inbreeding coefficient for inbred bulls was 3.43%. Animals were bred up to 18 months of age. Following slaughter, the mass and the yield of the head, liver, lungs, heart, kidneys, and brain were determined, and 4 types of fat were separated and weighed: perirenal, from the stomach, intestines, and pericardial. Inbred animals are more prone to the accretion of internal adipose tissue. Inbred bulls have 1.8 points more of it. Fat is more intensely accumulated around inbred bulls' multichambered stomachs and kidneys. Intensive fat accumulation was observed around the hearts and intestines of outbred bulls. Adipose tissue around the heart and intestines is more variable in inbred and outbred animals – from the forestomach and kidneys. The weight of inbred bulls' liver is less by 22.4%, kidneys – by 62.5%, heart – by 11.1%, and head – by 23.8% compared to outbred ones. The weight of their lungs is more by 10.5%. At the same time, inbred bulls tend to have brain weight gain of 12.5% and testicles – by 8.3%. Thus, inbreeding application in *Ukrainian beef breed*s with a small population size affects the growth of internal organs and the intensity of accumulation and distribution of interior fat. Due to more intensive accumulation of internal adipose tissue, inbred bulls have increased expenditure of forage energy for its formation. They are characterized by an increased yield of low-value raw fat, making them less efficient than outbred bulls for beef production.

Keywords: inbreeding, outbreeding, internal organs, adipose tissue, bulls.

INTRODUCTION

In the genetic progress of the beef breed, the importance is devoted to substantiating the selection of parent pairs. Spontaneous inbreeding is inevitable with a considerable (approximately 90%) use of natural mating during its breeding **[1]**. A significant number of scientific works currently devoted to a significant number of scientific works is devoted toA significant number are presently dedicated to determining inbreeding problems in pure breeding. Applying inbred mating almost always leads to a negative effect – inbred depression. Inbreeding in beef cattle herds negatively affects the reproductive capacity, weight, and linear growth, milk, and beef productivity of animals – the signs that most often affect the economy of this cattle rearing business. Functional disorders cause inbred depression in animals associated with the influence of genetic factors **[2]**. To understand the mechanisms of its manifestation, it is appropriate to conduct a detailed study of changes in the animal bodies, particularly to analyze the influence of close breeding on individual organs and systems.

Ukrainian beef breed was created by complex reproductive crossbreeding of Kian (K 3/8), Charolais (W 3/8), Simmental (C 1/8), and grey Ukrainian (SU 1/8) cattle **[3]**. In the course of its breeding, to consolidate the desirable traits, inbred mating is used quite often. Inbreeding reduces the productivity of beef cattle **[4]**. Inbreeding depression is manifested mainly by its traditional signs. If the inbreeding coefficient exceeds 7-11%, it negatively affects weaned calves' weight, increasing to 18 months of age and manifesting meat forms **[5]**. The regression coefficients between inbreeding and the weight of calves at the age of 210 days are negative **[6]**. An increase in inbreeding by 1% in Brahman and Tropical Composite cattle breeds is associated with a decrease in live weight

by 0.514 and 0.579 kg at the age of 1 year **[7]**. Inbreeding depression is 0.016 kg for the live weight of newborn calves, 0.418 kg – at the age of 200 days, 0.689 kg – at the age of 400 days, and 0.967 kg – at the age of 600 days. The decrease in the live weight of newborns by 0.103 kg is also observed with the increase in the inbreeding coefficient by 1% **[8]**, at weaning – by 2.03 kg **[9]**, at the age of 365 and 550 days – 0.29 kg **[10]**. The study **[11]** results demonstrated that the decrease in the live weight of beef cattle at different ages is observed in the range of 0.04 to 2.07 kg, with an increase in inbreeding by 1%. The increase in the inbreeding coefficient in animals leads to a decrease in the average daily gain of live weight before weaning and up to one year of age **[12]**. For the characteristics of weight gain, the average inbreeding depression is 0.269% per 1% of inbreeding **[13]**. It is insignificant and about 0.01% **[14]**. Inbreeding increases beef viscosity but does not significantly affect its other qualities or characteristics of carcasses, food consumption, and digestion **[15]**.

Brännäng was the first to study the distinctive features of fat deposition in the depot that are not part of the carcass **[16]**. The amount of adipose tissue in the animal body varies depending on the species **[17]**, breeds and pedigrees **[18]**, **[19]** lines **[20]**, age **[21]**, gender **[22]**, feeding conditions **[23]**, and animal housing **[24]**. Proceeding to the problem of growth of the internal adipose tissue that has low nutritional value in inbred and outbred animals, it should be noted that it is extremely insufficiently studied. Adipose tissue is studied under the skin between the muscles and in the muscles **[25]**. Inbred animals have less fat in carcass **[26]**. Over the past 10 years, almost no one has researched fat distribution in fat depots in inbred and outbred animals of some breeds. Therefore, this factor is quite significant, as the amount and distribution of fat can significantly impact the weight of the carcass after slaughter. The low-fat cost from various fat depots also does not stimulate the study. This kind of information would be beneficial in explaining the differences between various levels of carcass yield.

In the Ukrainian beef cattle breed, inbreeding depression is an escalating problem, as it negatively affects animal health and productivity. In cows, it is manifested by weight gain. However, producing ability **[27]**, although more enhancers in growth rate originate from inbred mating. Many issues of inbreeding influence the growth of internal adipose tissue and organs that are not part of the carcass but affect its yield and beef productivity remain insufficiently studied. Disclosure of the peculiarities of their growth is necessary to produce beef efficiently and purposefully at a higher yield of valuable components.

This paper aims to study inbreeding's influence on the distribution of internal adipose tissue and organ weight in the Ukrainian beef breed bulls.

Scientific Hypothesis

Previous studies have shown that inbreeding negatively affects cattle's live weight and growth rate, and its influence on carcass quality and feed digestion has not been confirmed. It was assumed that inbred animals should have a proportionally lower weight of internal organs while maintaining their relative yield to the pre-slaughter live weight. At the same time, the growth rate is closely related to the metabolic processes in the body, so inbreeding influences the development of individual organs that are actively involved in them, and fat deposition may differ from the general trend of decrease in live weight of animals.

MATERIAL AND METHODOLOGY

Samples

Two groups of experimental bulls were formed for the study using the method of balanced analogue groups. In the first group - inbred animals (5 animal units), the average value of the inbreeding coefficient (F_x) was 3.43%. The second group - is outbred bulls (5 animal units).

Chemicals

Formaldehyde (CH_2O, producer (Inter-Synthesis) Limited Liability Company, Ukraine, chemically pure for analysis).

Formalin (water solution formaldehyde, producer (Inter-Synthesis) Limited Liability Company, Ukraine).

Animals and Biological Material

The study was conducted with the use of the bulls of the Ukrainian beef breed (Figure 1). The experiment was conducted at the Volia breeding plant in the Zolotoninskyi district of the Cherkasy region. The bulls were bred from birth to slaughter at 18 months. Following slaughter, offal (head with horns, liver, lungs, heart, kidneys, brain, and testicles) and internal fat (pericardial, intestinal, multichambered stomach, perirenal) were selected for weighing.

Figure 1 Bull of the Ukrainian meat breed.

Instruments

Static scales 4BDU-1500X-P (Axis, Ukraine). Scale division ≥0.5 kg, weighing rang 10-1500 kg. GOST 29329-92 **[28]**. Weighing of bulls before slaughter.

Scales Prok (Ukraine). Weighing range of up to 150 kg. GOST 29329-92 **[28]**. Weighing of offal and internal fat.

Laboratory Methods

Method of forming the balanced analogue groups **[29], [30]**.

Determination of the inbreeding coefficient by Wright's method modified by Kyslovskyi **[31]**.

DSTU 4673: 2006. Cattle for slaughter. Specifications **[32]**.

DSTU 3938-99. Beef industry. Livestock slaughter products. Terms and Definitions **[33]**.

There are rules for pre-slaughter veterinary inspection of animals and veterinary and sanitary examination of beef and beef products (2002) **[34]**.

Description of the Experiment

Sample preparation: The study was conducted for 2 calendar days during the slaughter of bulls. During two calendar days, 140 samples were taken, 70 samples each day.

Number of samples analyzed: 140 samples from two conducted experiments (70 in each) were used in the study of the samples.

Number of repeated analyses: The weight of by-products was determined for each of the slaughtered bulls, including heads with horns 10 times, liver 10 times, lungs 10 times, heart 10 times, kidneys 10 times, brain and testicles 10 times, which amounted to 70 replicates.

Number of experiment replication: The study was repeated 10 times, with the experimental data processed using mathematical statistics methods.

Design of the experiment: The experiment was conducted at the Volia breeding plant in the Zolotoninskyi district of the Cherkasy region. Two groups of newborn well-grown inbred and outbred bulls of 5 animal units were formed in the herd of the Ukrainian beef breed. The animals were tested for the probability of origin by blood group factors. The bulls were grouped by the method of balanced analogue groups. The inbreeding coefficient was determined using a complete five rows of pedigrees according to Wright's method modified by Kyslovskyi. The selected animals at the age of up to 6-7 months were bred near suckler cows. After the weaning, they were accustomed to a typical diet and husbandry until 8 months of age. The animals were housed tethered with individual control of the amount of the feedstuff fed and consumed. Intensive breeding of bulls was carried out from 8 to 18 months. The general level of their feeding was expected to receive the average daily gains from 1000 to 1200 g. During this period, the animals consumed feedstuff of their production with the same rations. The weight of the feed consumed by each bull was counted every decade (two days in a row) by weighing the feed and the orts. During 8 to 18 months, each inbred bull consumed only 2,999, outbred 2,903 feed units (Table 1). The share of the concentrated feed in the diet was 45.6% and 45.8%, roughage feed – 19.0 and 19.2, succulent feed – 15.4 and 13.3, green feed – 20.0, and 21.7%. There was no significant difference in animal feed consumption between the groups.

Table 1 Feed consumption by inbred and outbred bulls at the age of 8 to 18 months, M ± m.

Feeds	Inbred (n = 5)		Outbred (n = 5)	
	Feed Units	%	Feed Units	%
Concentrated	1.369 ±50.9	45.6 ±0.56	1.328 ±55.8	45.8 ±0.10
Roughage	570 ±86.7	19.0 ±1.73	558 ±35.2	19.2 ±1.11
Succulent	461 ±51.4	15.4 ±0.50	387 ±31.5	13.3 ±0.41
Green	599 ±26.8	20.0 ±0.24	630 ±66.9	21.7 ±1.44
Total feed units	2.999 ±88.0	100.00	2.903 ±104.8	100.00

At 18 months, bulls were slaughtered at Cherkasy beef-processing-and-packing plant. Weighing of the animals and the slaughter products was performed individually. The pre-slaughter weight was determined after 24 hours of fasting. Following slaughter, the weight of offal was determined, including the weight of heads with horns, liver, lungs, heart, kidneys, brain, and testicles. Various types of adipose tissue were studied in the animal bodies, including adrenal, intestinal, multichambered stomach, and pericardial adipose tissue. Renal fat was removed from the kidneys and the inner side of the carcass in the lumbar and pelvic areas. The fat that covers the stomach is omentum fat. The intestine fat was isolated in the pericentric mesenterium. Subsequently, the average values by group and the yield of internal fat and offal to pre-slaughter weight were determined.

Statistical Analysis

The obtained data were processed by variation statistics according to the methods adopted in breeding and biology **[35]**. Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. Values were estimated using mean and standard deviation. We calculated the arithmetic mean (unweighted) value (M) and the arithmetic mean error (± m), which allowed us to estimate with some probability the deviation of the arithmetic mean deviation of the Fulton fattening factor. The statistical reliability of research results was ensured by analyzing samples with the number of fat samples from 5 to 10 samples per bull.

RESULTS AND DISCUSSION

Accumulation of a bull's internal fat is important due to its connection with feed costs. It has been proven that visceral fat accumulation is connected with the increased feed costs for live weight gain **[36]**. At the same time, the value of beef fat in the food and processing industries is decreased. Current trends in healthy feeding aim to reduce the caloric content of foods and partially replace solid animal fats with triglycerides containing polyunsaturated fatty acids due to the introduction of primary products of plant origin. In particular, the beef fat in cutlets for burgers is replaced by tiger nut oil emulsions. In sausages, the fat is recommended to be replaced by jelly-containing emulsified systems with the inclusion of peanut and linseed oil **[37]**. The cutlets are recommended to be prepared with olive oil **[38]**, so it is important to understand all the factors contributing to an increase in the yield of internal adipose tissue of slaughter animals.

In the case of such feeding, the bulls at the age of eighteen-month demonstrate different levels of internal adipose tissue deposition (Table 2). Samples of internal adipose tissue are shown in Figure 2ab. Inbred animals are more prone to increased deposition than outbred ones. Their bodies contain 69.8% of the total amount of internal adipose tissue. This is 1.8 points more in comparison with outbred animals. The increased amount of internal fat in the bodies of inbred animals is explained by its biological susceptibility to better reserving nutrients in intensive feeding and using them in periods of adversity.

Internal fat is deposited unevenly on various organs of the animal's body. The largest amount is in the intestines and forestomach of the animals of both groups. The smallest amount is formed around the heart.

Inbreeding in beef bulls causes significant differences in the distribution of internal adipose tissue. Inbred bulls have a relatively larger amount of fat in kidneys and stomachs in comparison with a relatively larger amount of fat than outbred bulls. In outbred bulls - around heart and intestines. Increased accumulation of adrenal adipose tissue in the bodies of inbred bulls may be one of the reasons for increased feed costs, as it contains the largest amount of refined fat compared to other depots **[39]**. According to certain data **[40]**, adrenal fat contains about 90% of extracted fat and the smallest amount of water and protein slaughter products.

Adipose tissue around the heart and intestines is more variable in inbred animals. In outbred animals - from forestomach and kidneys. In the bodies of inbred animals, adipose tissue is deposited twice more evenly on the forestomach and kidneys. In outbred animals - around the heart and intestines. The susceptibility of individual animal units may explain significant variability in inbred bulls related to the amount of pericardial fat to obesity and the manifestation of heart failure that is often observed during intensive beef cattle breeding **[41]**.

Table 2 Distribution of internal adipose tissue in inbred and outbred bulls at 18 months.

Adipose tissue	Inbred (n = 5)		Outbred (n = 5)	
	M ± m	Cv, %	M ± m	Cv, %
Total adipose tissue, kg	21.2 ±1.09	10.4	17.5 ±1.54	17.5
Internal fat, kg	14.8 ±1.10	14.9	11.9 ±1.24	20,9
Internal fat before pre-slaughter live weight, %	2.7 ±1.19	14.1	2.1 ± 018	17.2
Including pericardial, kg	1.2 ±0.52	90.8	1.5 ±0.42	55.0
% related to the internal fat	7.4 ±2.87	78.0	12.9 ±4.9	38.6
% related to the total fat	5.2 ± 2.13	81.7	8.7 ±1.84	42.3
From intestines, kg	5.6 ±0.41	14,7	6.3 ±0.40	12.6
% related to the internal fat	38.5 ±4.11	21.4	54.5 ±3.53	12.9
% related to the total fat	26.6 ±2.45	18.5	36.8 ±3.54	19.2
Fat from forestomach, kg	4.1 ±0.40	19.2	2.0 ±0.27	27.2
% related to the internal fat	27.9 ±0.48	11.9	16.9 ±0.70	22.4
% related to the total fat	19.4 ±1.46	15.0	13.2 ±3.72	56.5
Pararenal, kg	3.9 ±0.59	30.2	2.0 ±0.60	60.6
% related to the internal fat	26.3 ±2.36	18.0	15.9 ±3.14	39.5
% related to the total fat	18.3 ±1.92	21.0	10.8 ±2.37	44.1

Following animal slaughter, some by-products that have nutritional value are produced. The liver, heart, tongue, kidneys, etc., are important protein sources. The liver, heart, tongue, kidneys, etc., are important protein sources, including key amino acids, vitamins, and mineral elements **[42]**. Cattle liver is the primary product for pasta and paste production **[43]**. The by-products such as kidneys, lungs, and heart contribute to increased absorption of non-heme iron in vegetables, food supplements, and other products of plant origin **[45]**, and their efficiency far exceeds the influence of beef **[44]**.

The weight of offal depends on many factors. In particular, it is known that the weight of testicles depends on age and puberty, and it is gained due to intensive feeding **[46]**. There is evidence that the weight of offal in the bodies of similar bulls at 18 months is affected by pre-slaughter weight, but their percentage yield changes insignificantly **[47]**. The total number of edible and inedible offal is significantly affected by the breed and the age of the animals **[48]**.

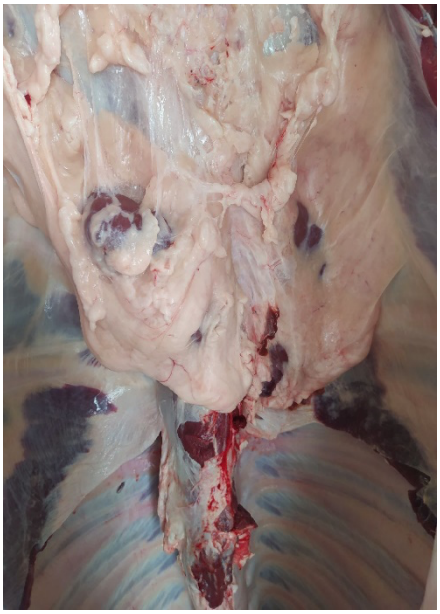

Figure 2a Samples of internal adipose tissue.

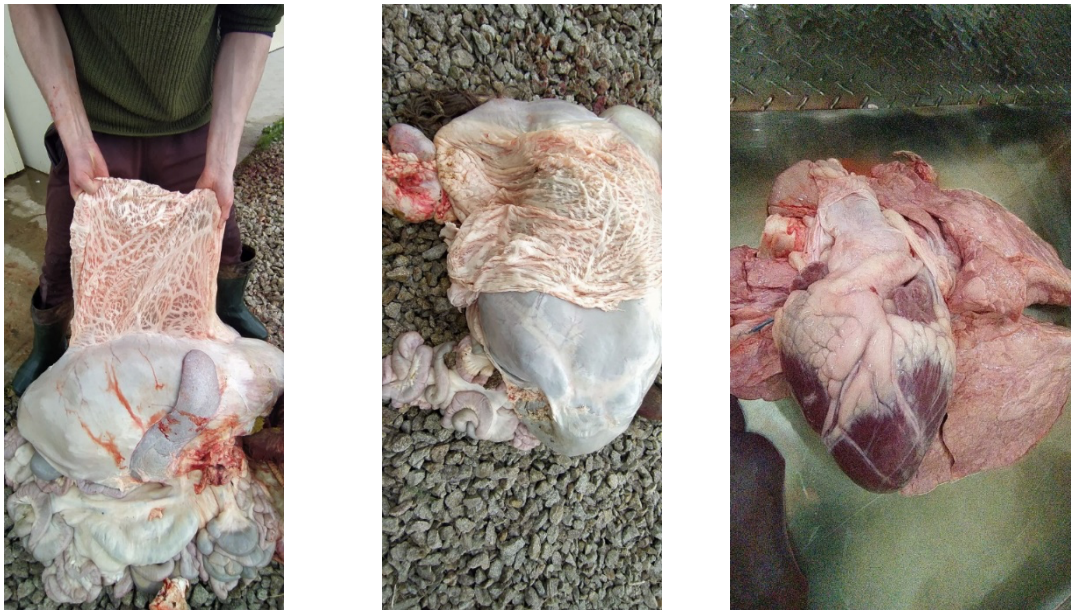

Figure 2b Samples of internal adipose tissue.

The internal organs of cattle are not only available primary products for the processing industry. They are indissolubly related to many valuable traits such as growth, health, and productivity. It was established **[49]** that 38 significant single nucleotide polymorphisms affect the weight of internal organs, which indicates the genetic condition of offal yield related to slaughter animals. The genetic condition of offal weight may be manifested in the course of inbreeding application due to the concentration of similar genes and the reduction of their diversity.

The average weight of by-products of inbred and outbred bulls is shown in Table 3.

Table 3 The weight of bull organs that are not part of the carcass.

Organ	Inbred (n = 5)		Outbred (n = 5)	
	M ± m	Cv, %	M ± m	Cv, %
Head with horns, kg	16.8 ±0.59	6.1	20.8 ±0.69	5.7
% of pre-slaughter live weight	3.3 ±0.10	5.3	3.6 ± 0.06	2.6
Liver, kg	5.8 ±0.12	3.6	7.1 ±0.50	12.2
% of pre-slaughter live weight	1.1 ±0.03	4.4	1.2 ±0.06	7.8
Lungs, kg	4.2 ±0.24	9.9	3.8 ±0.32	14.6
% of pre-slaughter live weight	0.8 ±0.05	10.2	0.7 ±0.06	15.4
Heart, kg	1.8 ±0.09	8.8	2.0 ±0.04	3.2
% of pre-slaughter live weight	0.4 ±0.01	7.4	0.4 ±0.01	4.2
Kidneys, kg	0.8 ±0.09	20,4	1.3 ±0.06	8.0
% of pre-slaughter live weight	0.2 ±0.03	38.5	0.2 ±0.03	22.2
Brain, g	431 ±57.1	22.9	383 ±14.8	6.7
Testicles, g	615 ±30.0	8.5	568 ±38.9	6.9

The weight of inbred bulls' liver is less by 22.4%, kidneys – by 62.5%, heart – by 11.1%, and head – by 23.8% compared to outbred ones. The weight of their lungs is more by 10.5%. At the same time, inbred bulls tend to have brain weight gain of 12.5% and testicles – by 8.3%. Similar data were published by Eisner **[50]**. According to him, the size of the heart, liver, brain, kidneys, pituitary gland, and pancreas in the bodies of inbred animals decreases, and the size of lungs, thyroid, and adrenal glands – increases. In studies on Holstein calves **[51]**, it was established that the relative weight of heads is larger in the animals that are significantly inferior to the animals of the same age-related to growth rate, or those that are characterized by higher gains and larger live weight at the time of slaughter. A similar result was obtained in our studies. Outbred bulls with larger live weights took precedence over the actual and relative head weights of inbred ones.

In contrast to inbreeding, in the course of crossbreeding, particularly Simmentals and Holsteins, no effect on the weight of by-products was found when breeding bulls in the same conditions and slaughtering at the same age **[52]**. Thus, in Ukrainian beef breed of small population size and with inbreeding application in combination with

the reduction in weight gain, reproductivity, and milk-producing ability of cows **[53]**, one of the most significant problems is the reduction of weight of liver, heart, kidneys, head, and increased content of internal fat.

Regardless of carcass composition, offal and internal fat taken together negatively affect the carcass yield. Approximately 1/4-1/3 of the basic components (water, protein, and fat) are in body parts that are not part of the carcass. The lowest price is for such parts **[54]**. Therefore, it is necessary to find the opportunity to change this ratio in the desired direction, as the cost of by-products is significantly lower than a carcass.

Internal fat is the most changeable and pliable tissue. After slaughter, it is removed from fat depots, as adipose tissue is not of great nutritional value. However, internal fat is associated with additional feed costs in animal breeding, which is unprofitable. In the course of inbreeding, the carcass yield is reduced due to internal fat accumulation. Excessive omental fat deposition in cattle under the same conditions is caused by several genetic loci **[55]**. The genes involved in proteolysis, transcription, translation, transport, immune function, and oxidative processes are different **[56]**. Inbreeding probably leads to the concentration of the relevant genes, which provokes an increase in internal fat deposition. Therefore, it is desirable to avoid close breeding in commercial beef cattle breeding and prefer outbred beef cattle production. Another disadvantage of excessive internal fat deposition is the negative correlation of its amount with intramuscular fat content **[57]**. Thus, the animals' marbling degree that isprone animals' accumulation will decrease. This negatively affects the cost of the most valuable cuts of the carcass. The maximum growth rate of adipose tissue of large and small glands and adrenal glands in bulls occurs in 7 to 12 months **[58]**. The most intensive adipose tissue growth around the heart is noted in 12 to 18 months of animal life. According to the data obtained in the study, inbreeding reduces heart size. Probably the less amount of fat deposited around the heart is due to this. This is compensated by an active adipose tissue accumulation in the forestomach and around the kidneys.

Peculiarities of fat distribution by fat depots may be considered in case of excessive generation of cattle waste. The formation of the larger amount of internal fat in the forestomach and kidneys of inbred bulls also leads to the assumption that the difference in internal fat content is mainly conditional on different periods of its accumulation commencement in this period on these organs, not rate. It was established **[59]**, **[60]** that adrenal fat is formed early, and subcutaneous and other fats are formed later. Analyzing the level of lipids in the body of *Aberdeen-Angus* and *white-headed Ukrainian cattle* and the crossbreeds of *Aberdeen-Angus* × *white-headed Ukrainian* **[61]**, it is noted that the crossbreeds showed true heterosis in internal adipose tissue accumulation per 1 kg of net weight, it is 113.2%. Topography of fat deposition in the cattle body is the trait primarily associated with animal precocity. Animals of precocious breeds are prone to early obesity. They also deposit much more fat on the outer parts of the carcass **[62]**. The patterns established in the study indicate the change in animal precocity under the influence of inbreeding. Thus, more fat is deposited on outbred animals' internal organs (heart, intestines), as they are relatively late-maturing cattle. Inbred animals, relatively more precocious bulls, accumulate more internal adipose tissue around the stomach and kidneys but have a less developed head, liver, heart, and kidneys.

CONCLUSION

Incombined with a decrease in pre-slaughter live weight of bulls, a decrease in pre-slaughter live weight of bulls, and a reduction in pre-slaughter live weight of bulls, inbreeding leads to the accumulation of internal adipose tissue. Inbred animals' total weight of internal fat is 24% higher, leading to inefficient feed consumption and an increased yield of low-value raw fat. Internal fat accumulation in outbred bulls is more often observed around the heart and intestines and in inbred animals – around the stomach and kidneys. Excessive accumulation of adrenal fat due to low moisture and protein content may negatively affect the redistribution of the feed energy consumed to grow more valuable parts of the carcass. Inbred bulls are also characterized by severe inhibition of the growth of internal organs such as the liver, heart, and kidneys, which reduces the weight of such offal following slaughter—still, the weight of their lungs, brain, and testicles increases. Regarding the results obtained, it is appropriate to breed outbred bulls of Ukrainian beef and apply inbreeding to a limited extent for breeding purposes.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

According to Protocol No. 10 of 18.04.2020 at the meeting of the Ethics Commission of the Faculty of Livestock Raising and Water Bioresources, National University of Life and Environmental Sciences of Ukraine, Act No. 3 and 4 were signed during the experimental research, i.e. in the process of the slaughter of cattle "all the rules of the current legislation of Ukraine were observed, following DSTU 4673: 2006.

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The influence of grain mixtures on the quality and nutritional value of bread

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ABSTRACT

The desire to survive in a competitive environment mobilizes managers to make unconventional decisions to increase their product range, quality, and safety. This study aims t to create a technology of bread with increased nutritional value using bioactivated cereal mixtures and develop new bread recipes. The experiment used bioactivated wheat and maize grains, flax, rye flour, 1st-graduate wheat flour, spontaneous fermentation starter, salt, and water. Vegetable components such as dried crushed hawthorn berries, jaggery, and barberry were also used. Standard, generally accepted chemical and organoleptic methods of examining raw materials, semi-finished and finished products were used. It was found that the best physical and chemical indices were possessed by testing the bread prepared with the addition of a 20% grain mixture. All experimental analyses improved several parameters compared to the control sample. The nutritional value of obtained products was increased from 0.5 to 3 times. According to the obtained results, it is possible to conclude the relevance of this topic is getting a new range of bread products with increased nutritional value.

Keywords: nutritional value, bread, grain, bioactivation, microbiological safety, grain mix

INTRODUCTION

Currently, a wide range of bread products are baked according to different recipes and correspond to the taste preferences of different population segments. The deterioration of the environment, the rapid pace of life, the reduction in the quantity and deterioration in the quality of food products lead to the maturing problem of developing food with increased nutritional value, and bread as one of the most widely distributed products is very important in the human diet. That is why the technology of obtaining grain bread as a healthy, "live" product is becoming increasingly popular.

An important feature of grain mixtures is their increased hydration capacity. The dough with such grain mixtures has a significant water-absorbing ability, allowing the binding free water in the dough. In turn, this leads to a decrease in the stove, a significant increase in the weight of the products, and a substantial reduction in moisture loss in the storage of finished products – to a slowdown in staling **[1]**, **[2]**, **[3]**. Bread with the addition of bioactivated grains and dry vegetable ingredients has many advantages compared to bread made from wheat flour, prepared according to a traditional recipe. So, it differs in that it contains almost entirely preserved proteins, fats, micro- and macronutrients, vitamins, and dietary fiber **[4]**, **[5]**.

Moreover, dry vegetable ingredients improve the color, taste, and aroma of the finished product and, importantly, further enrich the bread contained in the composition of many valuable nutrients **[6]**, **[7]**, **[8]**. In choosing grains for bio-activation and their further use, the composition of grain mixtures was because they have a high nutritional and biological value. Grain bioactivation is a controlled process of grain moisture saturation that occurs in the presence of water, heat, and air and is the beginning of germination. High-molecular substances are transformed into easily accessible forms. Due to this, bioactivated grain is a source of biologically active substances **[9]**, **[10]**. We have developed a technology and a range of bakery products made from bioactivated grain. However, despite the advantages of bread made from bioactivated grain, which is characterized by an

increased content of dietary fiber, minerals and vitamins compared to traditional types of bread, it has reduced protein content and a lack of lysine **[11]**, **[12]**, **[13]**. Bioactivated grains with sprouts (no longer than 5 mm in length) contain sufficient antioxidants, which in low concentrations slow down or prevent oxidative processes **[14]**, **[15]**. In addition, in germination in the grain, the enzyme systems are activated. There is a breakdown of complex nutrients into simpler, easily digestible by the human body **[16]**, **[17]**. A mixture of each component enriches it with certain useful substances for humans, and the product acquires a preventive orientation. The naturalness in the ratio of the natural raw materials components of food nutrients contributes to the increase in the nutritional value of products and their better absorption **[18]**, **[19]**, **[20]**.

Grain mixtures differ from other food groups by their low water content, energy saturation, transportability, long shelf life (up to one year), and the presence of functionally active ingredients in their composition. According to the grain, the mixture's rationally and purposefully formulated recipe, and you can get a product with an exceptional value proven in this article.

Scientific Hypothesis

The increased nutritional value of bread will depend on the beneficial properties of grain mixtures.

MATERIAL AND METHODOLOGY

Samples

The objects of the study were: bioactivated grains of wheat and corn, flax, rye flour, wheat flour of the 1st grade, spontaneous fermentation starter, salt, and water. Some herbal ingredients were used, such as dry crushed hawthorn berries, horseradish, and barberry. All analyzes were carried out in an accredited laboratory of the Almaty Technological University.

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Animals and Biological Material

Animal and biological materials were not used in this study.

Instruments

We used automatic fat extractor SER 148/3 (Velp Scientifica, Italy), Kjeldahl VELP UDK 129 (Velp Scientifica, Italy), atomic absorption spectrometer KVANT-Z-ETA-T (OJSC Kortek, Russia), convection oven UNOX XB693 (UNOX, Italy).

Laboratory Methods

In work, the following indicators of the raw materials used and the resulting assortments of bakery products were investigated: organoleptic indicators by GOST 5667-85 **[21]**, mass fraction of moisture by GOST 21094-75 **[22]**, mass fraction of fat by GOST 5668-68 **[23]**, a mass fraction the proportion of protein by GOST 10846-91 **[24]**, mass fraction of sugar by GOST 5672-68 **[25]**, mass fraction of carbohydrates by GOST 25832-89 **[26]**, the mass fraction of ash by GOST 5901-2014 **[27]**, mass fraction of porosity by GOST 5669-96 **[28]**, acidity content by GOST 5670-96 **[29]**, iron content by GOST 26928-86 **[30]**, vitamin A content by GOST R 54635-2011 **[31]**, and others. Some standards generally accepted chemical and organoleptic methods were used to study raw materials, semi-finished products, and prepared products.

Description of the Experiment

The methodological basis of the study was a systematic analysis of the technology used in the production of bakery products enriched with useful herbal ingredients. The following main tasks were performed sequentially: 1) selection and justification of the method for introducing herbal ingredients into the recipe for bakery products and 2) improvement of the technology used in bakery products by incorporating proper plant ingredients. The theoretical basis of the research consisted of general scientific and unique research methods, methods of system analysis, and experimental planning.

Number of samples analyzed: We analyzed 3 bread samples.

Number of repeated analyses: All tests were performed in triplicate.

Number of experiment replication: 2 times.

Design of the experiment: Bioactivation was carried out under the following modes such as washing the grain in clean drinking water, washing the grain 5% for disinfection, washing the grains the second time, soaking the grain in water for 24 hours at a temperature of 22-25 °C, bioactivation the grains at a temperature of 20-30 °C, dispersing the bioactivated grains to a humidity of 13-14% and crushing the bioactivated grains to pass a sieve

with round holes with a diameter of 1.5 mm, with a residue on the sieve of 4.0%. The main controlled indicator of wet sprouted grain was the presence of a germ root no longer than 5 mm in length in 90% of seeds.
After the bioactivation process of grains and drafting of compounding of grain mixtures, compounding of bread were made with an increasing food value and the physicochemical indices of baked foods are certain. The research applied the generally accepted and special methods of estimating raw material properties and the quality of baked foods.

Statistical Analysis

Data processing and calculations were performed using the Statistica 12.0 (StatSoft, Tulsa, OK, USA) sequential regression analysis program. The program first gives the values of all the regression coefficients and their confidence intervals (errors), then checks the significance of the regression coefficients. After removing all the insignificant coefficients, the remaining significant coefficients and their confidence errors are printed. Also, the data were analysed using MS Excel for Windows version 10 Pro, 2010. The data collected during the study were subjected to independent testing, and questionnaires were conducted to assess the organoleptic characteristics of control and test samples. The analysis process used absolute and relative statistical indicators and tabular and graphical methods for presenting the results. The student's t-test was used to evaluate bread yield, crumb porosity, and crumb moisture. To check the quality of the obtained regression equations, the multiple correlation coefficient R, the coefficient of determination R2, the Fisher Criterion F and the Durbin-Watson criterion d were calculated. On their basis, response surfaces and lines of equal levels (isolines) of indicators of the content of grain mixtures on technological indicators of the quality and safety of bread were obtained and built, depending on various combinations of the studied parameters: the dependence of the content of grain mixtures on the content of sourdough.

RESULTS AND DISCUSSION

Physical and chemical indices and the quality indicators of two samples of rye flour and wheat flour of the 1st grade were studied further and shown in Table 1 and Table 2.

Table 1 Physical and chemical indices of the medium rye flour.

Indicators	Sample 1	Sample 2
Humidity, %	13.5 ±0.3	13.7 ±0.5
Ash content, %	1.43 ±0.11	1.40 ±0.10
Acidity, deg.	2.0 ±0.07	2.0 ±0.04
Falling number, sec	158 ±0.2	155 ±0.3
Whiteness, the device unit	6 ±0.09	6 ±0.08
Pest infestation	Does not found	
Mineral impurity	The crunch is not felt	

Note: ± standard deviation.

Table 2 The 1st grade wheat baking flour's quality indicators.

Indicators	Sample 1	Sample 2
Humidity, %	13.7 ±0.1	14.0 ±0.4
Ash content, %	0.54 ±0.18	0.60 ±0.12
Acidity, deg.	2.0 ±0.04	2.0 ±0.03
Falling number, sec	250 ±0.3	246 ±0.8
Whiteness, the device unit	52.4 ±0.07	53.2 ±0.07
Amount of gluten, %	63 ±0.3	62 ±0.05
Gas-forming capacity, cm^3	1264 ±0.9	1300 ±0.2
Pest infestation	Not present	
Mineral impurity	The crunch not detected	

Note: ± standard deviation.

Lean on the research's result, it can be concluded that the flour samples used in this research correspond to the requirements of state standards. Pest infestation and crunch in flour were not detected in all samples.

Rye sourdoughs were prepared from medium rye flour to baking bread. The starter culture was prepared firstly for these purposes. The resulting thick starter culture was fed and brought to readiness by fermentation.

Some laboratory research has been carried out on the quality of rye sourdough. The results of the research are shown in the following Table 3. Following physical and chemical indices as humidity, acidity, and lifting force of the "ball" were studied.

Table 3 Physical and chemical indices of the rye sourdough's quality.

Names of the indices	Received results
Length of soaking, h	20
Temperature, ºC	26-30
Mass fraction of moisture, %	48
Acidity, deg.	13.5
Lifting force of the "ball", min	18

As a result of sourdough's quality analysis, lifting force indicators can be noted as good. The acidity and humidity correspond to the used grade of rye flour and the type of sourdough.

Wheat and maize grains were used for bioactivation. Grains for bioactivation should be of good quality, mature, and without impurities, and when submerged in water, 90-95% of the grains should sink to the bottom. One hundred large grains are selected and lowered into the water to do this. The surfaced grains are removed and replaced with other, good-quality grains. After that, the grains are laid out, covered with a wet cloth and put in a warm place at a temperature of 20-25 °C. After 72 hours, check the germinating of the grain, i.e. the presence of roots and seedlings. After that, the number of germinating grains is calculated as a percentage of the number of 100 grains initially taken for bioactivation.

The grain chosen for bioactivation was cleared of impurities. The water used in bioactivation should be potable and clean of impurities. Washing is carried out at room temperature.

Bioactivation was carried out as follows: washing grain in clean drinking water, washing grain with 5% for disinfection, washing grain a second time, and soaking grain in water for 24 hours at 22-25 °C. After washing, the grains are soaked in water at 20-25 °C. Then soak in water for 2-3 hours. After 4-6 hours, 90-95% of the grains should emerge. The bio-activated grains are then crushed until they pass through a 1.5 mm round hole sieve with a 4.0% residue on the sieve. A germinal root with a length of no more than 5 mm in 90% of the seeds was regarded as the main indicator to be monitored for moist germinated grains.

After the bioactivation process of grains and drafting of compounding of grain mixtures, compounding of bread were made with an increasing food value and the physical and chemical indices of baked foods are certain. The research applied the generally accepted and special methods of estimating raw material properties and quality of baked foods.

Three types of grain mixes were developed to further increase the nutritional value of bread: grain mixes "Kopzhasar", "Khanshaiym", and "Arman". The recipe and the percentage of ingredients to each other are shown in Tables 4-6.

The bread recipe was developed by using grain mixtures. Samples of dry bioactivated grains were stored at a temperature of 17±3 °C to determine the laboratory's acceptable shelf life and relative humidity of 75%. Under such conditions, all samples were kept for 12 months. No significant losses were observed, and no changes in organoleptic parameters were observed. After this period, deterioration in the quality of the grains was observed: the appearance of an unpleasant, musty smell, plaque on the grains, and deterioration in taste. Thus, bioactivated grains are conveniently stored and transported over long distances for no more than 12 months, with all the conditions being met.

Table 4 Recipes of control sample and grain mixture "Kopzhasar" and their nutritional values.

Name	Control	Consumption of raw materials, %					
		Kopzhasar					
Variants' number	0	1	2	3	4	5	6
Rye medium flour mixture and wheat baking grade 1	200	181	167	155	135	115	95
Bioactivated wheat	-	10	15	20	25	30	35
Bioactivated corn	-	3	5	10	15	20	25
Flax seeds	-	1	3	5	10	15	20
Dzhigida	-	3	5	10	15	20	25
Barberry	-	-	-	-	-	-	-
Sea-buckthorn	-	-	-	-	-	-	-
Hawthorn	-	-	-	-	-	-	-
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Table 5 Recipes of grain mixture "Arman" and its indicators of nutritional values.

Name	Consumption of raw materials, %					
	Arman					
Variants' number	7	8	9	10	11	12
Rye medium flour mixture and wheat baking grade 1	175	155	135	115	95	75
Bioactivated wheat	10	15	20	25	30	35
Bioactivated corn	5	10	15	20	25	30
Flax seeds	-	-	-	-	-	-
Dzhigida	-	-	-	-	-	-
Barberry	5	10	15	20	25	30
Sea-buckthorn	-	-	-	-	-	-
Hawthorn	5	10	15	20	25	30
Salt	1.5	1.5	1.5	1.5	1.5	1.5

Table 6 Recipes of grain mixture "Khanshaiym" and its indicators of nutritional values.

Name	Consumption of raw materials, %					
	Khanshaiym					
Variants' number	13	14	15	16	17	18
Rye medium flour mixture and wheat baking grade 1	175	155	135	115	95	75
Bioactivated wheat	10	15	20	25	30	35
Bioactivated corn	-	-	-	-	-	-
Flax seeds	5	10	15	20	25	30
Dzhigida	-	-	-	-	-	-
Barberry	-	-	-	-	-	-
Sea-buckthorn	5	10	15	20	25	30
Hawthorn	5	10	15	20	25	30
Salt	1.5	1.5	1.5	1.5	1.5	1.5

Then, guided by this recipe, we selected the most optimal dosage options for grain mixtures compared with the quality indicators of the control sample of bread. For a full and fair assessment of the quality, organoleptic and physical and chemical indicators of the quality of bread were studied.

After moisture was determined by the physical and chemical indices of the selected dosages of grain mixtures, acidity, protein, fatty acids, fiber, minerals, and vitamins were examined (Table 7).

Table 7 Chemical indices of the grain mixtures per 100 g of product.

Name of the indicators	Name of grain mixtures		
	Kopzhasar	**Arman**	**Khanshaiym**
Mass fraction of moisture, %	14.5 ±0.08	14.0 ±0.1	14.5 ±0.11
Protein, g	42.0 ±0.06	17.6 ±0.12	33.2 ±0.04
Dietary fiber, g	54.3 ±0.3	68.7 ±0.5	47.8 ±0.9
Carbohydrates, g	150.1 ±0.2	134.0 ±0.4	94.6 ±0.1
Fat, g	97.6 ±0.11	99.4 ±0.08	52.5 ±0.15
Vitamin B1, g	2.0 ±0.04	0.8 ±0.07	1.7 ±0.02
Vitamin B2, g	0.3 ±0.02	0.41 ±0.02	1.0 ±0.05
Vitamin B6, g	1.5 ±0.08	1.0 ±0.04	1.2 ±0.08
Iron, mg	15.0 ±0.11	38.7 ±0.12	12.0 ±0.08
Magnesium, mg	256.4 ±0.4	307.2 ±0.3	183.4 ±0.7
Potassium, mg	1566.0 ±0.15	2075.1 ±0.18	1814.0 ±0.11

Note: ± – standard deviation.

Thus, the optimal formulations of grain mixtures were selected, and grain mixtures' physical and chemical parameters were determined. Grain mixtures can be distinguished visually because it is easy to distinguish the colors and structure of the components in the composition.

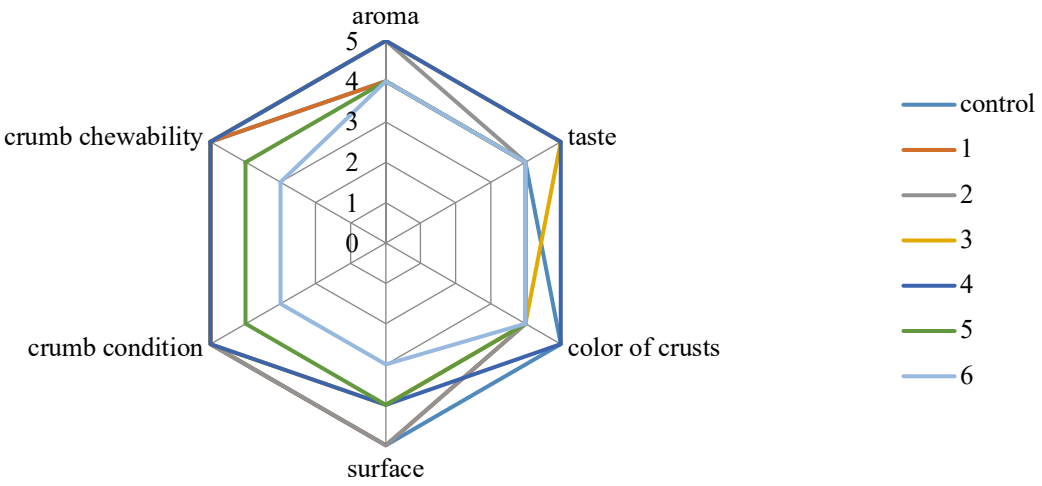
During the study of the quality indicators of prepared bread using promising phyto-enriching agents, namely, grain mixtures, the dough was prepared in a non-paired way. The bread dough was kneaded by hand, following the calculated values of the components according to the recipe (Table 7).

In recipes, all components are mixed until a homogeneous dough consistency is obtained. The dough was fermented in a thermostat for 150-180 minutes at 30-35 °C. After 60 and 120 minutes, the test was wrapped. The final acidity of the test was not more than 3 degrees. The fermented dough was divided into dough pieces weighing 350 g, rounded, and placed in moulds and sheets. The duration of proofing of the dough at a temperature of 37-38 °C and relative humidity of 75-80% was 40-50 minutes. The products were baked in a humidified baking chamber for about 30-35 minutes at 230-240 °C.

The test properties were evaluated by organoleptic and physical and chemical indices that were determined following standard methods for determining the quality of raw materials and products.

Organoleptic parameters were determined on a five-point scale. For this case, the finished bread was cooled for 1 hour at room temperature, and then the organoleptic parameters were evaluated. Figure 1 shows the organoleptic indicators in the five-point rating system. Samples of bread without bioactivated grains and sourdough were taken as a control version of loaves of increased nutritional value.

In some variants, the colour of the bread was rated higher than the previous value, while the proportion of rye-wheat bread decreased. The colour was less bright and greyish in the lower fractions of the flour content. In general, there is a noticeable similarity in the indicators. Colour with a decrease in the amount of flour became more intense and attractive, to a point after which the colour changed to dark, closer to black, inclusions of grain mixtures became more noticeable, and the crumb became denser less attractive to the consumer.



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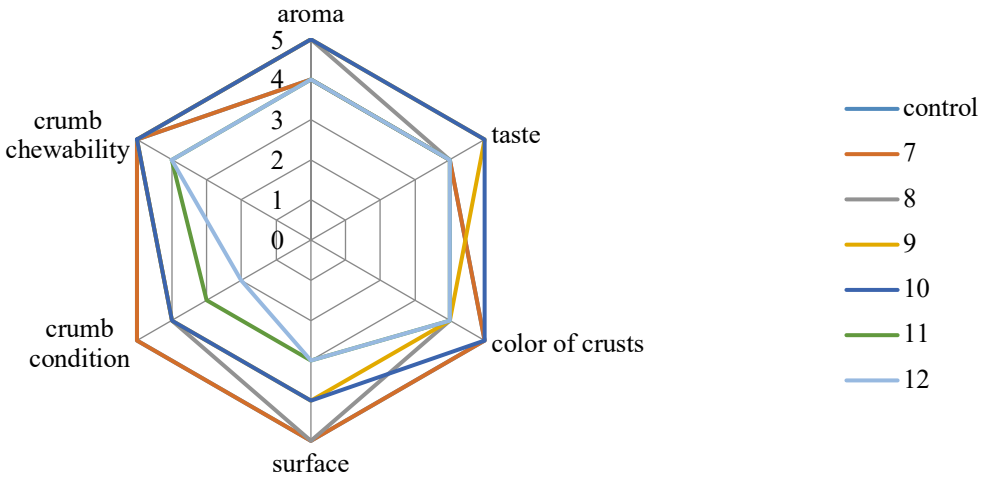

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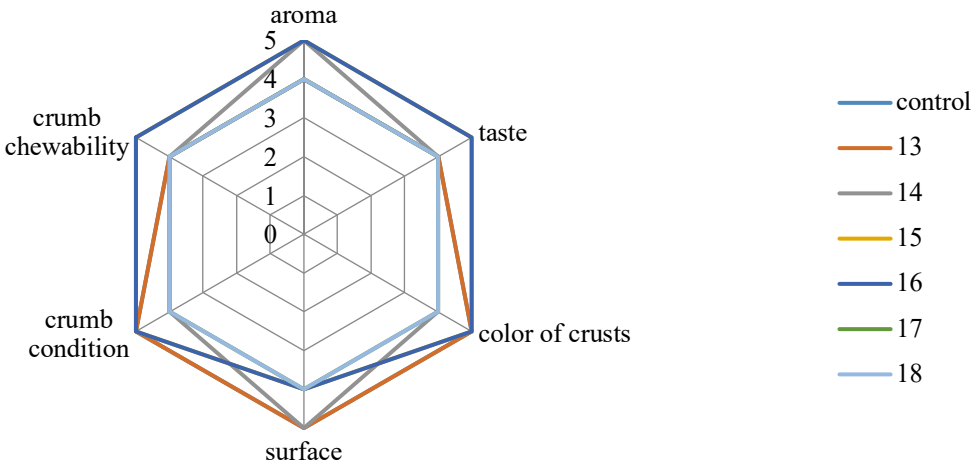

c)

Figure 1 The effect of grain mixture on the organoleptic indicators of bread: a – with a grain mixture "Kopzhasar"; b – with a grain mixture "Arman"; c – with a grain mixture "Hanshaiym".

After the organoleptic estimation of all samples, the results were calculated in the arithmetic mean and clearly expressed in Figure 1. Table 8 shows the characteristics of organoleptic indicators.

The bread samples had the correct shape, the colour of the crust was smooth, dark brown, the crumb was elastic, dark-coloured with grain inclusions, and the taste and aroma were pleasant, characteristic of the appearance and content of the grain mixture. Samples of grain bread can be characterized as products with a good volume, regular shape, and a slightly convex crust. Grain inclusions in all the studied samples make the developed bread attractive from a consumer point of view.

Generally, all studied bread samples had positive organoleptic indicators. According to organoleptic studies, samples of grain bread with 15 and 20% inclusion of grain mixtures have the best results.

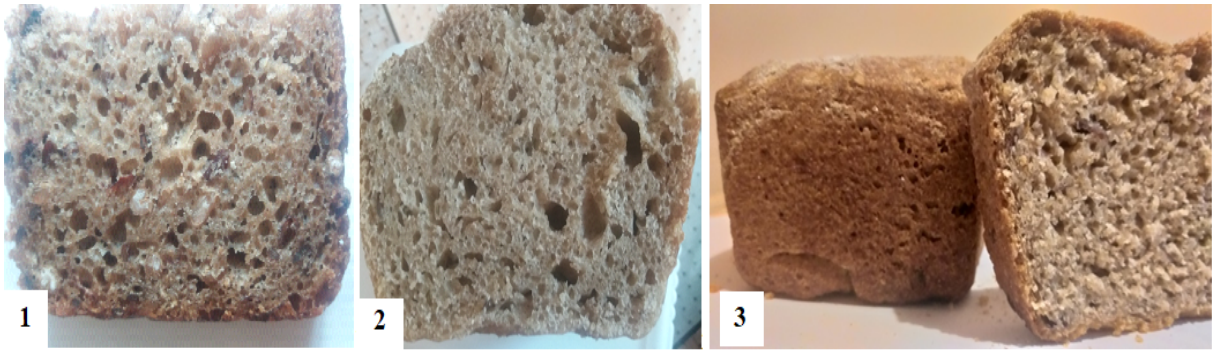

Figure 2 Obtained bread samples: 1 – bread with a grain mixture "Kopzhasar", 2 – bread with a grain mixture "Arman", 3 – bread with a grain mixture " Khanshaiym».

Microbiological parameters were determined, and the results are shown in Table 8.

Table 8 Effect of fermentation on the microflora of bread with grain mixture.

Indicators	Control sample	A grain mixture Kopzhasar	A grain mixture Arman	A grain mixture Hanshaiym
Mesophilic anaerobic and facultative microorganisms, CFU/g	$3.2x10^4$	$3.3x10^4$	$3.0x10^3$	$3.0x10^3$
Mold fungi, CFU/g	28.0	29.6	29.5	30.0
E. coli bacteria, CFU/g	Does not found	Does not found	Does not found	Does not found
Pathogenic microorganisms, salmonella, g	Does not found	Does not found	Does not found	Does not found
S.aureus staphylococci, g/cm	Does not found	Does not found	Does not found	Does not found

Adding starter cultures, rather than using yeast in the preparation of the dough, has a positive effect on the structure of the dough and, consequently, on the prepared bread. Lactic acid bacteria found in sourdough have many advantages, as they can reduce the growth and development of bacteria and mold fungi in bread. This significantly reduced microorganisms' content, which was proved by our microbiological study (Table 8).

According to the requirements, negative microbiological activity can be contained in the grain no more than 50 CFU/g. The microbiological studies also showed no pathogenic microorganisms of the salmonella group, staphylococci, or bacteria of the coli group in bread by adding grain mixtures. The physicochemical parameters of bread were studied (Table 9).

Table 9 Results of physical and chemical parameters in grain loaves per 100 g of bread, %.

Indicators	Control sample	A grain mixture Kopzhasar	A grain mixture Arman	A grain mixture Hanshaiym
protein, g	6.30 ±0.08	10.11±0.02	11.70 ±0.04	11.00 ±0.081
carbohydrates, g	30.0 ±0.04	45.1 ±0.07	43.2 ±0.09	45.0 ±0.05
fat, g	0.20 ±0.01	0.60 ±0.04	0.81 ±0.07	0.74 ±0.03
dietary fiber, g	1.30 ±0.04	2.71 ±0.08	3.10 ±0.02	2.65 ±0.07
Minerals, mg:				
calcium	25.0 ±0.04	34.0 ±0.02	43.6 ±0.08	38.5 ±0.11
magnesium	73.3 ±0.09	83.3 ±0.02	86.7 ±0.04	85.0 ±0.01
phosphorus	210.0 ±0.07	255.0 ±0.08	283.0 ±0.11	231.0 ±0.07
iron	3.25 ±0.02	3.38 ±0.01	3.68 ±0.02	3.38 ±0.09
Vitamins, mg:				
thiamine	0.310 ±0.01	0.49 ±0.08	0.51 ±0.04	0.51 ±0.05
riboflavin	0.11 ±0.02	0.14 ±0.02	0.20 ±0.04	0.14 ±0.07
Antioxidant activity	1.70 ±0.08	3.57 ±0.02	4.00 ±0.08	4.63 ±0.06
Content of polyunsaturated fatty acids	Does not found	33.60 ±0.08	34.8 ±0.01	38.50 ±0.02

Note: ± – standard deviation.

The analysis of the obtained physical and chemical parameters data shows that the obtained bread samples are rich in minerals and vitamins. The amount of chemicals in bread using grain mixture increased by up to 70% compared to the control sample. In comparison with the control sample, the studied samples showed an increase in the content of protein, phosphorus, dietary fiber, and antioxidant activity in grain bread using various starter cultures.

Judging by the taken results, the antioxidant activity increases in bread with bioactivated grains, depending on the recipe of the grain mixture, by 2-2.5 times. The study's results on the content of polyunsaturated fatty acids look to more potential due to the high content. They were not found in the control sample in the grain mixture, while in bread with sprouted grains, their content increases by 33-34 times. This is due to the content of large amounts of flax seeds in grain mixtures.

To sum up, the consumption of 100 g of grain bread with the addition of grain mixtures "Kopzhasar" and "Arman" satisfies the daily need of a healthy person in polyunsaturated fatty acids by 30%, and the other nutrients are satisfied from 1 up to 30%.

The results show that changing the recipe and using bio-activated grains and starters can slow down the process of moisture loss and thus the drying of bread during the storage time studied. These processes occur most intensively in the first 24 hours of storage.

Mathematical models adequate to the set experiment with some accuracy and simplicity make it possible to see a mathematical description of the simulation of the influence of grain mixtures' content on the bread's technological parameters. Start with it is necessary to establish the setting and the choice of the experimental model for determining the influence of the content of grain mixtures and leaven on the technological parameters of the bread.

To denote the process in question, whose mechanism of functioning is complex and unknown, we use the "black box" concept in the framework (Figure 3). The idea of a "black box" is when you need to determine what is required at the entrance to the system and what should be at the exit from it, no matter what is inside the system.

Our process, which is influenced by random influences W, has some "input" to input information about the regulated parameters of grain mixture content and an "output" to control the results characterized by optimization criteria. The state of the outputs Y is presumably functionally dependent on the state of the inputs X:Y = f(X). However, the type of dependence of the results on the inputs is unknown.

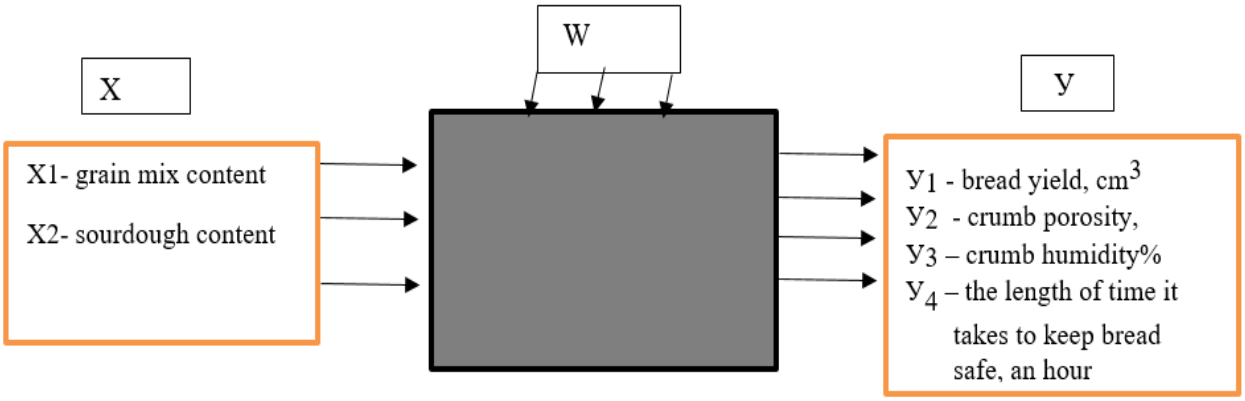

Figure 3 Model for determining the effect of grain mixture and sourdough content on bread quality and safety.

To solve the set tasks, the + planning of multifactorial experiments, statistical processing of the results and search optimization were planned: the optimization parameters and the most important factors that can affect the indicators of bread quality were selected, the research plan was defined, and a mathematical model based on the results was developed. This model was used to study the influence of controllable factors on the output parameters of the process in the stationary area of the factor space.

Table 10 Researched parameters of grain mixtures content's influence on bread's technological parameters and their levels of variation in laboratory conditions.

Adjustable parameters: coded (natural)	Coded levels			Variation interval
	–1	0	+1	
X1 - the content of grain mixtures, %	0.25	0.643	1.0	0.375
X2- the content of rye sourdough, g	318	150.0	339	10.5

The laboratory research on the effect of grain mixture content on bread's quality and safety parameters was carried out according to the scheme of two-factor planning of experiments.

If the type of dependence of the response on the studied parameters is unknown, the regression equation is shown as a polynomial of the second degree. The central point, together with other points of the plan, allows to estimate coefficients of the full quadratic regression model from k =2 coded variables $x1$, $x2$ in the area of factor space by this formula:

$$Y = b_0 + \sum b_i x_i + \sum b_{ij} x_i x_j , \quad (1)$$

The quadratic regression equation (1) has linear (main) effects $x1$,…, xk. The second-order terms $x\ i\ x\ j$ at ixj account for interaction effects, i.e. effects of joint action xi and xj on the value Y, and terms $x\ i\ x\ j$ at $i=j$ (i.e. $xi2$ - squared arguments) – non-linearity of response function Y at change of *i-th* argument. In this case, the effect of the *i-th* factor on the studied indicator of the impact of grain mixture content on technological indicators of bread is estimated by regression equation coefficients (1).

To begin with, it is necessary to calculate the statistical characteristics of the main indicators of the impact of the content of grain mixtures on technological indicators of quality and safety of bread and then analyze the obtained set of experimental data (Table 11).

According to the data of the experiment in Table 11, for each indicator, the following parameters are estimated: the mean *(M)* and the error *(m)*, the median (*med*) and mode (*mod*), the standard deviation s and the variance $s2$, the smallest (*min* – minimum) and the largest (*max* – maximum) values, the spread R, the skewness A and the excess E and the variation coefficient V.

Table 11 Statistical characteristics of indicators of the influence of grain mixtures on the quality and safety of bread.

Statistical characteristics	Unit designation	Parameter		Y1	Y2	Y3	Y4
		X1	X2				
Scope of observations	*N*	4	4	4	4	4	4
Arithmetic mean reading	*M*	0.643	327.100	2330.000	68.250	46.375	45
Standard error	*m*	0.247	6.024	60.553	3.326	1.068	10.247
Standard error, % of arithmetic mean	*m, %*	3.641	1.845	2.607	4.873	2.303	25.237
Median	*med*	0.643	321.131	2380.000	70.200	46.250	42
Mode	*mod*	1.000	337.000	#N/D	#N/D	#N/D	#N/D
Standard deviation	*s*	0.433	12.455	121.106	6.463	2.136	20.494
Sampling variance	*s2*	0.188	147.000	1434.667	44.250	4.563	420
Excess	*E*	-6.000	-6.000	3.642	-1.700	-0.543	0.343
Skewness	*A*	0.000	0.000	-1.87 877724461	-0.482	0.292	0.753
Spread	*R*	0.750	21.000	260.000	15.763	5.000	48
Minimum	*min*	0.250	318.000	2150.000	60.000	44.000	24
Maximum	*max*	1.000	339.000	2410.000	75.000	49.000	72
Coefficient of variation, %	*V*	69.28	3.45	5.20	9.75	4.61	45.765

The statistical characteristics of Table 12 provide a quantitative view of the empirical data (the position of the mean, its dispersion – scatter, skewness) and, as a first approximation, test the assumptions underlying the regression analysis. The resulting measures' standard errors are small and less than 4% of the corresponding mean values. Approximate equality of the mean and median is observed.

There is no mode for Y1, Y2, Y3 and Y4, while the kurtosis and skewness values are negative; the minimum and maximum values are approximately equidistant from the mean, and the coefficients of variation are less than 9 % for the resultant indicators. This indicates the closeness of empirical and normal or generalized-normal distributions.

After that, we began to study the influence of a set of production factors on each of the indicators. That is, a system of regression equations is determined that reflects this value:

$$F_i\left(\bar{y}\right) = f\left(x_{m+1}, x_{m+2}, ..., x_n\right) \qquad \left(i = 1, 2, ..., m\right), \tag{2}$$

Consequently, there are m indicators that act as optimality criteria, that is, y1, y2, ..., ym and n factors affecting these indicators - xm + 1, xm + 2, ..., xn (in our case, m = 6, n = 7). The form of communication between effective indicators and factors is assumed to be non-linear.

stepwise regression methods chose the optimal set of components of the model (2). The most common and effective methods are Forward, Backward and Stepwise.

The approximation of the optimality criteria Yi by a polynomial provides a good indication of the shape of the response surface.

As a result of the implementation of computational procedures implemented in the computer program Excel, Statistica 12, calculated four b-coefficients of nonlinear regression for the coded variables x1, x2, their standard errors, Student's t-tests to test the significance regression components, p probability levels, upper and lower 95 % confidence limits.

Table 12 Results of regression analysis of regulated parameters of grain mixture content on technological indicators of bread: coded variables.

Factor	Regression coefficient	Standard error	Student's t-test	*p*-value of significance	95% confidence limits	
					bottom	top
Y1 – bread yield, cm^3						
–	2330	60	38.35465	0.01639	1567.628	3092.372
*x*1	-50	60	-0.83333	0.557716	-812.372	712.3723
*x*2	70	60	1.166667	0.451125	-692.372	832.3723
*x*1*x*2	60	60	1.178667	0.735047	-0.099	0.0713
Y2 – crumb porosity, %						
–	68.25	2.25	30.33333	0.02098	39.66104	96.83896
*x*1	-0.75	2.25	-0.33333	0.795167	-29.365	27.83896
*x*2	5.25	2.25	2.333333	0.354650	-23.339	33.42655
*x*1*x*2	2.25	2.25	1.96234	0.066305	-0.0020	0.0556
Y3 – moisture content of crumb, %						
–	46.375	0.875	53	0,01201	35.25707	57.49293
*x*1	0.125	0.875	0.513221	0.909666	-10.9929	11.24293
*x*2	1.625	0.875	1.857143	0.314453	-9.49293	12.74293
*x*1*x*2	0.875	0.875	1.96234	0.3213321	-0.0020	0.0556

Thus, the following regression equation can be written in coded and natural values of factor X (Table 13).

Table 13 Equation of regression in coded and natural values of factor X.

The equation in coded values	The equation in natural values
Y1 - bread yield, cm^3 (3)	
$Y1 = 2330 - 50\ X1 + 70\ X2 + 60\ X1X2$	$Y1=3351.9-5139.05*X1-2.85714*X2+15.2381*X1*X2$
Y2 - crumb porosity, % (8)	
$Y2 = 68.25 - 0.75\ X1 + 5.25\ X2 + 2.25\ X1*X2$	$Y2=22.5714-189.714*X1+0.14286*X2+0.57143*X1*X2$
Y3- crumb moisture content, % (4)	
$Y3 = 46.375 + 0.125\ X1 + 1.625\ X2 + 0.875\ X1*X2$	$Y3=40.95238-72.6667*X1+0.015873*X2+0.222222*X1*X2$
Y4 - the length of time it takes to keep bread safe, an hour (5)	
$Y4 = 45.3\ X1 - 15\ X2 - 9\ X1*X2$	$Y4=40+758.8571*X1+0*X2-2.28571*X1*X2$

Checking the correctness of the calculations. If we substitute the natural values of factors X1 and X2 in equation (3-5), the value of Yi at each level will be the same as the corresponding coded values of factors X1 and X2 in equation (3-5) (Table 13).

The analysis of the obtained values of the Student's t-test and appropriate levels of significance *p* confirms a significant impact on the resulting indicators of the content of grain mixtures on technological indicators of bread: x_1 – grain mixture content, %; x_2 – water content, g. Thus, the linear components x_1 and x_2 with $p < 0.06$ proved significant. x_1 – grain mixture content, %; x_2 – negative impact on the resulting criteria Y_1 and Y_2.

Thus, based on the data received in experiments, by the method of least squares, the regression equations (3-5), depending on two investigated parameters x_1, and x_2 presented in the standardized scale, were calculated.

Table 14 Values of input factors in natural and coded form.

Number of an experiment	Values of X1 at X2 equal to				Yi	Relative change, %
	in kind values		in coded values			
Y1 – bread yield, cm^3						
1	1	339	1	1	2413	0
2	0.25	318	-1	-1	2370	0
3	1	318	1	-1	2532	0
4	0.25	339	-1	1	2734	0
Y2 – crumb porosity, %						
1	1	339	1	1	74	0
2	0.25	318	-1	-1	67	0
3	1	318	1	-1	62	0
4	0.25	339	-1	1	73	0
Y3 – humidity of crumb, %						
1	1	339	1	1	75	0
2	0.25	318	-1	-1	63	0
3	1	318	1	-1	62	0
4	0.25	339	-1	1	77	0
Y4 – the length of time it takes to keep bread safe, an hour						
1	1	339	1	1	23	0
2	0.25	318	-1	-1	41	0
3	1	318	1	-1	78	0
4	0.25	339	-1	1	33	0

To check the quality of the obtained regression equations (3-5) we calculated the multiple correlation coefficient *R*, the coefficient of determination *R2*, the Fisher Test F and the Durbin-Watson test *d* (Table 14). The values of statistical criteria given in Table 14 indicate that the obtained regression equations with 95% confidence probability reliably and adequately describe the impact of the studied parameters of grain mixtures content on technological indicators of bread.

Sufficiently high values of the multiple correlation coefficient (R = 0.8202 - 0.9206) indicate a very close relationship between the resulting indicators y_1, y_2, and y_3. The included in the study regulated parameters of grain mixtures content on technological indicators of bread. The coefficient of determination (R^2 = 0.643 - 0.8475) describes 64.3% and 84.6% variation of the corresponding response in the experimental data.

Fisher criterion F values calculated significance levels p <0.3905 indicate the obtained equations' sufficiently good approximating ability.

The serial correlation coefficients are weak and insignificant for the regression residuals of the equations. As evidenced by the values of the Durbin-Watson criterion *d*, we can assume that there is no serial correlation.

Thus, reliable and adequate regression equations of controlled parameters, fully characterizing the content of grain mixtures on safe storage time and technological parameters of bread, were obtained. Further, we present the values of estimated parameters (regression coefficients) – b_0, b_1, b_2, b_{12} obtained in Statistica 12.

The resulting loss is 0.000000000. The regression coefficient is 1. Explained variance (adequacy variance) is 100%, i.e. the hypothesis of equation adequacy to experimental data is accepted.

Then the analysis of response surfaces for indicators of grain mixture content on technological indicators of bread was carried out. First, a complete factor experiment 2^2 was conducted to study the dependence. For this purpose, planning matrices of the complete factor experiment were made (Tables 16-19).

Table 15 Statistical indicators of quality and characteristics of the adequacy of regression models.

Statistical quality indicators and adequacy criteria	Response		
	Y1	**Y2**	**Y3**
Multiple correlation *R 7*	0.8202	0.945310	0.854240
Determination coefficient *R2*	0.676540	0.847458	0.776256
Normalized *R-square*	0.018182	0.542373	0.345642
Standard deviation	120	4.5	1.75
Number of degrees of freedom *df*: k_1; k_2	2	2	2
Fisher criterion *F*	1.027778	2.777778	1.71234
Significance *F*	0.572078	0.390567	0.473016
Durbin-Watson criterion *d*	2.253	2.378	2.274
Coefficient of serial correlation *r*	-0.133	-0.231	-0.124

Note: k_1 and k_2 are the number of degrees of freedom for numerator and denominator, respectively.

Table 16 Planning matrix of the full factor experiment for two factors for Y1.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	z_1	z_2	X0	X1	X2	Y1
1	1	343	0	1	1	2452
2	0.25	342	0	-1	-1	237425
3	1	342	0	1	-1	2113
4	0.25	379	0	-1	1	23736

Table 17 Planning matrix of the full factor experiment for two factors for Y2.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	z_1	z_2	X0	X1	X2	Y2
1	1	3321	0	1	1	76
2	0.25	3524	0	-1	-1	65
3	1	3432	0	1	-1	61
4	0.25	343	0	-1	1	70

Table 18 Planning matrix of the full factor experiment for two factors for Y3.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	z_1	z_2	X0	X1	X2	Y3
1	1	342	0	1	1	51
2	0.25	347	0	-1	-1	47.5
3	1	315	0	1	-1	42
4	0.25	346	0	-1	1	48

Table 19 Planning matrix of the full factor experiment for two factors for Y4.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	z_1	z_2	X0	X1	X2	Y4
1	1	342	0	1	1	25
2	0.25	340	0	-1	-1	42
3	1	342	0	1	-1	73
4	0.25	347	0	-1	1	37

Substitute different values of *y* into the obtained expression and make the corresponding Table 20.

Table 20 Equal level lines for Y1.

	2410	2310	2210	2110	2010
-1	3	-7	-17	-27	-37
-0.75	1.7	-2.3	-6.3	-10.3	-14.3
-0.5	1.355	-1.125	-3.625	-6.125	-8.625
-0.25	1.227243	-0.59054	-2.40922	-4.22743	-6.04543
0	1.142853	-0.28543	-1.71445	-3.14243	-4.57143
0.25	1.0882786	-0.08843	-1.26443	-2.44143	-3.61742
0.5	1.05	0.05	-0.95	-1.95	-2.95
0.75	1.021739	0.152145	-0.71535	-1.58673	-2.45667
1	1	0.230743	-0.53843	-1.30769	-2.07642

The color marks show the value of the corresponding indicator by means of intensity. According to them, it is possible to determine the range of values of the variables, where the indicator of the quality of the bread is of the most significant importance.

To get a clearer idea of how the quality indicators of bread are related to the dosage of the grain mixture, volumetric graphs are built. The same levels of the displayed values of the quality indicators of bread are highlighted in the volumetric drawings using a "wire" mesh, different shading, and shading. The levels have the same values where these surface chart elements are the same.

The constructed surface diagram allows you to find the best combination of mixture components, which is difficult to identify in any other way from the available values.

Response functions are best represented graphically. Figures 4-6 show response surfaces and lines of equal levels (isolines) of grain mixture content indicators on technological indicators of bread quality and safety depending on different combinations of the studied parameters: X1 – grain mixture content, %, X2 – sourdough content, g.

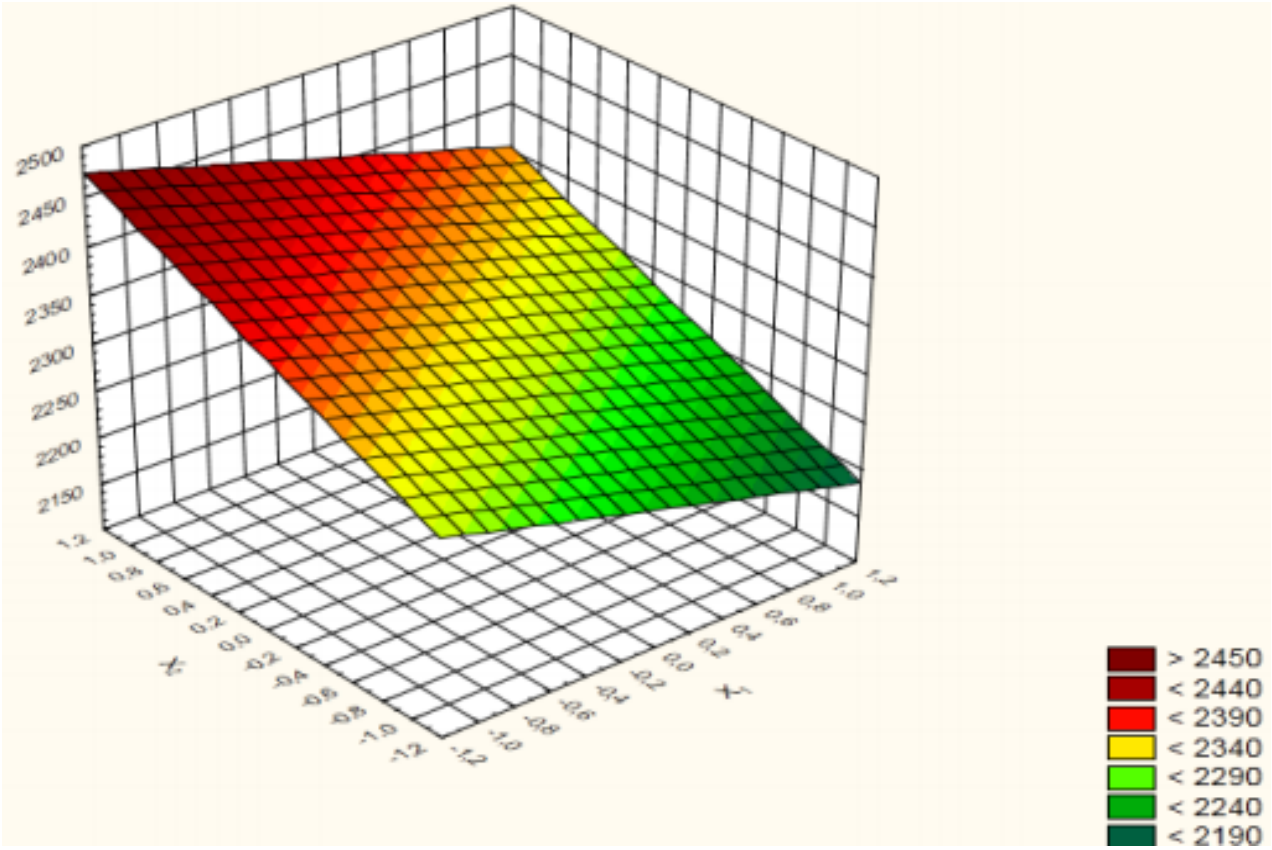

Figure 4 Response surface of grain mixture and starter content on technological indicators of bread for y_1.

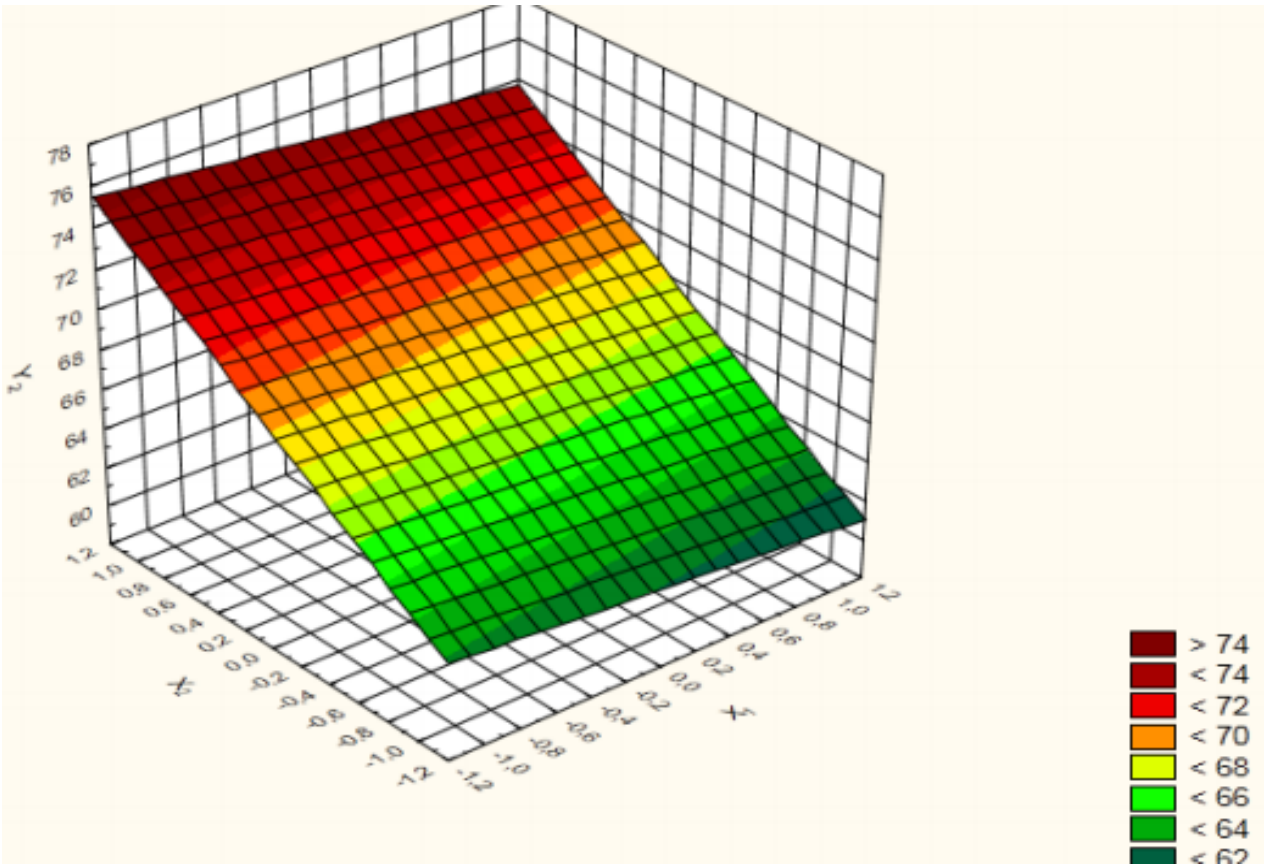

Figure 5 Response surface of grain mixture and starter content on technological indicators of bread for y_2.

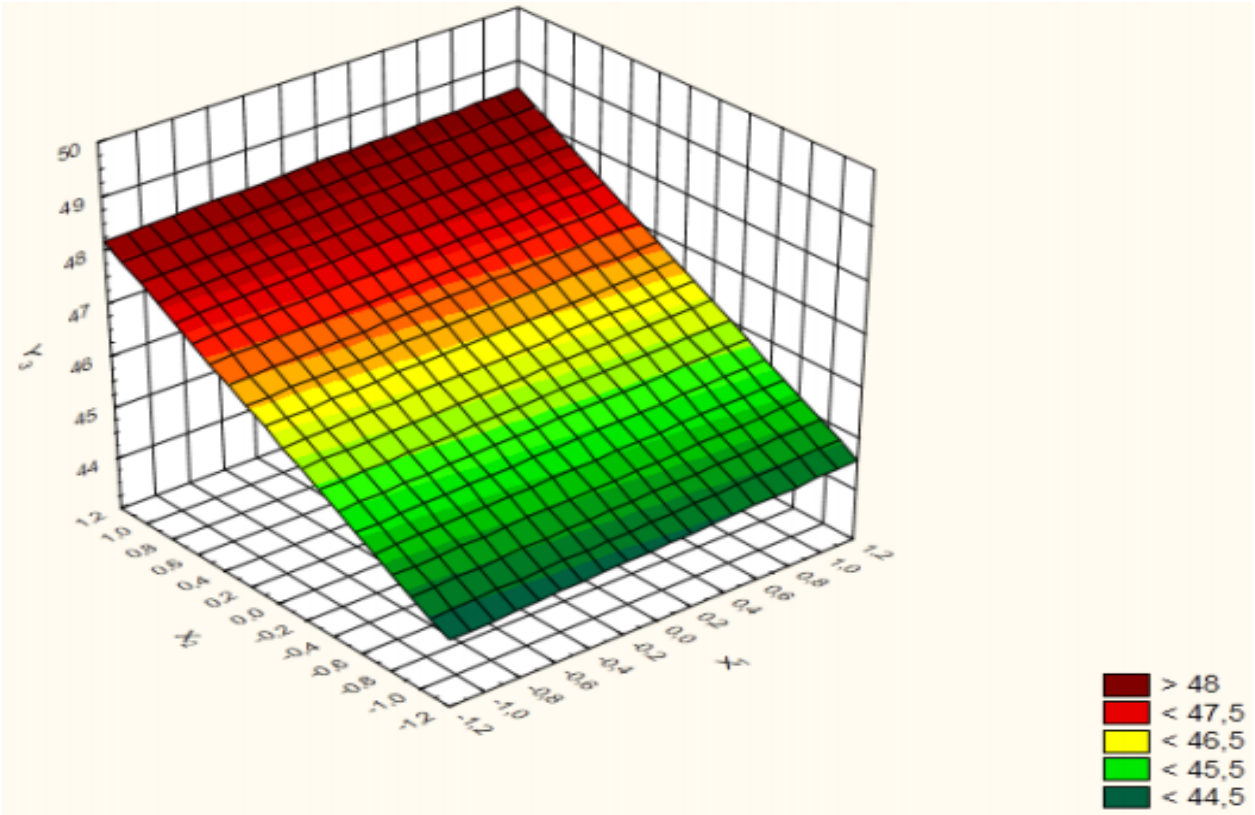

Figure 6 Response surface of grain mixture and starter content on technological indicators of bread for y_3.

After that, the changes occurring in bread samples during storage were investigated. Changes in the bread of increased nutritional value with grain mixtures compared with the control sample were investigated. The researched bread samples were stored at 20 ±5 ºC and 70 ±5% relative humidity. Assessment of bread quality was carried out 6, 24, and 48 hours after baking. The dynamics of changes are presented in Figure 7. Also, organoleptic analysis of the degree of freshness using an eight-point evaluation scale was carried out.

According to the data obtained, shown in Figure 7, the most significant difference was noticed at the end of the study period, when the control sample received 0 points (the control sample began to go moldy and lost its consumer appeal), and the bread using grain mixtures in the same study period of 72 hours showed 4 points, and no signs of mold and spoilage were noticed, only the consumer turgor was lost, the appearance of staling processes, etc. After baking, all the samples received the highest score, and a few hours after the end of the shelf life, all the samples showed such a big difference in scores.

A long-time of samples freshness explains due to the feature of the formulations of different samples of bread, especially in the presence of starter cultures and the presence of bioactivation grains due to the high moisture content, lactic acid bacteria, the activity of enzymes, beans, etc.

DISCUSSION

The quality indicators of bread crumbs using grain mixtures significantly differed from the control sample in terms of specific volume and porosity structure. According to organoleptic parameters, the experimental samples of grain bread, compared to the control sample, were more attractive in appearance, porosity, crumb color, taste, and aroma. It was found that the introduction of a grain mixture in the amount of 5-25% of the total mass of flour has a positive effect on the quality of bread. At the same time, the physical and chemical indicators of the quality of bread decrease with an increase in the organoleptic characteristics of the finished products. It was found that the best physical and chemical parameters were obtained from bread samples prepared with the addition of 20% of the grain mixture. In general, all types of experimental analyses performed showed an improvement in a number of indicators compared to the control sample. The nutritional value of the resulting products was increased from 0.5 to 3 times. The content of polyunsaturated fatty acids, vitamins, and minerals increased. Mathematical

modelling analyses proved the positive effect of the addition of grain mixtures on the structure and technological parameters of the bread.

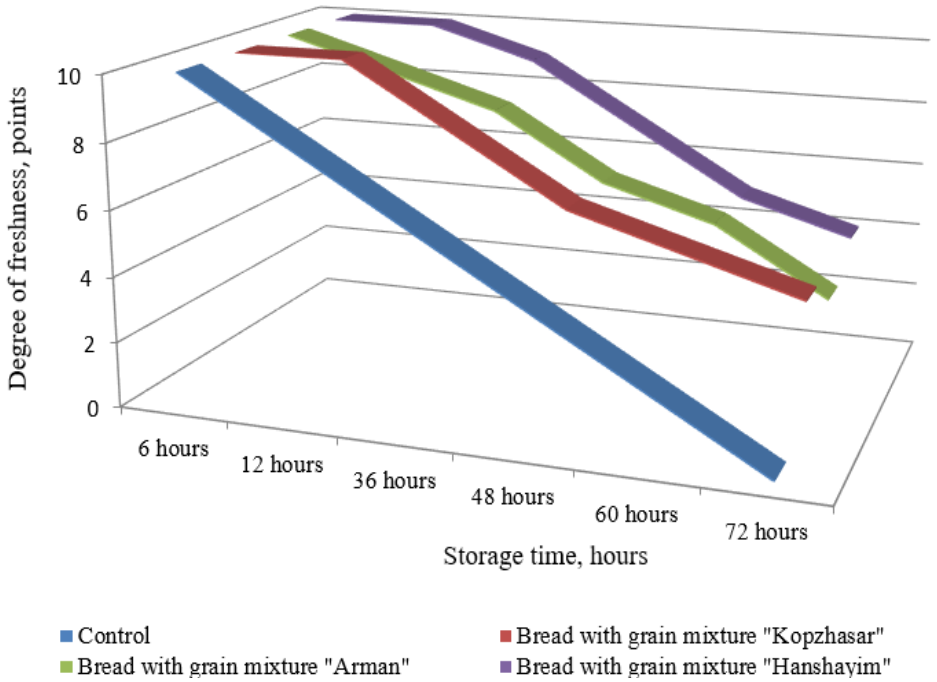

Figure 7 Changes in the degree of freshness of the tested bread samples.

CONCLUSION

The additive of grain mixtures and rye sourdough impact bread quality has been researched. The surface response of the content of grain mixtures and sourdough on the bread's technological parameters was obtained as bread's yield, crumb porosity, crumb moisture, and the duration of safe storage of the bread. All researched indicators showed an excellent result demonstrating the prospects and effectiveness of adding grain mixtures to the quality of the bread. Consequently, it has been experimentally proved that the introduction of grain mixtures into the bread recipe significantly increases the nutritional value of bread. Generally, the technology of bread using grain mixes and starter cultures is relevant for the modern world. It offers great opportunities to expand the range and ensure the microbiological safety of grain bakery products, especially with the addition of bioactivated grains.

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This article does not contain any studies that would require an ethical statement.
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Factorial analysis of taste quality and technological properties of cherry fruits depending on weather factors

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ABSTRACT

The results of researching the fund formation of dry soluble substances, sugars, and titrated acids in cherry fruits of 10 studied varieties under the Southern Steppe Subzone of Ukraine are given. According to the content of biochemical quality indicators, the following varieties were selected: Modnytsya (the content of dry soluble substances is 17.1%), Ozhidaniye (the content of sugars is 11.7%), and Solidarnost (the content of titrated acids is 1.79%). The cherry fruits units. By conducting a two-factor dispersion analysis, the feasibility of forecasting the content of the principal components of the chemical composition (dry soluble substances, sugars, titrated acids) in the cherry fruits was determined by average values and a factor that maximally impacts the accumulation of the studied indicators was identified during the studies. The dominant influence of weather conditions during research years was determined. Therefore, the taste qualities of the cherry fruits were proposed to forecast by average varietal value. The average and strong correlation dependences of influencing 19 weather factors on the content of the studied biochemical indicators in the cherry fruits were determined. The accumulation dependence models of dry soluble substances, sugars, and titrated acids were built based on the principal component and least-squares methods. The first-rank weather indicators with the maximum influence particles were identified for the studied biochemical quality indicators. The average monthly air temperature in June maximally impacted the fund accumulation of dry soluble substances in the cherry fruits (delta = 9.9%), the content of sugars - the average monthly precipitation in June (delta = 8.5%), on the content of titrated acids - the total number of precipitation days in June (delta = 18.62%). At the end of the flowering phase before fruit ripening and in the last month of fruit formation, humidity indicators had the greatest influence on the accumulation of the studied biochemical indicators in the cherry fruits (June).of Melitopol purpura and Modnytsya have maximum indicators of the sugar-acid index in the range of 8.9-9.3 relative

Keywords: cherry, biochemical quality indicators, factor analysis, components method, weather factor

INTRODUCTION

Cherry is a widespread crop, the fruits of which are valued for their pleasant taste and dietary properties in fresh and processed forms. Tartan cherry is native to Europe and popular in the USA **[1]**. The annual world production of stone fruit crops, particularly cherries, is 950 thousand tons on average. Ukraine is one of the countries producing stone fruits, namely cherry and wild cherry **[2]**, **[3]**. As of 2017, Ukraine ranks third place in terms of production volume, which is 172 thousand tons **[4]**. Compared to the wild cherry, the cherry has a characteristic astringent taste due to the higher acid/sugar ratio **[5]**, is rich in polyphenolic compounds such as flavonoids **[6]**, and has much more hydroxycinnamic acids, procyanidins, flavonol glycosides and flavonols **[7]**, **[8]**. The crop's popularity and high taste qualities are due to the high content of dry substances. Dry substances of the fruits are divided into insoluble and soluble in water. Insoluble substances are mainly those that build cell walls and mechanical tissue elements. The content of dry insoluble substances in some fruit products is small, on average 2-5%. The content of dry soluble substances in the cherry fruits is 14.4-21.6% on average. These include carbohydrates, nitrogenous substances, acids, tannins, other phenolic substances, soluble forms of pectins and vitamins, enzymes, mineral salts, etc. This compound group is represented by carbohydrates, mainly sugars **[9]**, **[10]**.

Scientists have determined that sugars in cherry fruits are 6.5-21.5%. They are represented by glucose, fructose, and sucrose. The quantitative ratio is dominated by glucose and fructose. Fructose is considered to be a particularly valuable and easily digestible sugar. It is 2 times sweeter than sucrose and 3 times sweeter than glucose. The richer its fruits, the sweeter they are **[11]**.

Organic acids are other important components of the chemical composition, which significantly impact the cherry fruits' quality, taste, and technological properties. Organic acids in the cherry are about 0.7-3%. The main acids are apple and lemon and a small amount of amber, salicylic, and ant. But it should be noted that the sour taste of the fruits is not due to the total supply of the acids but the titrated acidity, that is, the content of free acids **[12]**. The fruit quality is an important economic characteristic of the crop. The main purpose of its fruits is technological processing, but fresh cherry fruits are also used as an anti-inflammatory agent. Their use prevents colon cancer, stomach ulcers, and bronchitis. The decisive criteria for choosing the pomological variety of the cherry when consumers purchase are the appearance and taste of the fruits **[13]**.

Such principal components of the chemical composition, like sugars and organic acids, are involved in forming the taste qualities of the crop. The importance of crop varieties with high taste qualities has recently increased for fresh consumption and processing **[14]**, **[15]**. It was established that the content of dry soluble substances is in the inverse average correlation dependence on the amount of precipitation for the cherry fruit of the Lotovka (-0.76) and Shpanka (-0.83) varieties **[16]**. Climatic growing conditions have a decisive impact on forming the taste qualities of the fruit crops. Therefore, when global climate changes are observed in today's conditions, the research of the formation mechanisms of the taste qualities and technological properties of the cherry fruits of the updated variety range, influenced by various weather indicators with the selection of the best varieties for further storage and processing, is relevant **[17]**. Between the hydrothermal coefficient and the content of dry soluble substances in both varieties, a significant inverse correlation was established with the correlation coefficients -0.96 and -0.91. The regression equation is derived, whereby using the hydrothermal coefficients can forecast the content of dry soluble substances in the fruits **[16]**. Other researchers note that the temperature increase and the humidity decrease lead to increased content of dry soluble substances, including sugars. The content of chemical components varies depending on the variety and ripeness stage **[18]**. The ripeness stage of the cherry fruits is determined by the optimal content of sugars, acids, and anthocyanins **[19]**, **[20]**. The dependence of the content of chemical composition components in the fruits on the pomological variety of the cherry and climatic growing conditions is known **[21]**. Indicators of the chemical composition of the fruits vary by research years, but the average value characterizes the biological characteristics of the varieties and the possibility of their use.

As the crop advances from north to south, the sugar content in the cherry fruits of the same varieties usually increases **[22]**. The fruit crops accumulate less dry substances, including sugars, with the maximum precipitation amount. Dry years are characterized by a low total supply of nutrients **[23]**. The cherry fruits contain dry substance not less than 14-15%, sugars not less than 9-10%, and total acidity, not more than 1-1.2%, and are used to prepare canned fruits.

Therefore, the content level of dry soluble substances, sugars, and titrated acids in the stonecrop fruits, in particular cherries and wild cherries, as well as their accumulation and further storage, depend on many factors **[24]**. The degree of stressful weather factors influences the formation of the taste and technological parameters in the cherry.

To further improve the cherry fruits' transportation, storage, and processing technology, it may be possible to forecast the content of dry soluble substances, sugars, and titrated acids in the fruits, depending on the various influences of certain weather factors. Therefore, the research aim was to develop a mathematical model for improving the forecast of dry soluble substances, sugars, and titrated acids in the cherry fruits depending on the weather factors of regions with hydrothermal parameters similar to the Southern Steppe Zone of Ukraine.

Scientific Hypothesis

By conducting a two-factor dispersion analysis, the feasibility of forecasting the content of the principal components of the chemical composition in the cherry fruits will be determined by average values, and a factor that maximally impacts the accumulation of the studied indicators will be identified during the studies. It is foreseen to select the cherry varieties with the best quality indicators for transportation, storage, and processing of the fruits while preserving their biological value.

MATERIAL AND METHODOLOGY

Samples

The research was conducted during 13 consecutive vegetation periods from 2008 to 2019. During the experiment, we used the meteorological data provided by the meteorological station at Melitopol, Ukraine. Cherry plantations, where the research was conducted, are located in the Southern Steppe Subzone of Ukraine, 46°50′25″ north latitude, 35°21′32″ east longitude.

The following indicators characterize Chernozem's southern loamy soil of the experimental areas: humus content is 3.1%, and soil reaction is 8.1 ±0.2.

The region's climate is Atlantic-continental, with high temperatures and insufficient moisture. Climatic conditions of the zone where the research was conducted are given in Table 1.

Table 1 Climatic conditions of the Southern Steppe Subzone of Ukraine.

No.	Climate	Parameters
1	Annual average air temperature	9.1-9.9 °C
2	Average monthly air temperatures in the warmest months	20.5-23.1 °C
3	Sum of active temperatures above 10 °C from April to October	3316 °C
4	Annual average precipitation	475 mm
5	Annual average relative humidity	73%
6	Annual average wind speed	3.7 m/s
7	Hydrothermal coefficient	0.22-0.77

In general, the research region is favorable for cherry growing according to meteorological indicators. However, it should be noted that the genetic properties of cherry fruits can vary in a wide range due to the influence of stressful weather factors. Therefore, the influence of weather factors on the biochemical indicators of the cherry fruits makes it possible to choose the cherry varieties with the optimum content of dry soluble substances, sugars, and titrated acids. This will make it possible to establish the production of high-quality fruit products.

The cherries were grown according to the generally accepted technology for the zone. The trees were planted from 1999-2001 according to the 6×4 m scheme. Spaces between rows were under black steam. The gardens were not irrigated.

Chemicals

Iron-blue (ferricyanide) potassium – K_3 [Fe $(CN)_6$] (red blood salt), 0.1 n solution of alkali (NaOH), methylene blue, phenolphthalein (producer by “Merck” (Germany).

Animals and Biological Material

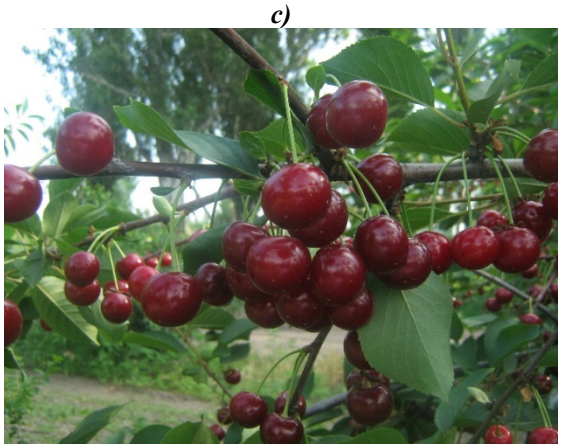
Ten cherry varieties of three terms of ripening were chosen: early – Ozhidaniye (Figure 1a); medium – Vstrecha (Figure 1b), Shalunya (Figure 1c), Seyanets Turovtseva (Figure 1d), Griot Melitopol (Figure 1e), Modnytsya (Figure 1f), Ekspromt (Figure 1g); late – Melitopol purpura (Figure 1h), Solidarnost (Figure 1i), Igrushka (Figure 1j).

a)

b)

c)

d)



e)

f)

g)

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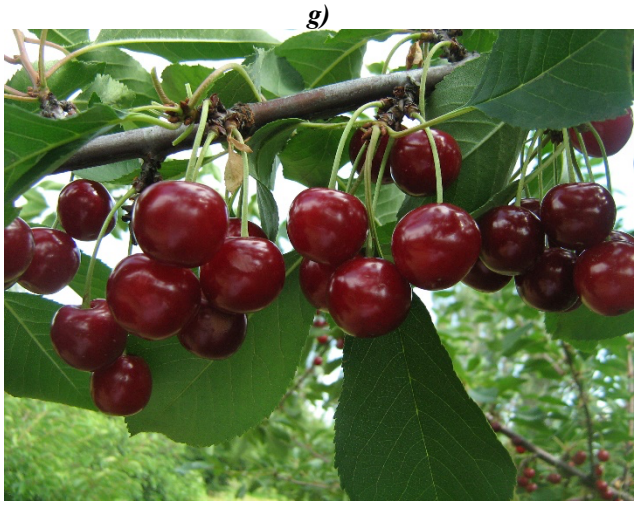
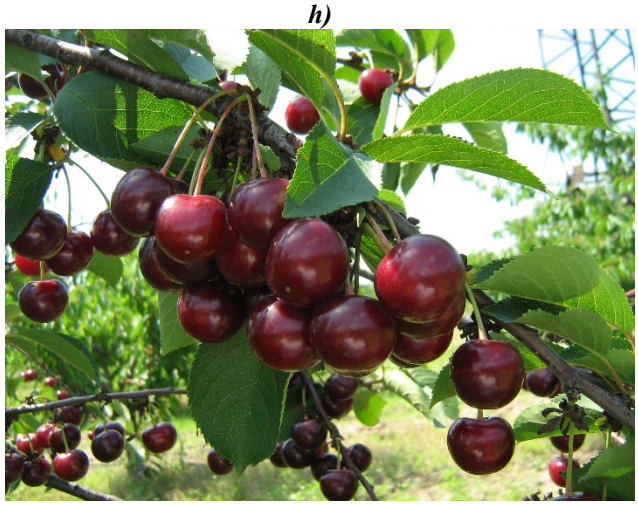


i)



j)

Figure 1 Photos of individual samples of sour cherries: a – Ozhidaniye; b – Vstrecha; c – Shalunya, d – Seyanets Turovtseva; e – Griot Melitopol; f - Modnytsya; g – Ekspromt; h – Melitopol purpurna; i – Solidarnost; j – Igrushka.

Instruments

Refractometer (IRF-454 B2M, manufacturer, open joint-stock company "KOMZ", Kazan).
Laboratory thermometer (TLS-200, manufacturer LLC "Inter-Synthesis", Ukraine).
Photo colorimeter (KFK-3, manufacturer LLC "Inter-Synthesis", Ukraine).
Flame spectrophotometer (Saturn-4, manufacturer "Inter-Synthesis" Limited Liability Company, Ukraine

Laboratory Methods

The content of dry soluble substances (SSC) was determined by the refractometer method **[25]**, the mass concentration of sugars was determined by the ferricyanide method **[25]**, and the mass concentration of titrated acids (TA) was determined by the titrimetric method.

Description of the Experiment

Sample preparation: Trees typical of a certain pomological variety, of the same age, with a medium intensity of fruiting were selected for conducting the research. The cherries of each pomological variety were harvested when the flesh was still dense enough, but the taste and colour were characteristic of this pomological variety. The calendar date of harvesting was determined by the following quality indicators of the fresh fruits: appearance, fruit size by the largest transverse diameter. The selected fruits corresponded to the indicators of the first commodity grade, in particular: shape and colour – typical of the pomological variety, fruits with a stalk, without mechanical damage to skin and flesh, without damage by pests and fungal diseases. The fruits were harvested from trees in 4 different places of the crown.

Number of samples analysed: To determine the content of dry soluble substances, sugars and titrated acids, a sample was taken for each pomological variety of 100 fruits from 6 trees that entered the full fruiting.

Number of repeated analyses: All measurements of an instrument and readings were performed 3 times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was also 3 times.

Design of the experiment: The number of repetitions of each experiment to determine one value was also 3 times. The content of dry soluble substances (SSC) was determined by the AOAC official method 920.151 **[26]**. The mass concentration of sugars was determined by the ferricyanide method **[25]**, the essence of which is the property of reducing monosaccharides to restore iron-blue (ferricyanide) potassium – K_3 $[Fe(CN)_6]$ (red blood salt) in iron-blue (ferrocyanide) potassium – K_4 $[Fe(CN)_6]$ (yellow blood salt) in an alkaline environment. Methylene blue was used as an indicator. When potassium ferricyanide was restored, the colour changed from blue to colourless or light yellow. The sucrose amount was determined by previously turning it into inverted sugar. The titrimetric method determined the mass concentration of titrated acids (TA). The essence is to neutralize organic acids in the experimental product with 0.1 n alkali solution. Titration is carried out before the transition of the solution from an acidic medium to an alkaline one. The moment of transition of the medium to an alkaline one is visually fixed by appearing the pink colour of the solution in the presence of a phenolphthalein indicator. Measurement instruments, auxiliary equipment, utensil, reagents and materials: a homogenizer; a blender or a mortar with a pestle; a 25-, 50- or 100-cm^3 pipette; an Erlenmeyer flask which can be connected to a reflux condenser; a 250-cm^3 graduated cylinder; a 250-cm^3 beaker with a magnetic or mechanical stirrer; a 50-cm^3 burette; a reflux condenser; analytical scales with weighing accuracy up to 0,01 g; a water bath. Reagents: only reagents of the established analytical purity and distilled or demineralized water or water of equivalent purity were used for the analysis; Sodium hydroxide NaOH with 0.1 mol/dm^3 (0.1N) concentration; phenolphthalein, a solution with 10 g/dm^3 (1%) mass concentration in ethyl alcohol with 95% volume concentration.**Statistical analysis**

The dependence models of the studied indicators were built according to the following scheme:

1st group of the indicators – the content of dry soluble substances, sugars, and titrated acids in different cherry varieties.

2nd group of the indicators – the hydrothermal coefficient, temperature difference for certain periods, the sum of active temperatures, and the sum of effective temperatures.

3. Correlation analysis of 1 and 2 groups of the indicators.

4. Selection of the most significant weather factors that impact the biochemical quality indicators.

5. Check the statistical hypothesis according to the Student criterion.

6. Construction of regression models based on the principal component method for each biochemical quality indicator of the cherry fruits.

7. Ranking and evaluating the weather indicators impacting the formation of the biochemical quality indicators of the cherry fruits.

In the works of many scientists **[27]**, **[28]**, **[29]**, **[30]** a general scheme of correlation-regression analysis was proposed for cases when the number of influence factors significantly exceeds the number of research options.

The instruments of a modern Data Mining computer technology with RStudio software environment were used to perform the statistical analysis.

RESULTS AND DISCUSSION

The chemical composition of the cherry fruits is formed mainly by carbohydrates, vitamins, and polyphenols. The group of carbohydrates includes sugars, pectin substances, and other compounds, which by 80 – 90 % are the quantitative composition of dry substances. Therefore, one of the important indicators that characterize the fruits is dry soluble substances. Their content is considered during the manufacture of processed products since raw materials and sugar consumption depends on this. The cherry fruits are characterized by a high content of dietary and medicinal substances that contribute to the body's functioning. They also contain simple sugars - glucose and fructose, improving heart function, ascorbic acid (5-10 mg/100 g of raw mass), vitamins A, B_1, B_2, and PP, as well as minerals - phosphorus, calcium, magnesium, iron. According to these indicators, the cherry ranks second after the apple **[16]**, **[31]**. Sugar and organic acids are the main nutritional and taste components of fruit juices that contribute to the formation of the main content of soluble solids and sensory properties **[32]**, **[33]**. When processing and storing the fruit juices, sugars and organic acids are less susceptible to change than other components such as pigments, antioxidants, and taste compounds. Dry soluble substances (DSS) are an important quality indicator of fruits. In the scientists' researches, DSS values ranged from 13.4 to 21.8%, significantly differing (p <0.05) among the varieties **[34]**, **[35]**. Except for Xiuyu and M-15 varieties, the DSS value for other varieties was above 14.0%, considered the "preferred line" for the cherries **[36]**.

Therefore, one of the important indicators that characterize the fruits is dry soluble substances. According to the research, this indicator in the cherry variety samples was 16.27% on average in the south of Ukraine (Table 2). The DSS highest average mass fraction varieties include Seyanets Turovtseva (17.02%) and Modnytsya (17.05%). Ekstrom variety (14.48%) had the lowest content of dry soluble substances.

Table 2 Content of dry soluble substances (DSS) in cherry fruits, % (2007-2019), $\bar{x} \pm s\bar{x}$, n = 5.

Pomological variety	Average content, %	Min content, %	Max content, %	Variation by years, Vp, %
Vstrecha	15.87 ±2.81	11.03	18.91	17.5
Ozhidaniye	16.31 ±2.02	10.31	18.27	12.5
Shalunya	15.94 ±2.70	11.28	19.19	17.2
Seyanets Turovtseva	17.02 ±3.53	10.23	21.49	20.6
Griot Melitopol	18.63 ±3.31	14.06	22.36	17.7
Melitopol purpura	15.79 ±2.81	11.43	19.98	17.8
Modnytsya	17.05 ±2.92	12.26	20.30	16.8
Ekspromt	14.48 ±2.53	10.03	17.26	16.9
Solidarnost	15.03 ±3.63	10.23	19.36	24.1
Igrushka	16.58 ±2.80	10.50	18.90	17.1
Average value	16.27 ±3.00	11.14	19.60	18.6
HIP_{05}	0.587	–	–	–

According to the research years, the average and significant variability of DSS content was found in the cherry varieties. The strong influence of abiotic factors on the DSS content in the cherry fruits was established for Seyanets Turovtseva and Solidarnost varieties with the variation coefficients of 20.6 and 24.1%, respectively. According to the research years, the most stable in terms of DSS content was the Ozhidaniye variety, with a variation coefficient of 12.5%. The fruits of the Modnytsya cherry variety were characterized by the optimum average DSS content (17.05%) and the average variability of this indicator (16.8%).

In fruits, the most common sugars are glucose, fructose, and sucrose, which are responsible for sweetness perception, while the main organic acids responsible for sourness perception are usually malic acid, critic acid, and tartaric acid, and so on. Glucose was the dominant sugar in the tested cultivars, followed by fructose and sucrose, which confirmed the previous research in Turkey **[37]**, Poland **[38]**, Hungarian **[39]**, and Italy **[40]**. Sorts *Ujfehertoi turbos*, *Erdi jubileum,* and *Erdi fubileum* had the highest glucose content, and Meili the lowest (12.239, 12.070, 10.475, and 6.399 g/100 g FW, respectively) **[33]**. By correlation analysis, the content of glucose was highly correlated with TSS and fructose (r = 0.892 and 0.836, respectively; p <0.001), similar to the tendency described for cherries, including sour **[39]** and sweet cherries **[40]**.

According to the research years, the average content of sugars in the cherry fruits of ten varieties was 11.28% (Table 3). The largest average mass fraction of sugars had the fruits of Griot Melitopol variety (12.19%), and the smallest – Ekspromt (10.35%). According to the research, the content variability of sugars in the cherry fruits of

different pomological varieties was at the medium and high levels with an oscillation range of Vp = 14.7-25.5%. The most stable content of sugars in the fruits had the Vstrecha variety (Vp = 14.7%). The fruits of the Ozhidaniye variety were characterized by the optimum average content of sugars (11.69%) and the indicator variability (16.8%). Our data are correlated with the results obtained by other researchers **[41]**, **[42]**.

Table 3 Content of sugars in cherry fruits, % (2007–2019), $\bar{x} \pm s\bar{x}$, n=5.

Pomological variety	Average content, %	Min content, %	Max content, %	Variation by years, Vp, %
Vstrecha	10.80 ±1.51	7.18	13.34	14.7
Ozhidaniye	11.69 ±1.90	6.65	14.03	16.8
Shalunya	10.84 ±1.92	7.45	14.04	17.6
Seyanets Turovtseva	11.55 ±2.43	8.03	15.07	20.8
Griot Melitopol	12.19 ±2.51	8.36	16.22	21.1
Melitopol purpura	11.33 ±2.20	7.15	14.65	19.5
Modnytsya	11.73 ±2.84	7.45	15.23	24.1
Ekspromt	10.35 ±1.73	6.14	12.65	16.4
Solidarnost	10.70 ±2.72	6.54	14.54	25.5
Igrushka	11.59 ±2.21	6.45	13.76	19.4
Average value	11.28 ±2.20	7.14	14.50	19.9
HIP_{05}	0.503	–	–	–

The important quality indicator of the cherry fruit is the content of titrated acids. Their formation is impacted on the weather growing conditions **[3]**, **[13]**, **[16]**, **[43]**, **[30]**. In particular, according to the literature data with significant moisture in 2018, compared to 2016 and 2017, the content of titrated acids is higher for the cherry fruits of the following varieties: Alpha by 14.5 and 15%, Pamyat Artemenka – 14 and 10%. Precipitations during the growing season and the ripening phase are strongly correlated with the content of titrated acids of the cherry fruits of Pamyat Artemenka and Alpha varieties with the correlation coefficients of r = 0.81 ±0.4 and r = 0.94 ±0.23 and r = 0.64 ±0.56 and r = 0.39 ±0.74 **[16]**.

During our research, the average value of titrated acids (TA) was 1.51% in the cherry fruits (Table 4). The maximum amount of TA was found in the cherry fruits of Griot Melitopol and Solidarnost varieties. Griot Melitopol variety of the 2014 harvest had the highest number of TA (2.06%). The cherry fruits of the Solidarnost variety (2019 harvest) also showed the maximum content of TA – 2.08%.

According to the research, the variability of TA content in the cherry fruits was at the medium and high levels (Vp = 14.9-26.7%). The most stable content of TA was in the cherry fruits of Solidarnost (Vp = 14.9%) and Ekspromt (15.7%), and the most variable - was in the cherry fruits of Melitopol purple (Vp = 24.5%) and Vstrecha (Vp = 26.7%). Solidarnost variety had the optimal indicator variability (Vp = 14.9%) and average content of TA (1.79%). The maximum sugar-acid index (SAI) was determined for the cherry fruits of Melitopol purple (8.9 relative units) and Modnytsya (9.3 relative units) varieties.

Table 4 Content of titrated acids (TA) in cherry fruits, %, (2008-2019), $\bar{x} \pm s\bar{x}$, n = 5.

Pomological variety	The average content of TA, %	Content of TA, %		Variation by years, Vp, %	SAI, relative units
		min	max		
Vstrecha	1.45 ±0.38	0.85	1.93	26.7	7.4
Ozhidaniye	1.51 ±0.31	1.01	1.92	21.1	7.7
Shalunya	1.49 ±0.34	1.04	1.91	22.6	7.2
Seyanets Turovtseva	1.62 ±0.30	1.03	2.03	18.7	7.1
Griot Melitopol	1.65 ±0.31	1.08	2.06	18.7	7.8
Melitopol purpura	1.26 ±0.31	0.92	1.82	24.5	8.9
Modnytsya	1.26 ±0.26	0.97	1.75	21.3	9.3
Ekspromt	1.40 ±0.22	1.05	1.72	15.7	7.3
Solidarnost	1.79 ±0.26	1.51	2.08	14.9	5.9
Igrushka	1.65 ±0.30	1.22	2.01	18.1	7.0
Average value	1.51 ±0.33	1.07	1.92	22.3	7.4
HIP_{05}	0.265	–	–	–	

The dominant influence of the weather conditions on all chemical composition components of the cherry fruits was found according to the results of the two-factor dispersion analysis (Table 5). The weather conditions of the

research years (factor A) had the following influence particles: DSS – 61.9%, sugars – 53.5%, and TA – 40.8%. The influence of varietal characteristics (factor B) on the quality indicators of the cherry was less significant.

The influence particle of factor B was 13.0% for DSS, 5.6% for sugars, and 17.3% for TA.

Table 5 Results of two-factor dispersion analysis.

Variation source	Square sum	Freedom degree	Dispersion	Fact	$F_{table.095}$	Influence, %
			Dry soluble substances			
Factor A (year)	2238.1	2	186.5	1435.7	1.8	61.9
Factor B (variety)	471.1	9	52.3	402.9	1.9	13.0
Interaction of AB	855.6	108	7.9	60.9	1.3	23.7
			Sugars			
Factor A (year)	1051.2	11	87.6	915.0	1.8	53.5
Factor B (variety)	111.6	9	12.4	129.5	1.9	5.6
Interaction of AB	757.7	108	7.0	73.2	1.3	38.6
			Titrated acids			
Factor A (year)	24.6	12	2.0	77.5	1.8	40.8
Factor B (variety)	10.5	9	1.1	44.0	1.8	17.3
Interaction of AB	9.1	108	0.0	3.2	1.3	15.1

The average varietal value of the studied indicators should be used when developing a mathematical model **[9]**, **[44]**, **[45]**.

The correlations between the fund of dry soluble substances, sugars, and titrated acids in the cherry fruits of the studied varieties (Y_1) and the complex weather conditions for 13 years (factors Xi) were determined.

The most influential weather factors were selected according to the calculated paired correlation coefficients$r_{Y_1X_i}$.

The significance of these correlation coefficients was tested using a statistical hypothesis H_0: $\rho = 0$ (where ρ – is the correlation coefficient of the general population) under the alternative hypothesis H_1: $\rho \neq 0$ at the significance level$\alpha = 0{,}05$.

The Student criterion was used for checking the statistical hypothesis.

The significant correlation coefficients were determined at the significance level of 0.05 and the number of freedom degrees $k = 11$, which had the intervals in the range of [-1; -0.55] and [0.55; 1].

According to the above algorithm, the regression models were built based on the calculated principal components and transformed into formula 3, as per formulas 1 and 2. The following regression models were obtained after normalizing the factors (reduction to uniform units of measurement of the studied indicators):

$$\hat{Y}_1 = 0{,}055\tilde{x}_1 + 0{,}2137\tilde{x}_2 - 0{,}0854\tilde{x}_3 + 0{,}3382\tilde{x}_4 + 0{,}2457\tilde{x}_5 - 0{,}1151\tilde{x}_6 + 0{,}2292\tilde{x}_7 - 0{,}075\tilde{x}_8 + 0{,}036\tilde{x}_9 - 0{,}1462\tilde{x}_{10} + 0{,}1094\tilde{x}_{11} - 0{,}1094\tilde{x}_{12} + 0{,}2362\tilde{x}_{13} + 0{,}2922\tilde{x}_{14} + 0{,}0088\tilde{x}_{15} + 0{,}2380\tilde{x}_{16} + 0{,}2706\tilde{x}_{17} + 0{,}3514\tilde{x}_{18} + 0{,}3279\tilde{x}_{19}$$

$$\hat{Y}_2 = 0{,}1928\tilde{x}_1 - 0{,}2231\tilde{x}_2 + 0{,}2887\tilde{x}_3 - 0{,}1492\tilde{x}_4 - 0{,}1771\tilde{x}_5 + 0{,}2721\tilde{x}_6 - 0{,}2420\tilde{x}_7 + 0{,}2759\tilde{x}_8 + 0{,}214436\tilde{x}_9 + 0{,}1774\tilde{x}_{10} + 0{,}2488\tilde{x}_{11} + 0{,}2712\tilde{x}_{12} - 0{,}1953\tilde{x}_{13} + 0{,}1482\tilde{x}_{14} + 0{,}20988\tilde{x}_{15} - 0{,}2305\tilde{x}_{16} - 0{,}2499\tilde{x}_{17} - 0{,}1745\tilde{x}_{18} - 0{,}2099\tilde{x}_{19}$$

$$\hat{Y}_3 = 0{,}3523\tilde{x}_1 + 0{,}2542\tilde{x}_2 + 0{,}3357\tilde{x}_3 - 0{,}2697\tilde{x}_4 + 0{,}3623\tilde{x}_5 + 0{,}3498\tilde{x}_6 + 0{,}3767\tilde{x}_7$$

The above models characterize the dependence of the accumulation indicators of dry soluble substances ($\hat{Y}_1$), sugars ($\hat{Y}_2$), and the content of titrated acids ($\hat{Y}_3$) on the weather factors (X_i).

The indicators Δ_j, that estimate the influence particle of certain weather factors on the content of dry soluble substances, sugars, and titrated acids, were determined according to the coefficients of the calculated regression models. The calculation results are shown in tables 6 and 7.

The values of the influence particle coefficients of the weather factors (Δ_i, %) for indicators of dry soluble substances and sugars) are in the range of 0.2-9.9%. We divided the weather factors into ranks depending on the coefficient values Δ_i(I = 1-19). The average monthly air temperature in June (X1) had the maximum influence on the fund accumulation of dry soluble substances and received 1 rank by the indicator value Δ_{X1}, which was 9.9%. For the accumulation of sugars, the average monthly precipitation in June was crucial at Δ_{X2} – 8.5%.

Table 6 Table of paired correlation coefficients and indicators $\Delta_i, \%$ – factor influences particles on the accumulation of dry soluble substances and sugar in cherry fruits.

(Xi)	Factors	Dry soluble substances			Sugars		
		Paired correlation coefficients ($r_{Y_jX_i}$)	Values of factor influence particle ($\Delta_i, \%$)	Rank	Paired correlation coefficients($r_{Y_jX_i}$)	Values of factor influence particle ($\Delta_i, \%$)	Rank
1	**Average monthly air temperature in June**	0.7689	9.9%	1	0.7462	7.4%	3
2	**Average monthly precipitation in June**	-0.6955	9.6%	2	0.8961	8.5%	1
3	**Average minimum relative humidity in March**	0.6932	2.3%	16	0.5899	3.6%	17
4	**Average minimum relative humidity in June**	-0.8298	7.8%	5	0.9111	8.5%	2
5	**Amount of precipitation during the period from the end of flowering to fruit ripening**	-0.6301	7.3%	6	-0.8305	7.1%	4
6	**Total number of precipitation days of more than 1 mm in March**	0.8983	2.7%	15	-0.6463	3.3%	18
7	**Total number of precipitation days with more than 1 mm in June**	-0.8311	8.9%	3	-0.7501	6.2%	5
8	**Average air temperature during the fruit harvest period**	0.6211	8.3%	4	-0.7089	4.2%	15
9	**Absolute maximum air temperature during the fruit harvest period**	0.7401	1.1%	18	-0.6496	3.9%	16
10	**Difference between absolute maximum and minimum air temperatures during the fruit harvest period**	0.5704	3.3%	12	-0.6720	4.5%	12
11	**Average minimum air temperature during the fruit harvest period**	0.7279	3.1%	13	0.6450	6.0%	8
12	**Average maximum air temperature during the fruit harvest period**	0.9047	5.0%	11	0.7104	6.1%	7
13	**Amount of precipitation during the fruit harvest period**	-0.7347	6.8%	8	0.7563	5.6%	10
14	**Number of precipitation days with more than 1 mm during the fruit harvest period**	-0.5913	2.7%	14	-0.5859	3.0%	19
15	**Amount of effective temperatures during the fruit harvest period**	0.5605	0.2%	19	-0.6644	5.1%	11
16	**Hydrothermal coefficient during the fruit harvest period**	-0.7884	7.1%	7	-0.7801	6.2%	6
17	**Absolute minimum relative humidity during the fruit harvest period**	-0.6504	1.4%	17	0.6252	4.5%	13
18	**Average minimum relative humidity during the fruit harvest period**	-0.6417	6.2%	10	0.6481	4.3%	14
19	**Average relative humidity during the fruit harvest period**	-0.7416	6.3%	9	-0.7775	5.6%	9

The indicators Δ_j, that estimate the influence particle of certain weather factors on the content of dry soluble substances, sugars, and titrated acids, were determined according to the coefficients of the calculated regression models. The calculation results are shown in Tables 6 and 7.

The values of the influence particle coefficients of the weather factors (Δ_i, %) for indicators of dry soluble substances and sugars) are in the range of 0.2-9.9%. We divided the weather factors into ranks depending on the coefficient values Δ_i (I = 1 – 19). The average monthly air temperature in June (X1) had the maximum influence on the fund accumulation of dry soluble substances and received 1 rank by the indicator value Δ_{X1}, which was 9.9 %. For the accumulation of sugars, the average monthly precipitation in June was crucial at Δ_{X2} – 8.5%.

2nd rank by influence degree on DSS accumulation (Δ_{X2} – 9.6%) and sugars (Δ_{X4} – 8.54%) received such weather indicators as the average monthly precipitation in June (X) and the minimum relative humidity in June (X). The total number of precipitation days with more than 1 mm in June (X7) for DSS and the average monthly air temperature in June (X1), while forming the fund of sugars, had the coefficient values Δ of 8.9% and 7.44% and took 3rd rank. The weather indicators, which received 4-19 ranks, had less influence on forming the fund of dry soluble substances and sugars in the cherry fruits. This is confirmed by the values of the influence particles Δ , which had a range of 0.2 to 7.1%. Of the 19 common weather factors that have an important role, while forming the fund of dry soluble substances and sugars, 12 factors are crucial during the fruit harvest period, 4 factors – are in June and 2 factors are important in March, and 1 factor - in the period from flowering to fruit ripening.

The fruit ripening stage, which takes place in June, is crucial for forming the fund of dry soluble substances and sugars in the cherry fruits of the studied varieties. Summarizing the obtained data, we may conclude that the most significant indicators (1-3 ranks), while forming the fund of dry soluble substances and sugars in the cherry fruits, were revealed the following:

Average monthly air temperature, average monthly precipitation, minimum relative humidity, and a total number of precipitation days in June. To analyze the influence of the weather factors on the accumulation of the fund of titrated acids, 7 indicators of the weather factors (X_i), a certain growing season that can significantly impact the accumulation of the fund of titrated acids in the cherry fruits, were found and selected (Table 7). The analysis of the values of the influence particle coefficients of the weather factors (Δ_i, %) on the accumulation of titrated acids in the cherry fruits) allowed to determine their oscillation range – 8.9-18.6%. Of the 7 weather factors that significantly impact the accumulation of titrated acids in the cherry fruits, 4 factors are crucial during the fruit harvest period, 2 factors are in June, and 1factor is important in the period from flowering to fruit ripening.

Table 7 Paired correlation coefficients and indicators Δ_i, % - factor influences particles on the content of titrated acids in cherry fruits.

Factor symbol (Xi)	Factors	Paired correlation coefficients ($r_{Y_jX_i}$)	Values of factor influence particle (Δ_i, %)	Rank
1	**Average monthly precipitation in June**	0.8507	17.7%	2
2	**Amount of precipitation during the period from the end of flowering to fruit ripening**	0.7838	15.7%	3
3	**Total number of precipitation days with more than 1 mm in June**	0.8621	18.6%	1
4	**Difference between absolute maximum and minimum air temperatures during the fruit harvest period**	-0.7415	11.5%	6
5	**Amount of precipitation during the fruit harvest period**	0.6119	8.9%	7
6	**Number of precipitation days with more than 1 mm during the fruit harvest period**	0.7754	15.7%	4
7	**Hydrothermal coefficient during the fruit harvest period**	0.6208	11.9%	5

For further research result analysis, the factors, depending on the coefficient values Δ_i (i = 1-7), were divided into ranks. The total number of precipitation days in June (X3) maximally impacted the accumulation of titrated acids in the cherry fruits and received 1st rank by the value of the index Δ_{X3}, which was 18.6%. 2nd rank by influence degree on the accumulation of titrated acids in the cherry fruits (Δ_{X1} – 17.7%) was received by such weather indicators as the average monthly precipitation in June (X1). During the growing season, from the end of flowering to fruit ripening (X2), while forming the fund of titrated acids, the precipitation had the coefficient value Δ of 15.7% and took 3rd rank. The remaining 4 weather factors (X1, X2, X3, X4) had a much smaller impact on the formation of the fund of titrated acids in the cherry fruits (Δ_X = 8.9-15.7%).

The above analysis confirms that the accumulation of DSS, sugars, and titrated acids in the cherry fruits were most influenced by the humidity indicators of the last fruit formation month (June) and the period from the end of flowering to fruit ripening.

CONCLUSION

1. Some varieties, namely Modnytsya (the content of DSS is 17.05 %, Vp is 16.8%), Ozhidaniye (the content of sugars is 11.69%; Vp is 16.8%), and Solidarnost (the content of TA is 1.79%, Vp is 14.9%), are the most suitable for growing in the Southern Steppe Subzone of Ukraine according to technological properties.
2. It was determined that the fruits of Melitopol purple and Modnytsya varieties have the maximum indicators of the sugar-acid index (8.9-9.3 relative units).
3. It is advisable to forecast the taste qualities of the cherry fruits according to the average varietal value based on the dominant influence of the weather conditions of the research years (factor A). Factor A had the influence particles - 61.9, 53.5, and 40.8%, respectively, on forming the foundation of DSS, sugars, and TA in the cherry fruits.
4. The correlation analysis of the influence of the weather factors on the content of DSS, sugars, and titrated acids in the cherry fruits was carried out. The average and strong correlation dependences were determined ($\left|r_{Y_j X_i}\right| \geq 0{,}55,\ i = 1-19, j = 1$ between 19 weather factors (Xi, i = 1-19) and the accumulation of the biochemical indicators in the cherry fruits.
5. The accumulation dependence models of the biochemical indicators (DSS, sugars, TA) in the cherry fruits were built based on the principal component and the least-squares methods.
6. The 1st rank weather parameters were determined due to the calculation of the influence particles of each weather factor on the biochemical indicators in the cherry fruits. It was found that the average monthly air temperature in June (Δ = 9.9%) had the maximum influence on the accumulation of dry soluble substances, the average monthly precipitation in June (Δ = 8.5%) on the content of sugars, the total number of precipitation days in June (Δ = 18.62%) on the content of titrated acids in the cherry fruits.
7. The air humidity indicators at the end of the flowering phase before fruit ripening and the last month of fruit formation had the greatest influence on the accumulated biochemical indicators in the cherry fruits (June).

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Manifestation of living and post-slaughter traits of productivity in inbred and outbred bull calves of Ukrainian meat cattle breed

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ABSTRACT
Selection in meat cattle herds requires caution due to the manifestation of inbred depression in traits that affect the economics of this livestock industry. This paper analyses the productivity of inbred and outbred bull calves of the Ukrainian meat cattle breed and justifies methods of pair selection in purebred herds with natural pairing. In bull calves, the growth of animals and traits of their meat productivity after slaughter were considered. Inbreeding was determined based on their pedigree. Inbred animals tended to have a growth rate of 10.2% from birth to 8 months of age. Afterwards, their average daily gain in live weight decreases sharply compared to outbred peers, who grow faster over a more extended period. From 8 to 18 months of age, it is probably (p >0.95) higher by 27.3% compared to inbred animals. Inbred bull calves have higher variability (Cv,%) in average daily gains. This indicates different adaptations to the environment during the suckling period and after weaning. Outbred animals tend to gain 2.3% of body weight at 12 months, 4.7 at 15 months, and 10.3% at 18 months. Its variability with age decreases by 7.4 points in inbred bull calves and 0.4 points in outbred ones, from 8 to 18 months. The inbred animals spent 29.5% more feed per kg of gain (p >0.95) than the outbred ones. Inbred bull calves vs outbred ones at 15 and 18 months of age tend to improve the expression of meat forms by 1.3 and 2.7%. They are relatively shorter and have a more rounded barrel. As a result, they have a shorter period of rapid growth. With the small size of the Ukrainian meat cattle population, one of the most important problems is reducing genetic variation in beef productivity traits and manifesting inbred depression in them. In purebred commercial herds, the mating of close animals should be avoided. To do this, an "order" for bulls should be made, and pairs should be selected without using inbreeding at different grades. Thus, outbred bull calves will reach live weight more quickly, spending less feed per growth unit, and have better basic slaughter traits.

Keywords: inbreeding, outbreeding, meat productivity, bull calves, selection, "order" for sires.

INTRODUCTION
The Ukrainian meat cattle breed was bred in 1993 by complicated reproductive crossbreeding of the Chianina (3/8), the Charolais (3/8), the Simmental (1/8), and the Ukrainian Grey (1/8) **[1]**. Animals of this breed are characterised by significant variability in growth, milk production, and reproductive ability. In terms of average daily gain and slaughter traits alone, they are not inferior to representatives of other meat cattle breeds. For this, cattle have attracted the attention of beef manufacturers. Close breeding and intensive selection by traits are often used when working with the Ukrainian meat cattle breed. This allows the formation of the desired type and reduced variability inherent in multibreeding. Among sires derived from close breeding, due to their more intensive selection, there are more prepotent improvers in terms of growth rate **[2]**. Females with a lower culling percentage show inbred depression in weight growth, reproductive capacity, and milk production. The most significant deterioration in productivity occurs in cows derived from intralineage inbreeding.

The decline in these traits is of great concern, as it is possible to lose the advantages of these cattle over other meat cattle breeds in Ukraine. The impact of inbreeding on the meat productivity of animals obtained through

complex reproductive crossbreeding remains insufficiently studied. Vital importance should be given to the justification of parental pair selection. There is a high risk of spontaneous inbreeding when purebred breeding is used, especially in natural mating conditions. Close breeding leads to negative effects, such as inbred depression on the viability and productivity of cattle **[3]**. It is relevant to investigate the manifestation of meat productivity traits in inbred and outbred bull calves based on the importance of their livestock in beef production and to substantiate methods of pair selection in purebred herds.

Analysis of literature sources. A high level of close breeding leads to a decrease in the genetic diversity of traits **[4]** and increased homozygosity **[5]** and is one of the most important issues in cattle populations. Inbreeding negatively affects the reproductive capacity of bull calves, especially their sperm morphometry **[6]**. The inbreeding coefficient correlates most negatively (p >0.99) with their length (r = -0.1449) and width (r = -0.2494). Inbred (Fx >3.5%) bull calves have a higher percentage of highly active but non-progressive spermatozoids **[7]**, which increases the interval between calving in cows.

In dams, the degree of inbreeding prolongs the age of first calving **[8]** and reduces the number of unfertilized oocytes **[9],** increasing dystocia in firstborns and stillbirths **[10]**, **[11]**. The 1% increase in the inbreeding coefficient leads to a decrease in live weight: newborns by 0.103 kg **[12]**, at 205 days by 0.24 kg **[13]**, at 365 days by 0.514 and 0.57 kg **[14]**. As for the impact of inbreeding on meat productivity, the published data are contradictory. In Carolino & Cama **[10]**, inbred depression with carcass traits is extremely low and insignificant.

It has been proven that inbred depression is affected not only by the inbreeding of the animal but also by its mothers **[15]**. Maternal and calf inbreeding increases prenatal neonatal mortality with 3.4% and 6.3% of firstborns, respectively **[16]**. Each 1% increase in the inbreeding rate of a cow and calf increases the age at first calving by 1.4 and 0.8 days, respectively. Parental inbreeding has limited effects **[17]**. The average inbred depression with a 1% increase in inbreeding is 0.269% for weight gain and 0.174% for reproductive traits **[18]**.

This review of literature sources highlights that inbred depression is a growing problem and a need for inbreeding management in beef cattle breeds originated in Ukraine.

Scientific Hypothesis

The study of inbreeding impact on productivity traits of bull calves of the Ukrainian meat cattle breed during life and after slaughter and justification of the methods of pair selection at these cattle breeding will help to prevent the appearance of inbred depression.

MATERIAL AND METHODOLOGY

Samples

The study was conducted on inbred (Figure1) and outbred bull calves of the Ukrainian meat cattle breed.

Animals and Biological Material

Inbred and outbred bull calves of the Ukrainian meat cattle breed were studied.

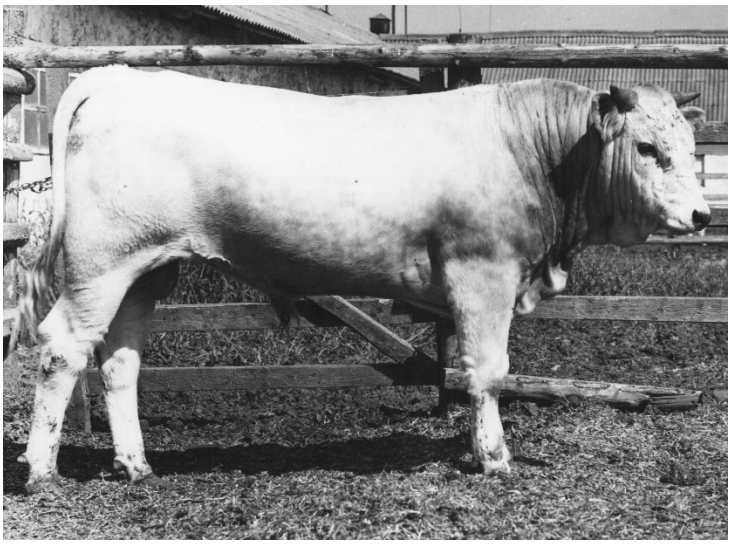

Figure 1 Inbred bull calves.

Instruments

Electronic analytical scale (KERN ABS 120-4, SE "Khimtex", Ukraine).
Laboratory ruler (ElizLabs 68933, Ukraine)
Measuring tape (Schweikin, Ukraine).

Laboratory Methods

Live weight was determined by individual weighing at the end of each month in the morning before feeding.
Net weight gain (Ng) for each day of life was determined according to ICAR **[19]** using the formula:

Ng = slaughter weight (carcass), kg x 1000 / age at slaughter, days

Fat tissue was divided into slaughter fat (subcutaneous) and intermuscular one following DSTU 3938-99 **[20]**.
Muscle tissue index (MTI) was determined by the ratio of muscle tissue to bone mass, adipose tissue and tendons and ligaments.

Description of the Experiment

Sample preparation: The work was carried out on the Ukrainian meat cattle breed animals. The slaughter of cattle was managed at Volia" breeding plant, Zolotonosha district, Cherkasy region. In the herd, five well-developed inbred and outbred newborn bull calves were selected by balanced analog groups. The difference in their live weight and age between the groups was up to 5%. Inbreeding was determined using five generations of pedigrees

Number of samples analyzed: Five well-developed inbred and outbred newborn bull calves were selected by balanced analog groups in the herd.

Number of repeated analyses: 5.

Number of experiment replication: 3.

Design of the experiment: The five well-developed inbred and outbred newborn bull calves selected for the study were tested for origin reliability by blood group factors. Until 6-7 months of age, they were raised near their mothers with the use of suckling. After weaning until they were 8 months of age, they were accustomed to a typical diet and housing conditions. Intensive bull calves were reared from 8- to 18 months of age on a leash. Their general level of feeding was calculated to produce an average daily gain of 1,000 to 1,200 g. During this period, the bull calves were fed a feed according to rations prepared as indicated in the standards. The weight of feed eaten by each bull calf was counted every decade (two days in a row) by weighing the given fodder and its residues. Their energy value (in-feed units) and costs per 1 kg of live weight gain were calculated based on the feed consumed. There was no significant difference in feed consumption between the groups from 8 to 18 months (Table 1).

Table 1 Feed consumption by inbred and outbred bull calves from 8 to 18 months, M ±m.

Feed	Inbred (n = 5)		Outbred (n = 5)	
	feed units	%	feed units	%
Concentrated	1369 ±50.9	45.6 ±0,56	1328 ±55.8	45.8 ±0.10
Rough	570 ±86.7	19.0 ±1,73	558 ±35.2	19.2 ±1.11
Juicy	461 ±51.4	15.4 ±0,50	387 ±31.5	13.3 ±0.41
Green	599 ±26.8	20.0 ±0,24	630 ±66.9	21.7 ±1.44
Total feed units	2999 ±88.0	100.0	2903 ±104.8	100.0
Feed costs per 1 kg of grain, feed units	10.1 ±0.36		7.8 ±0.67*	

Note: *) $p > 0.95$.

The live weight of animals and their average daily weight gain from birth to 18 months of age and meat productivity at 18 months after slaughter were determined. Live weight was determined by individual weighing at the end of each month in the morning before feeding. At 8-, 12-, 15-, and 18 months of age, bull calves were weighed on two consecutive days with average weight calculation. The expression of meat forms at 15 and 18 months was assessed using a 60-point scale. The animals were slaughtered at the Cherkasy meat processing plant. Before that, they were weighed before and after 24 hours of starvation with free access to water (pre-slaughter live weight). After slaughter, the slaughter weight (paired carcass) was determined. The slaughter yield (carcass yield) was calculated regarding pre-slaughter live weight.

After cleaning the carcass, the absolute weight of the cut-offs was weighed, and their share of the slaughter weight (carcass) was determined. Boning of the left half-carcasses of bull calves was made. After that, bones,

muscle tissue, including the highest, first, and second grades following GOST 7595-79 **[21]**, tendons and ligaments, and fat tissue were weighed.

They were weighed to compare subcutaneous and intermuscular fat tissue. The weight of total fat tissue was determined as the amount of internal fat and fat from the carcass. After boning, the muscular-bone ratio and the muscular tissue index were determined. Muscle-bone ratio (MBR) was calculated as the ratio of muscle tissue to bone The Muscle Tissue Index (MTI) was determined by the ratio of muscle tissue to the weight of bones, fat tissue, and tendons and ligaments **[22]**. After slaughtering, the hide was removed and weighed to determine its net weight, without any residual muscle or fat tissue (if its weight exceeded 500 g), blood clots and contamination, and adhering dung. The length of the hide was measured along the spine from the upper edge of the neck in the middle between the horns to the line that connects the ends of the ischial tuberosities. The width was measured along the line in the middle third of the hide. Before measuring, the hide was spread out on a table; creases and other irregularities were straightened out without stretching the length and width.

Statistical Analysis

The obtained data were processed using variational statistics methods. We determined the average values by groups, the error of the average, and the difference between the average and its probability. To characterize the degree of variability of traits, the coefficient of variability (CV,%) was calculated as the ratio of the average square deviation to the average value for the group. The statistical analysis data were produced by Microsoft excel and Statistica 15. The accuracy of the experimental data was determined using the Student's test for a confidence probability of ≤ 0.05 based on the number of parallel determinations of at least 5. Linear programming problems were solved using the MS Excel spreadsheet processor "Search for a solution" setting Excel Solver

RESULTS AND DISCUSSION

Inbred bull calves are born with a lower live weight than outbred ones (Table 2). Sumreddee, et al. **[15]** and Hidalgo, et al. **[12]** also established the manifestation of inbred depression by neonatal weight. There is a tendency to increase the average daily weight gain by 10.2% from birth to 8 months in inbred bull calves. This was probably due to better milk production in their mothers, which balances the detrimental effects of inbreeding on litter growth rate during the suckling period. Due to faster growth during the suckling period, inbreds tend to increase live weight by 8.1% at 8 months of age. This contradicts the data of Hidalgo, et al. **[13]** according to which inbred litter weight at 240 days of age decreases by 0.685 kg and Fx growth by 1%. Thereafter, the average daily gain decreases compared to outbred animals. Inbred and outbred bull calves maintain a high growth rate until 18 months of age. Outbred cattle grow faster for a longer period. **[22]**, **[23]**, **[24]**. The average daily live weight gain from 8 to 18 months is probably 27.3% ($p > 0.95$), higher than inbred peers. An advantage in growth rate from 8 to 18 months in outbred animals is also observed in OTHER studies **[25]**, **[26]**, **[27]**, **[28]**.

Table 2 Weight growth of inbred and outbred bull calves.

Trait	Inbred (n = 5)		Outbred (n = 5)	
	M ±m	Cv,%	M ±m	Cv,%
An average daily gain in the period (month) from – to:				
-//- newborns - 8	922 ±59.2	12.8	837 ±26.0	6.2
-//- 8-12	1066 ±86.3	16.2	1301 ±63.7	9.8
-//- 12-15	1097 ±112.9	20.6	1255 ±121.7	19.4
-//- 15-18	703 ±176.8	50.0	1115 ±200.0	35.9
-//- 8-18	967 ±62.6	12.9	1231 ±47.2*	7.7
Live weight at age, (months):				
newborns	31.4 ±1.8	11.4	32.4 ±1.2	7.1
-//-8	255 ±13.3	10.4	236 ±6.9	5.8
-//-12	385 ±10.9	5.7	394 ±9.5	4.8
-//-15	485 ±10.7	4.4	508 ±12.6	5.0
-//-18	553 ±11.6	4.0	610 ±20.5	6,7
The expression of meat forms (points) at age, (months)				
-//- 15.	54.1 ±1.2	4.4	53.4 ±2.9	10.7
-//- 18.	56.3 ±1.8	3.1	54.8 ±2.3	8.3

Note: *) $p > 0.95$.

The trend of a 2.3% live weight preference in outbred animals begins to manifest at the age of 12 months. At 15 months of age, the difference is already 4.7%, and at 18 months, it is 10.3%. Inbred bull calves have higher average daily gain variability than outbred ones. This indicates their unequal adaptation to environmental conditions during the suckling period and after weaning. The coefficient of variation of live weight in inbred bull calves at 15 and 18 months of age tends to decrease compared to outbred ones. Its variability decreases with age in inbred bull calves more significantly (by 7.4 points), and only by 0.4 points in outbred ones.

Consequently, related to high variability in growth rate, inbred animals are prone to stabilization of live weight at older ages. The inbred animals spent 29.5% (p >0.95) more feed per kg of body weight gain during The 8 to 18-month test period compared to outbred peers (see Table 1). According to Carolino and Cama **[10]** inbred depression CONCERNING feed consumption efficiency is insignificant. At the age of 15 and 18 months, inbred bull calves also tend to have a better expression of meat forms than outbred ones by 1.3 and 2.7%, respectively. This contrasts to data obtained on THE NELORE breed in Brazil **[29]**, **[30]**.

Outbred bull calves have an advantage over inbred peers in pre-slaughter live weight by 9.1% (p >0.95), slaughter weight by 12.3 (p >0.95), and net gain by 8.9%, and slaughter yield (carcass) by 1.8 points (Table 3). Carcasses of inbred bulls have 20.5% more fat and muscle tissue cut, and they have a significantly lower coefficient of their variability.

Table 3 Traits of the slaughter of inbred and outbred bull calves.

Trait	Inbred (n = 5)		Outbred (n = 5)	
	M ±m	Cv, %	M ±m	Cv, %
Pre-slaughter live weight, kg	518 ±6.6	2.5	565 ±15.4*	5.5
Slaughter weight (carcass), kg	309 ±4.1	2.6	347 ±10.5*	6.0
Slaughter yield (carcass), %	59.7 ±1.06	3.6	61.5 ±1.11	3.6
Net gain, g	583 ±16.2	5.6	635 ±19.2	4.2
Trimmings, kg	5,3 ±0.25	9.6	4.4 ±0.70	31.7
Trim, % of the half-carcass weight	3.4 ±0.21	12.1	2.6 ±0.38	29.1

Notes: *) p >0.95.

Inbred bull calves are inferior to outbred peers in terms of weight of half-carcasses by 7.1% (Table 4). In absolute terms, more muscle tissue is obtained from outbred animals. Bull calves from close breeding predominate by 14.3% of outbred peers in terms of fat tissue content in the carcass. The latter feeds are used for growth, not for fat deposition. At one and a half years of age, the carcasses of inbred animals have less fat tissue under the skin. They have 3.3 times as much fat tissue deposited between the muscles. Significant accumulation of fatty tissue between the muscles in inbred cattle markedly affects their exterior, which is less angular and has better meat form development. Fat tissue between the muscles plays a significant role by shifting them slightly **[31]**, **[32]**.

Inbred bull calves are inferior to outbred ones in muscle content but have higher amounts of top and second-grade pulp. According to the sausage classification, second-grade beef includes a large amount of fat between the muscles not separated during trimming in inbred bull calves is higher (p >0.95) by 6.3 points. Premium grade beef, including muscle tissue without fat, tendons, and ligaments, was 5.5 points higher in inbred animals (p >0.95).

Fat tissue in cattle carcasses plays an important role **[33]**, **[34]**. Its low content worsens the taste of beef. An excessive amount of fat tissue under the skin and between the muscles reduces the marketability of carcasses because its excess is cut off and disposed of. The formation of such carcasses requires an increase in feed consumption. A rather high proportion of fat tissue (35.9%) between muscles in inbred animals, serving as a cushion for blood vessels, nerves, and lymphatic glands, is subject TO excessive waste formation. Outbred bull calves at this age have a higher deposition of fat under the skin (19.5%).

Usually, inbred cattle have less fat under the skin and more between the muscles. In the subcutaneous tissue, reserve fat is deposited, supporting, protective, and heat-insulating function. Reserve fats in the body serve as an energy reserve and depot for water. The maximum growth of subcutaneous tissue in animals occurs in the period from 7 to 12 months, and fat tissue between muscles – between 12 and 18 months of life **[35]**, subcutaneous fat and fat between muscles in the form of large layers appears at the age of 18 months **[36]**.

Inbreeding of 18-month-old bull calves of the Ukrainian meat cattle breed has the maximum positive effect on the growth of fat tissue between the muscles, with the highest natural growth and the highest natural growth. Subcutaneous fat tissue, which has a relatively low nutritional value, is more depressed during inbreeding.

Table 4 Morphological composition of half-carcasses of inbred and outbred 18-month-old bull calves.

Trait	Inbred (n = 5)		Outbred (n = 5)	
	M ±m	Cv, %	M ±m	Cv, %
Half-carcass weight, kg	157.1 ±4.53	5.8	168.2 ±4.29	5.1
Bone weight, kg	27.6 ±0.98	7.1	27.3 ±1.25	9.2
Bone weight, %	17.6 ±0.59	6.8	16.2 ±0.66	8.2
Muscle tissue, kg	116.8 ±2.74	4.7	130.6 ±3.76*	5.8
-// -, %	74.3 ±1.57	4.2	77.7 ±0.78	2.0
including the highest grade, %	20.4 ±2.57	25.2	14.9 ±1.22*	16.4
First grade, %	37.5 ±2.99	16.0	49.4 ±1.05*	4.3
Second grade, %	42.1 ±1.15	5.5	35.8 ±1.70*	9.5
Tendons and ligaments, kg	6.3 ±0.93	29.6	4.7 ±0.21	8.8
Tendons and ligaments, %	4.0 ±0.45	21.8	2.8 ±0.08	6.1
Fat tissue in the carcass, kg	6.4 ±0.26	8.2	5.6 ±1.02	35.8
-// -, %	4.1 ±0.17	8.5	3.3 ±0.57	34.4
Including slaughter fat, kg	4.1 ±0.26	12.5	4.9 ±1.07	43.7
- //- , % to total fat	19.3 ±1.25	12.7	27.4 ±5.62	41.02
- //- , % to fat in the carcass	64.1 ±3.90	12.1	87.5 ±3.93	9.39
Including intermuscular fat, kg	2.3 ±0.35	30.6	0.7 ±0.11	26.5
- //- , % to total fat	11.0 ±2.01	36.9	4.6 ±0.58	25.1
- //- , % to fat in the carcass	35.9 ±3.9	7.8	12.5 ±4.95	68.0
Muscle-bone ratio	4.3 ±0.18	8.5	4.8 ±0.20	8.5
The muscle tissue index (MTI)	2.9 ±0.14	9.6	3.4 ±0.11	6.4

Notes: *) p >0.95.

According to Zhao, et al. **[5]**, homozygosity is significantly negatively associated with slaughter fat and its thickness in the back of the barrel. However, fat tissue between the muscles improves the nutritional value of beef and is not delayed in development due to a significantly higher growth rate.

The results of this study were compared with those reported by Carolino & Cama **[10]**. Carolino & Cama **[10]**. evaluated the effect of inbreeding depression using carcass information collected through the Carnalentejana, DOP certification program on 7701 animals slaughtered between 1995 and 2004 under the certification program. The retail meat yield as a percentage of carcass weight and percentage of meat cuts, including rump steak, were taken into account. The results clearly show an adverse effect of inbreeding on most meat cattle traits.

The aimed at the quantitive evaluation of the effect of inbreeding on carcass quality, growth rate, and conformation characteristics in purebred Charolais, Limousin, Simmental, Hereford, and Angus meat cattle populations based on Irish commercial and breeding herds. Inbred animals have lower carcass weight and fat in it. The effects of inbreeding are more expressed in British breeds. The effect on carcass weight ranges from -0.87 kg (Charolais) to -1.90 kg (Hereford), with a 1% increase in inbreeding. Continental animals suffer more from inbreeding due to the expression of meat forms and conformation of carcasses than British ones. Inbred animals are smaller and narrower due to poorly developed muscles. Inbreeding increases the viscosity of meat but does not significantly affect other meat qualities or carcass traits **[37]**, **[38]**, **[39]**, **[40]**.

In our study, the relative bone weight in the carcasses of inbred animals is higher by 1.4 points. The beef yield and quality analysis by muscle tissue index and muscle-bone ratio show a pronounced tendency to improve these traits in outbred bull calves. For every kilogram of bones, they have 11.6% more pulp. Outbred cattle have a 17.2% better MTI than inbred cattle.

From animals of both groups, heavy hides with a large area are obtained (Table 5). Inbred bull calves have a shorter length of 26.3% (p >0.99) and an area of 7.0% (p >0.95). The size of the hide was also affected by measurements of the exterior of the animals. Inbred bull calves are shorter and more rounded. Inbred bull calves also have a 15.3% lighter hide (p >0.95).

Thus, one of the most important problems in the Ukrainian meat cattle breed with its small population size is reducing genetic diversity and the occurrence of inbred depression and traits of meat productivity. This has also been confirmed in small populations as well as in breeds with large numbers. In commercial herds, it is recommended to avoid mating close animals **[8]**, **[6]**. It is important to intensively use many young sires of high breeding value **[13]**. A special feature of stock breeding in commercial herds should be grouped or line-group selection, with strict adherence to the cross of the highest producing lines to prevent inbreeding during further breeding **[41]**, **[42]**.

Table 5 Hide sizes in inbred and outbred bull calves.

Hide traits	Inbred (n = 5)		Outbred (n = 5)	
	M±m	Cv, %	M±m	Cv, %
Weight, kg	44.5 ±1.45	5.7	51.3 ±2.44*	8.2
-"-,% of fat weight	8.6 ±0.31	6.2	9.0 ±0.30	5.9
Length, m	1.9 ±0.03	2.5	2.4 ±0.07**	5.2
Width, m	2.2 ±0.08	6.2	1.9 ±0.06*	5.4
Area, m^2	4.3 ±0.06	2.2	4.6 ±0.05*	1.8

Notes: *) p >0.95; **) p >0.99.ffff.

Selection begins with an "ordering" of sires. When making a plan for their use, the improvement type of selection is taken as the basis. When making a line-group selection plan in commercial herds, there is no need to consider the mothers' origin of each dam, as a large group is assigned to one sire. With this approach, the origin of the breeding stock is taken into account according to the ancestors of the parents and the pedigree on both sides. This is done by determining the close relations between the bulls, who have offspring in the herd. They are common ancestors for most dams and occur in various combinations in their pedigrees.

CONCLUSION

Inbreeding reduces the meat productivity of bull calves compared to outbreeding. It contributes to the appearance of animals prone to higher fat deposition in the carcass, obtaining higher grades of muscle tissue from them, leading to increased feed consumption for gain. Inbred bull calves tend to increase the cut-off of slaughter fat and meat from carcasses. Preference should be given to outbred cattle with a higher and more stable growth rate from 8 to 18 months. Animals of this type have a longer, higher-legged barrel and a higher final live weight. They maintain high gain rates for a long time and reach the maximum live weight later than inbred ones.

Inbred animals tended to have a growth rate of 10.2% from birth to 8 months of age. Afterwards, their average daily gain in live weight decreases sharply compared to outbred peers, who grow faster over a more extended period. From 8 to 18 months of age, it is probably (p >0.95) higher by 27.3% compared to inbred animals. Inbred bull calves have higher variability (Cv, %) in average daily gains. This indicates different adaptations to the environment during the suckling period and after weaning.

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Incorporation of catechin extracts from gambier products and pasak bumi in the production of functional instant green robusta coffee

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ABSTRACT

The research was used to produce functional instant green coffee through gambier catechin extract and pasak bumi powder. This involved using a non-factorial completely randomized design with 5 treatments and 3 replications. The treatments consist of 5 formulations (F), including the instant green coffee (%), gambir catechin extract (%), and pasak bumi powder (%) where F1 was at 100:0:0, F2 was 80:15:5, F3 was 70:20:10, F4 was 60:25:15, and F5 was 50:30:20. The results showed the functional instant green coffee produced has a water content of 3.84 – 4.81%, soluble speed of 26.78 – 29.33 seconds, and total phenol of 16.79 – 169.48 mg/L, and IC50 of 44.68 – 207.59 ppm. The addition of gambier catechin extract and pasak bumi powder to the formulation was observed to have significantly increased the functional properties and water content. Moreover, the soluble speed of the instant coffee fulfils the quality requirements of the Indonesian National Standard (SNI) number 2983 of 2014.

Keywords: gambier, instant, catechin, green coffee, pasak bumi

INTRODUCTION

Humans accept coffee from both the sensory and functional aspects despite numerous pieces of information on its effects on body health. It has been reported that both robusta and arabica generally contain functional compounds in chlorogenic acid. This compound was also discovered by **[34]** to be present in coffee as an antioxidant, with robusta reported by **[38]** to contain higher content at 43. 63% than arabica, which has 36.18%. According to **[14]**, roasting can reduce robusta caffeine and chlorogenic acid levels by 13 – 25% and 37 – 59%, respectively. Several studies have been conducted to maintain the antioxidant properties of coffee such as the addition of herbal cereals in **[29]**, optimisation of roasting temperature to reduce damage to chlorogenic acid compounds in **[8]** and **[3]**, and the use of a spontaneous fermentation with *Wickerhamomyces anomalous* (Strain KNU18Y3) on green coffee beans in **[7]**.

Green coffee is currently gaining popularity among world coffee lovers, and it is mainly different from the ordinary types due to the effect of the bean processing method on its functional properties and aroma. According to **[6]**, green robusta has better functional properties than roasted coffee, as indicated by their total phenol contents of 208.89 mg/L and 119.22 mg/L, respectively. **[18]** also showed that green robusta contains 81.6% antioxidant compounds and has higher caffeine content and high antioxidant properties. It is important to add bioactive compound materials in its production process to increase its antioxidant properties and reduce caffeine levels. One source of these bioactive compounds is catechin and pasak bumi extract.

Catechin is a product from the aqueous extraction of the leaves and twigs of the gambier plant (*Uncaria gambir Roxb*), which have been discovered to contain more than 52.25% catechin compounds **[37]**. This extract was further reported by **[11]** to be an antioxidant with an IC_{50} of 2.74 g/mL, while **[30]** also showed its ability to form canna-based edible films, which are antioxidants. According to **[13]** and **[36]**, the roots of the pasak bumi plant also contain eurikomanone, quassinoids, flavonoid, phenolic, and terpenoid compounds which are observed to have antioxidant potentials.

Scientific Hypothesis

The addition of gambir catechin extract has a significant effect on increasing the functional properties of instant green coffee, especially its antioxidant activity.

MATERIAL AND METHODOLOGY

Samples

Instant coffee powder made from green robusta coffee powder 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, Na_2CO_3, and nutrient broth (NB) 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 green coffee powder from JagadRaye Coffee 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 **[2]**: measurement of water content using the gravimetric method. Soluble speed [2]: Dissolve 100 g of instant coffee in 200 mL of water. Then the length of time instant coffee dissolves in water is calculated as the speed at which it dissolves in water using a stopwatch. Total phenol **[31]**: Determination of total phenol content was carried out by means of a spectrophotometric method using Folin-Ciocalteu reagent. Antioxidant activity **[17]**: Antioxidant testing using the DPPH method (2,2 diphenyl-1-picrylhydrazyl) was used.

Description of the Experiment

Sample preparation: The instant green coffee powder, gambier catechin extract, and instant pasak bumi powder with a size of 80 mesh are mixed. Each treatment is put into a cup and then brewed with 100 mL of hot water at 80 °C and stirred using a magnetic stirrer.

Number of samples analyzed: A non-factorial completely randomized design was used in this study. A total of five treatments are carried out using the percentage ratio of instant green coffee: gambier product catechin extract: instant pasak bumi. F1 = (100:0:0), F2 = (80:15:5), F3 = (70:20:10), F4 = (60:25:15), and F5 = (50:30:20).

Number of repeated analyses: Three repetitions for each treatment factor. The total sample analysed was 15 samples.

Number of experiment replication: Each treatment was repeated 3 times.

Design of the experiment:

Instant green coffee

Green coffee beans were dried to a moisture content of 12% and ground using a grinder. The powder was filtered using an 80-mesh sieve, after which water was added at a temperature of 100 °C and a ratio of 1:2, stirred, left for 10 minutes, and later filtered using a filter cloth to obtain the filtrate. Moreover, maltodextrin (10% w/w) and egg white (20% w/w) were added to the filtrate, mixed using a mixer for 10 minutes at high speed to form foam, and spread out on an aluminium pan lined with Polypropylene plastic. The mixture was dried in a carbine dryer at 60 °C for 4 hours, blended, and filtered using an 80-mesh filter to obtain a green coffee powder.

Gambier product catechin extract

The catechin extract was prepared using the maceration method. This involved blending the dried gambier sticks until smooth and sieved through an 80-mesh sieve. The 100g gambier powder was macerated using ethanol for 1 day (24 hours) at a ratio of 3:1. Moreover, the catechin extract was filtered using Whatman filter paper No. 41 and evaporated at 85 °C with a rotary vacuum evaporator to vaporise the ethanol and remove the aroma. The catechin extract was later dried using an oven at a temperature of 85 °C for approximately 20 hours, blended, and sifted again.

Instant pasak bumi powder production

The instant pasak bumi powder was prepared. This involved the filtration of the powder using an 80-mesh sieve after which water was added at 1:2 and a temperature of 100 °C; the mixture was stirred, left for 10 minutes, and filtered again using a filter cloth to obtain the pasak bumi filtrate. Moreover, maltodextrin (10% w/w) and egg

white (20% w/w) were added to the filtrate, mixed using a mixer for 10 minutes at high speed to form foam, and spread out on an aluminium pan lined with Polypropylene plastic. The mixture was dried in a carbine dryer at a temperature of 60 °C for 4 hours, blended, and filtered using an 80-mesh filter to obtain a green coffee powder.

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

Water content

The water content of the functional instant green coffee produced ranged from 3.84 to 4.81% with the highest and lowest recorded in F5 and F1 treatments respectively as indicated in the following Figure 1.

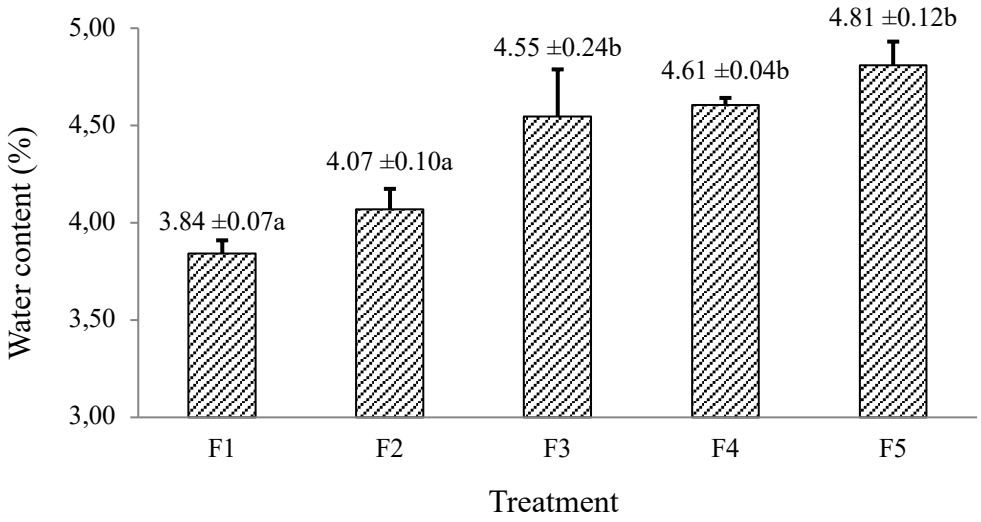

Figure 1 Effect of formulation on the water content of functional instant green coffee. Note: F1 = 100% green coffee instant: 0% gambir catechin extract: 0% instant pasak bumi; F2 = 80% green coffee instant: 15% gambir catechin extract: 5% instant pasak bumi; F3 = 70% green coffee instant: 20% gambir catechin extract: 10% instant pasak bumi; F4 = 60% green coffee instant: 25% gambir catechin extract: 15% instant pasak bumi; F5 = 50% green coffee instant : 30% gambir catechin extract: 20% instant pasak bumi.

The diversity analysis in Figure 1 showed that the formulation treatment significantly affects the water content of functional instant green coffee. Moreover, the F3 treatment with 20% gambier catechin extract and 10% pasak bumi was observed to have increased the water content. This is associated with the fact that the catechin extract and pasak bumi contain phenolic compounds with a hydroxyl group (OH) that can bind water. It is also important to note that the existence of more OH groups usually leads to more water being bound. Meanwhile, the water content in foodstuffs comprises both bound and free water.

This instant coffee fulfils the quality requirements of the Indonesian National Standard (SNI) No. 2983 of 2014 which states that the maximum water content is 5%. The values obtained in this research were observed to be higher than the 1.57 – 1.61% reported by **[21]** for instant coffee from Tungkal Jambi and the 2.34% by **[39]** for cold-brewed instant coffee. Meanwhile, the values are in the same range as 4.4.% found by **[15]** for instant coffee produced from micro-size coffee combined with *Bacillus coagulans*.

Soluble Speed

This is one of the quality requirements for instant coffee according to SNI No. 2983 of 2014, which is set at a maximum of 30 seconds. The values obtained in this research were between 26.78 – 29.33 seconds, as indicated in Figure 2 and this means the requirements are satisfied. Meanwhile, the values are higher than the 152.26 seconds **[19]** for instant coffee made from robusta coffee incorporating maltodextrin but lower than the 11.48 – 13.95 seconds reported by **[28]** while studying instant robusta with coconut sugar and cane sugar.

The diversity analysis showed that the formulation treatment significantly affects the soluble speed of functional instant green coffee. A higher concentration of gambier catechin extract in the formulation was found to cause a reduction in the soluble speed as indicated in Figure 2. This is because the catechin compounds in gambier products are semi-polar and a higher concentration of catechin usually leads to higher semi-polar nature of instant coffee, thereby, causing a reduction in the solubility of the product in water. This phenomenon was also reported in **[24]**.

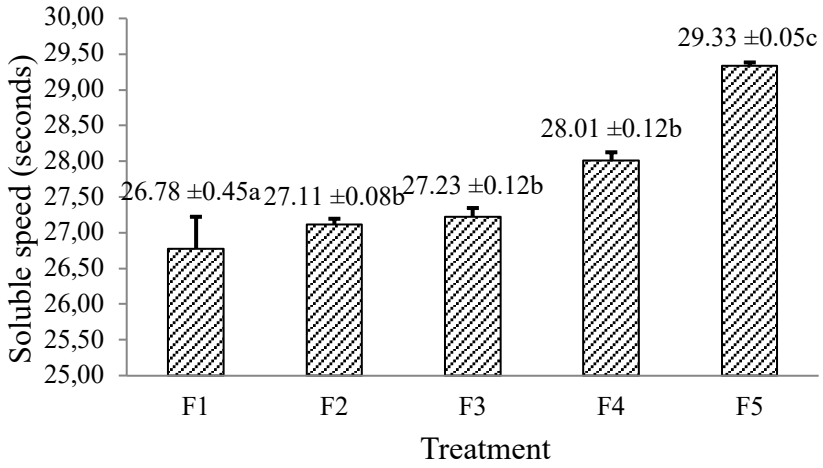

Figure 2 Effect of formulation treatment on the soluble speed of functional instant green coffee.

Total Phenol

The total phenol of the functional instant green coffee produced ranged from 16.79 to 169.48 mg/L as indicated in Figure 3.

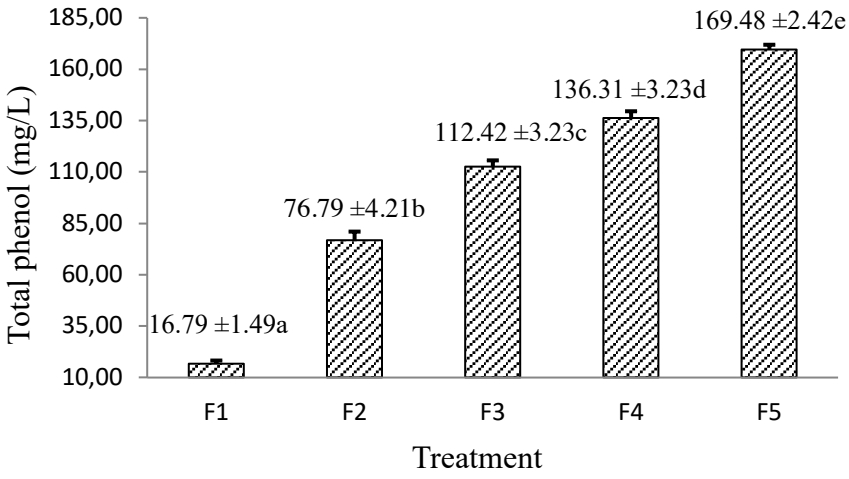

Figure 3 Effect of formulation treatment on total phenol of functional instant green coffee.

These values are slightly lower than 171.633 mg/L reported by **[4]** and higher than 16.26 – 30.65 mg/L and 42.4 – 59.8 mg/L recorded by **[32]**, **[33]** and **[5]**, respectively. However, this coffee has a total phenol content similar to the results of research by **[9]**, which is 29.23 – 158.19 mg/mLGAE, **[22]** regarding cinnamon coffee of 34.46 mg/mLGAE, oven-roasted coffee, which is 16 – 66 mg/mLGAE **[1]**, famous brand coffee circulating in Indonesia is 46.27 mg/mLGAE **[16]** and roasted arabica coffee is 49.90 mg/mLGAE **[23]**. Compared with the research of **[6]**, this total phenol is much lower, i.e. unroasted coffee contains 208.89 mg/mLGAE of total phenol and 119.22 mg/mLGAE in roasted coffee.

The diversity analysis showed the significant effect of the formulation treatment on the total phenol of functional instant green coffee. It was discovered that a higher concentration of gambier catechin extract and pasak bumi in

the formulation increased the total phenol. This is, therefore, associated with the polyphenolic compounds in the catechin extract and pasak bumi. The result is in line with the findings of **[20]** and **[29]** that gambier contains polyphenol compounds in the form of catechins by 50%. In comparison, **[40]** found phenolic compounds of catechins and tannins at 65.6 – 74.2% and 11.32 – 17.76%, respectively. Moreover, **[10]** showed that pasak bumi contains several secondary metabolites: alkaloids, terpenoids, sterpenoids, steroids, flavonoids (phenols), and saponins.

Antioxidant Activity

The antioxidant activity of functional instant green coffee was measured using IC_{50} such that a higher IC_{50} value indicates lower antioxidant activity and vice versa. The values were observed to be from 44.68 – 207.59 ppm as shown in Figure 4, and are the same as the findings of **[25]** that the encapsulated green coffee extract has 87.65 ppm and **[39]**, which showed that green coffee brewed with cold water has 71.97 – 83.21 ppm. However, the values are higher than the 25.187 ppm reported for green coffee extract dried using the foam mat method by **[26]**, **[27]**, and **[18]** reported that robusta green coffee contains antioxidants with an IC_{50} of 81.6 µg/mL and lower than 167.426 to 294.710 ppm recorded for green coffee from Ethiopia by **[35]** and **[12]** reported that robusta coffee contains antioxidants with an IC_{50} of 2210 µg/mL.

The diversity analysis showed that the formulation treatment significantly affects the IC_{50} of functional instant green coffee, as indicated in Figure 4. This was observed because a higher concentration of gambier catechin extract and pasak bumi powder in the formulation caused a reduction in the IC_{50} and a higher antioxidant activity. This is associated with flavonoid compounds that are considered antioxidants in the gambier catechin extracts and pasak bumi powder. Moreover, it also indicates consistency with the total phenol data recorded in Figure 3, which showed the same trend. Phenol is also an antioxidant, which means a higher content of this compound can increase the antioxidant properties of the product, as indicated by a decrease in IC_{50}.

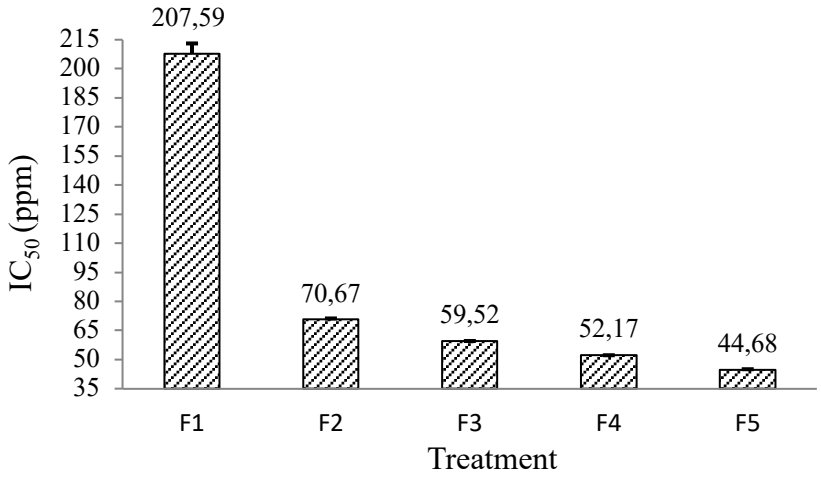

Figure 4 Effect of formulation treatment on IC_{50} of functional instant green coffee.

CONCLUSION

Added catechin extract of gambier and pasak bumi in instant green coffee significantly increases total phenol content and IC_{50}. Besides that, there was also a change in the physical properties of instant green coffee, namely an increase in water content and speed of dissolving. The functional instant green coffee produced has a water content value of 3.84 – 4.81%, soluble speed of 26.78 – 29.33 s, total phenol of 16.79 – 169.48 mg/L and an IC of IC_{50} of 44.68 – 207.59 ppm.

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

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Mixing of flour mixture components in the production of pasta from nontraditional raw materials

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ABSTRACT

Along with a balanced amino acid composition and high protein digestibility, food products should contain complex carbohydrates and ballast substances (dietary fibers) that ensure the normal functioning of the digestive organs. In this regard, fresh raw foods for dietary pasta are of interest for modern pasta production. It is to such raw materials for the manufacture of dough that flour and starch from some cereals, triticale flour, and stale deformed bread are referred. When forming pasta dough from nontraditional flour raw materials, an important technological value for giving the best rheological properties of the dough is the uniform distribution of the binder component in the mixture – dry wheat gluten (DWG). As an object of experimental research to study the mixing process, a loose flour mixture of cereals (barley, oats, corn, buckwheat, millet, peas, and soybeans) was used, compiled based on calculating the recipe using computer programs. The prepared mixtures are a valuable source of nutrients and minerals. At the same time, to improve the technological properties of the dough as a biologically active additive, 25% DWG is introduced to improve the rheological properties of the dough.

Keywords: dry wheat gluten, flour mixture, mixing, pasta, pasta dough recipe

INTRODUCTION

In the healthy nutrition policy, much attention is paid to nutrition physiology. Pasta made from nontraditional raw materials, in comparison with other types of flour products, will have several advantages: high digestibility of basic nutrients, high consumer properties (each category of persons can satisfy their taste needs), long shelf life, low cost and accessibility for all segments of the population **[1]**, **[2]**, **[3]**, **[4]**. However, such products are not produced in our country, and manufacturers must use baking flour from soft wheat, the protein of which has a deficiency of the most important essential amino acids **[5]**, **[6]**, **[7]**. At the same time, the further development of pasta production will be directed towards expanding the range for the use of new types of raw materials, such as nontraditional poly-cereal raw materials, as well as improving the technological processes of mixing, pressing, forming the dough, drying, and cooking pasta **[8]**.

Therefore, pasta production based on nontraditional poly-cereal raw materials is one of the promising directions for creating functional products.

Scientific Hypothesis

The main scientific hypothesis is to increase the nutritional value and consumer properties of traditional pasta by using nontraditional poly-cereal raw materials; develop a mathematical model of the process of mixing poly-cereal flour components; study the main technological parameters of the process of making pasta of increased nutritional and biological value based on nontraditional poly-cereal raw materials, depending on the proposed formulations.

MATERIAL AND METHODOLOGY

Samples

As an object of experimental research to study the mixing process, a loose flour mixture of cereals (barley, oats, corn, buckwheat, millet, peas, and soybeans) was used, compiled based on calculating the recipe using computer programs.

Chemicals

The chemical composition (mass fraction of protein, starch and fiber content, ash content) was determined using the Infrascan-105 "KAN" analyzer.

Animals and Biological Material

The animal and biological materials weren't used in this research.

Instruments

The Chopin Alveograph (France), Farinograph-AT company Brabender (Germany), Case drying SESh-3M (Russian Federation), and The Infrascan-105 "KAN" analyzer (Russian Federation) were used in this research.

Laboratory Methods

Experimental studies on the processes of mixing flour mixture components, pressing pasta dough, and drying finished pasta were carried out under the conditions of the International Research Center "Technology of Food and Processing Industries" of the Kazakh National Agrarian Research University; LLP "Kazakh Research Institute of Processing and Food Industry" and the Astana branch of LLP "Kazakh Research Institute of Processing and Food Industry".

The methodology and materials for assessing the quality and technological properties of poly-cereal raw materials in pasta production are described below.

Evaluation of quality, technological properties, and determination of Kazakhstan's food safety indicators of grain raw materials was carried out following the requirements of existing GOST standards.

Poly-cereal mixtures for the production of a promising range of pasta based on nontraditional raw materials were made following the results of automated calculation using our software **[7]**, **[20]**, **[22]**. This software is designed to compound composite mixtures from whole grains of cereals and legumes based on flour.

Study of the rheological properties of the test. The main technological properties that determine the efficiency of the technological process of pressing pasta dough are elastic-plastic properties: viscosity, elasticity, and extensibility of the dough. In this regard, we studied the rheological properties of pasta dough made in accordance with the developed recipe based on poly-slag raw materials. The prepared mixture will differ in terms of the properties studied. In this regard, to give the test the best elastic-plastic properties, dry wheat gluten was introduced into the formulation to improve the binding of all components introduced into the mixture formulation.

Determination of the rheological properties of flour using Chopin's Alveograph (France). The essence of the method consists of kneading the dough at constant humidity and a solution of sodium chloride, after which the dough is installed in a special compartment of the Alveograph, where the extensibility of the dough is determined during the inflating of dough balls, and automatic plotting with curves showing the rheology of flour.

Determination of water absorption and rheology of flour. The study was carried out on a Brabender Pharynograph (Germany). The principle of operation is to mix the dough in a dough mixer with the addition of the necessary amount of water to obtain the desired consistency. The results of the study were recorded in the observation log.

On the Farinograph device, water absorption, the dough's formation time, the dough's stability, the degree of dilution of the dough, and the degree of quality according to the Farinograph were also studied.

The production of pasta was carried out on a laboratory screw pasta press.

The study of pasta and the kinetics of drying was carried out following the technological process of pasta production after pressing the pasta dough.

The method of experimental research was as follows. The selected pasta sample was sent for drying in the drying cabinet SESh-3M at the temperatures of the drying agent at 40, 50, and 60 °C and at intervals of every 5 minutes, the humidity values of the experimental suspension of pasta were determined. In the course of experimental studies, the time (duration) of the convective effect of the drying agent on pasta, and the mass fraction of moisture of the experimented product was recorded. Then, on the data obtained, diagrams and drying kinetic curves were constructed, which characterize the drying process of pasta.

The assessment of the quality of consumer properties of pasta was carried out following GOST R 51865-2002, according to which the assessment is descriptive. It also provides a score analysis of the quality of pasta, which facilitates a comparative assessment of products, more objectively reflecting their consumer advantages and quality changes during their long-term storage.

The evaluation of the developed batch of pasta was carried out according to the following indicators: appearance, colour, smell, taste, consistency, and state of the cooking water. Each indicator is characterized by five to six descriptive categories with the assignment of these points. The total maximum score is 100 points.

Description of the Experiment

Sample preparation: To conduct further experimental studies to substantiate the technology of pasta production based on nontraditional grain raw materials, raw materials of plant origin have been identified as the object of research, which can be conditionally divided into three groups:

- flour mixtures (3 recipes), compiled according to a scientifically based recipe and compiled based on the calculation of the recipe using computer programs;
- pasta dough (3 types), obtained based on nontraditional poly-cereal raw materials;
- pasta (3 types) from dough made based on the developed recipe.

To form a uniform and homogeneous flour mixture, the kinetics of mixing was studied on a laboratory kneading machine.

Number of samples analyzed: We analyzed 36 samples.

Number of repeated analyses: Repeated analyses 9.

Number of experiment replication: Triple.

Statistical Analysis

Experimental studies were conducted to study the effect of the duration of kneading pasta dough on the rheological properties of the dough according to the following indicators: the dependence of the elasticity of the dough (P, mm×H_2O); the extensibility of the dough (L, mm); specific work (W, e.a.); the coefficient of elasticity (Ie, %) on the rotational speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of pasta dough from nontraditional raw materials, compiled according to recipes No.1, No.2, No.3 and a control sample of pasta dough from wheat flour of the 1st grade. Conventional and special methods of mathematical statistics, methodical approach, and methods of comparative analysis were used to conduct experimental studies.

RESULTS AND DISCUSSION

When forming pasta dough from nontraditional flour raw materials, an important technological value for giving the best rheological properties of the dough is the uniform distribution of the binder component in the mixture – dry wheat gluten (DWG) **[7]**, **[9]**, **[10]**. In general, the efficiency of the mixing process depends on the design, technological and kinematic parameters: **[11]**, **[12]**, **[13]**:

$$E_c = \Psi\left(K_3 \frac{W_1 \ldots W_{n-1}}{W_n} \cdot t \cdot n \right) \quad (1)$$

Where:

K_3 is the filling factor; $W_1 \ldots W_{n-1}$, W_n is the ratio of components in the mixture; n – is the rotation frequency of the working body; t – is the mixing time.

Applying probability theory to the kinetics of mixing and emulsification, the magnitude of the interface at a certain time will be equal to **[11]**, **[12]**, **[13]**:

$$S = S_p\left(1 - e^{-tc}\right) \quad (2)$$

Where:

S is the size of the interface; t – is the mixing time; S_p – is the maximum possible surface; $(1 - e^{-tc})$ – maximum possible partition surface; $c = \ln \frac{1}{(1-\varepsilon)}$.

Equations (1 and 2) are valid for all mixing systems since when all devices work, the goal is to increase the interface of the phases. It is possible to move the components of the interface to the individual components of the loading volume **[11]**, **[12]**, **[13]**.

The part of the total number of elementary volumes, which consists of equal volumes containing at least one of the elements of the surface, the section obtained by mixing over time is determined by the formula **[11]**, **[13]**.

$$P_\tau = 1 - e^{-RS_p\left(1-e^{-\pi}\right)} \tag{3}$$

Where:
R is the proportionality coefficient.

The value of the fraction of the total number of volumes V, which consists of volumes V_o containing one of the components of the mixture, is determined by the formula **[11]**, **[12]**, **[13]**:

$$\left(P_t\right)_E = 1 - \left[e^{-RS_p\left(1-e^{-\pi}\right)}\right]^{\frac{V}{V_0}} \tag{3}$$

Where:
V is the total volume of the mixture; V_o is the volume of samples taken from the mixture.

Thus, in the case when $(P_t)_E$ is taken as the final value for a satisfactory mixing result, the desired time can be obtained by solving equation (3) relatively **[10]**, **[17]**.
In practice, the mixing efficiency is estimated by the coefficient of variation of the distribution of the key component in the micro-volumes of the mixture **[11]**, **[12]**, **[13]**:

$$V_c = \frac{100}{x}\sqrt{\frac{\sum_{i=1}^{i=n}(x_i - x)^2}{n-1}} \tag{4}$$

Where:
x is the arithmetic mean of the quantity values, that is, the average content of the key component in the samples; x_i is the value of a random variable in the i-th experiment; n is the number of samples.

At the same time, statistical methods are used to predict the quality of mixing **[14]**, **[15]**.
The process of mixing a multicomponent bulk mixture is a probabilistic process, the study of which involves statistical methods and probability theory. Fischer F. K. gives the diffusion mixing equation for a horizontal cylindrical mixer **[16]**, **[17]**, **[18]**, **[19]**:

$$\frac{dW}{dt} = D_o\frac{d^2W}{dz} + D_2\left(\frac{d^2}{dr} + \frac{1}{r} + \frac{dW}{dr}\right) \tag{5}$$

Where: W is the probability distribution density, meaning the particle concentration; D_0 and D_z are the coefficients of axial and radial diffusion; r and z are the distance in the radial and axial directions; t is the mixing time.

The longitudinal mixing of particles obeys the following law **[16]**, **[17]**, **[18]**, **[19]**:

$$\frac{dc}{dt} = -\omega\frac{dc}{dx} + D_l\frac{d^2c}{dx^2} \tag{6}$$

Where:
D_l is the coefficient of longitudinal mixing.

The model is called two-component if mixing occurs simultaneously in the longitudinal and transverse directions **[16]**, **[17]**, **[18]**, **[19]**:

$$\frac{dc}{dt} = -\omega \frac{dc}{dx} + D_l \frac{d^2c}{dx^2} + \frac{Dr}{r} \cdot \frac{dc}{dr}\left(r \frac{dc}{dr}\right) \quad (7)$$

Where:
r is the radius of the apparatus; D_r is the coefficient of transverse mixing.

Due to various physical and mechanical properties, the mixing of bulk components is simultaneously accompanied by the opposite process to the above – segregation of the finished mixture. Segregation is the concentration of particles having a similar characteristic (mass, size, shape, etc.) under the influence of gravitational and inertial forces. The end of the mixing process must be established at the moment when the phenomenon of segregation has not begun to manifest itself **[12]**, **[20]**.

To better represent the physical picture of mixing, a graph of the dependence of the coefficient of variation (V_c) on the mixing time (t) is plotted. The curve characterizing the mixing process is called the "mixing curve". The analysis of the mixing kinetics (Figure 1) shows the presence of three zones **[7]**, **[22]**:
- I zone – zone of intensive mixing as a result of shear and convective processes;
- II zone – zone of delayed diffusion mixing;
- III zone – is a manifestation of the segregation process, increasing the coefficient of variation.

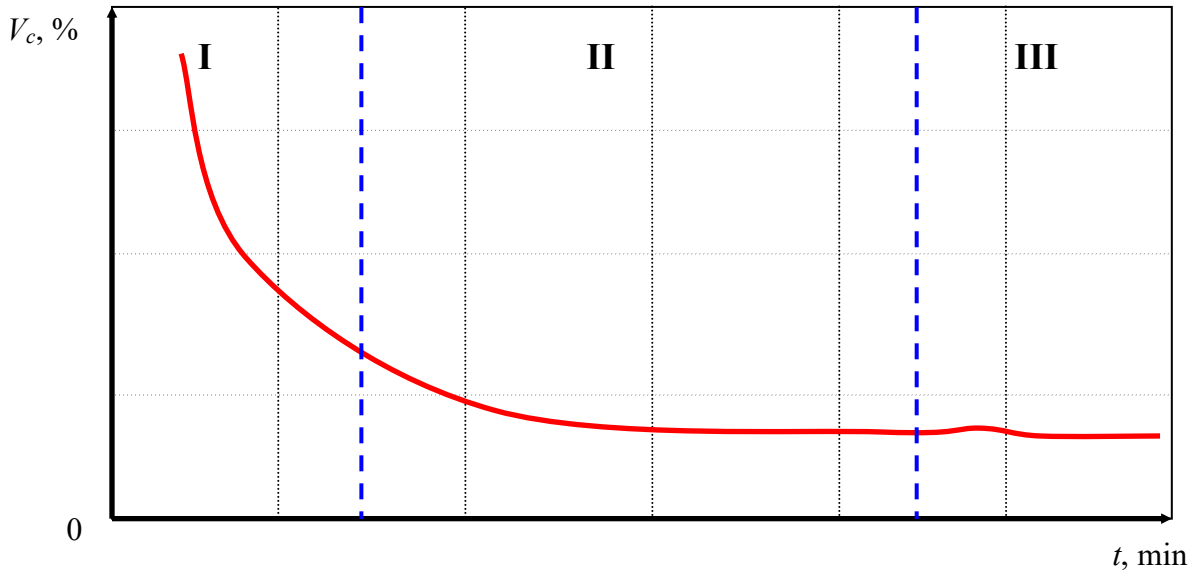


Figure 1 Kinetics curve of mixing of bulk components in a mixture.

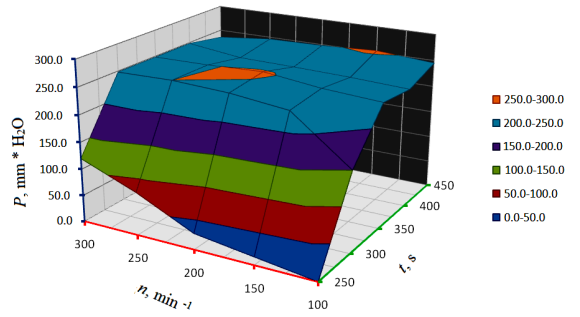
Moreover, unlike diffusion mixing, the first two processes (shear and convective) do not depend on the characteristics of the mixed components. During the mixing of the components, the one at which $V_c \rightarrow$ min should be taken **[7]**, **[22]**. Given this physical picture of mixing, it is necessary to distinguish between two main parameters – the quality of the process and the duration of the operation until the specified quality is achieved.
The above theoretical prerequisites for mixing the components were laid down as the basis for several experimental works. To conduct experimental studies on the study of the dough kneading process, a recipe for pasta made from nontraditional raw materials was developed – No. 1, No. 2, and No. 3. The calculated indicators of the selected recipes are indicated in Table 1. **[20]**, **[21]**, **[22]**.

Experimental studies were conducted aimed at studying the effect of the duration of kneading pasta dough on the rheological properties of the dough according to the indicators: dough elasticity (P, mm×H_2O), dough extensibility (L, mm), specific work (W, e.a.), elasticity coefficient (Ie, %), at fixed values of the rotational speed of the working organ (n, min^{-1}) and the time spent on the process of kneading pasta dough (t, sec). Based on experimental data, a graph was constructed of the dependence of the change in the values of the rheological properties of the pasta dough on the rotation frequency of the working organ and the time spent on kneading the pasta dough **[7]**, **[22]**.

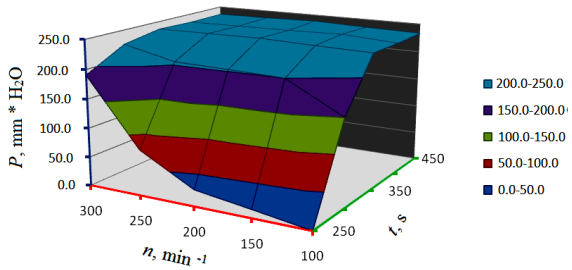
Table 1 Recipe of the flour mixture for the production of pasta dough +25% DWG.

Name of raw materials	Values, %	Estimated nutritional value of the mixture
Recipe No1		
corn	33.3333333333333	protein – 18.028%;
oats	33.3333333333333	starch – 60.256%;
millet	16.6666666666667	fiber – 8.076%;
soy	16.6666666666667	fats – 8.61%;
		ash – 3.664%;
		energy value – 405.847 kcal
Recipe No2		
corn	50.0	protein – 17.824%;
oats	16.6666666666667	starch – 63.076%;
buckwheat	16.6666666666667	fiber – 6.684%;
soy	16.666666666666	fats – 8.348%;
		ash – 2.946%;
		energy value – 408.028 kcal
Recipe No3		
barley	16.0	protein – 18.5%;
corn	25.0	starch – 56.7%;
oats	15.0	fiber – 13.23%;
buckwheat	27.3	fats – 7.76%;
peas	16.7	ash – 5.34%;
		energy value – 406.07 kcal

Figure 2 shows the three-dimensional dependence of the change in the elasticity values of the dough (P, mm×H_2O) on the rotation speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of the pasta dough from unconventional raw materials compiled according to experimental recipes No. 1 – No. 3 and a control sample of pasta dough.



recipe No. 1



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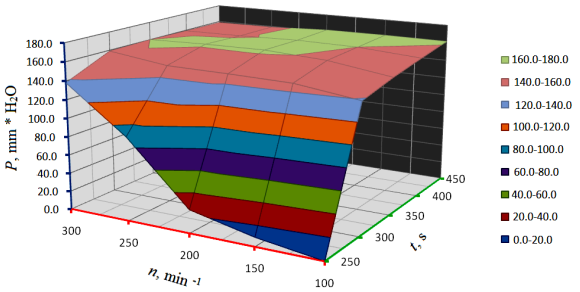

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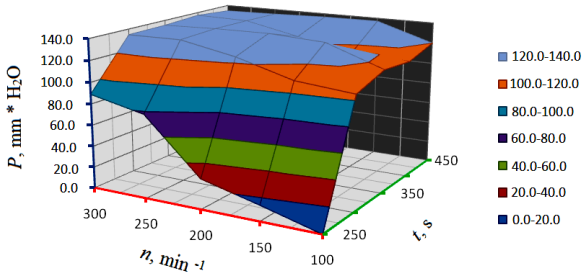


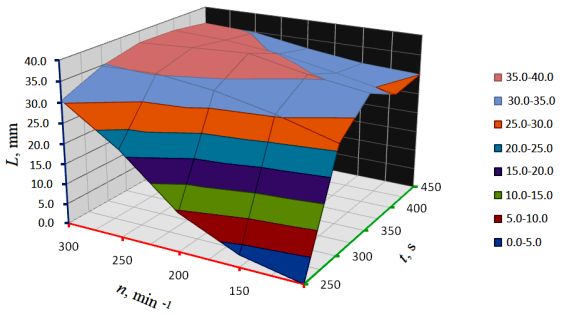
control sample of pasta dough from wheat flour of the 1st grade

Figure 2 Three-dimensional dependence of the change in the elasticity values of the dough (P, mm×H_2O) on the rotation speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of pasta dough from nontraditional raw materials.

The analysis of the three-dimensional dependencies of the kneading of the pasta dough showed that with an increase in the rotation speed of the working body of the kneading unit and the processing time of the pasta dough of the loose flour mixture, the values of the elasticity of the dough increase to specific values. Similar dynamics were observed in all formulations No. 1 – No. 3 and a control sample of pasta dough made based on wheat flour of the 1st grade. At the same time, prolonged processing of the pasta dough leads to a decrease in the experimental values of the elasticity of the dough (P, mm×H_2O), since prolonged processing of the object of study leads to the destruction of the structure of the pasta dough, thereby reducing the experimental values. The maximum values of the dough elasticity were reached at n = 200 min^{-1} and the duration of kneading t = 350 sec. The minimum values of the elasticity of the dough were observed at n = 100 min^{-1} and the time of kneading the dough t <200 sec.

As a result of experimental studies conducted to study the rheological properties of pasta dough from nontraditional flour raw materials in terms of the elasticity of the dough (P, mm×H_2O), it was found that the best characteristics were pasta dough made according to recipe No1 **[7]**, **[22]**. For example, the maximum elasticity values of pasta dough were 253.0 mm×H_2O with a kneading duration of 350 seconds and a rotation speed of n = 200 min^{-1}. At the same time, the value of the elasticity index of the control sample of pasta dough based on wheat flour of the 1st grade was 137.0 mm×H_2O with a kneading duration of 350 seconds and a rotation speed of n = 200 min^{-1}. The maximum elasticity values of pasta dough No. 2 and No. 3 of the sample were 238.0 mm ×H_2O and 162.0 mm×H_2O, respectively, with a kneading duration of 350 seconds and a rotation speed of n = 200 min^{-1}.

Next, three-dimensional surfaces were constructed to change the values of dough extensibility (L, mm) from the rotation speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of pasta dough from nontraditional raw materials, compiled according to recipes No. 1 – No. 3 and a control sample of pasta dough from wheat flour of the 1st grade (Figure 3).



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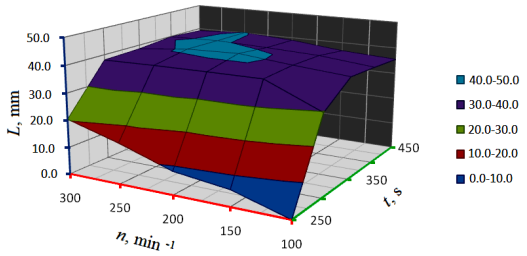

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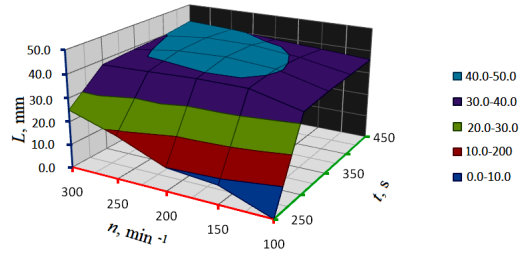

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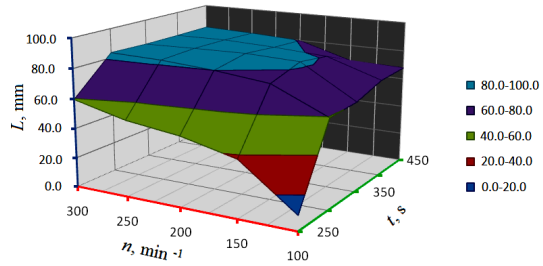


control sample of pasta dough from wheat flour of the 1st grade

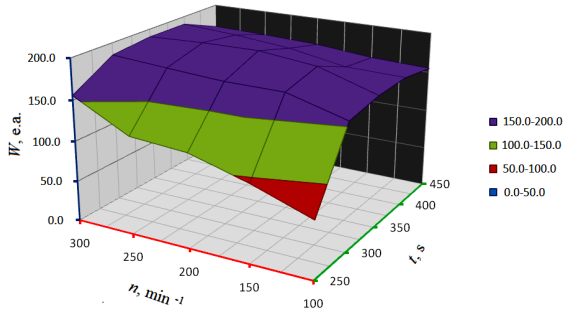
Figure 3 Three-dimensional dependence of the change in extensibility values (L, mm) on the rotation speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of pasta dough from nontraditional raw materials.

The analysis of the three-dimensional dependencies of the kneading of the pasta dough showed that with an increase in the rotation speed of the working organ of the dough mixing plant and the processing time of the

pasta dough, the values of the extensibility of the dough increase to the maximum values. Similar dynamics were observed in all formulations No. 1 – No. 3 and a control sample of pasta dough made based on wheat flour of the 1st grade. At the same time, prolonged processing of the pasta dough leads to a decrease in the experimental values of extensibility (L, mm), since prolonged processing of the object of study leads to the destruction of the gluten structure in the pasta dough, thereby reducing the experimental values. The maximum values of the elasticity of the dough were reached at n = 200 min^{-1} and the duration of kneading t = 350 sec. The minimum values of the test extensibility were observed at n = 100 min^{-1} and the time of kneading the dough t <200 sec.

As a result of the experimental studies conducted to study the rheological properties of pasta dough from nontraditional flour raw materials in terms of the extensibility of the dough (L, mm), it was found that the control sample of pasta dough made based on wheat flour of the 1st grade had the best characteristics **[7]**, **[22]**. The maximum values of the extensibility of the control sample of the pasta dough were 84.0 mm with a kneading duration of 350 seconds and a rotation speed of the working body of the kneading unit n = 200 min^{-1}. At the same time, the minimum values of the extensibility index of 38.5 mm corresponded to the pasta dough compiled according to recipe No1 at the average values of the rotation speed of the working body of the kneading unit n = 200 min^{-1} and the duration of kneading t = 350 sec. The maximum values of the extensibility index of the pasta dough in test samples No. 2 and No. 3 were 41.0 and 42.2 mm, respectively.

Next, three-dimensional surfaces were constructed to change the values of specific work (W, e.a.) from the rotation frequency of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of pasta dough from unconventional raw materials compiled according to recipes No. 1 – No. 3 and a control sample of pasta dough from wheat flour of the 1st grade (Figure 4).



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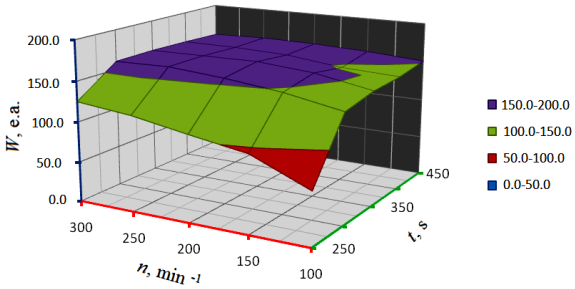

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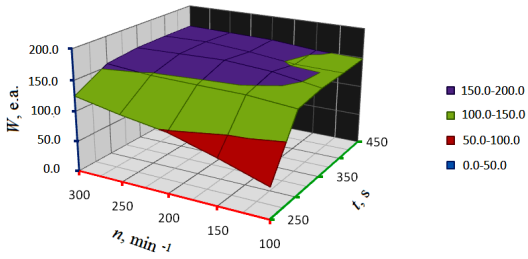

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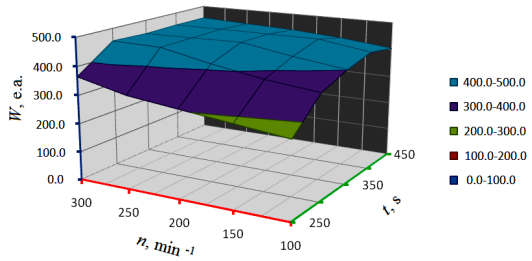


control sample of pasta dough from wheat flour of the 1st grade

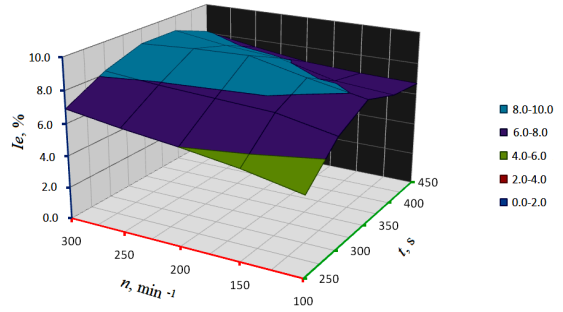
Figure 4 Three-dimensional dependence of the change in the values of specific work (W, e.a.) on the rotation speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of the control sample of the pasta dough.

The analysis of the three-dimensional dependencies of the kneading of the pasta dough showed that with an increase in the rotation speed of the working body of the dough mixing plant and the processing time of the pasta dough, the values of the specific work of the dough increase to certain values. The physical pattern of changes in the values of W (e.a.) is identical in all formulations No. 1 – No. 3 and the control sample of pasta dough from wheat flour of the 1st grade. At the same time, prolonged processing of the pasta dough leads to a

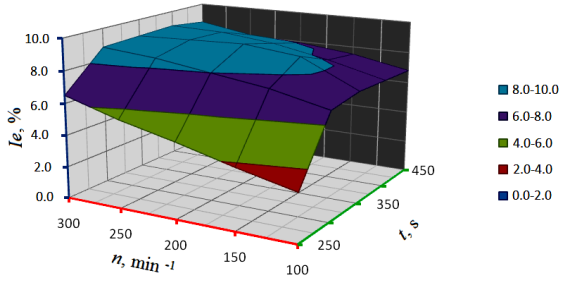
decrease in the experimental values of the specific work (*W*, e.a.), since prolonged processing of the object of study leads to the rupture of gluten, and hence the structure of the pasta dough, thereby slightly reducing the experimental values of the rheological properties of the pasta dough. The maximum values of the specific work of the test were reached at n = 200 min^{-1} and the duration of kneading t = 350 sec. The minimum values of the elasticity of the dough were observed at n = 100 min^{-1} and the time of kneading the dough t <200 sec.

As a result of the experimental studies conducted to study the rheological properties of pasta dough from nontraditional flour raw materials in terms of the specific work of the dough (*W*, e.a.), it was found that the control sample of pasta dough made based on wheat flour of the 1st grade had the best characteristics **[7], [22]**. For example, the maximum value of the specific work of the pasta dough was 455.0 units (e.a.) with a kneading duration of 350 seconds and a rotation speed of n = 200 min^{-1}. At the same time, the test's specific work index values for experimental samples No. 1, No. 2, and No. 3 were 193.0, 162.0, and 164.0 e.a. respectively, with a kneading duration of 350 seconds and a rotation speed of n = 200 min^{-1}.

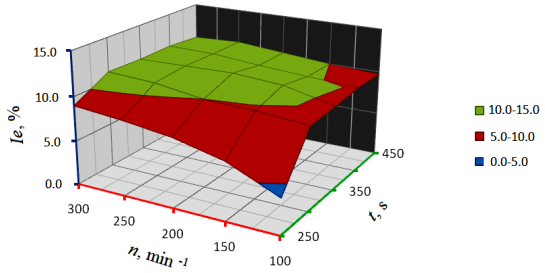
Next, three-dimensional surfaces were constructed to change the values of the elasticity coefficient (*Ie*, %) from the rotation speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of pasta dough from nontraditional raw materials compiled according to recipes No. 1 – No. 3 and a control sample of pasta dough from wheat flour of the 1st grade (Figure 5).



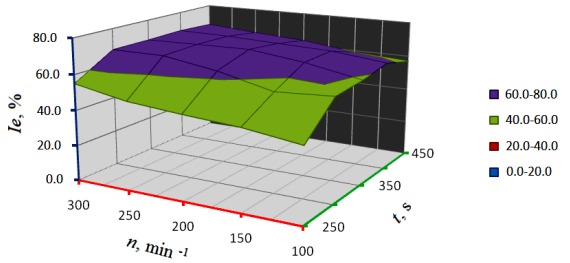
according to recipe No.1



according to recipe No. 22



according to recipe No. 33



according to the control sample of pasta dough from wheat flour of the 1st grade

Figure 5 Three-dimensional dependence of the change in the values of the elasticity coefficient (*Ie*, %) on the rotation speed of the working body (n, min^{-1}) of the kneading machine and the time spent on the kneading process (t, sec) of pasta dough from nontraditional raw materials.

The analysis of the three-dimensional dependencies of the pasta dough kneading showed that with an increase in the rotation speed of the working body of the dough mixing plant and the processing time of the pasta dough, the values of the elasticity coefficient increase to maximum values. Similar dynamics were observed in all formulations No. 1 – No. 3 and a control sample of pasta dough made based on wheat flour of the 1st grade. At the same time, prolonged processing of the pasta dough leads to a slight decrease in the experimental values of the elasticity coefficient (*Ie*, %), since prolonged processing of the object of study leads to the destruction of the structure of the pasta dough, thereby reducing the experimental values of rheological properties. The maximum value of the elasticity coefficient (*Ie*, %) was achieved at n = 200 min^{-1} and the duration of kneading t = 350 sec. The minimum values of the elasticity coefficient of the dough were observed at n = 100 min^{-1} and the duration of kneading the dough t <200 sec.

As a result of experimental studies conducted to study the rheological properties of pasta dough from nontraditional flour raw materials in terms of the coefficient of elasticity (*Ie*, %), it was found that the best characteristics were pasta dough made according to recipe No. 1 **[7]**, **[22]**. For example, the maximum value of the coefficient of elasticity of a control sample of pasta dough based on wheat flour of the 1st grade was 68.9%, with a kneading duration of 350 seconds and a rotation speed of $n = 200$ min^{-1}. At the same time, the values of the coefficient of elasticity of the pasta dough of experimental samples No. 1, No. 2, and No. 3 were within the same limits. They amounted to 9.3%, 9.1%, and 11.2%, respectively, with a kneading duration of 350 seconds and a rotation speed of $n = 200$ min^{-1}. The minimum values were 4.8%, 3%, and 4% for experimental samples No. 1, No. 2, and No. 3 with a kneading duration of 250 seconds and a rotation speed of $n = 100$ min^{-1}.

Thus, according to the results of the experimental studies, it was established (Figure 2) that the maximum value of elasticity (*P*, mm×H_2O) of pasta dough is 253.0 mm×H_2O with a kneading duration of 350 seconds and a rotation speed of $n = 200$ min^{-1}. The value of the elasticity index for the control sample of pasta dough based on wheat flour of the 1st grade was 137.0 mm×H_2O with the same kneading duration and rotation frequency. The maximum elasticity values of pasta dough No. 2 and No. 3 of the sample were 238.0 mm×H_2O and 162.0 mm×H_2O, respectively, with a kneading duration of 350 seconds and a rotation speed of $n = 200$ min^{-1}.

The results of the study of the rheological properties of the pasta dough from nontraditional flour raw materials in terms of the extensibility of the dough (*L*, mm) showed (Figure 3) that the control sample of the pasta dough had the best characteristics. The maximum extensibility values of the control sample of the pasta dough were 84.0 mm with a kneading duration of 350 seconds and a rotation speed of the working body of the kneading unit $n = 200$ min^{-1}. At the same time, the minimum values of the extensibility index of 38.5 mm corresponded to the pasta dough compiled according to recipe No. 1 with the above average values of the rotation speed of the working body of the kneading unit and the duration of kneading. The maximum values of the indicator (*L*, mm) in test samples No. 2 and No. 3 were 41.0 and 42.2 mm, respectively.

The results of the study of the rheological properties of pasta dough from non-traditional flour raw materials in terms of the specific work of the dough (*W*, e.a.) showed (Figure 4) that the control sample of the pasta dough also had the best characteristics. The maximum value (*W*, e.a.) was 455.0 units with a kneading duration of 350 seconds and a rotation speed of $n = 200$ min^{-1}. For experimental samples No. 1, No. 2 and No. 3, the maximum value (*W*, e.a.) was 193.0, 162.0 and 164.0 e.a., respectively, at the above values of the kneading duration and rotation frequency.

The results of the study of the rheological properties of pasta dough from non-traditional flour raw materials in terms of the coefficient of elasticity (*Ie*, %) showed (Figure 5) that the pasta dough made according to recipe No. 1 had the best characteristics. The maximum value of the elasticity coefficient for the control sample of the pasta dough was 68.9% with a kneading duration of 350 seconds and a rotation speed of $n = 200$ min^{-1}. The maximum values of the pasta dough indicator (Ie, %) for experimental samples No. 1, No. 2, and No. 3 were 9.3%, 9.1% and 11.2%, respectively. At the same time, the kneading duration was 350 seconds at a rotational speed of $n = 200$ min^{-1}. For these samples, the minimum values of the elasticity coefficient were 4.8%, 3% and 4%, respectively, with a kneading duration of 250 seconds and a rotation speed of $n = 100$ min^{-1}.

CONCLUSION

The analysis of the theoretical foundations of the mixing process showed that during the mixing of the components, one should take the one at which the coefficient of variation $V_c \rightarrow min.$ Given this physical picture of mixing, it is necessary to distinguish between two main parameters – the process's quality and the operation's duration until the specified quality is achieved.

In existing mixers, the mixing process is carried out on the principle of a random process with the expectation of the probability of a favorable outcome, which is a significant disadvantage of mixer designs.

Analyzing the presented three-dimensional surfaces, we can confidently judge the kinetics of the process of kneading pasta dough from nontraditional flour raw materials in pasta production according to the recipes we developed. In the first stage, we observe a prolonged pasta dough formation until a thick mass is formed. Further, a uniform redistribution of all particles in the total mass was observed, while the process of water absorption by starch grains of the flour mixture is actively underway.

It should be noted that further intensification of the processing of pasta dough from nontraditional flour raw materials leads to the destruction of the protein structure, resulting in mechanical denaturation of gluten.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement:

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

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The effect of incorporation of gambier filtrate and rosella flower petals extract on mechanical properties and antioxidant activity of canna starch based active edible film

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ABSTRACT

The research objective was to analyse the incorporation effect of gambier filtrate and rosella flower petals extract on mechanical properties and antioxidant activity of canna starch-based active edible film. This research used an experimental method consisting of two treatments, namely gambier filtrate (A): A1 = 3, A2 = 4, and A3 = 5 (% v/v), as well as rosella flower petals extract (B): B1 = 2, B2 = 4 and B3 = 6 (% v/v) and each treatment was replicated three times. The results showed that the two treatment interactions significantly influenced elongation percentage, water vapour transmission rate, and antioxidant activity. The edible film's thickness, tensile strength, and water vapour transmission rate were 0.096-0.124 mm, 1.89-3.38 MPa, and 12.99-17.04 $g.m^{-2}.d^{-1}$, respectively. The edible film contains an antioxidant compound of the strong category with IC50 values of 34.53 to 48.02 ppm. Treatment of A3B2 [gambier filtrate 5% (v/v) and rosella flower petals extract 4% (v)] was the best treatment. This edible film is generally suitable for application as a packaging material for food having high lipid content to inhibit the oxidation process of that food.

Keywords: antioxidant, edible film, gambier, thickness, rosella.

INTRODUCTION

Due to chemical, biochemical and microbiological reactions, food materials will be accelerated with the existence of oxygen gas, water vapour, sunlight, and temperature. Oxygen gas and sunlight are external factors that cause rancidity reactions in food having high lipid content. To avoid this reaction, packaging materials are required that inhibit oxygen gas and sunlight and inhibit rancidity reaction with the availability of antioxidant compounds in that packaging materials. The edible film is one of the food packaging materials that can be formulated by adding antioxidant compounds from synthesis and natural materials and has barrier properties against oxygen gas and sunlight.

The study results by **[12]** showed that polysaccharides edible film incorporated with essential oil and the herbal extract could improve the mechanical properties of edible film and increase the shelf life of meat and sensory quality to increase nutritional value through inhibition of oxidation reaction. **[8]** showed that potato starch edible film added with potato skins had an antioxidant property with antioxidant activity and phenolic compound content of 24-55% and 10-22 mg $GAE.g^{-1}$, respectively. Some authors **[3]** reported that adding turmeric extract on alginate edible film could produce an edible film with an antioxidant property with a DPPH value of 38.28ppm.

Gambier extract is produced from the gambier plant (*Uncaria gambir* Roxb) by processing the leaves and young twigs using hot water, pressing liquid precipitation and drying the sediment **[22]**. Gambier extract contains a catechins compound with 98% **[23]**. Moreover, **[22]** showed that the catechins compound in gambier extract had semi-polar properties and contained compounds with antioxidant and antibacterial properties. The extract was applied to inhibit the oxidation reaction in cassava chips **[7]**. Gambier extract was also used by **[26]** in canna starch-based edible film, but its antioxidant activity was still low. Besides gambier extract, rosella

flower petals extract is produced from the flower petals of the rosella plant (*Hibiscus sabdariffa*), which are dried at 40 °C, and crushed in a blender, and extracted. Rosella flower petals extract also contains anthocyanin with a strong antioxidant property with IC50 values of 50 to 100 ppm **[5]**. Seaweed syrup added with rosella flower petals extract contains an anthocyanin compound of 0.625 g.100 mL^{-1} **[13]**.

Edible film development conducted by researchers currently continues to increase from year to year through the use of natural materials containing antioxidant and antibacterial properties such as curcumin **[24]**, black chokeberry extract **[15],** and some plants extract containing phenolic compounds **[31]**. But until now, no edible film is incorporated with two natural materials with antioxidant properties such as gambier filtrate and rosella flower petals extract. This research objective was to analyse the incorporation effect of gambier filtrate, and rosella flower petals extract on mechanical properties and antioxidant activity of canna starch-based active edible film.

Scientific Hypothesis

The addition of gambir catechin extract has a significant effect on increasing the functional properties of edible film.

MATERIAL AND METHODOLOGY

Samples

The edible film is made from biopolymer materials such as canna starch, glycerol, and CMC with incorporated gambier filtrate and rosella flower petals extract.

Chemicals

Olive oil from PT HNI, Indonesia, carboxymethyl cellulose (CMC), 2,2–diphenyl-1-picrylhydrazyl (DPPH), and nutrient agar (NA) obtained from the Laboratory of Chemical Agricultural Products, Faculty of Agriculture, Sriwijaya University, Indonesia.

Biological Material

Gambier (*Uncaria gambir* Roxb) extract from Babat Toman Village, Banyuasin District, South Sumatra, Indonesia. Rosella (*Hibiscus sabdariffa*) flower petals from PT HNI Indonesia. Canna (*Canna edulis* Ker) starch from Industri Lingkar Organik Sleman, Yogyakarta, Indonesia.

Instruments

Drying oven, magnetic stirrer, incubator, vacuum pump (model; DOA-P504-BN), spectrophotometer, haze meter (serie NDH – 200, Nipon Denshoku Kogyo Co., Ltd.), micrometre (Roch, A281500504, Sisaku SHO Ltd, Japan), testing machine MPY(type: PA-104-30. Ltd. Tokyo, Japan), water vapour transmission rate tester of Bergerlahr cup method, hot plate (Torrey Pines Scientific brand) and analytical balance (Ohaus Corp. Pine Brook, N. J. USA).

Laboratory Methods

The edible film-making process was done according to the modified procedure by **[26]**. Parameters of thickness, percent elongation, tensile strength, and water vapour transmission rate of the edible film were measured referring to **[1]** by using the tool haze meter (serie NDH – 200, Nipon Denshoku Kogyo Co., Ltd.), micrometre (Roch, A281500504, Sisaku SHO Ltd, Japan), testing machine MPY(type: PA-104-30. Ltd. Tokyo, Japan), water vapour transmission rate tester of Bergerlahr cup method, hot plate (Torrey Pines Scientific brand), respectively, while for the antioxidant activity parameters measured using the 2,2–diphenyl-1-picrylhydrazyl (DPPH) method **[20]**.

Description of the Experiment

Sample preparation:

Gambier filtrate production

Gambier extract is crushed until fine using mortar and subsequently is sieved using an 80 mesh siever. Weighing of fine gambier extract 40 (% w/v) and then put it into a volumetric glass and added with aquadest until 100 mL boundary mark. The suspension was stirred using a magnetic stirrer for 10 minutes, filtered and using Whatman No. 1 filter paper, and centrifuged at 1000 rpm, followed by the filtrate.

Edible film production

Canna starch as much as 4 g is put into Beaker glass of 250 mL in size, and aquadest water is added up to the mark of 100 mL. Starch suspension is stirred by using a magnetic stirrer while being heated by using a hotplate at a temperature of 65 °C until perfect gelatinisation is obtained. Gelatinised starch suspension is added with 1% glycerol (v/v), in which the stirring process and heating are maintained. Suspension is added with gambier filtrate according to treatments 3, 4, and 5% (v/v) until homogenous mixture and then added with rosella flower petals extract according to treatments 2, 4, and 6% (v/v). After homogenizing edible film suspension, CMC as much as 1% (w/v) is added gradually while maintaining temperature and stirring. Subsequently, olive oil as

much as 1% (v/v) is added while stirring. Edible film suspension is vacuum treated using a vacuum pump for 1 hour. Edible film suspension of as much as 40 mL is poured into a petri dish with a diameter of 15 cm and then dried within a drying oven at 60 °C for 24 hours. The edible film is released from the petri dish and put into a desiccator for 1 hour. Finally, the edible film is ready to be analysed.

Number of samples analyzed: The number of analysed was 9.

Number of repeated analyses: Three repeated analysed were performed for each treatment factor. The total sample analysed was 27 samples.

Number of experiment replication: The number of experiment replication as many as 9 samples.

Design of the experiment: Treatment factors consisted of gambier filtrate (A): A1 = 3; A2 = 4 and A3 = 5 (% v/v) and rosella flower petals extract (B): B1 = 2, B2 = 4 and B3 = 6 (% v/v).

Statistical Analysis

This research used a factorial, completely randomised design. Treatments with significant effects were further tested using the HSD test at α = 5%. Research results were analysed using the analysis of variance (ANOVA) method with the aid of the SAS program-Windows version 9.

RESULTS AND DISCUSSION

The edible film had thickness in the range of 0.096 to 0.124 mm, and these values fulfilled the Japan Industrial Standard (JIS, 1975) of 0.25 mm maximum. The highest thickness of this edible film is similar to the thickness of tapioca starch-based edible film, which is incorporated with kelakai leaves extract with a magnitude of 0.124 mm **[21]**and the lowest thickness is similar to the thickness of edible alginate film, which is incorporated with curcumin extract with a magnitude of 0.096 mm **[3]**. This result is lower than the thickness of the edible film obtained from the study, with an average value of 0.26 mm **[27]**. This is also higher than the thickness of edible film made from catfish surimi with a size of 0.049 mm **[30]**.

Treatment of gambier filtrate at 5% (v/v) concentration combined with rosella flower petals extract at 2% (v/v) concentration (A_3B_1) produced the highest thickness. In contrast, the lowest thickness was found in treatment of gambier powder filtrate at 3% (v/v) concentration combined with rosella flower petals extract at 6% (v/v) concentration (A_1B_3). The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film was presented in Figure 1.

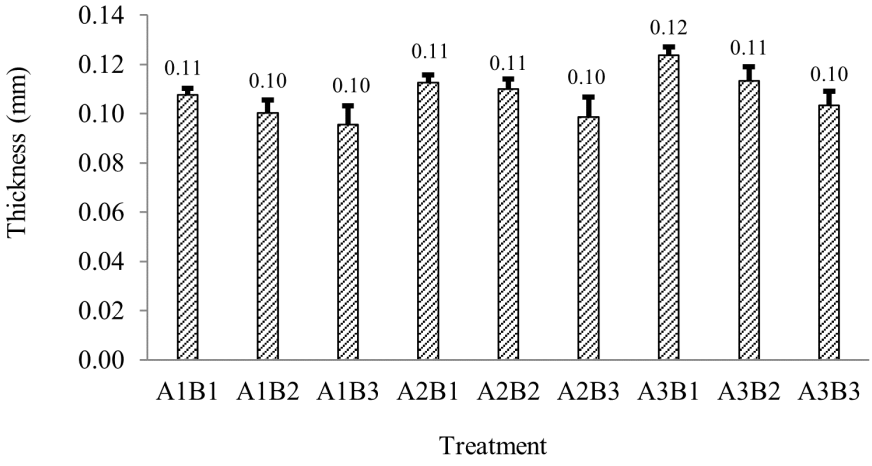

Figure 1 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the thickness of the active edible film.

Treatment of gambier filtrate and rosella flower petals extract significantly affected edible film thickness, but their interactions had no significant effect. Results of honestly significant different (HSD) test for the impact of gambier filtrate concentration on active edible film thickness were shown in Table 1.

Active edible film thickness increases according to the increase of gambier filtrate concentration. It is known that gambier filtrate contains a catechin compound with semi-polar characteristics, which includes solids that are insoluble in water—the amount of these solids affected the increase of active edible film thickness. The results of this study are the same as those produced by **[28],** which explains that the edible film thickness of sugar palm fruit had increased according to the rise of plasticiser concentration in which plasticiser is polymers

that make up the edible film matrix that affects on the increase of total soluble solids within edible film suspension.

Table 1 Results of HSD test for the effect of gambier powder filtrate concentration on active edible film thickness, elongation percentage, tensile strength, water vapor transmission rate, and antioxidant activity.

Treatment	Thickness (mm)	Elongation percentage (%)	Tensile strength (MPa)	Water vapor transmission rate ($g.m^{-2}.day^{-1}$)	Antioxidant activity (IC_{50}) ppm
A_1 (3% v/v)	0.101 ±0.006a	17.94 ±2.94a	3.27 ±0.19a	16.50 ±0.22a	45.99±2.06a
A_2 (4% v/v)	0.107 ±0.007b	22.85 ±2.58b	2.44 ±0.13b	15.32 ±2.19ab	39.51±1.33b
A_3 (5% v/v)	0.113 ±0.010c	32.00 ±6.86c	1.96 ±0.06c	14.27 ±2.08b	35.61 ±1.29c

Note: Numbers followed by the same letter at the same column are not significantly different (p >0.05).

The HSD test in Table 2 showed that concentration increase of rosella flower petals extracts had decreased active edible film thickness. This is because rosella is hydrophilic or polar which affects the decrease in the thickness of the edible film. This statement is supported by **[2]** which states that rosella flower petals extract contains anthocyanin compounds, which are polar molecules.

Table 2 Results of HSD test for the effect of rosella flower petals extract concentration on active edible film thickness, elongation percentage, water vapor transmission rate, and antioxidant activity.

Treatment	Thickness (mm)	Elongation percentage (%)	Water vapor transmission rate ($g.m^{-2}.day^{-1}$)	Antioxidant activity (IC_{50}) ppm
B_1 (2% (v/v)	0.115 ±0.008a	19.90 ±5.01a	14.25 ±2.17a	41.97 ±5.58a
B_2 (4% (v/v)	0.108 ±0.006b	25.88 ±5.99b	15.49 ±2.19ab	40.27 ±5.43b
B_3 (6% (v/v)	0.099 ±0.004c	27.01 ±10.51c	16.35 ±0.31b	38.88 ±4.73c

Note: Numbers followed by the same letter at the same column are not significantly different (p >0.05).

Elongation percentage

The produced elongation percentage of active edible film was in the range of 14.90 to 38.62%. This elongation percentage was lower than the JIS standard (1975) which sets out of minimum 70%. Still, it was higher compared to millet starch edible film added with clove essential oil with a magnitude of 5.67% **[11]** and edible films based on the pumpkin with a magnitude of 13.13 – 14.47% **[16]** as well lower than a composite edible film of palm starch and chitosan which is incorporated with olive oil with a magnitude of 224.6% **[10]** and edible films based on alginate namely 27.67 – 43.57% **[18]**. The highest and the lowest elongation percentages of the active edible film were found on A_3B_3 and A_1B_1 treatments, respectively. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film was shown in Figure 2.

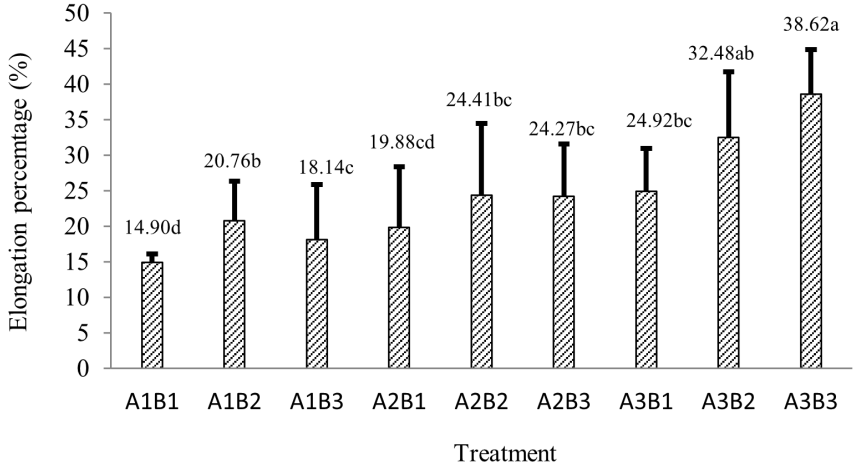

Figure 2 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the elongation percentage of the active edible film.

Edible film elongation percentage was significantly affected by treatments of gambier filtrate, rosella flower petal extract and their interaction. HSD test at a 5% level (Table 1) showed that the higher the gambier filtrate concentration, the higher the edible film elongation percentage. It is previously mentioned that the catechin compound has semi-polar characteristics and part of the catechin with polar characteristics affects the addition of hydrophilic compound in the edible film suspension. which causes the increase in edible film elongation percentage increased. Active edible film elongation percentage increased according to the rise of rosella flower petal extract concentration (Table 2). This was also influenced by the addition of hydrophilic compound as in gambier filtrate because rosella flower petal extract contains water-soluble anthocyanin compound. **[11]** showed that the elongation percentage of millet edible film had decreased with the increase of clove essential oil concentration. It is known that clove essential oil has hydrophobic characteristics and this can be interpreted that the hydrophobic component decreases edible film elongation percentage. In contrast, the hydrophilic component increases the edible film elongation percentage.

This edible film is formed by several materials consisting of canna starch, glycerol, gambier filtrate, rosella flower petals extract, CMC and olive oil. Edible film matrix is formed by complex bonds amongst these constituent materials. This complex bond consist of canna starch-glycerol-gambier filtrate-rosella flower petals extract-CMC-olive oil. Constituent materials of this edible film are divided into three hydrophilic components: canna starch, glycerol, gambier filtrate and rosella flower petals extract; CMC as an emulsifier as olive oil as a hydrophobic component. The hydrophilic component was more dominant in forming of edible film matrix than other components. This cause interaction treatment of A_3B_3 had produced the highest elongation percentage.

Tensile strength

The produced tensile strength of the active edible film was in the range of 1.89 to 3.38 MPa. A1B1 treatment (gambier filtrate of 3% v/v and rosella flower petals extract of 2% v/v) produced the active edible film with the highest tensile strength. In contrast, the lowest was found in A_3B_3 treatment (gambier filtrate of 5% v/v and rosella flower petals extract of 6% v/v). The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film was shown in Figure 3.

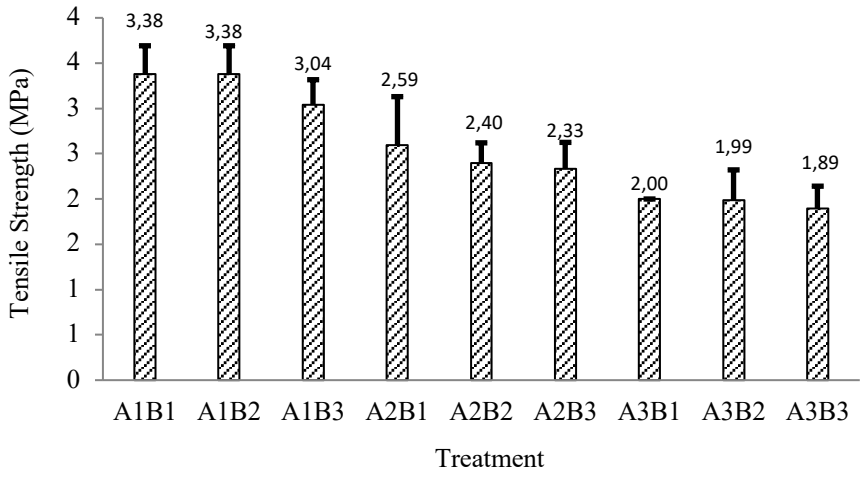

Figure 3 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the tensile strength of the active edible film.

Analysis of variance results showed that gambier filtrate treatment had a significant effect on the tensile strength of the active edible film. In contrast, the treatment of rosella flower petals extract and both treatment's interactions had no significant effect on the tensile strength of the active edible film. HSD test at the 5% level in Table 1 showed that the higher the gambier filtrate concentration, the lower the tensile strength of the active edible film. This is related to the catechin compound that has semi-polar characteristics as mentioned previously. The tensile strength of the edible film is influenced by its constituent components in which components having hydrophilic characteristics such as sorbitol will decrease the tensile strength of the edible film. In contrast, a hydrophobic component or non-polar component will increase the tensile strength of the edible film. In addition, the tensile strength of the edible film is inversely proportional to the elongation percentage, namely, the higher the tensile strength, the lower the elongation percentage (Table 1). This is the

general theory that applies to edible film as stated by **[29]** that increasing the elongation percentage of the edible film will cause lower tensile strength of the edible film.

The tensile strength of edible film according to the standard of JIS 1975 (Japanese Industrial Standart) is a minimum of 0.39226 MPa. The tensile strength of the produced edible film from several treatments combination was in the range of 1.89 to 3.38 MPa and all the produced edible films fulfilled the JIS standard. These tensile strength values are higher compared to the tensile strength of edible film from sweet potato starch as reported by **[6]** with a magnitude of 0.75 MPa. They are lower compared to the tensile strength of edible film from breadfruit starch, as reported by **[34]**, with a magnitude of 93.43 MPa.

Water vapour transmission rate

The water vapour transmission rate of the produced active edible film was in the range of 12.85 to 17.04 $g.m^{-2}.d^{-1}$ and higher than the JIS 1975 standard with a maximum value of 10 $g.m^{-2}.d^{-1}$. The water vapour transmission rate of this active edible film was higher (12.99 to 17.04 $g.m^{-2}.d^{-1}$) than alginate edible film added with turmeric extract (1.37 $g.m^{-2}.d^{-1}$) as reported by **[3]**. It was lower than canna-based edible film added with gambier extract (20.23 $g.m^{-2}.d^{-1}$) as written by **[26]**. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapour transmission rate of the active edible film was shown in Figure 4.

Analysis of variance results showed that treatments of gambier filtrate and rosella flower petals extract and their interaction had a significant effect on the water vapour transmission rate of active edible film. Further test in Table 1 showed that the water vapour transmission rate of the active edible film had decreased with gambier filtrate concentration. This is influenced by semipolar characteristics of catechin compounds within gambier filtrate. The addition of essential oil from lemon and bergamot to protein isolate edible film could decrease water vapour transmission rate **[4]**. In addition, the water vapour transmission rate of edible film decrease with the increase of edible film thickness (Table 1). This is because the thicker the edible film, the more difficult for water vapour to penetrate the edible film.

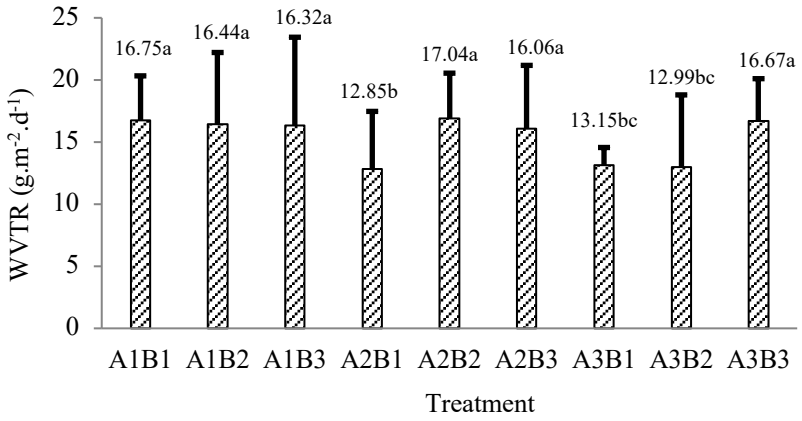

Figure 4 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the water vapor transmission rate of the active edible film.

The HSD test at the 5% level in Table 2 showed the opposite results with rosella flower petals extract. The higher the concentration of rosella flower petals extracts, the higher the water vapour transmission rate of active edible film. It is previously mentioned that rosella flower petals extract has hydrophilic characteristics, which make it easier for water vapour to penetrate the edible film. **[17]** reported that adding a hydrophobic component in form of sunflower oil to green bean starch edible film could decrease water vapour transmission rate. The opposite is true for the addition of a hydrophilic component.

Figure 4 shows that treatments A2B1, A3B1, and A3B2 had lower water vapour transmission rates than other treatments. This is due to the gambier filtrate's influence containing semipolar catechin compounds which the rosella flower petal extract is polar. Thus, the combination of higher gambier filtrate than rosella flower petal extract, the lower the water vapour transmission rate of the edible film produced.

Antioxidant activity

The produced active edible film had an antioxidant activity with IC_{50} values in 34.53 to 48.02 ppm. The higher the IC_{50} value, the lower the antioxidant properties, and *vice versa*. The most increased antioxidant activity was found in the A_3B_3 treatment, whereas the lowest was found on the A1B1 treatment. The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity (IC_{50}) of the active edible film was shown in Figure 5.

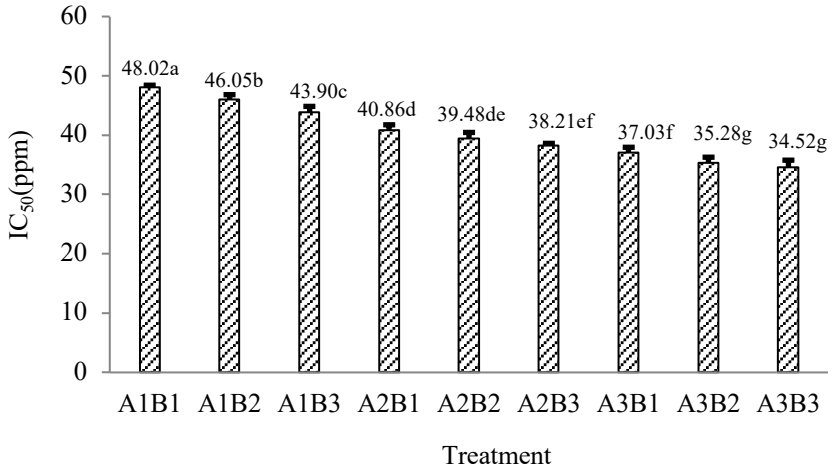

Figure 5 The effect of interaction treatment of gambier filtrate and rosella flower petal extract on the antioxidant activity (IC_{50}) of the active edible film.

The IC_{50} value of this edible film was similar to edible film incorporated with turmeric extract with an IC_{50} value of 38.28 ppm as reported by **[3]**. **[19]** had said that polyvinyl alcohol edible film added with curcumin had an antioxidant activity of 35.16 ppm and **[15]** had reported that edible alginate film incorporated with black chokeberry extract had the antioxidant activity of 32.96 ppm. However, the IC_{50} value of this edible film was higher than potato starch edible film included with *Salvia officinalis* essential oil with a magnitude of 68.35 ppm as reported by **[23]**, **[32]**, **[33]** with IC_{50} of 50.42-77.41 ppm and **[14]** with IC_{50} of 87.41 ppm.

The IC_{50} value of the active edible film is significantly influenced by treatments of gambier filtrate and rosella flower petals extract and their interaction. The increase of gambier filtrate concentration results in the increase of the antioxidant activity of active edible film, as presented in Table 1. The IC_{50} value had decreased with the rise of gambier filtrate concentration. The increase in antioxidant activity is due to catechin compound content within gambier filtrate. **[25]** had described that gambier extract has potential as a drug that contains antioxidant, anthelmintic, antibacterial and antidiabetic. Results of the HSD test at a 5% level (Table 2) showed that the increase of rosella flower petals extracts results in the growth of antioxidant activity of active edible film as indicated by the decrease of IC_{50} value. This is due to the anthocyanin compound available in rosella flower petals extract. **[5]** reported that rosella flower petals contain an anthocyanin compound with antioxidant characteristics with IC_{50} values in the range of 50 to 100 ppm.

CONCLUSION

The mechanical properties of the active edible film fulfilled JIS 1975 standard, especially in terms of thickness, tensile strength, and water vapour transmission rate. However, the elongation percentage has not met the standard. The active edible film has antioxidant characteristics of the strong category with IC50 values in the range of 34.53 to 48.02 ppm. In general, this edible film is feasible to be applied as packaging material for high lipid foods to inhibit the oxidation process in those foods.

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

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The potential of goat meat as a nutrition source for schoolchildren

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ABSTRACT

The issue of rational nutrition of children is still extremely relevant and an effective factor in ensuring the preservation of the life and health of children. Pathological conditions associated with intolerance to certain components of food are increasingly common. Biologically complete products play an important role in the organization of rational nutrition of children, which can be created only in industrial production conditions. When assessing the chemical composition of experimental goat meat samples (Zaanenskaya, Alpine, Nubian), no abnormal deviations were detected, and all indicators were in the generally accepted contents of this type of animal muscle tissue. The mineral composition showed that goat meat is rich in such elements as potassium – 1693.22 – 4125.83 mg/kg; sodium – 852.27 – 1518 mg/kg, magnesium – 125.33 – 295.8 mg/kg; calcium – 79.27 – 160.79 mg/kg, iron 11.42-87.52 mg/kg. The vitamin composition of goat meat showed that the content of pantothenic acid (B5) was 0.53 – 0.62 mg / 100g, pyridoxine (B6) 0.52 – 0.64 mg/100g tocopherol 0.27 – 0.33 mg/100g. The mass fraction of goat meat proteins was 2.1 ±0.3 – 2.4 ±0.4%. The study of the dynamics of changes in the composition of protein fractions based on the results of comparative studies of the ratio of sarcoplasmic proteins showed the content of water-soluble (1.75 – 4.06%), salt-soluble (1.75 – 2.44%), alkali-soluble (11.15 – 15.10%) proteins. The salt-soluble fraction reflects the total changes in the state of protein fractions, the solubility of which was not the same for the rocks under consideration (the highest concentration was determined in the Nubian rock).

Keywords: goat meat, nutritional value, nutrition of schoolchildren, fractional composition of proteins, moisture binding ability

INTRODUCTION

Meat and meat products are important nutrients for the human body. At the moment , the following types of meat are widely in demand in the republic of Kazakhstan: beef, horse meat, lamb, poultry. In recent years, the volume of meat and livestock produced has shown steady growth. Thus, it shows an increase in demand and stable growth of the meat and meat products market. Currently, in the republic of Kazakhstan, as of 2021, the indicator of the number of goats is 3 million 93 thousand heads. In 2021, more than 8 thousand tons of goat meat were sold for slaughter. It should be noted that Kazakhstan shows a leading position in the export of lamb and goat meat outside the EU countries, which is 91.9%. In particular, supplies to the EU for $ 3.4 million, to Uzbekistan for $ 1.6 million are provided, exports to Iran amounted to 112 thousand us dollars. Considering that from 2003 to the present, the development of goat breeding has almost doubled, as well as the growing interest of consumers primarily in healthy and proper nutrition, favourable conditions are emerging for the development of this market segment. With an annual increase in the number of goats in the country amounting to 7.1%, by 2050, the number will reach 6 million 278 thousand goats. With the increase in livestock, it is expected to achieve the production of goat meat of 17.1 thousand tons **[1]**.

Goat meat belongs to non-traditional raw materials. Currently, goat meat does not have a wide range of consumers in the Republic of Kazakhstan. However, active scientific research on using this type of raw meat in the industry is already underway in the world and has great prospects in the domestic market **[2]**, **[3]**, **[4]**, **[5]**, **[6]**, **[7]**, **[8]**. Regarding taste, goat meat is not inferior to mutton **[9]**. Goat meat has a moderately pronounced salty taste and is not sweet, like beef **[10]**. Young goat meat is lighter than other types of meat, and it has a pale pink color. The meat of old animals is brick-red and darkens in the air **[11]**. Goat fat is pure white **[9]**.

Table 1 Nutritional value of different types of meat (per 100 g) **[12]**.

Indicator	Goat Meat	Chicken	Beef	Lamb
Energy value, kcal	143	190	210	206
Proteins, g	27	25	27	26
Fats, g	3.1	7.4	9.3	9.5
Saturated fats, g	0.9	2.0	3.5	3.5
Cholesterol, mg	75	89	86	92

The meat of goats aged 4-6 weeks, young animals and castrated goats are eaten. The meat of young animals aged six to ten months is considered the best. The meat of adult goats is sharper **[12]**. Nevertheless, the manual of the XIV century on home economics, "Le Ménagier de Paris" states that the best, sweet and fatty meat is obtained from six-seven-year-old castrated goats: it makes an excellent pate **[13]**. The meat of adult uncastrated goats has a pronounced specific odour **[10]**, a possible unpleasant odour in females and young animals may be due to improper processing of the carcass **[14]**.

Compared to other types of red meat, goat meat is leaner. It has less cholesterol and fat than lamb and beef **[15]**, it is less caloric than beef or chicken, and contains a lot of protein **[10]**. Goat meat is rich in unsaturated fatty acids, minerals, and amino acids **[13]**. Goat meat is well digested and digested, it is hypoallergenic and suitable for children's and dietary nutrition **[9]**. Goat meat is a source of B vitamins, pantothenic, folic, para-aminobenzoic acids and choline. Regarding the content of vitamins A, B1 and B2, goat meat significantly exceeds the meat of other farm animals **[16]**. The use of goat meat is not prohibited by any religious norms, Muslims and Jews can eat it. It positively impacts our multinational and multi-confessional society **[10]**.

Scientific Hypothesis

The study of the physic-chemical composition, the fractional composition of proteins allows us to obtain data on the technological properties of goat meat for use in the production of meat products.

MATERIAL AND METHODOLOGY

Samples

The research objects were the meat of goats aged 9-10 months, obtained from 3 breeds: Nubian, Zaanen and Alpine (*m. L. dorsi*, shoulder blade), grown in the breeding farm "Zerenda" located in Kazhymukan auls, Tselinograd district, Akmola region, Kazakhstan. The meat was bought in a specialized meat market.

Chemicals

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

Instrument

The content of mineral elements was determined using the Spectr AA 220 FS (VARIAN B.V, USA) atomic absorption spectrophotometer. The MOD MARS 6 (CEM Corporation, USA) microwave sample preparation system was used for sample preparation. The vitamin composition was determined using a high-performance liquid chromatograph "Agilent-1200" (Agilent Technologies, USA).

Laboratory Methods

Laboratory studies of meat raw materials were carried out based on the NAO "S. Seifullin KATU" (Nur-Sultan, RK) and the FGBNU "V. M. Gorbatov Food Systems Research Center" of the Russian Academy of Sciences (Moscow, RF). The following were investigated: the total chemical composition (moisture, fat, protein, ash) GOST 25011-2017, BCC (Grau-Hamm method), mineral composition (GOST R 55484-2013), vitamin composition (GOST 32307-2013), the fractional composition of the protein fraction (GOST 25011-81) **[17]**, **[18]**, **[19]**, **[20]**, **[21]**, **[22]**, **[23]**.

Description of the Experiment

Sample preparation: The objects of research were samples of goat meat of three breeds, namely the Zaanen, Alpine, and Nubian goat breed. The primary 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.

Number of samples analyzed: we analyzed 27 samples.

Number of repeated analyses: All instrument measurements were performed twice.

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

Design of the experiment: To determine magnesium, a lanthanum solution is added to an aliquot of a sample solution of a suitable volume selected with a pipette. The resulting solution is diluted with a solution of nitric acid with a mass fraction of 0.65% so that the mass concentration of magnesium is within the range of the linearity of measurements by the AAC method for this element. The typical measurement range for magnesium is from 0.05 to 0.4 mg/dm^3. If necessary, the lower limit of the measurement range may be smaller, depending on the mass concentration of magnesium in the sample solution. Lanthanum solution. They are added in the volume necessary to obtain a mass concentration of lanthanum in a solution for measurements by the AAC method of 10 g/dm^3 (for example, when diluting the sample solution after mineralization to 10 cm, 2 cm^3 of a lanthanum solution of a mass concentration of 50 g/dm^3 is added).

Calibration solutions of magnesium are prepared with mass concentrations of 0.05, 0.1, 0.2 and 0.4 mg/dm^3. To do this, 0.25, 0.5, 1.0 and 2.0 cm^3 of a standard magnesium solution are added to measuring flasks with a capacity of 50 cm^3, respectively. 10 cm^3 of a lanthanum solution of a mass concentration of 50 g/dm^3 is added to each flask, and the volume of contents in the flasks is brought to the mark with a solution of nitric acid. Calibration solutions are prepared on the day of the analysis.

The atomic absorption spectrometer is set up on the day of the test in accordance with the device's operating instructions. A wavelength of 285.2 nm and an optical slit width of 0.7 nm is set to determine magnesium.

Statistical Analysis

The statistical evaluation of the results was carried out 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.

RESULTS AND DISCUSSION

Table 2 Physico-chemical parameters of the studied goat meat samples.

Name of the indicators to be determined	Unit of measurement	Test results		
		Zaanen breed	Alpine breed	Nubian breed
Physico-chemical indicators				
Mass fraction of moisture	%	79.9 ±8.0	79.5 ±8.0	77.0 ±7.7
Mass fraction of fat	%	2.1 ±0.3	2.1 ±0.3	2.4 ±0.4
Mass fraction of protein	%	17.0 ±2.6	17.5 ±2.6	19.3 ±2.9
Mass fraction of ash	%	0.92 ±0.14	0.80 ±0.12	1.21 ±0.17
Vitamins				
B3	mg/100 g	5.20 ±1.04	6.76 ±1.35	5.62 ±1.12
B5	mg/100 g	0.62 ±0.12	0.53 ±0.11	0.59 ±0.12
B6	mg/100 g	0.64 ±0.16	0.64 ±0.16	0.52 ±0.13
D3	mg/100 g	<1.0	<1.0	<1.0
E	mg/100 g	0.32 ±0.06	0.27 ±0.05	0.33 ±0.07
Minerals				
Potassium	mg/kg	2470.10 ±370.52	1693.22 ±253.98	4125.83 ±618.87
Sodium	mg/kg	852.27 ±136.36	1005.83 ±160.93	1518.21 ±242.91
Magnesium	mg/kg	148.71 ±22.31	125.33 ±18.80	295.88 ±44.38
Zinc	mg/kg	37.95 ±7.43	25.14 ±5.13	15.78 ±3.44
Iron	mg/kg	27.28 ±6.18	87.55 ±12.83	11.42 ±4.00
Manganese	mg/kg	0.52 ±0.10	0.27 ±0.05	0.21 ±0.04
Calcium	mg/kg	148.32 ±25.21	160.79 ±27.33	79.27 ±19.82

The study of the dynamics of changes in the composition of protein fractions based on the results of comparative studies of the ratio of sarcoplasmic proteins, based on the extraction of sarcoplasmic proteins from muscle tissue with a buffer solution of low ionic strength and obtaining fractions of water-soluble, salt-soluble and alkali-soluble proteins, followed by determination of their amount by the Kjeldahl method, with the release of non-protein, peptide and residual nitrogen are presented in Table 2.

The musculature of an animal is not something given once and for all. It develops as the animal grows, changes following the current needs of the body and atrophies with ageing and decreased motor activity. The mobility of parts of the animal's body is given by the contractile ability of the muscular system, based on the delightful contractile proteins – aggregates of their molecules change their sizes when interacting. The main proteins of contractile structures are actin and myosin. Strands of these proteins form cellular structures capable of pulling together the poles of the cell to which they are attached. At the same time, the shortening of microfilaments (filamentous structures of the cytoskeleton) does not occur due to the shortening of the protein molecules (actin and myosin), but due to their mutual sliding inside the actomyosin complex and a decrease in the total length of microfilaments. Proteins of one type seem to move between proteins of another type, and the tissue contracts with some effort, ensuring that the work of shifting body parts is done. This work can be expressed by reducing the length of the muscle (dynamic work) or in tension (static work), counteracting its stretching **[24]**. The movement of the threads of the actomyosin complex requires energy expenditure and the formation of bonds between its components. In this connection, muscle tissue proteins have multifunctional properties. The study of the fractional composition of muscle tissue protein with fattening variation allows us to judge the most effective diet, achieving the maximum desired effect (Table 3).

Table 3 Fractional composition of goat meat proteins.

Fractional composition of protein	Unit of measurement	Zaanen	Alpine	Nubian
Water-soluble proteins	%	4.06	2.81	1.75
Salt - soluble proteins	%	1.75	2.13	2.44
Alkali - soluble proteins	%	11.15	12.55	15.10
Moisture binding capacity	%	73.45	74.42	73.94

When analysing the results, it was revealed that the largest amount of protein was contained in the meat of the Nubian breed.

Depending on the extraction conditions, three groups of proteins are distinguished:

- water-soluble proteins consisting mainly of sarcoplasmic proteins (myogen, globulin, myoglobulin, nucleoproteins);
- salt-soluble proteins consisting mainly of myofibrillary proteins (myosin, actin, actomyosin, as well as so-called regulatory proteins: tropomyosin, troponin);
- alkali-soluble proteins consist mainly of stroma proteins, including collagen, elastin, and glycoproteins – mucin and mucoid **[25]**, **[26]**.

The salt-soluble fraction reflects the total changes in the state of protein fractions, the solubility of which was not the same for the rocks under consideration (the highest concentration was determined in the Nubian rock). It should also be noted that the salt-soluble fraction decreases significantly with an increase in the duration of cultivation. The water-soluble fraction in the maximum concentration is determined in the Zaanenskaya rock, almost more than two times than in the Nubian rock.

It should be noted that the myogen and myoglobulin proteins of the water-soluble fraction are part of the proteins extracted by saline solution. The alkali-soluble fraction includes collagen and elastin, a significant part of water-soluble and salt-soluble proteins. Consequently, the content of water-soluble, salt-soluble, and alkali-soluble proteins cannot definitively judge the percentage of these fractions in meat during cultivation.

When assessing the chemical composition of experimental goat meat samples, no abnormalities were detected, and all indicators were in the generally accepted contents of this animal muscle tissue.

The content of the vitamin composition was also within the same limits for all breeds. To assess the nutritional value of this type of meat, it is recommended to conduct a comparative analysis with similar samples of lamb meat.

It should be noted that significant differences were found when studying the mineral composition of different breeds of goat meat. Particular attention should be paid to the content of minerals such as potassium and sodium in the Nubian rock, which was found to be significantly higher than in the other two. At the same time, significant iron content was determined in the Alpine rock, more than 2 – 3 times relative to other rocks. To test the hypothesis

of increased iron content in the meat of this breed or the presence of this artifact due to the animal's characteristics (or poor exsanguination), it is necessary to conduct a more detailed study with a larger sample.

It should also be noted that the calcium content in the meat of all three breeds is determined above industrially kept animals, such as beef and lamb, which is also possibly due to the best conditions for keeping animals that participated in this experiment.

As a result of the conducted studies on the mineral composition of goat meat, it is possible to draw a general conclusion that, according to this indicator, meat can be classified as high-containing concerning the main essential elements. Moreover, recommend this raw material to produce baby food products, which have increased requirements for the composition of vitamins and minerals.

There were no significant differences in moisture-binding capacity (WCC). In general, it is essential to note that WCC, regardless of the feeding diet, gives significantly better values with proper maintenance and slaughter of animals. It can be concluded that the selected fattening technologies in the experiment and the absence of stress (the WCC indicator under stress will be less than 65%) gives such results.

Goat meat, which has a better ability to concentrate meat juice inside the muscle fiber, is more valuable because of its technological characteristics. Therefore, it can also be recommended for the preparation of delicatessen products and dried products.

Karami M. et al. determined the effect of the goat diet on the fatty acid profile and the resulting meat quality. Twenty-four young goats of the Kachagan breed with an average live weight of 14.2 ±1.46 kg were selected for the study. Palm oil in the amount of 3% was added to the diet of one group, and rapeseed oil in similar concentrations was added to the feed of the second group of animals. Blood sampling and weighing of animals were performed before the experiment and after 33, 66 and 102 days. At the end of the experiment, it was shown that adding 3% rapeseed oil to the diet of goats improves the fatty acid profile of meat by increasing the concentration of omega-3 fatty acids, thereby making it more beneficial to health. At the same time, no such effect was observed from palm oil **[27]**. Lushnikov V. V. Yusova O.V. conducted studies of subcutaneous fat of young goats aged 4, 6, and 8 months of Russian and Zaanen breeds. The study found a significant amount of valuable polyunsaturated fatty acids. The optimal value was noted in goats aged 6 months **[28]**. Uzakov Ya. M. et al. conducted a comparative analysis between mutton and goat meat. According to the data obtained, mutton contains more dry matter (1.5-2%), which is explained by the high-fat content in mutton. According to amino acids, goat meat contains more arginine (1.88 ±0.05 and 1.62 ±0.04), lysine (1.84 ±0.04 and 1.65 ±0.03), histidine (2.01 ±0.06 and 1.71 ±0.03), methionine (1.40 ±0.04 and 1.23 ±0.04) and aspartic acid (1.20 ±0.04 and 1.06 ±0.03), but at the same time, a lower content of the following amino acids was noted: leucine (1.36 ±0.04 and 1.64 ±0.06), glycine (0.81 ±0.04 and 0.99 ±0.06) **[29]**. At the moment, Kazakhstan has 180 million hectares of pasture lands. Of these, 18.7 million hectares. foothill and 8,9 million hectares of mountain pastures. At the same time, it is worth noting the low use of pastures (about 10-15%). Optimal pastures for goats are foothills and low mountains. The low use of pastures opens up the possibility of breeding goats in industrial quantities. Goats are spread throughout the world. They live in small or large herds and in different areas and environments. Because of its distinctive taste and desired chemical composition, goat meat is increasingly consumed in Serbia. Animal foods are rich in protein, vitamins and minerals, but contain very little fat, especially cholesterol. This review paper aims to highlight some health benefits, nutritional values and potential use of goat meat. The chemical composition of goat meat affects race, gender, productivity and adaptability to stress, environment, management, diet, weight at slaughter, health condition, and slaughter and procedures with the carcasses after slaughter. The average chemical composition of lean goat meat contains about 75.42% water, 3.55% fat, 19.95% protein and 1.06% mineral matter. The energy value is about 580 kJ per 100 g. Goat meat has about the same nutritional value as well as sheep meat. Due to the low content of saturated fatty acids and cholesterol, goat meat in the human diet is a healthier alternative than other red meat. Polyunsaturated fatty acids are prevalent in goatmeat, and a diet rich in unsaturated fatty acids is correlated with a reduced risk of stroke and coronary disease. In addition, essential amino acids such as lysine, threonine and tryptophan are present in goat meat. Regardless of the nutritional value, goat meat is still less appreciated due to its specific smell and taste, even if the animal is older **[30]**. In general, goat meat is not inferior to other meat types regarding nutritional and biological value–it has a high protein content (up to 29%), and it is a good source of minerals, vitamin B-complex, and essential amino acids. However, the meat of older and culled goats is less juicy, less tender, has a characteristically different odour and taste compared to kids' goat meat (and meat of other animals), and thus tends to be less desirable. Different meat products could be produced using goat meat (including culled goat meat): dry-fermented sausages (e.g., sucuk), dry-cured meats (Violino di capra–goat prosciutto), frankfurters, mortadella, etc. without adverse effects on products' technological properties. The negative impact of goat meat on the properties of meat products is mainly associated with using goat fatty tissue. However, this could be overcome by using fatty tissue of other animals (e.g., pork back fat or beef fatty tissue) **[31]**. Herzegovinian dry smoked goat meat is a traditional cured

meat product made of the whole carcass of adult castrated bucks, dry salted and cold smoked. It has been traditionally produced in Herzegovina for centuries, especially in the wider area of the Stolac municipality. This study aimed to determine the quality parameters of Herzegovinian dry smoked goat meat. For the research, the samples were made into eight separate anatomical units (neck, sirloin, leg, loin, flank, breast, shoulder, hindshank), on which the tests were performed. Sensory, physical, and chemical tests were performed on the examined samples. Also, its hydrolytic and oxidative changes (acid and peroxide number, TBARS value) were determined to monitor changes in fats. The sensory evaluation determined that a "pleasant" aroma characterized the examined samples. Chemical tests revealed significant differences in the values of the examined parameters between samples from different anatomical regions. The least hydrolytic and oxidative changes were found in the breast samples with the highest fat content. PCA analysis revealed a positive correlation between moisture content and pH value and a negative correlation of these parameters with fat content. Furthermore, a significant positive correlation was found between NaCl content, ash, peroxide number, and TBARS values. Fat content was characteristic in the breast samples, moisture in the shoulder samples, and protein in the hindshank samples, while NaCl and ash content were characteristic in the neck samples **[32]**. Two variants of sucuk were made: one of beef meat and beef tail fat and another of goat meat and goat tail fat with meat/fat ratio of 75/25 and the same ingredients. After filling, the sausages were hung to dry in a traditional smoking house (without possibly controlling the temperature or humidity). Weight loss, pH, non-protein nitrogen content, basic chemical composition, instrumental colour measurement and sensory evaluation were made for both variants. Both variants had an almost identical weight loss (36.98 beef sucuk and 36.25 goat sucuk). Changes in pH value and non-protein nitrogen content had the same tendency, and end values did not differ. The basic chemical composition at the end of production indicates that both variants were of excellent quality. L* and b* values did not differ, but there was a significant difference in a* value (11.72 beef and 14.15 goat). In terms of appearance, texture and taste, assessors gave poorer grades to goat sucuk, but these grades do not indicate that the product is unacceptable (they were more than 5). It is possible to replace goat tail fat with beef fat to appease the specific flavour of the product and to make it more acceptable to consumers who may not be used to such flavour **[33]**. The quality of fresh goat meat can be defined strictly in terms of physical and chemical properties or consumer perception. In Serbia, there is not enough information about the quality of goat meat and goat meat products, such as smoked ham. This study aimed to determine differences in the basic chemical composition, colour, fatty acids composition, and volatile compounds in fresh meat and smoked ham (*musculus gluteus superficialis*). The meat was obtained from the population of White Serbian goat, five or six years old. ISO methods were implemented to determine the quality of these parameters. A statistically significant difference (p <0.05) was determined between values of protein, fat, moisture, ash, pH value, fatty acids and volatile compounds determined in fresh meat and finished product (smoked ham). It is assumed that the complex chemical and biochemical processes occurring during production (growing, curing, smoking, drying) resulted in statistically significant differences between the quality parameters in fresh meat and smoked ham. There was a statistically significant difference (p <0.05) between the values of capric acid, lauric acid, myristic acid, pentadecanoic acid, pentadecenoic acid, palmitic acid, palmitoleic acid, hep tadeca-noic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and gadoleic acid identified in the thigh meat prepared for curing and smoking in compared to the value of the fatty acids identified in the final product (smoked ham) **[34]**. The potential of goats to produce high-quality meat is mainly reflected in their healthy fats, low-calorie intramuscular fats, saturated fats, and, especially, their high ratios of unsaturated (UFA) and saturated (SFA) fatty acids, as well as hypocholesterolemic and hypercholesterolemic fatty acids. This study aimed to collect and compare meat quality parameters for domestic Balkan, Alpine and Saanen goats of the same age. Samples for all tests were taken from musculus gluteus superficialis. Chemical composition, pH value, fatty acid composition, the content of volatile compounds, colour, and overall sensory quality (appearance, texture and smell) were determined. In chemical composition, moisture, fat, protein, and ash varied significantly between the examined groups as opposed to pH values. Furthermore, among all the examined groups, a significant difference was found between fatty acids and volatile compounds. The determined ratio of polyunsaturated fatty acids (PUFAs) to SFAs was 0.089, 0.085 and 0.071 for Balkan, Alpine and Saanen goat meats, respectively. Regarding that ratio, Saanen goat meat had the most favourable characteristics. Saanen goat meat showed the highest nutritional value. On the other hand, Balkan goat meat had the lowest intramuscular fat content. Measurements of the meat colour from all three groups and overall acceptability showed significant differences between breeds. Obtained results point to the impact of breed on goat meat's chemical composition and fatty acid profile **[35]**. Goats provide valuable products that are appreciated by consumers looking for food that is not only tasty but also healthy, and probably, one of them is goat meat. Breeding of local breeds such as the native Carpathian goat has been gaining importance in recent years, which creates an opportunity for the development of the goat meat market. This study aimed to investigate the influence of goat breed on the basic chemical, fatty and amino acid composition, colour and sensory evaluation of meat. The research material consisted of Carpathian goats from the

NRIAP experimental plant in the southern part of Poland and goats from a farm keeping Saanen goats in south-eastern Poland. Ten male goat kids from each breed were taken to the NRIAP farm. The meat quality obtained from the leg (m. *biceps femoris*) of male goat kids about 150 days old at slaughter was analysed. The meat of the Carpathian goat was characterized by a lower content of protein and cholesterol (p <0.01), and a higher content of fat and general collagen compared to the meat from Saanen goats (p <0.05). Cholesterol content in goat meat of both breeds was similar and ranged from 55.08 mg/100g (Carpathian) to 56.79 mg/100g (Saanen). Despite the higher collagen content, the goat meat of Carpathian breeds was characterized by lower shear force, less hardness (p <0.05) and chewiness, which was more delicate. A higher content of monounsaturated acids characterized the fat of Carpathian goat breeds, mainly C 18:1n:9, and a more favourable (lower) saturation index, S/P (p <0.05) **[36]**. This study aimed to evaluate the effect of the production system on growth performances and meat quality of suckling Messinese goat kids. At birth, 102 suckling kids were divided into two homogeneous groups for sex and body weight (3.4 kg); animals of the SES group were fed exclusively with spontaneous pasture and kept in the stable during the evening; animals of the ES group were fed exclusively with spontaneous pasture, characterized by the presence of *Quercus suber*, and kept exclusively outdoors. From birth to weaning, kids were weighed every 10 days. Carcase yields and meat quality traits on the *Longissimus dorsi* muscle were studied at slaughter. Data were subjected to ANOVA. ES group showed the highest final body weight (10.53 kg vs. 9.40 kg) **[37]**. Dietary fats are fatty acids that may play positive or negative roles in preventing and treating diseases. In nature, fatty acids occur in the form of mixtures of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), so their nutritional and/or medicinal values must be determined. Herein, we do not consider the classic indices, such as ∑SFA, ∑MUFA, ∑PUFA, ∑n-6 PUFA, ∑n-3 PUFA, and n-6 PUFA/n-3 PUFA; instead, we summarize and review the definitions, implications, and applications of indices used in recent years, including the PUFA/SFA, index of atherogenicity (IA), the index of thrombogenicity (IT), the hypocholesterolemic/hypercholesterolemic ratio (HH), the health-promoting index (HPI), the unsaturation index (UI), the sum of eicosapentaenoic acid and docosahexaenoic acid (EPA + DHA), fish lipid quality/flesh lipid quality (FLQ), the linoleic acid/α-linolenic acid (LA/ALA) ratio, and trans fatty acid (TFA). Of these nutritional indices, IA and IT are the most commonly used to assess the composition of fatty acids as they outline significant implications and provide clear evidence. EPA + DHA is commonly used to assess the nutritional quality of marine animal products. All indices have their advantages and disadvantages; hence, a rational choice of which to use is critical **[38]**. This study investigated the relationship between muscle fiber characteristics and fatty acid composition of four major muscles in Korean native black goat (KNBG). *Longissimus lumborum* (LL), *psoas major* (PM), *semimembranosus* (SM), and *gluteus medius* (GM) were obtained from five male KNBGs of 36 months of age and subjected to histochemical analysis to determine fatty acid composition and meat quality traits. There were significant (p <0.05) differences in fiber number percentage (FNP) and fiber area percentage (FAP) of fiber types among these four muscles. PM had the highest FNP of type I and the lowest FNP of type IIB, while SM had the highest FNP of type IIB. The highest fat content was observed in LL, while SM had the lowest fat content. The proportions of SFA and MUFA were significantly (p <0.05) different among four muscles due to differences in the majority of fatty acids, such as oleic (C18:1) and palmitic (C16:0) acids. The PUFA/SFA ratio differed significantly (p <0.05) among four muscles, and the highest PUFA/SFA ratio was observed in PM. Results suggested that LL and PM might be healthful because of the higher desirable fatty acid value and PUFA/SFA ratio. Also, data showed that correlations between muscle fiber types and fatty acids proportion of goat muscles were reversed with those of cattle muscles **[39]**. The arguments included in the article were based on a small number of information preserved in the sources, which concern a seasonal presence of the Wallachian shepherds in the areas situated north of the line designating the scope of permanent (year-round) rural settlements founded on the Wallachian law. It was practised in forests belonging to the king and in private estates throughout all seasons. This research resulted in the thesis statement that groups of the Wallachian shepherds led seasonal grazing of their herds in the submontane areas in the 15th century. Various factors, primarily of an economic nature, made these pastoral activities disappear or, at least, made them significantly limited at the turn of the 16th and 17th centuries. Pastoralism of a transhumance type existed throughout the period under discussion, in modern sources referred to as koszarnictwo (transhumance herding), consisting of the period under discussion, in modern sources referred to as koszarnictwo (transhumance herding), consisting of periodic migrations of pastoral groups from permanent villages. Similarly to what I have claimed in my previously published research on the Wallachian pastoralism in the Carpathian areas, there are no indications in the sources which would justify a thesis for a long time widespread in historical studies on the presence of a nomadic phase in the history of the Wallachian colonization in the Polish areas. Also, in the case of the studied areas, its existence can be given no confirmation **[40]**. To assess the development of kid's bodies during birth – 6 months kids were ed at birth, at 28 days, at weaning (60 days) and 6 months, registering total weight gain and average daily gain achieved by them in stages throughout the period monitored. The research considered two farms of goats from the Carpathian breed

differentiated by the technology of rearing and exploitation practised, which were situated in the South Muntenia Region (Giurgiu and Prahova counties). Prahova County exploits animals belonging to Carpathian breed Prahova County farm exploits Carpathian breed animals and practicing an extensive operating system or pastoral, and the farm from Giurgiu County exploits the same breed using the same breed semi-intensive operating system. The best result in terms of quantitative parameters of meat production (average daily gain, average total gain) was achieved by practising semi-intensive operating systems **[41]**. Five types of meat were produced: traditional milk capretto (MC), heavy summer capretto (HSC), summering (SCh), fall (FCh) and late fall chevon (LFCh). HSC was the most tender meat, having fewer cooking losses than MC and redder chevon types. The instrumental profile corresponded with the appearance and texture attributes perceived by panellists. With kids' ageing, meat lost its milk aroma (MC) and sweet taste (HSC). It acquired an increasing intensity of goat flavour and livery notes, partially related to the feeding regime and fatty acid profile. A niche market preferred chevon over capretto; while the cluster of consumers unfamiliar with chevon showed a decrease in pleasantness when tasting chevon, the familiar group reduced their ratings only for meat from the oldest kids **[42]**. Collagen constitutes 20-30% of proteins in the organism of mammals and birds, and it is a major component of the intramuscular connective tissue. In the muscles, collagen is mainly stored in epimysium, perimysium, and endomysium. There are more than 20 genetic types of collagens in the skeletal muscles; among them, collagen type I and type III are a significant portion. The morphology, composition, and quantity of the connective tissue in the muscles depend predominantly on their type and the animal's species, breed, and age. Owing to differences in the methods of determining collagen, the content of this protein can differ in individual muscles. High content of this incomplete protein in the muscles' connective tissue significantly impacts the meat's tenderness and decreases its quality. The cross-linking of collagen in the muscles that are highly active in live animals increases with the age of animals and causes the meat to become hard. Lower content of collagen was found in the muscles with longer sarcomeres and in the meat from late maturing and castrated animals **[43]**. The possibility for improvement of carcass traits and quality of kid meat of the autochthonous Balkan goat breed by crossing with the Saanen breed was investigated in this study. The trial was carried out on one group of Balkan goat kids and three groups of kids cross of Balkan and Saanen goats with different proportions of Saanen genes: 25, 50 and 75%. Each group had 16 male kids, slaughtered at an average body weight of 18 kg. With the increase in the proportion of Saanen genes, the age of kids that reached preslaughter weight decreased, the chilling loss increased, and the proportion of fat tissue (kidney and pelvic fat) in the carcass side decreased ($P \leq 0.05$). The crossing also increased the proportion of carcass parts of the first category (leg and loin section) and muscle tissue in those parts. The highest proportion of muscle tissue in the thigh (74.91%) and loin section (75.66%) was determined in kids from the group with 75% of Saanen genes, and kids from this group also had the highest proportion of intramuscular fat (2.48%) in samples of m. longissimus dorsi. Slight differences between kid groups were established in indicators of technological meat properties, such as water binding capacity and tenderness, with the increase in the proportion of Saanen genes in the genotype. The sensory score for roasted meat was high, and scores for tenderness and juiciness were slightly higher in kid crosses with 50% and 75% of Saanen genes ($p \leq 0.05$). Results presented in this study confirm the positive effect of crossing the Saanen breed with the Balkan breed on carcass traits and for obtaining meat of more desirable quality **[44]**. Little is known about the fatty acid composition of the major muscles in goats from different breeds. Forty male suckling kids, 20 Criollo Cordobes and 20 Anglo Nubian, were slaughtered at 75 days of age and their longissimus thoracis (LT) fatty acid composition (semitendinosus (ST) muscles were analysed to clarify the effects of genotype and muscle type on goat kid meat. Genotype greatly influenced the fatty acid composition of goat kid meat **[45]**. Forty suckling kids, 20 intact males and 20 females were randomly assigned to two groups: I (60 ±2 days old and live weight ≤11 kg) and II (90 ±3 days old and live weight >11 kg). Sex significantly influenced meat colour, WarnerBratzler shear force, cooking losses, water holding capacity and intramuscular fat content, while the age/weight significantly influenced cholesterol and tenderness. The main fatty acids identified from the intramuscular fat were oleic (30.1-32.6%), palmitic (19.6-21.0%) and stearic (13.5-16.3%). Levels of saturated and unsaturated fatty acids ranged from 40.1% to 41.9% and from 57.6% to 59.1%, respectively. Meat from CC kids is pale red, tender, and juicy, and the intensity of flavour and aroma were medium-high **[46]**. Thirty-two male goats were divided into four racial groups: eight pure Boer breeds, eight 3/4 Boer + 1/4 SPRD crossbred, eight 1/2 Boer + 1/2 SPRD crossbred and eight 1/2 Anglo Nubian + 1/2 SPRD crossbred. All goats were reared under the feedlot system and slaughtered at the average age and live weight of 223 days and 29 kg, respectively. The chemical composition was determined, including moisture, protein, ash, fat, cholesterol, phospholipids, and fatty acids. The breed types had no significant effect on moisture, protein, ash, fat, cholesterol, and phospholipids contents. However, the percentages of oleic and stearic acids and the MUFA/SFA ratio showed significant differences between the four breed groups, ranging from 0.72 for 3/4 Boer + 1/4 SPRD crossbred to 0.95 for 1/2 Boer + 1/2 SPRD crossbred. The oleic acid (C18:1) was found in the highest percentage in the fatty acid profile in goat meat, particularly for 1/2 Boer + 1/2 SPRD and 1/2 Anglo + 1/2 SPRD

genotypes. The crossing of exotic Boer and Anglo Nubian breeds with the natives SPRD resulted in goat meat of high quality, even at a ratio of 50%, since the goat meat showed low cholesterol percentage and high protein and unsaturated fatty acids contents **[47]**. Some quality traits of meat from purebred French Alpine kids and Boer crossbreeds aged 50 days were evaluated in the study. Samples of m. quadriceps femoris were taken to determine the chemical composition and physicochemical properties of meat, as well as a water-to-protein ratio, energy value, levels of cholesterol and amino acids in a protein, and fatty acid concentration in intramuscular fat. It was found that meat from crossbred kids, compared to meat from purebred kids, contained more intramuscular fat, cholesterol and vitamin A, had a higher calorific value, a brighter colour, a lower water-holding capacity, a higher level of physiological maturity (measured as the value of a water-to-protein ratio), and got higher scores for tenderness and juiciness. The meat protein from crossbred kids had a more desirable essential amino acid/non-essential amino acid (EAA/NEAA) ratio. At the same time, intramuscular fat contained less OFAs and had more desirable unsaturated fatty acid/ saturated fatty acid (UFA/SFA) and DFA/OFA (UFA+C 18:0 /SFA-C 18:0) ratios. Due to a high protein content (19.44 and 19.74%), low levels of fat (1.67 and 1.96%) and cholesterol (48.76 and 56.63 mg/100g), a low energy value (96.36 and 101.47 kcal/100g), a high concentration of essential amino acids, a desirable fatty acid profile and high scores for sensory properties, meat from purebred French Alpine kids and (especially) Boer crossbreds may be recommended as a valuable component of a low-fat diet **[48]**. The effect of two different rearing systems of goats, such as grown under confinement and raised on the field, was evaluated on the muscles of an intact male goat. The physicochemical properties such as pH, water activity (Aw), and chemical composition, including moisture, protein, ash, calcium, iron, phosphorus, cholesterol, phospholipids and fatty acids, were determined. The rearing system had no significant effect on protein content and water activity. Concentrations of ash and fat were significantly (p <0.05) different, being higher in goats raised under confinement, while goats raised in the field had a higher percentage of moisture and phospholipids. However, pH, iron, phosphorus, and cholesterol contents were significantly higher for animals raised under confinement. The predominant fatty acid in goat meat in both rearing systems was oleic (C18:1), ranging from 36.23 to 43.56%. Higher contents of saturated fatty acids (SFA) and lower contents of monounsaturated fatty acids (MUFA) were found to be significantly ($p \leq 0.05$) different in goats raised in field. This resulted in a greater ratio of unsaturated fatty acids (UFA) and SFA, and that of MUFA and SFA in goat meat of animals raised under confinement compared to that of the goats raised on the field **[49]**. The weight at slaughter (LWS) for kid goats in Mediterranean countries is lower than in Arabian or African countries. Logically, increasing LWS could increase a farmer's profit margin. Forty-five twin male kids from the Canary Caprine Group breed were used to compare carcass and meat quality at 6, 10 and 25 kg LWS. Dressing percentage based on full weight was lower for 25 kg LWS compared with LWS of 6 and 10 kg, although based on empty body weight, dressing percentage for 25 kg LWS was similar to that with 6 and 10 kg LWS. However, the dressing percentage based on empty body weight was lower for 6 vs. 10 kg LWS. There were no significant differences among LWS in percentage contributions to the whole carcass of primal cuts, excluding the neck (lower proportion in 25 kg LWS kids). LWS did not affect tissue distribution in the carcass except for intermuscular fat (higher for 25 vs. 6 kg LWS). Few differences between LWS were observed concerning meat quality parameters. Results suggest that increasing LWT from 6 to 10 and 25 kg for kids artificially reared does not negatively affect carcass or meat quality yet would result in more edible meat (pounds) to be marketed **[50]**. The effect of castration and slaughter age on fat, cholesterol, phospholipids and fatty acid contents was determined for native Brazilian goat meat muscles. Groups of castrated and intact "Mestiço" goats were slaughtered at 175, 220, 265 and 310 days of age. Castration and slaughter age significantly affected total cholesterol and fatty acids contents. The cholesterol content increased with the advance in slaughter age. Meat from castrated goats had a higher cholesterol content than that from intact. Goat muscle contained mostly C18:1 (38-44%), C18:0 (23-25%), C16:0 (18-21%), and C18:2 (4-6%) fatty acids. There were no differences in saturated, unsaturated and polyunsaturated fatty acids levels among the four groups slaughtered at different intervals. Castrated goat meat contained significantly greater unsaturated and polyunsaturated fatty acids than that of intact. Total fat and phospholipids percentages ranged from 3.0 to 3.4 g/100g and 10.6 to 11.1 mg/100g, respectively, for intact and castrated goats **[51]**. Twenty Boer x Spanish goats, in the age range of 90-118 days, were assigned to two dietary treatments, with 10 animals fed a grain ration (G) and 10 grazed in rangeland. The grain ration contained sorghum grain (67.5%), cottonseed hulls, dehydrated alfalfa meal, cottonseed meal, soybean meal, molasses, and mineral and vitamin supplements. Animals were slaughtered at the age range of 206-234 days. Intramuscular fat (IF) and the diet specimens - representative samples of G and the parts of range plants (RPs) that goats were expected to have consumed - were analysed for fatty acid composition. The percentage of 16:0 was higher in RPs than in G, but not different between IF from range goats and that from grain-fed goats. Total unsaturated fatty acid (UFA) percentage was higher in G than in RPs. The major UFAs were 18:2 and 18:3 in RPs, and 18:1 and 18:2 in G. In IF, 18:1 constituted more than two-thirds of UFAs, regardless of diet type **[52]**.

CONCLUSION

When assessing the chemical composition of experimental goat meat samples (Zaanenskaya, Alpine, Nubian), no abnormal deviations were detected, and all indicators were in the generally accepted contents of this type of animal muscle tissue. The mass fraction of goat meat proteins was 2.1 ±0.3-2.4 ±0.4%. The study of the dynamics of changes in the composition of protein fractions based on the results of comparative studies of the ratio of sarcoplasmic proteins showed the content of water-soluble (1.75 – 4.06%), salt-soluble (1.75 – 2.44%), alkali-soluble (11.15 – 15.10%) proteins. The salt-soluble fraction reflects the total changes in the state of protein fractions, the solubility of which was not the same for the rocks under consideration (the highest concentration was determined in the Nubian rock). There were no significant differences in moisture-binding capacity (WCC) (73.45; 74.42; 73.94%). Generally, it is important to note that goat meat, which has a better ability to concentrate meat juice inside the muscle fiber, is more valuable in terms of its technological characteristics. Therefore, it can also be recommended to produce food for schoolchildren.

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Investigation of internal organs and additive tissue of hybrid hypophthalmichthys (*Hypophthalmichthys spp.)* as a promising raw material for the production of dietary nutritional products

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ABSTRACT
Preservation of the nutritional value of fish and the useful qualities of its rich composition is extremely important. The urgent task of the food industry is to develop and create quality food products that meet modern production trends and compete in domestic and foreign markets. This scientific paper describes studies aimed at assessing the specific weight (%) of essential nutrients (glycogen, proteins, and lipids) in particular organs and tissues of different size and mass groups of the hybrid of silver and bighead carp experimental ponds and reservoirs of Ukraine. To achieve the goal in the research process, fish farming, biochemical (study of total protein, lipids, and carbohydrates), and statistical (mathematical processing of research results) research methods were used. In all size and mass groups of the hybrid of silver and bighead carp from ponds and reservoirs in 2017, 2018, and 2019, mostly satisfactory values of general metabolism indicators were found - glycogen, proteins, and lipids in the liver, gills, and muscles of fish. In annual fish of winter ponds, total protein and glycogen content in all organs and tissues was slightly reduced. The organisms of biennial fish from feeding ponds were characterized by fluctuations in the content of glycogen in the liver (it was the highest in fish, 3.28 – 3.33%). Significant fluctuations in the total protein content of muscle, liver, and gills and a slight excess of glycogen in the liver and lipids in the gills of three-year-olds were observed in the reservoirs. The difference found in the availability of essential nutrients in the body of the studied fish indicates a change in the intensity and direction of their metabolic processes. However, their physiological condition at the time of the study was within normal limits.

Keywords: pond, reservoir, proteins, lipids, glycogen.

INTRODUCTION
The accumulation of glycogen, proteins, and lipids in fish is influenced by many biotic and abiotic factors **[1]**. The above factors can be significantly influenced by a number of factors, hydrochemical state of water, temperature, gas regime of the reservoir, seasons, and meteorological factors **[2]**. Significant dependencies can be made by the species, age, and sex of fish, stocking density and the presence of invasions, and the level of contamination of the reservoir with various nutrients or toxic substances (heavy metals, petroleum products, and their derivatives) **[3]**, **[4]**. Oil and petroleum products are the most common toxicants in the aquatic environment **[5]**. Their characteristic feature is that these substances are slowly destroyed under natural conditions, sometimes change their chemical form, and gradually accumulate in various ecosystem components **[6]**. Oil harms fish's metabolism, inhibiting the activity of various localized enzymes that carry out the hydrolysis of protein and carbohydrate components of food, which leads to an increase in total protein. Accumulating heavy metals in fish can cause significant disturbances in cellular metabolism **[7]**, causing oxidative damage to proteins and nucleic acids. Biogenic elements, which include nitrogen compounds, enter the body through the gill apparatus and intestines of fish, damaging their epithelial layer. In addition, they can adversely affect other internal organs, including the liver, kidneys, and spleen of fish. In the future, this can lead to metabolic disorders, which can be reflected in the neurohumoral regulation of metabolism and enzymatic activity, changing the content of energy-

intensive compounds. This can affect fish survival, the quality and quantity of gametes, and hence the species diversity of ichthyofauna **[8]**. The toxic effect of phenolic compounds on fish is manifested in the disruption of protein biosynthesis, barrier functions of membranes, etc., which affects the processes of balance, respiration, and motor activity. In fish it causes anxiety and, subsequently, seizures and death. That is why phenols are classified as toxic paralytics **[9]**. Chromium has mutagenic and teratogenic properties, as its effects on fish can cause significant changes in physiological, histological, biochemical, and genetic parameters **[10]**. One of the main components in the body of fish is protein, and its content depends on the rate of linear growth. In addition, in fish, proteins can be used as alternative energy sources, which, if necessary (with the participation of aminotransferases) play a role in the processes of adaptation to the negative factors of the environment **[11]**. Studies of internal organs, particularly the liver, provide objective information that can be used to determine the general physiological state of the body **[12]**. It is known that the liver is an important organ of metabolism **[13]**. Among its main functions are the processes of digestion and the processes of protein metabolism (synthesis and decomposition). The cleavage of proteins into amino acids is subsequently broken down to form ammonia or urea or is involved in protein-synthetic processes. The level of lipid accumulation is directly dependent on the fatness of fish, and the direction of lipid metabolism varies depending on the stage of ontogenesis, sex, and phase of the reproductive cycle. For example, the lipid content in the liver of males and females differs and depends on the stage of maturity of the gonads **[14]**. Lipids are the basis for all intracellular membranes and play a significant role in cell metabolism **[15]**. Violation of homeostasis of the body always indicates the presence of pathology or stress. The total lipid content indicates the activity of anabolic and catabolic processes and the mobilization of lipids as a source of energy **[16]**. After all, lipids can be used in adaptive rearrangements of structural components of cells, tissues, and organs **[17]**. For aquatic organisms, the accumulation of many lipids provides vital support. It determines the survival of individuals when changing environmental factors in their combination, taking into account the peculiarities of the annual cycle of the organism **[18]**. Glycogen is a polysaccharide that stores carbohydrates, especially in liver and muscle tissue. In addition, it is a labile, readily available energy-intensive compound that can be converted to glucose when urgently needed (e.g., toxic substances entering fish tissues). Glycogen is also a source of chemical energy and a regulator of blood pressure. The content of glycogen in the tissues of fish's liver may decrease under the influence of pollution or deficiency of water-soluble oxygen caused by significant energy expenditure to overcome stress **[19]**. Under adverse conditions, detoxification and antioxidant protection systems change in fish tissues. Stimulation of detoxification mechanisms requires additional energy expenditure **[20]**, usually accompanied by suppression of energy metabolism. However, in the end, the effective work of regulatory and coordinating mechanisms ensures the organism's adaptation to changing conditions **[21]**.

Scientific Hypothesis

The difference in the availability of essential nutrients (glycogen, proteins, and lipids) in the body of the silver and bighead carp hybrid may indicate a change in the intensity and direction of their metabolic processes. At the same time, among the relevant studies, this area remains insufficiently studied. However, previously extensive work has been carried out, including collecting ichthyological material with a subsequent study of the chemical composition of their organs and tissues.

MATERIAL AND METHODOLOGY

The research was conducted in the spring, summer, and autumn periods from 2017 to 2019 in ponds based on the training, research, and production laboratory of fish farming of the National University of Life and Environmental Sciences of Ukraine (TRPLF NULES of Ukraine) of Ukraine, village Nemishayevo, Kyiv region (Polissya zone); State Enterprise "Experimental Farm" Nyvka " of the Institute of Fisheries of the National Academy of Agrarian Sciences (SEEF "Nyvka" IF NAAS) of Ukraine, Kyiv; (ponds are on the border of the zones (it is along the Nivka River that the Forest-Steppe is divided to the south and Polissya to the north)); Bila Tserkva Experimental Hydrobiological Station of the Institute of Hydrobiology of the National Academy of Sciences (BEHS IHB NAS) of Ukraine, Bila Tserkva (Forest-steppe zone), Kosiv (Kyiv region) and Velykoburlutsky (Kharkiv region) reservoirs (Forest-steppe zone).

Samples

Collection of ichthyological material was carried out during stocking and catching fish in ponds and reservoirs. The number of stocks in the ponds was 6, and the number of catches was 18; Velykoburlutsky Reservoir had 1 stocking, catch – 2, Kosiv Reservoir – 2 stocks, and 2 catches. The material for the study was a youth of the year, annuals, biennials, and triennials of the hybrid of silver and bighead carp (Figure 1, Figure 2, and Figure 3).

Figure 1 Biennial of silver and bighead carp hybrid caught in the autumn from a feeding pond No. 1 of TRPLF NULES of Ukraine.

Figure 2 Catch of silver and bighead carp hybrid biennials in the wintering pond No. 119 of SEEF "Nyvka" IF NAAS.

Figure 3 Collection of ichthyological materials (biennials of the hybrid of silver and bighead carp) in the spring during the capture of the winter pond No. 119 of SEEF "Nyvka" IF NAAS.

Chemicals

Potassium hydroxide (KOH), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Anthrone ($C_{14}H_{10}O$), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Concentrated sulfuric acid (H_2SO_4), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Sodium hydroxide (NaOH), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Sodium carbonate (Na_2CO_3), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Potassium sodium tartaric acid ($KNaC_4H_4O_6 \times {}_4H_2O$), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Folin–Ciocalteu reagent (FC), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Vanillin reagent (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Animals and Biological Material

45 specimens of the hybrid of silver and bighead carp of different size and mass groups (youth of the year, annuals, biennials, triennials) caught from ponds, and 35 specimens from reservoirs were processed.

Instruments

Net of grids with a mesh step from 30 to 100 mm (producer "CrayFish" Limited Liability Company, Finland).
Electronic laboratory scales (TBE-0.15-0.001-a-2, producer «Inter-Synthesis» Limited Liability Company, Ukraine).
Technical electronic scales (BTHE-6-H1K-1, producer "Inter-Synthesis" Limited Liability Company, Ukraine).
Counting chamber of Najotta (producer "Laboratory equipment" Limited Liability Company, Ukraine).
Binocular microscope (XSP-139B LED Ulab, producer "Laboratory equipment" Limited Liability Company, Ukraine).
Apstein's grid (producer "ADS-Lab" Limited Liability Company, Ukraine).
Bogorov counting chamber (producer "ADS-Lab" Limited Liability Company, Ukraine).
Stereoscopic microscope (MBS-9, producer "Laboratory equipment" Limited Liability Company, Ukraine).

Laboratory Methods

The content of total protein in organs and tissues was determined by Lowry et al. (1951) **[22]**, lipids using the standard commercial kit "Total Lipids" (Philisit-Diagnostics, Ukraine), and glycogen - using anthrone reagent.

Description of the Experiment

Sample preparation: When determining the chemical composition in the organs and tissues of fish from reservoirs in different periods, selected 5 specimens of fish differing in weight and age.

Number of samples analyzed: 135 samples of tissues and organs (liver, white muscles, and gill petals) were taken from the fish caught in the ponds, and 105 samples were taken from the reservoirs to determine the number of proteins, fats, and carbohydrates.

Number of repeated analyses: In the experimental ponds and the Kosiv Reservoir, the repetitions of the experiments were twofold, and in the Velykoburlutsky Reservoir - one-time.

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

Design of the experiment: The content of total proteins in tissue samples was determined by the method of Lowry et al. (1951) **[22]**. Briefly, 0.1 g of tissue and organ was hydrolyzed for 1 hour in 10 mL of 10% NaOH at a temperature of 60 °C. To 0.1 mL of the hydrolysate was added 10 mL of solution No. 3, and staining was carried out for 15 minutes. Then, the sample added 1.0 mL of Folin's reagent diluted 1:1 with distilled water. The staining was carried out for 30 minutes. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 720 nm against control. The amount of protein was set according to the calibration schedule. Solution No. 3 was prepared from solutions No. 1 and No. 2 in a ratio of 9:1. Solution No. 1 was prepared based on 0.1 n NaOH with the addition of 20 g Na_2CO_3 and 0.5 g of potassium, and sodium tartaric acid. Solution No. 2 contained 1 g $CuSO_4$ per 1 liter of distilled water.

The content of total lipids was determined using a phosphorovaniline reagent. Briefly, 100 mg of tissue was hydrolyzed in 1.5 mL of concentrated sulfuric acid for 15 minutes. About, 0.1 mL of the hydrolysate was added with 3 mL of vanillin reagent (10 mmol L^{-1} of vanillin and 11.5 mmol L^{-1} of phosphoric acid). The solution was stained for 40 min. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 530 nm against control. The amount of lipid was set according to the calibration schedule.

The content of glycogen was determined by the anthrone method. Briefly, 0.1 g of tissue was hydrolyzed for 1 hour in 3 mL of 30% KOH at a temperature of 100°C, then 0.9 mL of distilled water and 3 mL of 0.2% anthrone were added to 0.1 mL of the hydrolysate. Then the sample was boiled at 100 °C for 10 minutes. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 620 nm against control. The amount of glycogen was established according to the calibration graph.

Statistical Analysis

The statistical evaluation of the results was carried out 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. We calculated the arithmetic mean (unweighted) value (M), and the arithmetic mean error (±m), which made it possible to estimate with a certain probability the deviation of the arithmetic mean deviation Fulton fatness rate. The statistical reliability of the results of the research was provided by analyzing samples with the number of fish from 10 to 25 specimens.

RESULTS AND DISCUSSION

One of the integral indicators of the physiological state of fish is metabolism, which is determined by the content of proteins, lipids, carbohydrates, etc. in the organs and tissues **[23]**.

The general indicators of metabolism of annual hybrids of white with variegated silver carp, which were caught from winter ponds in the spring of 2017 – 2018, are shown in Table 1.

Table 1 General indicators of metabolism of annual hybrids of white with variegated silver carp caught from winter ponds, M ±m, n = 5.

Cloth	Glycogen, %		Albumen, %		Lipids, %	
	Spring, 2017 ye.	Spring, 2018 ye.	Spring, 2017 ye.	Spring, 2018 ye.	Spring, 2017 ye.	Spring, 2018 ye.
Pond No 101 SE DG "Nivka" IRG NAAS						
Muscles	4.3 ±0.02	0.45 ±0.01	13.69 ±0.04	13.62 ±0.03	0.38 ±0.01	0.35 ±0.01
Cv	8.33	6.58	0.68	0.47	8.32	6.48
Liver	2.11 ±0.02	2.07 ±0.02	12.78 ±0.02	12.87 ±0.01	3.56 ±0.03	3.59 ±0.02
Cv	2.28	2.23	0.41	0.18	1.98	1.10
Branchia	0.46 ±0.01	0.40 ±0.02	12.29 ±0.02	12.32 ±0.03	0.42 ±0.01	0.40 ±0.01
Cv	6.97	10.32	0.36	0.47	9.12	4.14
Rate No 2. NNVLR NULES of Ukraine						
Muscles	0.37 ±0.01	0.33 ±0.01	13.30 ±0.02	13.35 ±0.01	0.28 ±0.01	0.30 ±0.01
Cv	7.21	9.25	0.28	0.17	5.26	4.98
Liver	1.92 ±0.01	1.89 ±0.02	12.29 ±0.02	12.35 ±0.01	2.87 ±0.02	2.90 ±0.02
Cv	1.72	1.92	0.34	0.18	1.88	1.64
Branchia	0.38 ±0.01	0.34 ±0.01	12.12 ±0.09	12.11 ±0.09	0.32 ±0.01	0.35 ±0.01
Cv	5.26	9.55	1.59	1.69	9.88	6.48
Rate No. 5 BEGS IGB NASU						
Muscles	0.28 ±0.01	0.30 ±0.01	11.19 ±0.04	12.60 ±0.31	0.26 ±0.01	0.28 ±0.01
Cv	10.75	11.10	0.80	6.01	6.34	5.05
Liver	1.77 ±0.01	1.83 ±0.02	10.92 ±0.08	12.19 ±0.07	2.78 ±0.01	2.83 ±0.02
Cv	1.29	1.99	1.61	1.29	1.03	1.77
Branchia	0.31 ±0.01	0.26 ±0.01	10.76 ±0.06	12.05 ±0.11	0.29 ±0.01	0.34 ±0.01
Cv	8.60	9.12	1.21	2.12	6.13	7.58

Note: Cv – In this table and in the following – the coefficient of variation.

The average values of the share of glycogen in most organs and tissues of annuals of the hybrid of silver and bighead carp in 2017, and 2018 were at the level of 0.5% and below (Figure 4). Exceptions were the indicators of the specific gravity of glycogen in the liver of bighead carp: in the winter pond No. 101 – above 2%; in winter ponds No. 2 and No. 5 – less than 2% in both years of research. Thus, a higher level of glycogen in the liver of annual fish is evident compared to its presence in the muscles and gills of the youth of the year of silver and bighead carp hybrids.

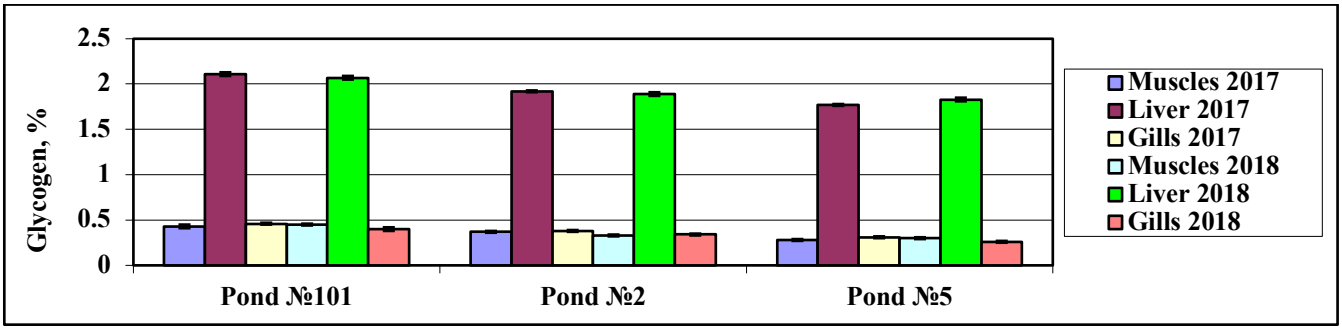

Figure 4 The average values of glycogen (%) in the organs and tissues of annuals of the hybrid of silver and bighead carp from winter ponds in 2017 and 2018.

The average values of the specific weight of protein in most organs and tissues of annuals of the hybrid of silver and bighead carp in 2017 and 2018 were almost evenly distributed in them, at the level of 10 – 14% (Figure 5). Exceptions were the indicators of the proportion of protein content in the winter pond No. 5 – they were lower (about 10%) and fluctuated over the years.

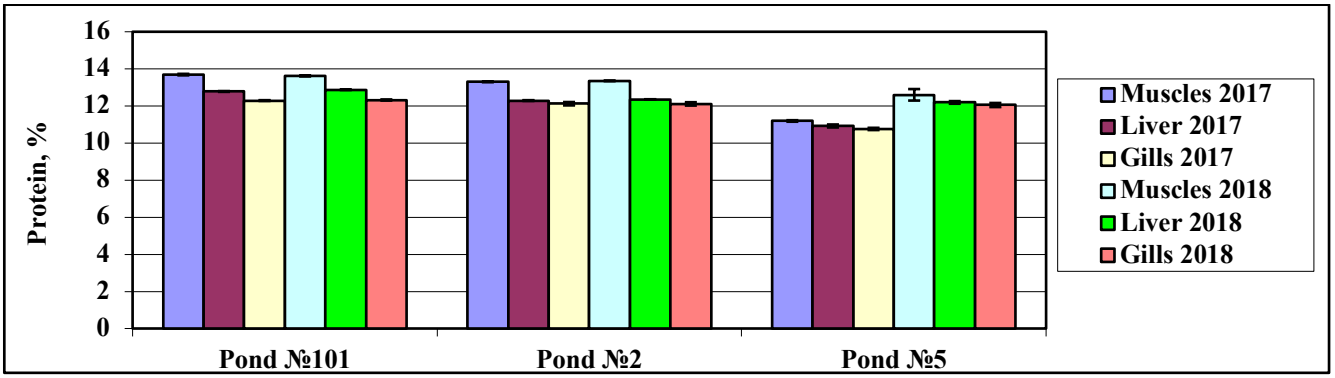

Figure 5 Average values of total protein (%) in the organs and tissues of annuals of silver and bighead carp hybrid from winter ponds in 2017 and 2018.

The average values of the specific weight of lipids in most organs and tissues of annuals of the hybrid of silver and bighead carp in 2017 and 2018 were, like glycogen, at 0.5% and below (Figure 6). Exceptions were the indicators of the proportion of lipids in the liver of bighead carp: in the winter pond No. 101 – above 3.5%; in winter ponds No. 2 and No. 5 – about 3% in both years of research. Thus, a higher level of lipids (as well as glycogen) is present in the liver of annual fish compared to its content in the muscles and gills of the youth of the year of the hybrids of silver and bighead carp.

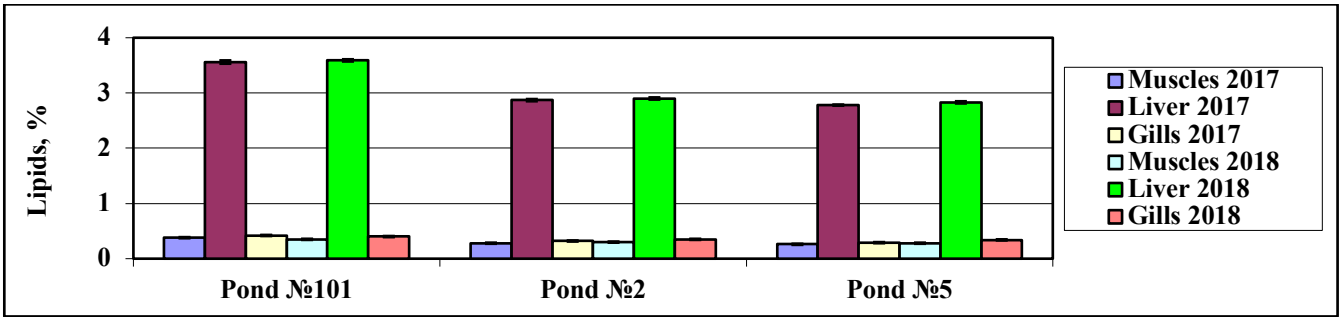

Figure 6 The average values of lipids in the organs and tissues of annuals of the hybrid of silver and bighead carp from wintering ponds in 2017 and 2018.

Thus, according to the results of studies conducted in 2017 and 2018, it was found that the concentration of glycogen, total protein, and lipids in fish organs and tissues of fish from SEEF "Nyvka" IF NAAS and TRPLF NULES of Ukraine was satisfactory. In annual fish from BEHS IHB NAS in 2017, the total protein and glycogen content in organs and tissues was slightly reduced, and the number of lipids was within normal limits.

The results indicate that the annuals of the hybrid of silver and bighead carp in the winter significantly reduce or stop the trophic activity and switch partially or completely to endogenous nutrition.

Due to the use of energy compounds by fish, there is a gradual decrease in their content during the winter. During this period, the most vulnerable are fish, especially those that did not have enough spare nutrients before winter. The data obtained probably indicates that the fish were not fully prepared for winter because they did not have enough energy compounds.

Winter is one of the most difficult periods in fish life, especially for the young, in the first year of life **[24]**. With the lack of feeding, the effective consumption of natural foods by annual herbivorous fish is almost stopped in late August or the first decade of September. Almost from the third decade of September, the youth of the year, in ponds without food, to ensure viability begin to use endogenous nutrients from their own "depot" **[25], [26]**. Adverse hydrological factors (low temperature, lack of oxygen, etc.) could also affect wintering conditions. The temperature largely regulates the intensity of metabolism and fish development rate **[27]**, **[28]**.

At low temperatures, lipids during critical physiological stress become the body's main energy source. Their synthesis provides the need for fish for lipids in the body due to lipids, which are part of the natural and artificial feed base.

Lipids of "peripheral" organs (muscles, gills, intestines) are mainly used, and lipids of a brain and a liver remain for maintenance of regulatory functions **[29]**, **[30]**, **[31]**. To stabilize the water temperature and create the desired wintering conditions, an important factor is the ice cover of the ponds, playing a significant role in heat exchange between the water column and atmospheric air. Direct ice cover ensures the stability of water temperature throughout the winter, which allows youth of the year to effectively use the accumulated nutrients without increased energy costs **[32]**, **[33]**, **[34]**. The specifics of the south of Ukraine against the background of a general warming of the atmosphere were the most sensitive to wintering of annuals of carp, which significantly affects the specific conditions of the zone, the water temperature under the action of periodic warming during wintering. Each such warming leads to increased mobility of youth the year in the winter. In the practical absence of food and the impossibility of their effective assimilation, mobility leads to deterioration of the general physiological state, reduced body resistance, overweight, and, as a result, low annual yield, which has been proven, quite reliably by previous studies **[35]**, **[36]**, **[37]**.

The resistance of fish to wintering conditions increases with age, and accordingly, fewer nutrients are used. Its condition after wintering is better compared to younger age groups of fish (Table 2).

Table 2 General indicators of metabolism of a two-year-old white hybrid with variegated silver carp caught from feeding ponds, M ±m, n = 5.

	Glycogen, %		Albumen, %		Lipids, %	
	Autumn, 2017 ye.	Autumn, 2018 ye.	Autumn, 2017 ye.	Autumn, 2018 ye.	Autumn, 2017 ye.	Autumn, 2018 ye.
Pond No. 101 SE DG "Nivka" IRG NAAS						
Muscles	0.68 ±0.01	0.66 ±0.01	14.53 ±0.03	14.57 ±0.03	0.57 ±0.02	0.55 ±0.02
Cv	2.64	4.05	0.39	0.53	7.30	6.58
Liver	3.33 ±0.03	3.28 ±0.03	13.97 ±0.04	13.93 ±0.02	5.65 ±0.04	5.69 ±0.02
Cv	1.77	1.22	0.63	0.39	1.41	0.80
Branchia	0.76 ±0.01	0.74 ±0.01	12.92 ±0.02	12.96 ±0.01	0.82 ±0.02	0.74 ±0.02
Cv	3.22	3.31	0.34	0.25	5.26	6.96
Rate No. 2 NNVLR NULES of Ukraine						
Muscles	0.55 ±0.03	0.57 ±0.02	14.30 ±0.09	14.21 ±0.07	0.46 ±0.01	0.45 ±0.01
Cv	11.64	7.55	1.33	1.07	6.57	4.97
Liver	2.87 ±0.06	2.84 ±0.05	12.85 ±0.03	12.83 ±0.02	4.68 ±0.01	4.64 ±0.01
Cv	4.61	3.80	0.56	0.32	0.68	0.71
Branchia	0.62 ±0.02	0.65 ±0.02	12.45 ±0.04	12.43 ±0.04	0.71 ±0.02	0.69 ±0.02
Cv	6.17	6.40	1.24	0.73	5.82	6.96
Rate No. 5 BEGS IGB NASU						
Muscles	0.43 ±0.01	0.42 ±0.01	14.03 ±0.14	14.22 ±0.22	0.39 ±0.01	0.41 ±0.02
Cv	5.20	4.02	2.19	3.41	5.88	8.46
Liver	2.51 ±0.06	2.61 ±0.02	12.77 ±0.01	12.81 ±0.03	4.61 ±0.14	4.66 ±0.06
Cv	5.02	1.63	0.18	0.51	6.92	3.07
Branchia	0.52 ±0.01	0.49 ±0.02	12.32 ±0.06	12.29 ±0.06	0.63 ±0.01	0.65 ±0.01
Cv	3.85	7.38	1.06	1.05	4.74	3.52

After wintering in annual local carp, obtained by crossing Nyvka plant line of small-scale intra-breed type of Ukrainian frame breed and Nyvka intra-breed type of Ukrainian scaly breed, it was found that fat consumption was 21.7 and 27.6%, respectively, for control and research group, protein consumption 11.1 and 13.3% **[30]**.

The average values of biochemical parameters of biennials of the hybrid of silver and bighead carp caught from feeding ponds in 2017 and 2018 were 0.5% and above (Figure 7).

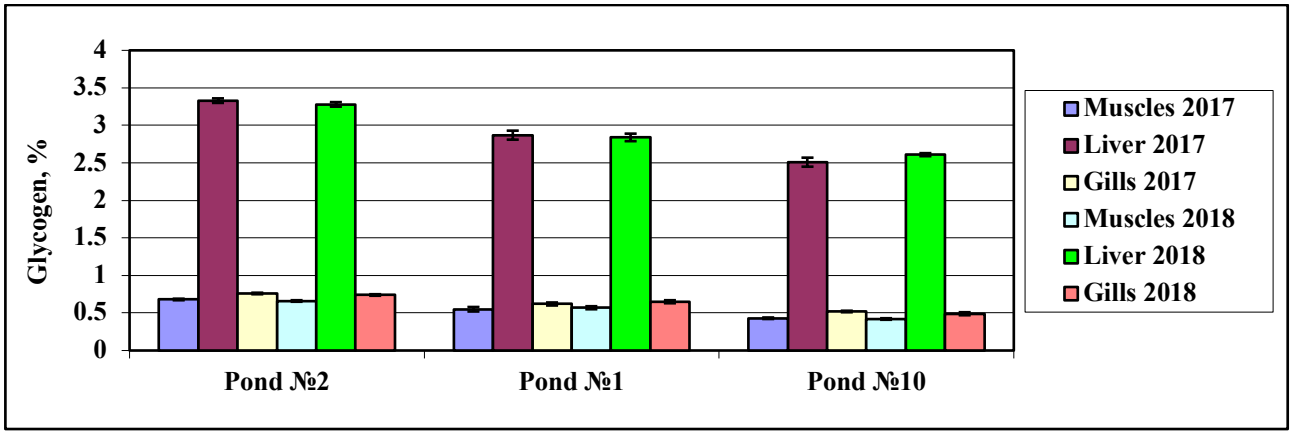

Figure 7 The average glycogen (%) values in the organs and tissues of biennials hybrid of silver and bighead carp from feeding ponds in 2017 and 2018.

Exceptions were indicators of glycogen content in fish's liver from feeding ponds: in pond No. 2, 3 – 3.5%; in pond No. 1, 2.5 – 3% and pond No. 10, the lowest about 2.5% in both years of research. Thus, in the liver of biennials, a higher level of glycogen is evident than its presence in the muscles and gills of young hybrids of silver and bighead carp.

The average values of the proportion of protein in most organs and tissues of biennials of the hybrid of silver and bighead carp in 2017 and 2018 fluctuated markedly by year and in particular organs and tissues: in muscle, the figures were at 14 – 14.5%; in the liver at the level of 12.5 – 14% (the highest pond fish No. 2); in gills – at least at the level of less than 12% (Figure 8).

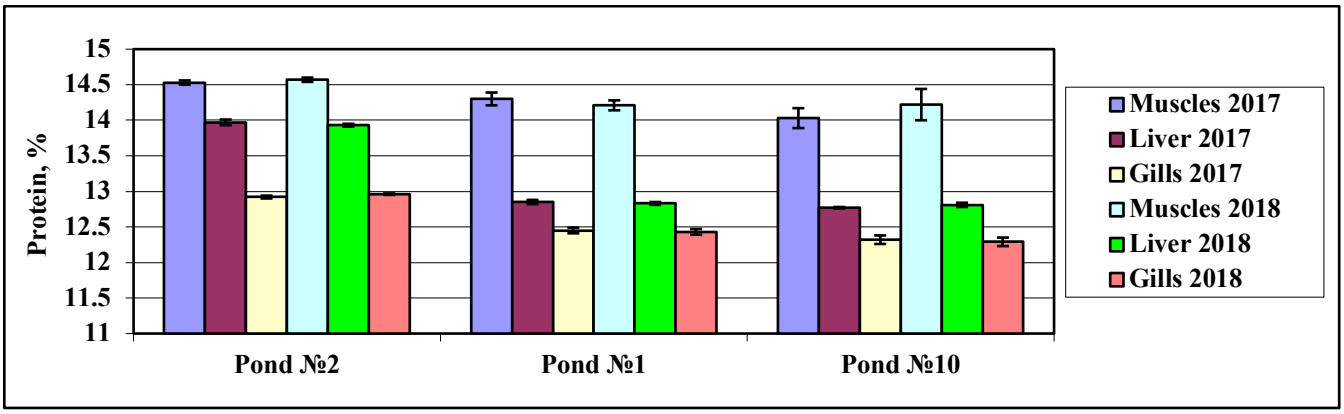

Figure 8 The average protein (%) values in the organs and tissues of biennials of the hybrid of silver and bighead carp from feeding ponds in 2017 and 2018.

The average lipids values in most organs and tissues of biennials of the hybrid of silver and bighead carp from feeding ponds for 2017 and 2018 were at 1% and below (Figure 9). Exceptions were indicators of lipid content in the liver of feeding ponds: pond No. 2 above 5 – 6%; ponds No. 1 and No. 10 4 – 5% in both years of research. Thus, a higher level of lipids is present in the liver of biennials and annuals compared to its content in the muscles and gills of the youth of the year hybrid of silver and bighead carp.

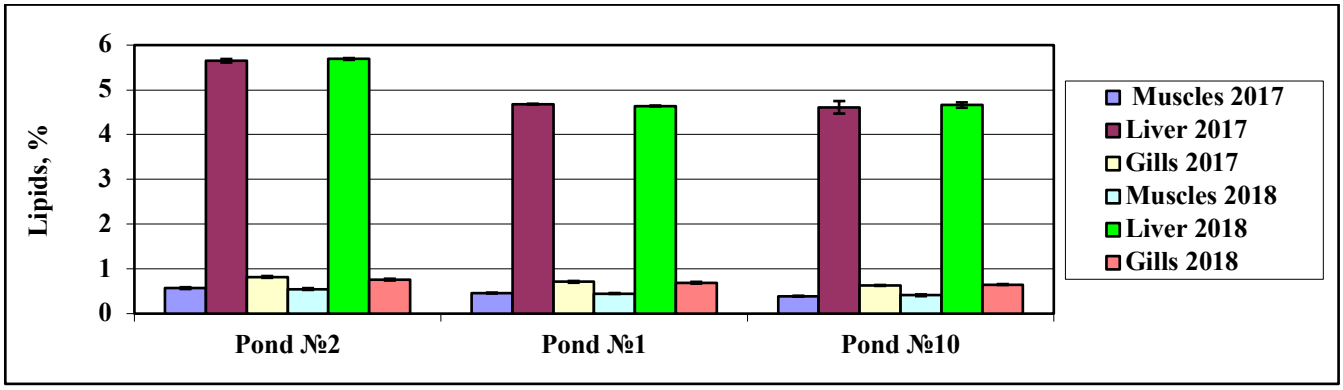

Figure 9 The average lipids (%) values in the organs and tissues of biennials hybrid of silver and bighead carp from feeding ponds in 2017 and 2018.

A study of biennials of Galician carp conducted by scientists at the „Korop" farm showed that skeletal muscle protein was 16.2% and lipid was 8.3%. This indicates a satisfactory physiological condition before landing on winter ponds **[38]**.

The results of studies of the level of energy compounds in the body of biennials of the hybrid of silver and bighead carp in research farms during 2017 and 2018 did not reveal significant physiological and biochemical status violations. It was recorded that the content of glycogen in the liver of biennials from SEEF "Nyvka" IF NAAS during 2017 and 2018 was the highest at 3.28 – 3.33%, compared with fish from other studied farms. All fish were characterized by fluctuations in the liver's glycogen content, which can be explained by the significant rate of mobilization of this substance to meet the energy problems of fish in a particular period and the short recovery time.

Table 3 shows the general metabolism indicators of biennials of white hybrids with variegated silver carp caught from winter ponds in the spring of 2018 – 2019.

Table 3 General indicators of metabolism of biennials of white hybrid with variegated silver carp caught from winter ponds, M ±m, n = 5.

Cloth	Glycogen, %		Albumen, %		Lipids, %	
	Spring, 2018 ye.	**Spring,** 2019 ye.	**Spring,** 2018 ye.	**Spring,** 2019 ye.	**Spring,** 2018 ye.	**Spring,** 2019 ye.
Pond No. 101 SE DG "Nivka" IRG NAAS						
Muscles	0.60 ±0.01	0.59 ±0.01	14.33 ±0.07	14.39 ±0.05	0.50 ±0.02	0.49 ±0.03
Cv	3.33	3.88	1.07	0.75	9.59	13.77
Liver	2.98 ±0.04	2.91 ±0.01	13.93 ±0.24	13.89 ±0.30	5.42 ±0.06	5.39 ±0.03
Cv	3.33	1.04	3.93	4.85	2.59	1.37
Branchia	0.66 ±0.04	0.60 ±0.03	12.87 ±0.28	12.95 ±0.12	0.75 ±0.02	0.70 ±0.02
Cv	12.08	12.87	4.91	2.10	4.84	5.24
Rate No. 2 NNVLR NULES of Ukraine						
Muscles	0.48 ±0.03	0.52 ±0.01	13.99 ±0.15	13.89 ±0.04	0.38 ±0.03	0.36 ±0.02
Cv	11.75	4.96	2.36	0.69	15.00	10.62
Liver	2.68 ±0.02	2.70 ±0.03	12.82 ±0.04	12.77 ±0.01	4.52 ±0.03	4.49 ±0.04
Cv	1.43	2.12	0.76	1.72	1.26	2.14
Branchia	0.50 ±0.02	0.56 ±0.02	12.38 ±0.04	12.36 ±0.06	0.64 ±0.01	0.62 ±0.01
Cv	7.10	7.47	0.79	1.09	4.66	4.23
Rate No. 5 BEGS IGB NASU						
Muscles	0.40 ±0.01	0.39 ±0.01	13.85 ±0.04	13.95 ±0.23	0.32 ±0.01	0.34 ±0.01
Cv	4.14	5.82	0.68	3.64	9.88	9.31
Liver	2.28 ±0.03	2.43 ±0.03	12.71 ±0.08	12.75 ±0.16	4.44 ±0.02	4.47 ±0.02
Cv	3.29	2.88	1.44	2.88	1.25	1.03
Branchia	0.49 ±0.01	0.45 ±0.01	12.27 ±0.05	12.23 ±0.05	0.59 ±0.01	0.60 ±0.01
Cv	9.82	6.65	0.85	0.85	4.48	3.33

The average glycogen values in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2018 and 2019 were about and above 0.5% (Figure 10). Exceptions were glycogen content in the liver of bighead carp wintering ponds: No. 119 about 3%; No. 1 more than 2.5%, and No. 11 more than 2% in both years of research. As a result, a higher level of glycogen was found in the liver of biennials than its presence in the muscles and gills of the youth of the year hybrids of silver and bighead carp.

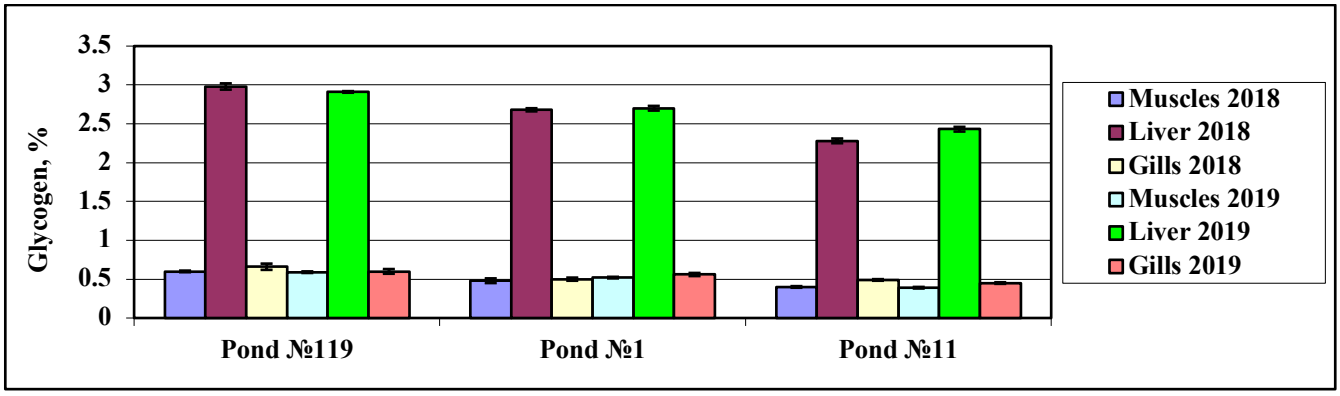

Figure 10 The average glycogen (%) values in the organs and tissues of biennials of the hybrid of silver and bighead carp from wintering ponds in 2018 and 2019.

The average values of the proportion of protein in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2018 and 2019, as well as biennials, fluctuated markedly over the years and in particular organs and tissues: in muscle levels of 14 – 14.5%; in the liver at the level of 12.5 – 14% (the highest pond fish No. 2); in gills at least at the level of less than 12.5 – 13% (Figure 11).

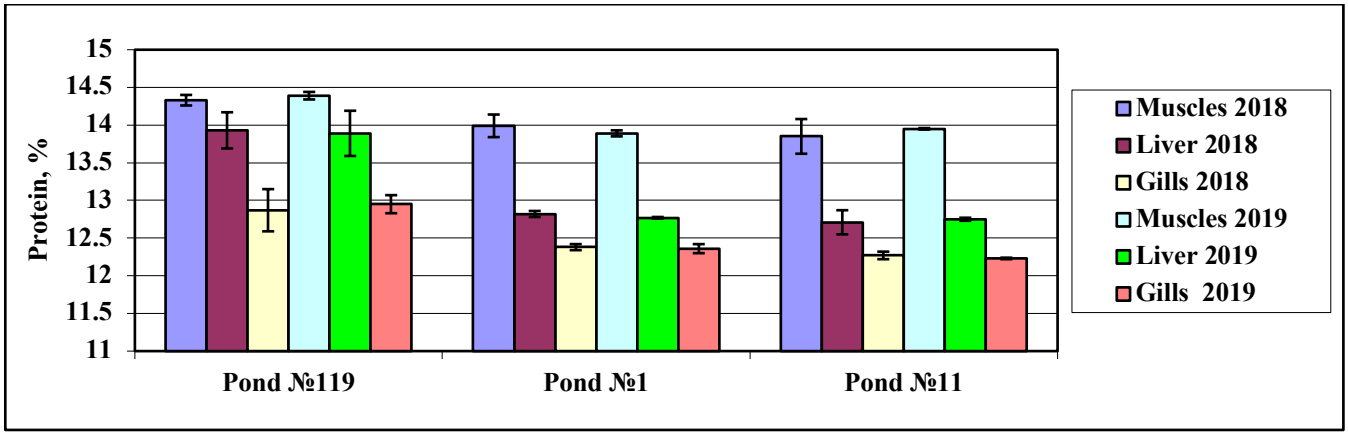

Figure 11 The average values of protein (%) in the organs and tissues of biennials of the hybrid of silver and bighead carp from wintering ponds in 2018 and 2019.

The average lipids values in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2018 and 2019, as in biennials, were at 1% and below (Figure 12).

In the muscles of the Clarias gariepinus, as well as in the hybrid of silver and bighead carp, there is a low content of total lipids, which is 1.26%, which indicates that they belong to the group of fish with low-fat content (less than 5%) and confirmed by literature data **[39]**. Other literature sources should note that catfish (along with Atlantic wolffish, spotted wolffish, many carps, some Salmonidae, and most flounders) are medium-fat fish and the content of lipids in muscle is from 2 – 6% **[40]**.

Exceptions were the indicators of lipid content in the liver of bighead carp from winter ponds: in pond No. 119, above 5%; in ponds No. 1 and No. 11, more than 4% in both years of research. Thus, higher lipids are found in the liver of biennials and annuals and biennials, compared to its content in the muscles and gills of the youth of the year of hybrid silver and bighead carp.

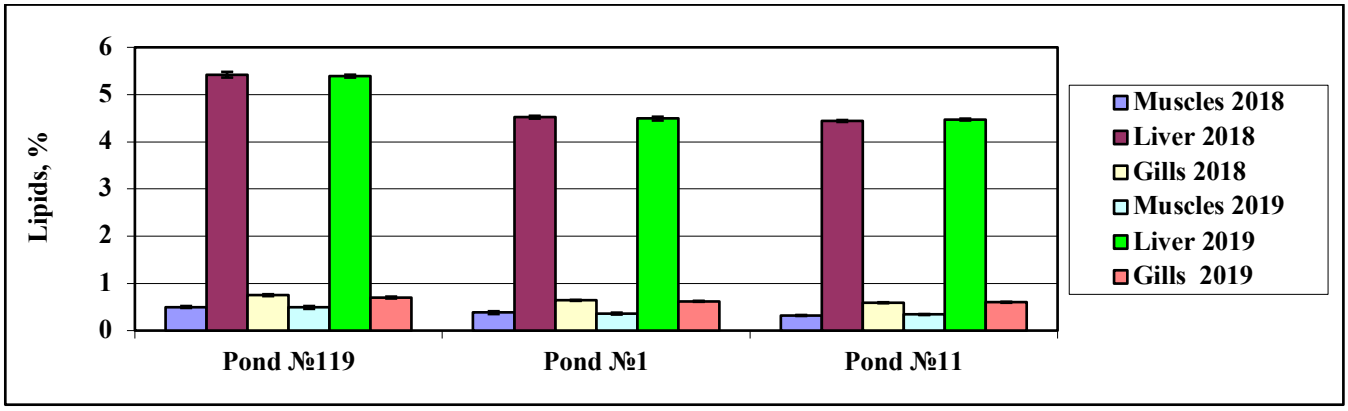

Figure 12 The average lipids (%) values in the organs and tissues of biennials of the hybrid of silver and bighead carp from wintering ponds in 2018 and 2019.

The results of the biennials of the hybrid of silver and bighead carp in 2018 and 2019 indicate that their physiological state at the time of the study was within the physiological norm. Table 4 shows the data on the general metabolism of different groups of white hybrids with variegated silver carp caught from the Kosiv Reservoir.

Table 4 General metabolism indicators of different groups of white hybrids with variegated silver carp caught from the Kosiv Reservoir, M ±m, n = 5.

Cloth	One-year	One-year	Three-year-olds (stocking in 2018)	Three-year-olds (stocking in 2018)
	Spring, 2018	Spring, 2019	Autumn, 2019	Autumn, 2019
Glycogen, %				
Muscles	0.53 ±0.03	0.38 ±0.02	0.76 ±0.02	0.80 ±0.03
Cv	10.99	14.15	6.22	8.29
Liver	2.19 ±0.02	2.10 ±0.01	5.38 ±0.06	5.51 ±0.04
Cv	2.20	1.51	2.50	1.65
Branchia	0.40 ±0.02	0.37 ±0.02	0.82 ±0.03	0.86 ±0.01
Cv	9.71	13.49	7.02	3.68
Albumen, %				
Muscles	13.48 ±0.07	13.41 ±0.05	20.04 ±0.21	22.16 ±0.11
Cv	1.24	0.82	2.42	1.14
Liver	12.67 ±0.08	12.59 ±0.04	18.00 ±0.19	18.56 ±0.21
Cv	1.35	0.68	2.38	2.52
Branchia	12.20 ±0.16	11.86 ±0.20	13.44 ±0.15	15.50 ±0.22
Cv	2.95	3.70	2.57	3.17
Lipids, %				
Muscles	0.40 ±0.01	0.36 ±0.01	0.70 ±0.02	0.82 ±0.02
Cv	7.91	9.23	4.98	6.80
Liver	3.11 ±0.03	2.91 ±0.07	5.79 ±0.04	7.26 ±0.09
Cv	2.16	5.49	1.65	2.63
Branchia	0.32 ±0.01	0.28 ±0.02	1.19 ±0.01	1.36 ±0.05
Cv	9.92	12.39	2.82	7.71

The average glycogen values in most organs and tissues of annuals and triennials of the hybrid of silver and bighead carp of the Kosiv Reservoir in 2018 and 2019 were up to 0.5% and 1%, respectively (Figure 13). Exceptions were glycogen content in the liver of bighead carp of the Kosiv Reservoir, which was slightly more than 2% in one-year-olds and 5 – 5.5% in triennials in both years of research. Thus, a higher level of glycogen in the liver of annual fish is evident compared to its presence in the muscles and gills of the youth of the year of the hybrid of silver and bighead carp.

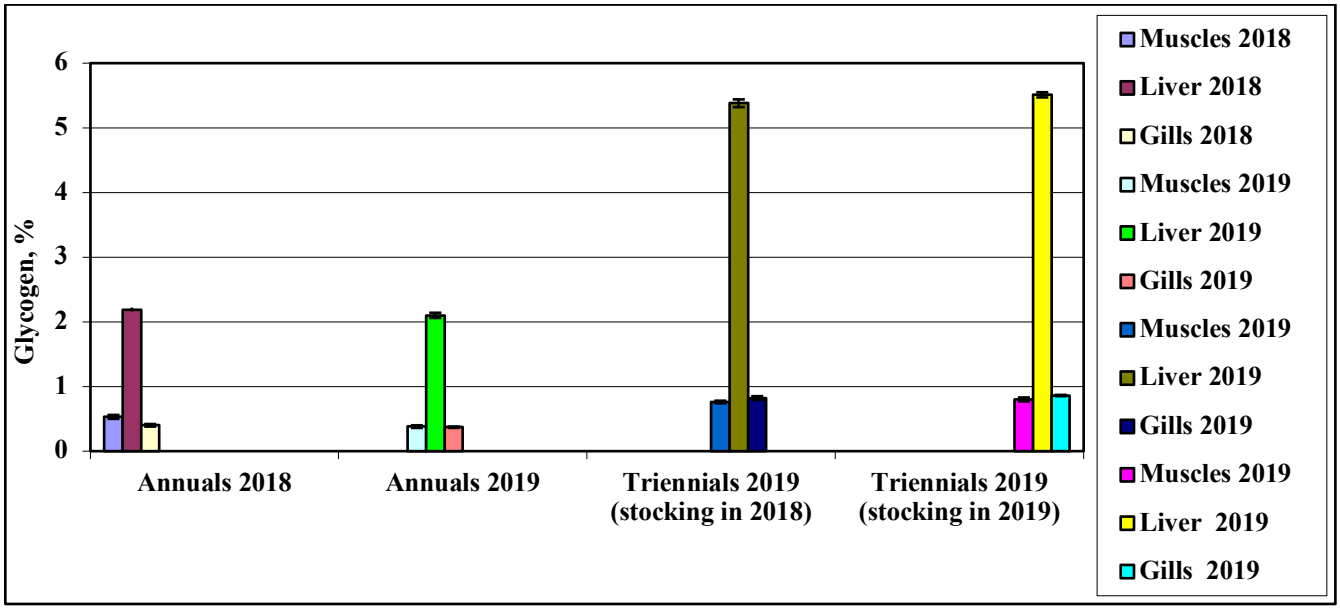

Figure 13 The average glycogen (%) values in the organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir during 2018 and 2019.

The average values of protein content in most organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir in 2018 and 2019 were lower in some organs and tissues in annuals, 12.5%, and higher in triennials, 15 – 22% (Figure 14). Moreover, the highest content was in the muscles of all age groups of fish, and the lowest protein content was in the gills.

The average lipid content in the muscles and gills of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir in 2018 and 2019 was at the level of annuals less than 0.5% and triennials about 1% or more (Figure 15). The lipid content in the liver was much higher: in annual fish, they were about 3%, and in triennials from stocking in 2018, about 6%, and from stocking in 2019, more than 7%.

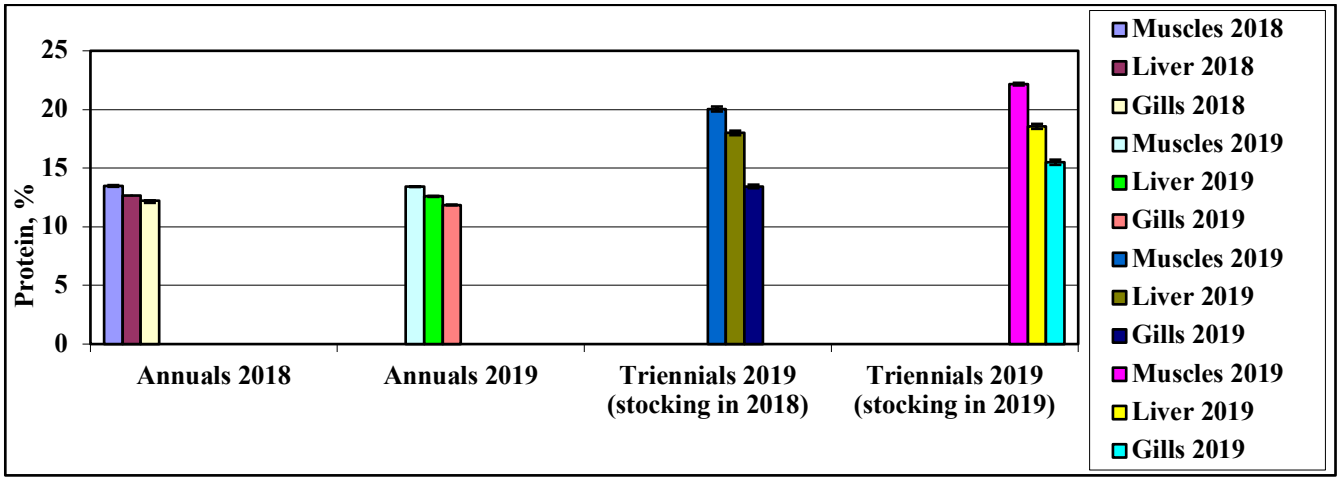

Figure 14 The average values of protein (%) in the organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir during 2018 and 2019.

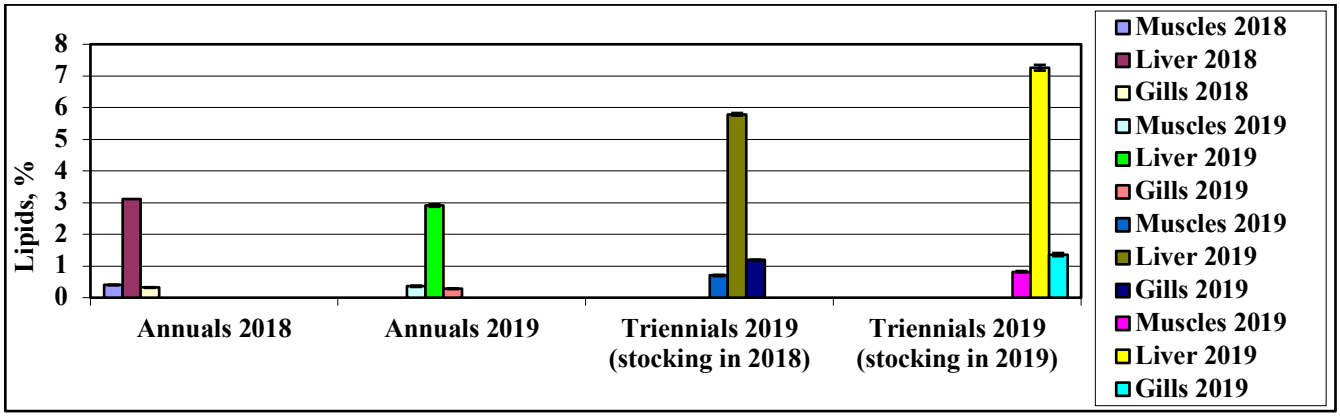

Figure 15 The average values of lipids (%) in the organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir during 2018 and 2019.

The results of the annuals of the hybrid of silver and bighead carp in 2018 and 2019 indicate that their physiological state at the time of the study was within the physiological norm.

The studied triennial hybrids of silver and bighead carp from the Kosiv Reservoir in 2019 were marked by significant fluctuations in total protein content in muscles (20.04 – 22.16%), liver (18.00 – 18.56%), and gills (13.44 – 15.50%). The amount of glycogen in the liver (1.79 – 1.84 times) and lipids in the gills (1.19 – 1.36 times) increased slightly. The obtained data can be explained by a certain heterogeneity of the general physiological state of fish. This can be caused by hereditary factors that determine a certain diversity of fish composition in reservoirs and the conditions of fish keeping.

Other scientists have shown that the total protein content in the white skeletal muscles of bream, roach, and zander in the fall significantly exceeded the protein content in the liver. Thus, the total protein content in the white skeletal muscles of bream from the Kyiv Reservoir exceeded the protein content in the liver by 63.8%, in roach muscles by 147.1%, and in zander muscles by 16.1%, and in perch muscles by 111.7%. Comparing the protein content in the liver of almost all species of fish, it can be noted that the fish from the Kremenchuk reservoir contains less protein than in Kyiv. First of all, this indicates that the conditions of this reservoir are favorable for the accumulation of lipids and glycogen in the liver **[41]**.

When comparing the lipid content of roach, bream, and zander, it is noticeable that at lower temperatures of the reservoir (Kyiv Reservoir), they accumulate fewer lipids in the liver but more in the muscles **[42]**.

The highest glycogen content in the liver was in zander and perch (10.8 – 11.0 %) and the muscles (5.6 – 6.2 %) of fish inhabiting the Kyiv Reservoir. Bream and roach from this reservoir contained 8.5 – 9.6 % liver and 3.6 – 5.1% muscles **[43]**. The general metabolism indicators of different groups of white hybrids with variegated silver carp, which were caught from the Velykoburlutsky reservoir, are presented in Table 5.

Table 5 General metabolism indicators of different groups of white hybrids with variegated silver carp caught from Velykoburlutsky reservoir, M ±m, n = 5.

Cloth	Autumn 2017 ye.	Autumn2018 ye.	Summer2019 ye.	Autumn 2017 ye.	Autumn 2018 ye.	Summer 2019 ye.	Autumn 2017 ye.	Autumn 2018 ye.	Summer 2019 ye.
	Age group								
	0+	1+	2+	0+	1+	2+	0+	1+	2+
	Glycogen, %			Albumen, %			Lipids, %		
Muscles	0.46 ±0.04	0.56 ±0.01	0.79 ±0.03	12.62 ±0.27	14.28 ±0.06	17.20 ±0.12	0.31 ±0.01	0.53 ±0.02	1.21 ±0.03
Cv	18.95	4.77	9.08	4.79	0.95	1.59	9.72	7.80	5.05
Liver	2.04 ±0.02	2.78 ±0.02	3.65 ±0.04	12.17 ±0.08	12.82 ±0.06	15.99 ±0.23	3.12 ±0.04	5.33 ±0.19	6.16 ±0.09
Cv	2.15	1.64	2.31	1.48	1.00	3.22	4.02	7.91	3.37
Branchia	0.81 ±0.04	0.93 ±0.02	1.20 ±0.02	11.90 ±0.25	12.37 ±0.04	13.80 ±0.29	0.33 ±0.01	0.81 ±0.02	1.47 ±0.08
Cv	13.97	3.68	3.20	4.65	0.67	4.72	10.05	4.61	11.70

The average values of glycogen in most organs and tissues of the youth of the year of the hybrid of silver and bighead carp of the Velykoburlutsky Reservoir in 2017, 2018, and 2019 increased from annuals to triennials and were, respectively, at the level: in muscle up to 0.5% or more; in gills 0.8% and more; the highest in the liver - 2.0 – 3.5% and more (Figure 16).

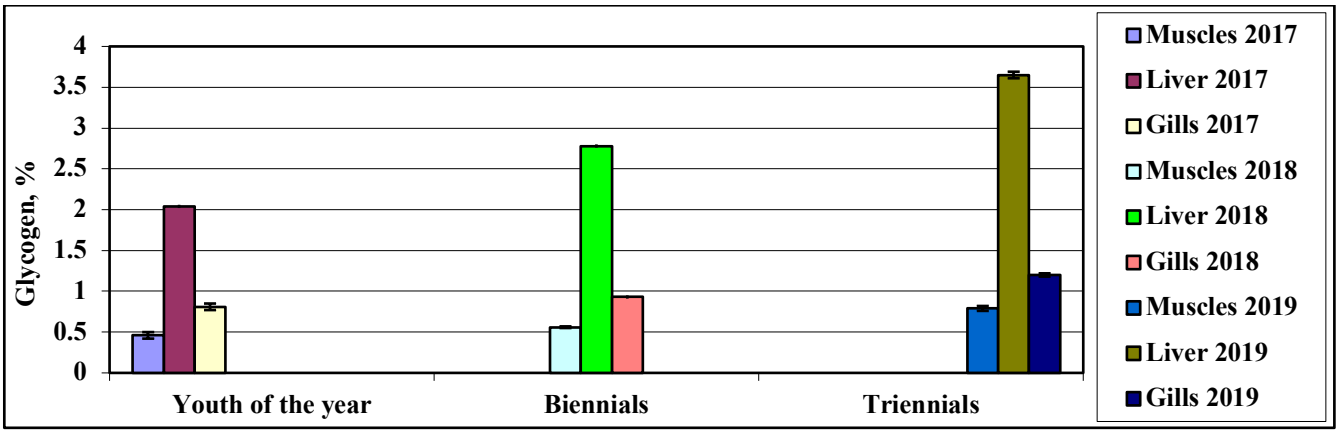

Figure 16 The average glycogen values in the organs and tissues of the youth of the year, biennials and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir during 2017, 2018, and 2019.

The average values of protein content in most organs and tissues of the youth of the year, biennials, and triennials of hybrid of silver and bighead carp from Velykoburlutsky Reservoir in 2017, 2018, 2019 also increased with age and fluctuated in some organs and tissues of fish: in the highest muscles more than 12 – 17%; in the gills 12 – 14%; in the liver 12 – 16% (Figure 17).

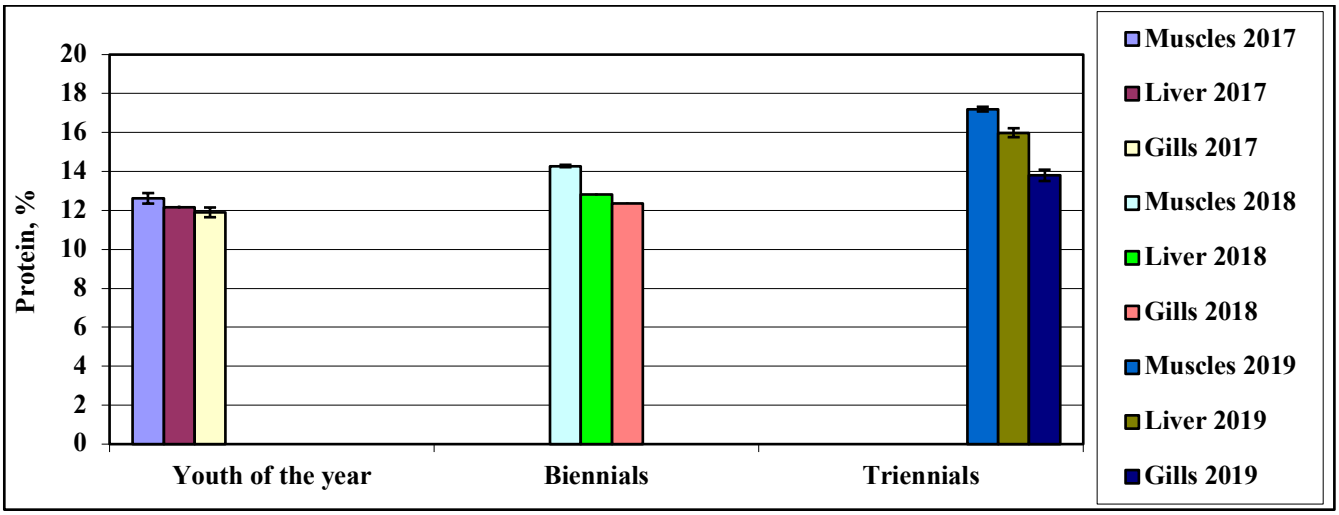

Figure 17 The average protein values in the organs and tissues of the youth of the year, biennials and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir during 2017, 2018, and 2019.

The average lipid content in the liver, muscles, and gills of the youth of the year, biennials, and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir in 2017, 2018, and 2019 gradually increased from the youth of the year to triennials. It was at a lower level in muscle 0.2 – 1.2% and gills 0.2 – 1.5% (Figure 18). Lipid content in the liver was much higher: in the year's youth, it was more than 3%, in biennials, 5.3%, and in triennials, more than 6%.

Satisfactory levels of essential nutrients were characteristic of the experimental groups from the Velykoburlutsky Reservoir, not considering the slight excess of glycogen content in the gills of triennials.

In the study of freshwater fish in the Kremenchuk reservoir, it was found that the protein content in the meat of bighead carp, on average from 16% to 18.7% (autumn catch), and carp meat was from 16% to 18.8% (spring catch), bream meat from 17% to 21.7% (autumn catch). Total fat content from 3.1% to 8%. These data indicate that this raw material is characterized by high protein content and medium fat **[45]**, **[46]**

In the Samarska bay of the Zaporizhzhya Reservoir, the protein content in the muscles of perch, zander, and roach decreased, but only 14% had a significant deviation of 14%. In the gills, a significant decrease in protein

content was observed in predatory fish by 24% in perch and 13% in zander. In the liver, the protein content was significantly reduced in all species of fish, perch by 30%, zander by 27%, bream by 30%, and roach by 34% **[47]**, **[48]**. Decreased protein content in fish may indicate the presence of stressors stimulating its proteolysis.

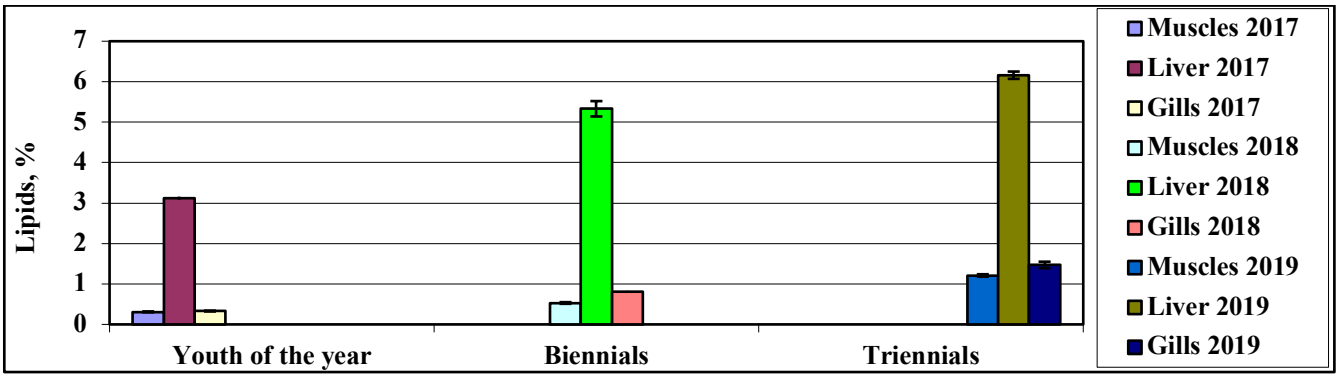

Figure 18 The average values of lipids in the organs and tissues of the year's youth, biennials, and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir during 2017, 2018, and 2019.

Fish from Samarska bay showed a significant increase in muscle lipid content: roach by 70%, bream by 67%, perch by 31%, and zander by 75%. Elevated levels of lipids in the muscle tissue of fish in Samarska bay may indicate a violation of metabolism due to unfavourable living conditions in this area **[49]**, **[50]**.

Reduced fat content was found in fish liver. Significant deviations were found in nonpredatory fish, in bream - by 18%, and in roach by 12%. A significant decrease in total fat content in the liver of the studied fish selected in the Samarska bay, indicates a general violation of lipid metabolism and the accumulation of reserve lipids.

In industrial fish species caught in the Samarska bay of the Zaporizhzhya Reservoir, there is a predominant decrease in glycogen content in the studied tissues and organs. Glycogen decreased by 20% in perch muscle and 29% in zander muscle. In the liver of perch, glycogen content decreased by 35%, in the liver of zander by 36%, roach by 20% **[44]**.

Thus, for the studied, different size and weight groups of the hybrid of silver and bighead carp, caught from ponds and reservoirs are mainly characterized by satisfactory values of overall metabolic rates.

CONCLUSION

It has been found that the liver of annuals and biennials of fish has a higher level of glycogen and lipids than its presence in the muscles and gills of bighead carp. In most organs and tissues of annual fish in 2017 and 2018, the average values of the specific weight of protein were almost evenly distributed in them, were at the level of 10 – 14%. The exceptions were the protein content in the winter pond No. 5 was lower (about 10%) and fluctuated depending on age. In 2017 and 2018, the average values of the specific weight of muscle protein varied between 14 – 14.5%; in the liver ,12.5 – 14% (the highest in fish pond No. 2); in the gills - less than 12%. In biennials caught from winter ponds in 2018, and 2019, muscle protein content was 14 – 14.5%; in the liver - at the level of 12.5 – 14% (the highest rates in fish caught from pond No. 2); in gills - at least at the level of less than 12.5 – 13%. The study of reservoirs showed significant fluctuations in protein content in the muscles (20.04 – 22.16%), liver (18.00 – 18.56%), and gills (13.44 – 15.50%) of fish. In the bighead carp triennials, the glycogen content in the liver (1.79 – 1.84 times) and lipids in the gills (1.19 – 1.36 times) was recorded.

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Ethical Statement:
In accordance with the protocols № 3/2017, № 5/2018 and № 1/2019 at the meeting of the Ethics Commission of the Faculty of Animal Husbandry and Aquatic Bioresources of the National University of Life and Environmental Sciences of Ukraine during the experimental catches signed Acts № 1/3, 2/5 and 1/1 ie in the process of catching hybrid hypophthalmichthys (*Hypophthalmichthys spp.)* "all norms of the current legislation of Ukraine according to DSTU 2284:2010 are observed".
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Milk and diary products – summary of European legislation, hygiene manuals, ISO standards and *Codex Alimentarius* standards

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ABSTRACT

European Union legislation laying down rules for the dairy sector. The legislation defines the conditions under which milk and milk products intended for human consumption can be imported into the EU. Milk and milk products must come only from third countries that appear on the list of authorized countries. Establishments, where milk and milk products are produced, must be approved for export. The TRACES system is used on imports and the consignment must be accompanied by a certificate. This system ensures product traceability and prevents the introduction of diseases. An important role is delegated to the designated border control posts (BCPs) where the appropriate customs and veterinary inspections are performed by government institutions of the country. The European Union has adopted legislation to ensure the safety of food placed on the market in EU member countries. This legislation sets general hygienic requirements for food production based on the good manufacturing practice and the HACCP system. The criteria for microorganisms, chemicals, and applicable food additives are set. Also, the legislation contains requirements for product labeling. Part of the legislation concerns the common organization of the market in milk and milk products. These regulations contain rules for direct payments, subsidies, define the school milk system, etc. Specific legislation creates rules for organic bio food production, for production and labeling of products with the Protected Geographical Indication, Protected Designation of Origin, and Traditional Specialty Guaranteed. There is also legislation that defines the labeling of products intended for specific populations, e.g. gluten-free foods, lactose-free foods, etc. Areas not regulated by the legislation include the labeling of products with certification marks designed to highlight the suitability of food for religious purposes or quality certification.

Keywords: milk, milk products, legislation, standards, *Codex Alimentarius*

INTRODUCTION

European Union legislation contains several pieces of legislation that comprehensively address the entire dairy sector. The legislation defines the conditions under which milk and milk products intended for human consumption can be imported into the EU. Milk and milk products must come only from third countries that appear on the list of authorized countries. Establishments, where milk and milk products are produced, must be approved for export. TRACES is used on import and the consignment must be accompanied by a certificate. This system ensures product traceability and prevents the introduction of diseases **[24]**. The European Union has adopted several pieces of legislation that establish rules for the production and marketing of safe food in individual EU countries. This legislation sets hygienic limits for the content of foreign substances, and microorganisms define requirements for traceability and labeling of products. Part of the legislation concerns the common organization of the market in milk and milk products. These regulations set out the rules for direct payments, and subsidies. Specific legislation creates rules for organic food production. The European Union also has specific legislation that defines the rules for labeling products with the Protected Geographical Indication, Protected Designation of Origin, and Traditional Specialty Guaranteed. There is also legislation in the EU that define the labeling of products intended for at-risk populations, e.g. gluten-free foods, lactose-free foods, etc. Areas not regulated by the legislation include the labeling of products with certification marks designed to highlight the suitability of a food for religious purposes or quality marks **[25]**. In this article, we have summarized the legislation

that applies in the European Union for milk and dairy products. In the text and the tables, in most cases, we list the legislation without amendments, which can be found directly in the Eurlex databases we have worked with. In this article, we have summarized the legislation that applies in the European Union, hygiene manuals, ISO standards and *Codex Alimentarius* standards for milk and dairy products.

GENERAL HEALTH RULES

Regulation (EU) No 182/2011 of the European Parliament and of the Council **2016/429 [1]** of 9 March 2016 on communicable animal diseases and amending and repealing certain acts in the field of animal health and Commission Delegated Regulation (EU) **2020/692 [2]**supplementing Regulation (EU) of the European Parliament and the Council **2016/429 [1]** lay down rules concerning the entry into, and the movement and treatment of, consignments of certain products of animal origin after they enter into the Union. The conditions for entry into the Union of raw milk, milk products, colostrum, and colostrum-based products shall be based on the animal health risks posed by those products. These risks are linked to the country or territory of origin or their zone and to the species of animals from which these products were obtained. In the case of milk and colostrum, two diseases are a cause for concern - foot-and-mouth disease and bovine fever virus infection, and therefore raw milk and colostrum should only enter from third countries or territories or zones free of these diseases. Consumer health protection is ensured by the application of several pieces of EU legislation: Regulation (EC) No **178/2002 [3]**, Regulation (EC) No **852/2004 [4]**, Regulation (EC) No **853/2004 [5]**, Regulation (EC) No **2017/625 [6]**, **2019/627 [7]** form the legal basis for the production, trade and official control of food of animal origin.

IMPORTS OF MILK AND DAIRY PRODUCTS FOR HUMAN CONSUMPTION INTO THE EU

Harmonized EU legislation makes it possible to apply the same requirements for the marketing of milk and milk products in all Member States and prevents milk and milk products that can transmit infectious diseases dangerous to livestock or humans from entering the EU.

These principles also apply to consignments that are under EU transit and/or temporary storage procedures. Depending on the risk they may pose, such consignments are exempted from public health requirements but must comply with veterinary requirements.

In general, the products must come from countries that are allowed to enter milk and dairy products into the EU. The establishment of origin must be approved and authorized as an establishment from which milk and milk products may be imported into the EU.

The third country of origin must have an approved residue control plan.

A non-EU country must meet certain requirements to obtain a marketing authorization for milk and dairy products. The most important aspects to consider before authorization are:

- the organization, structure, competencies, and powers of the veterinary services,
- third-country legislation,
- non-EU country rules on animal disease prevention and control,
- the health status of livestock, other domestic animals and wildlife,
- the regularity and speed of information on infectious animal diseases provided by the third country to the European Commission and the World Organization for Animal Health (OIE),
- hygiene requirements for the production, handling, storage, and dispatch of products of animal origin.

AUDIT

Before a non-EU country obtains authorization to place milk and dairy products on the EU market, the European Commission can carry out an audit to verify that all the criteria set out in EU legislation are properly met.

AUTHORIZED THIRD COUNTRIES AND ESTABLISHMENTS

Based on the principles contained in EU legislation and the results of the Commission's audit, a non-EU country may be included in the list of third countries eligible for the entry of milk and milk products into the EU.

The list of these third countries provides Commission Implementing Regulation (EU) **2021/405 [8]** of 24 March 2021 establishing the lists of third countries or regions thereof from which, by Regulation (EU) No **2017/625 [6]** authorizes the entry into the Union of certain animals and goods intended for human consumption. Before exporting milk and dairy products to the EU, the country must be listed.

All imports of milk and milk products into the EU must come from an approved establishment that has been authorized and listed for this purpose. Third countries are responsible for updating the lists of installations and informing the Commission of any changes. Lists of establishments in non-EU countries authorized to produce fresh meat are published on the Commission's website.

CERTIFICATE

Consignments of milk and milk products entering the EU must be accompanied by a CHED certificate. The model certificate is set out in the Commission Implementing Regulation (EU) **2020/2235 [9]** (Chapter, 33, 34, 25 - sample certificates).

PUBLIC HEALTH

Public health requirements must be met. For example, a non-EU country is required to have an approved monitoring plan for "residues". Products placed on the market in the EU must comply with the requirements of food law, namely Regulation (EC) No **178/2002 [3]**.

BORDER CONTROL

Regulation of the European Parliament and the Council (EU) **2017/625 [6]** lays down the principles governing the organization of veterinary checks on products of animal origin entering the EU from outside the EU at border inspection posts.

Milk and milk products entering the EU are checked at the EU Border Control Station (BIP) by the Commission Implementing Regulation (EU) **2019/1014 [10]** laying down detailed rules concerning minimum requirements for border inspection posts, including inspection centers. Member States' official veterinarians ensure that milk and milk products comply with all requirements laid down in EU legislation.

TRACES NT SYSTEM

TRACES NT (Trade Control Expert System - New Technology) is the European Commission's online system for sanitary and phytosanitary certification required for the import of animals, animal products, food, and feed of non-animal origin and plants into the European Union, and for intra-EU trade, and animal exports and certain animal products from the EU. Exporters from third countries who plan to export products of animal origin to the EU must be registered in this system through the European Commission, which must be contacted for this purpose by the competent authority of the third country.

Detailed rules for operations to be carried out during documentary, identification, and physical checks on an imals and goods subject to official controls at border inspection posts and following those controls are defined in the Commission Implementing Regulation (EU) **2019/2130 [11]**.

From 14 December 2019 (date of application of the Regulation on official controls - Regulation (EU) **2017/625 [6]**, the use of the Common Health Entry Documents (CHED) has become mandatory for the entry of animals and goods into the EU under Article 47 of this Regulation. The CHED document has several variants (A, P, PP, D), while the CHED-P document is required for the import of dairy products. CHED-P is a common medical entry document for consignments of products of animal origin, germinal products, and animal by-products (EC, 2022).

It is issued by the veterinary authorities in TRACES after an inspection. The first part must be completed by the importer to notify in advance of the import or transit of animal products in the EU 24 hours before the arrival of the goods. The second part of the form is filled in by the relevant veterinary and food administration, which confirms that it has carried out checks at the border inspection post and authorizes the entry of the products into the EU.

TRACEABILITY

The food traceability requirement is defined by Regulation (EC) No **178/2002 [3]** as amended. This Regulation contains general principles for the traceability of food.

Traceability means the ability to find and trace food, feed, food-producing animals, or substances that are intended or intended to be added to food or feed at all stages of production, processing, and distribution.

Stages of production, processing, and distribution mean any stage, including import, from, and including primary food production to and including storage, transport, sale, or delivery to the final consumer and, where relevant, import, production, storage, transport, distribution, sale, and feed delivery.

Food and feed business operators (FBOs) must:

1. Ensuring the traceability of food, feed, food-producing animals, and any substances which are intended to be added to or intended to be added to food or feed must be established at all stages of production, processing, and distribution.
2. Be able to identify any person who supplies them with food, feed, a food-producing animal, or any substance intended to be added to food or feed or which is intended to be added to food or feed. To this end, such operators must have systems and procedures in place that allow this information to be made available to the competent authorities upon request.

3. Have systems and procedures in place to identify other businesses to which their products are supplied. This information shall be made available to the competent authorities upon request.
4. Foods or feedingstuffs which are placed on the market or are likely to be placed on the market in the Community must bear an appropriate label or marking enabling them to be traced through appropriate documentation or information by the relevant requirements of the more specific provisions.

FOOD LABELING

Milk and milk products must be labeled by:

- Regulation (EU) No **931/2011 [13]** on the traceability of animal products,
- EC Regulation No **1169/2011 [14]** on the provision of information to consumers,
- EC Regulation No **1308/2013 [15]** on the common organization of the markets in agricultural products.
- The products must bear an oval veterinary mark.
- Nutrition and health claims may be made on products by Regulation (EC) No 258/97 of the European Parliament and the Council **1924/2006 [16]**.
- The product ca contains several symbols present in Table 1 **[17]**.

The traceability requirements for food of animal origin are set out in Implementing Regulation (EU) No **1095/2010 [12], 931/2011 [13]**.

To ensure traceability, the following are required:

- the name and address of the FBO supplying the food, and
- the name and address of the FBO to which the food was delivered.

Regulation (EU) No **931/2011 [13]** applies to all FBOs at all stages of the production, processing, and distribution of food of animal origin, including primary producers, retailers, wholesalers, intermediaries, storers, and transporters of food of animal origin.

All products of animal origin placed on the market have been marked with a health mark or identification mark.

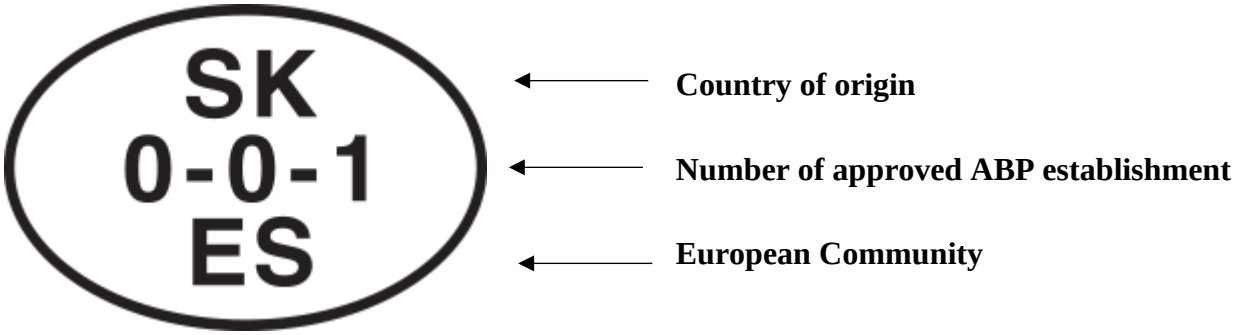

Figure 1 Example of the European union health and identification mark. Note: SK – Slovakia, ES – European Community .

Table 1 Food Packaging Symbols Explained.

Symbols	Definition
	This symbol is often used on containers, such as Tupperware, to show that the product is suitable for food use. It may or may not have the word "food" below the cup and fork.
	Recycling – this logo is used internationally to show that the product can be recycled. This is not an indication that the packaging has been made from recycled material. If you see a number in the middle of this image, that is to indicate the percentage of recycled material that makes up that product.
	The Green Dot In Continental Europe the 'Green Dot' trademark indicates that a fee has been paid to fund the recycling of the product. It is a financial symbol principally and indicates that the producer is part of PRO Europe's packaging recovery organisation, a company that seeks to promote economic producer responsibility. In the UK it is often used incorrectly on products to suggest they are recyclable. This is a misuse of the symbol.
	Plastic recycling – another widely used symbol to show that the plastic used in the packaging can be recycled. The PET refers to Polythene Terephthalate which is commonly used in this application. The number inside (1 – 7) defines the resin used in making the packaging.
	Keep Britain Tidy – this symbol is included to remind consumers to dispose of their waste in an appropriate manner. It does not necessarily relate to the type of packaging used, but is rather a campaign against littering.
	The Forest Stewardship Council The FSC looks after our forests and can be found on all wood and paper products as well as products such as latex that are derived from trees. The FSC logo can be found on products such as decking, charcoal, and kitchen utensils. When you buy a product with the FSC label, it's a guarantee that the trees used were replaced or allowed to grow naturally, that the rights of indigenous people are protected, and that the homes of wildlife are conserved.

PRODUCTION

The production of dairy products is subject to the general hygiene requirements set out in several European regulations(EC): Regulation (EC) No **178/2002 [3]**, Regulation (EC) No **852/2004 [4]**, Regulation (EC) No **853/2004 [5]**. The processed milk must meet the hygienic requirements, which are the total number of microorganisms, the number of somatic cells, the absence of residues of veterinary drugs, and not exceeding the maximum permissible amounts of certain contaminants. The purchase of milk takes place in the form of a contractual relationship with the primary milk producer. In production, input, inter-operational, and output control is performed by the HACCP plan. The controls are also focused on the pasteurization regime, reaching the prescribed temperature and time of pasteurization as well as on the inactivation of the enzyme alkaline phosphatase. From a microbiological point of view, the legislation sets limits specifically for the production process and final products. The products must not be contaminated with pathogenic microorganisms, foreign contaminants, and physical objects. The quality requirements for raw milk as well as the final product are determined by each producer, taking into account existing requirements that apply specifically to specific products. For example, in the case of sour milk products, the legislation determines the number of living microorganisms that must be present in the product, in the case of butter, the amount of fat in the product is determined, and so on. Food additives may be added to milk products by Regulation (EC) No 258/97 of the European Parliament and the Council. in the case of butter, the amount of fat in the product is determined, etc. Food additives may be added to milk products by Regulation (EC) No 258/97 of the European Parliament and the Council. in the case of butter, the amount of fat in the product is determined, etc. Food additives may be added to milk products by Regulation (EC) No 258/97 of the European Parliament and the Council **1333/2008 [18]**. Food additives are substances that are not normally consumed as food as such but are intentionally added to food for technological purposes. Packaging materials must be suitable for use in the food industry. The production of

specific PGI, PDO and TSG products is carried out in such a way that the registered product specification **1151/2012 [19]**, **668/2014 [20]**. Other legislation is listed in the overview tables.

COMMON MILK MARKET ORGANIZATION

The common organization of the market in agricultural products is governed by Regulation (EU) No 182/2011 of the European Parliament and the Council **1308/2013 [15]**. For example, the regulation defines and catalogs milk and selected dairy products, addresses the contractual arrangements for the supply of milk, the obligations of first-time raw milk buyers, and obliges the Commission to report to the European Parliament on the situation in the dairy market.

Other European Union legislation in this area, which is listed in Table 2, also applies.

Table 2 Legislation for the common organization of the market in milk and milk products.

Prescription number	Name of the regulation
595/2004	Commission Regulation (EC) No Commission Regulation (EC) No 595/2004 of 30 March 2004 laid down detailed rules for the application of Council Regulation (EC) No 1234/2007 1788/2003 establishing a levy on the milk and milk products sector.
565/2013	Commission Implementing Regulation (EU) No 565/2013 of 18 June 2013 amending Regulations (EC) No 1731/2006, (ES) No 273/2008, (ES) No 566/2008, (ES) No 867/2008, (ES) No 606/2009 and Implementing Regulations (EU) No 543/2011 and (EU) Amending Regulation (EC) No 1333/2011 as regards the notification obligation under the common organization of agricultural markets and repealing Regulation (EC) No 491/2007.
1307/2013	Regulation (EU) No 182/2011 of the European Parliament and of the Council Commission Regulation (EC) No 1307/2013 of 17 December 2013 laid down rules for direct payments to farmers under support schemes within the framework of the common agricultural policy and repealing Council Regulation (EC) No 1257/1999 637/2008, and Council Regulation (EC) No 73/2009.
1308/2013	Regulation (EU) No 182/2011 of the European Parliament and of the Council Regulation (EC) No 1308/2013 of 17 December 2013 establishing a common organization of the markets in agricultural products and repealing Council Regulations (EEC) No 2454/93 922/72, (EHS) No 234/79, (ES) No 1037/2001, and (ES) No 1234/2007.
906/2014	Commission Delegated Regulation (EU) No Regulation (EU) No 906/2014 of the European Parliament and of the Council of 11 March 2014 1306/2013 as regards public intervention expenditure.
907/2014	Commission Delegated Regulation (EU) No Regulation (EU) No 907/2014 of the European Parliament and of the Council of 11 March 2014 1306/2013 as regards paying agencies and other bodies, financial management, clearance of accounts, guarantees, and use of the euro
1097/2014	Commission Implementing Regulation (EU) No 1097/2014 of 17 October 2014 amending Regulation (EU) No 479/2010 on notifications by the Member States in the milk and milk products sector.
2016/ 1238	Commission Delegated Regulation (EU) 2016/1238 of 18 May 2016 supplementing Regulation (EU) No 182/2011 of the European Parliament and the Council 1308/2013 as regards public intervention and private storage aid.
2016/ 1240	Commission Implementing Regulation (EU) 2016/1240 of 18 May 2016 laying down rules for the application of Regulation (EU) No 1308/2013 of the European Parliament and the Council about public intervention and aid for private storage
2016/2080	Commission Implementing Regulation (EU) 2016/2080 of 25 November 2016 opened the sale of skimmed milk powder by a tendering procedure.
2017/1185	Commission Implementing Regulation (EU) No 2017/1185 of 20 April 2017 laying down detailed rules for the application of Regulation (EU) No 182/2011 of the European Parliament and the Council 1307/2013 and (EU) No 1308/2013 as regards the provision of information and the submission of documents to the Commission and amending and repealing several Commission Regulations.
2017/40	Commission Delegated Regulation (EU) 2017/40 of 3 November 2016 supplementing Regulation (EU) No 182/2011 of the European Parliament and the Council Amending Commission Implementing Regulation (EU) No 1308/2013 as regards Union aid for the supply of fruit and

	vegetables, bananas and milk in educational establishments and amending Commission Implementing Regulation (EU) No 1308/2013 907/2014.
2018/147	Council Regulation (EU) 2018/147 of 29 January 2018 amending Regulation (EU) No 182/2011 1370/2013 as regards the quantitative limit for the purchase of skimmed milk powder.
2018/149	Commission Delegated Regulation (EU) 2018/149 of 15 November 2017 amending Commission Delegated Regulation (EU) 2016/1238 as regards compositional and quality requirements for milk and milk products eligible for public intervention and private storage aid.
2018/150	Commission Implementing Regulation (EU) 2018/150 of 30 January 2018 amending Implementing Regulation (EU) 2016/1240 as regards methods for analyzing and evaluating the quality of milk and milk products eligible for public intervention and private storage aid.
2018/150	Commission Implementing Regulation (EU) 2018/150 of 30 January 2018 amending Implementing Regulation (EU) 2016/1240 as regards methods for analyzing and evaluating the quality of milk and milk products eligible for public intervention and private storage aid.
2018/1879	Commission Implementing Regulation (EU) 2018/1879 of 29 November 2018 amending Implementing Regulation (EU) 2016/2080 as regards the date of storage of skimmed-milk powder sold under a tendering procedure.
2018/765	Commission Implementing Regulation (EU) 2018/765 of 23 May 2018 amending Implementing Regulation (EU) 2016/2080 as regards the date of storage of skimmed-milk powder sold under a tendering procedure.
2020/532	Commission Implementing Regulation (EU) 2020/532 of 16 April 2020 laying down derogations from Implementing Regulations (EU) No 809/2014, (EU) No 180/2014, (EU) No 181/2014, (EU) 2017/892, (EU) 2016/1150, (EU) 2018/274, (EU) 2017/39, (EU) 2015/1368, and (EU) 2016/1240, as regards certain administrative and on-the-spot checks applicable under the common agricultural policy.
2020/591	Commission Delegated Regulation (EU) 2020/591 of 30 April 2020 established a temporary special aid scheme for the private storage of certain cheeses and fixed the amount of aid in advance.
2020/ 597	Commission Implementing Regulation (EU) 2020/597 of 30 April 2020 granting private storage aid for butter and fixing in advance the amount of aid.
2020/598	Commission Implementing Regulation (EU) 2020/598 of 30 April 2020 granting private storage aid for skimmed-milk powder and fixing the amount of aid in advance.
2020/599	Commission Implementing Regulation (EU) 2020/599 of 30 April 2020 authorizing the conclusion of agreements and decisions on production planning in the milk and milk products sector.
2020/600	Commission Implementing Regulation (EU) 2020/600 of 30 April 2020 laying down derogations from Implementing Regulation (EU) 2017/892, Implementing Regulation (EU) 2016/1150, Implementing Regulation (EU) No 615/2014, Implementing Regulation (EU) 2015/1368 and Implementing Regulation (EU) 2017/39 as regards certain measures to address the COVID-19 pandemic crisis.
2020/760	Commission Delegated Regulation (EU) 2020/760 of 17 December 2019 supplementing Regulation (EU) No 182/2011 of the European Parliament and of the Council amending Regulation (EC) No 1308/2013 as regards the rules for administering import and export tariff quotas subject to licenses and supplementing Regulation (EU) No 1308/2013 of the European Parliament and the Council 1306/2013 as regards the lodging of security in the administration of tariff quotas.
2020/1471	Commission Implementing Regulation (EU) 2020/1471 of 12 October 2020 fixing the interest rates to be used for calculating the costs of financing intervention measures comprising buying-in, storage, and disposal for the EAGF 2021 accounting period.

FOOD HYGIENE

In the field of food hygiene, the legislation is listed in Table 3.

Table 3 Food hygiene legislation.

Prescription number	Name of the regulation
2020/2235	Commission Implementing Regulation (EU) 2020/2235 of 16 December 2020 laying down detailed rules for the application of Regulations (EU) 2016/429 of the European Parliament and the Council and (EU) 2017/625 as regards model animal health certificates, model official certificates, and model animal health certificates / official certificates for the entry and movement of consignments of certain categories of animals and goods into the Union, the official certification of such certificates and repealing Regulation (EC) No 1774/2002; Implementing Regulations (EU) No 599/2004 636/2014 and (EU) 2019/628, Directive 98/68 / EC and Decisions 2000/572 / EC, 2003/779 / EC, and 2007/240 / EC.
2019/627	Commission Implementing Regulation (EU) 2019/627 laying down uniform practical arrangements for carrying out official controls on products of animal origin intended for human consumption by Regulation (EU) 2017/625 of the European Parliament and the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls.
2019/1139	Commission Implementing Regulation (EU) 2019/1139 of 3 July 2019 amending Regulation (EC) No Amending Regulation (EC) No 2074/2005 as regards official controls on the food of animal origin related to food chain information and fishery products and reference to recognized testing methods for the detection of marine biotoxins and testing methods for raw and heat-treated cow's milk.
2017/625	Regulation (EU) 2017/625 of the European Parliament and of the Council on official controls.
16/2012	Commission Regulation (EU) No Amending Annex II to Regulation (EC) No 16/2011 of the European Parliament and of the Council 853/2004 as regards requirements for frozen foodstuffs of animal origin intended for human consumption.
931/2011	Commission Implementing Regulation (EU) No 931/2011 of 19 September 2011 on traceability requirements laid down in Regulation (EC) No 931/2011 of the European Parliament and the Council 178/2002 about the food of animal origin.
765/2006	Commission Decision 2006/765/EC repealing certain implementing rules concerning food hygiene and health conditions for the production and placing on the market certain products of animal origin intended for human consumption.
2074/2005	Commission Regulation laying down implementing measures for certain products under Regulation (EC) No 1234/2007 853/2004 and for the organization of official controls under Regulations (EC) No 854/2004 and 882/2004 derogating from Regulation (EC) No Amending Regulation (EC) No 852/2004 853/2004 and 854/2004.
2073/2005	Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.
853/2004	Regulation (EC) No Laying down specific hygiene rules for food of animal origin.
852/2004	Regulation (EC) No 852/2004 on food hygiene. Commission Communication on the implementation of food safety management systems taking into account essential requirements programs (NCPs) and HACCP-based procedures, including simplification/flexibility in implementation for certain food business operators. *Codex Alimentarius* standards - Regulation (EC) No 852/2004 on food hygiene takes into account the international food safety standards contained in the *Codex Alimentarius*. The European Commission's guidance document on the implementation of certain provisions of Regulation (EC) No 852/2004 on food hygiene sets out certain standards developed by the *Codex Alimentarius*.
178/2002	Regulation (EC) No 178/2002 laid down the general principles and requirements of food law, and guidelines for the implementation of Articles 11, 12, 16, 17, 18, 19, and 20.
315/93	Council Regulation (EEC) No Council Regulation (EEC) No 315/93 of 8 February 1993 laid down Community procedures for contaminants in food.
1881/2006	Commission Regulation (EC) No Regulation (EC) No 1881/2006 of 19 December 2006 set maximum levels for certain contaminants in foodstuffs.

OTHER LEGISLATION VALID IN THE DAIRY SECTOR

Dairy companies produce food waste and the EU has addopted health rules concerning animal by-products and derived products not intended for human consumption which should be taken into account (Table 4).

Table 4 Additional legislation for the dairy sector.

Prescription number	Name of the legislation
1069/2009	Regulation (EC) No 1/2003 of the European Parliament and the Council Commission Regulation (EC) No 1069/2009 of 21 October 2009 laying down health rules concerning animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1829/2003 1774/2002.

LEGISLATION FOR PGI, PDO, AND TSG

Products with a protected geographical indication, a protected designation of origin, and guaranteed traditional specialties are marked with quality marks by EU legislation (Table 5).

Table 5 Legislation for PGIs, PDOs, TSGs.

Prescription number	Name of the legislation
1151/2012	Regulation (EU) No 182/2011 of the European Parliament and of the Council 1151/2012 of 21 November 2012 quality systems for agricultural products and foodstuffs.
668/2014	Commission Implementing Regulation (EU) No 668/2014 of 13 June 2014 laying down rules for the application of Regulation (EU) No 182/2011 of the European Parliament and of the Council 1151/2012 on quality systems for agricultural products and foodstuffs.

Figure 2 Example of the European PDO, PGI and TSG marks. Note: Protected geographical indication (PGI); Protected designation of origin (PDO); and Traditional speciality guaranteed (TSG).

ORGANIC AGRICULTURAL PRODUCTION

Since 1 January 2022, Regulation (EU) **2018/848** of the European Parliament and of the Council of 30 May 2018 is the applicable legislative act, also known as the basic act, laying down the rules on organic production and labelling of organic products, repealing and replacing Council Regulation (EC) No **834/2007** of 28 June 2007. The new regulation provides for transitional periods for the implementation of certain new provisions, in particular on trade. Please refer to section 2 of Chapter IX of Regulation (EU) 2018/848, where provisions under previous Council Regulation (EC) No **834/2007** and Commission Regulation (EC) No **889/2008** may apply for a limited period.

It is on the basis of Regulation (EU) 2018/848 that the Commission adopts further detailed secondary legal acts. The types of secondary legal acts are the following:

- delegated acts, also known as Commission Delegated Regulations, which are acts of general application to supplement ("Commission Delegated Regulation supplementing") or amend ("Commission Delegated Regulation amending") certain non-essential (in the sense of complementary) elements of the legislative act;
- implementing acts, also known as Commission Implementing Regulations, which are used where uniform conditions for implementation are needed.

Delegated acts amending the basic act are progressively incorporated into the so-called "consolidated" text of the legislative act and become part of it. Please note that the consolidated version of Regulation (EU) **2018/848** is made available only for informative purposes, but has no legal effect. The authentic versions of the relevant acts, including their preambles, are those published in the Official Journal of the European Union

Table 6 Legislation for organic farming.

Prescription number	Name of the legislation
834/2007	Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labeling of organic products and repealing Regulation (EEC) No 2454/93, 2092/91.
889/2008	Commission Regulation (EC) No 889/2008 of 5 September 2008 laid down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labeling of organic products about organic production, labeling, and control.
848/2018	Regulation (EU) No 2018/848 of the European Parliament and of the council of 30 May 2018 on organic production and labeling of organic products and repealing council regulation (EC) No 1782/2003, 834/2007.
2021/1165	Commission Implementing Regulation (EU) 2021/1165 of 15 July 2021 authorizing certain lists of products and substances for use in organic farming.
2021/1378	Commission Implementing Regulation (EU) No 2021/1378 of 19 August 2021 laying down certain rules concerning the certificate issued to operators, groups of operators, and exporters in third countries involved in imports of organic products and products from conversion into the Union, establishing a list of recognized public inspection bodies and private inspection bodies by Regulation (EU) No 2018/848 of the European Parliament and the Council.
2021/2119	Commission Implementing Regulation (EU) No 2021/2119 of 1 December 2021 laying down detailed rules concerning certain records and declarations required of operators and groups of operators and the technical means for issuing certificates by regulation (EU) 2018 of the European Parliament and the Council / 848 and amending Commission Implementing Regulation (EU) 2021/1378 as regards the certification of operators, groups of operators and exporters in third countries.
2021/2307	Commission Implementing Regulation (EU) No 2021/2307 of 21 October 2021 laying down rules concerning the documents and notifications required for organic products and products of conversion intended for import into the Union.
2021/2325	Commission Implementing Regulation (EU) No 2021/2325 of 16 December 2021 establishing, according to Regulation (EU) No 2018/848 of the European Parliament and the council, the list of third countries and the list of public inspection bodies and private inspection bodies recognized under article 33 (2). 2 a 3 Council Regulation (EC) No 834/2007 to import organic products into the Union.
2020/427	Delegated Regulation (EU) 2020/427 of 13 January 2020 amending Annex II to Regulation (EU) 2018/848 on certain detailed production rules for organic products (OJ L 87, 23.3.2020)
2020/1794	Delegated Regulation (EU) 2020/1794 of 16 September 2020 amending Part I of Annex II to Regulation (EU) 2018/848 on the use of in-conversion and non-organic plant reproductive material (OJ L 402, 1.12.2020)
2021/642	Delegated Regulation (EU) 2021/642 of 30 October 2020 amending Annex III to Regulation (EU) 2018/848 on certain information to provide on the labelling of organic products (OJ L 133, 20.4.2021)
2021/716	Delegated Regulation (EU) 2021/716 of 9 February 2021 amending Annex II to Regulation (EU) 2018/848 on organic production rules on sprouted seeds and chicory heads, on feed for certain aquaculture animals and on aquaculture parasite treatments (OJ L 151, 3.5.2021).

2022/474	Delegated Regulation (EU) 2022/474 of 17 January 2022 amending Annex II to Regulation (EU) 2018/848 on specific requirements for the production and use of non-organic, in-conversion and organic seedlings and other plant reproductive material.
2020/2146	Delegated Regulation (EU) 2020/2146 of 24 September 2020 supplementing Regulation (EU) 2018/848 on exceptional production rules in organic production (OJ L 428, 18.12.2020)
2021/1189	Delegated Regulation (EU) 2021/1189 of 7 May 2021 supplementing Regulation (EU) 2018/848 on the production and marketing of plant reproductive material of organic heterogeneous material of particular genera or species (OJ L 258, 20.7.2021)
2020/464	Implementing Regulation (EU) 2020/464 of 26 March 2020 laying down certain rules for the application of Regulation (EU) 2018/848 on the documents needed for the retroactive recognition of periods for the purpose of conversion, the production of organic products and information to be provided by EU countries (OJ L 98, 31.3.2020)
2021/1165	Implementing Regulation (EU) 2021/1165 of 15 July 2021 authorising certain products and substances for use in organic production and establishing their lists (OJ L 253, 16.7.2021)
2021/715	Delegated Regulation (EU) 2021/715 of 20 January 2021 amending Regulation (EU) 2018/848 on the requirements for groups of operators (OJ L 151, 3.5.2021)
2021/1006	Delegated Regulation (EU) 2021/1006 of 12 April 2021 amending Regulation (EU) 2018/848 on the model of certificate attesting compliance with the rules on organic production (OJ L 222, 22.6.2021)
2021/1691	Delegated Regulation (EU) 2021/1691 of 22 September 2021 amending Annex II to Regulation (EU) 2018/848 on the requirements for records keeping from operators in organic production (OJ L 334, 22.9.2021)
2021/771	Delegated Regulation (EU) 2021/771 of 21 January 2021 supplementing Regulation (EU) 2018/848 laying down specific criteria and conditions for the checks of documentary accounts in the framework of official controls in organic production and the official controls of groups of operators (OJ L 165, 11.5.2021)
2021/2304	Delegated Regulation (EU) 2021/2304 of 18 October 2021 supplementing Regulation (EU) 2018/848 with rules on the issuance of complementary certificates certifying the non-use of antibiotics in organic production of animal products for the purpose of export (OJ L 461, 27.12.2021)
2021/279	Implementing Regulation (EU) 2021/279 of 22 February 2021 laying down detailed rules for implementation of Regulation (EU) 2018/848 on controls and other measures ensuring traceability and compliance in organic production and the labelling of organic products (OJ L 62, 23.2.2021)
2021/1935	Implementing Regulation (EU) 2021/1935 of 8 November 2021 amending Implementing Regulation (EU) 2019/723 on the information and data on organic production and labelling of organic products to be submitted by means of the standard model (OJ L 396, 10.11.2021, p. 17–26)
2021/2119	Implementing Regulation (EU) 2021/2119 of 1 December 2021 on records and declarations required from operators and groups of operators and on the technical means for the issuance of certificates in accordance with Regulation (EU) 2018/848 and amending Implementing Regulation (EU) 2021/1378 of 19 August 2021 on the issuance of the certificate for operators, groups of operators and exporters in third countries (OJ L 430, 2.12.2021)
2021/1697	Delegated Regulation (EU) 2021/1697 of 13 July 2021 amending Regulation (EU) 2018/848 on the criteria for the recognition of control authorities and control bodies competent to carry out controls on organic products in third countries, and on the withdrawal of their recognition (OJ L 336, 23.9.2021)
2021/1698	Delegated Regulation (EU) 2021/1698 of the 13 July 2021 supplementing Regulation (EU) 2018/848 with procedural requirements for the recognition of control authorities and control bodies that are competent to carry out controls on operators and groups of operators certified organic, and on organic products in third countries, and with rules on their supervision and the controls and other actions to be performed by those control authorities and control bodies (OJ L 336, 23.9.2021)
2021/1342	Delegated Regulation (EU) 2021/1342 of 27 May 2021 supplementing Regulation (EU) 2018/848 with rules on the information to be sent by third countries and by control authorities and control bodies for the purpose of supervision of their recognition under Article 33(2) and (3)

	of Regulation (EC) No 834/2007 of 28 June 2007 for imported organic products and the measures to be taken in the exercise of that supervision (OJ L 292, 16.8.2021)
2021/2305	Delegated Regulation (EU) 2021/2305 of 21 October 2021 supplementing Regulation (EU) 2017/625 with rules on the cases where and conditions under which organic products and in-conversion products are exempted from official controls at border control posts, the place of official controls for such products and amending Commission Delegated Regulations (EU) 2019/2023 and (EU) 2019/2124 (OJ L 461, 27.12.2021)
2021/2306	Delegated Regulation (EU) 2021/2306 of 21 October supplementing Regulation (EU) 2018/848 with rules on the official controls in respect of consignments of organic products and in-conversion products intended for import into the EU and on the certificate of inspection (OJ L 461, 27.12.2021)
2022/760	Delegated Regulation (EU) 2022/760 of 8 April 2022 amending Delegated Regulation (EU) 2021/2306 as regards the transitional provisions for certificates of inspection issued in Ukraine (OJ L 139, 18.5.2022)
2021/1378	Implementing Regulation (EU) 2021/1378 of 19 August 2021 laying down certain rules in accordance with Regulation (EU) 2018/848 concerning the certificate issued to operators, groups of operators and exporters in third countries involved in the imports of organic and in-conversion products into the EU and establishing the list of recognised control authorities and control bodies for the purpose of compliance (OJ L 297, 20.8.2021)
2021/2307	Implementing Regulation (EU) 2021/2307 of 21 October 2021 on documents and notifications required for organic and in-conversion products intended for import into the EU (OJ L 461, 27.12.2021)
2021/2325	Implementing Regulation (EU) 2021/2325 of 16 December 2021 establishing, pursuant to Regulation (EU) 2018/848 the list of third countries and the list of control authorities and control bodies that have been recognised under Article 33(2) and (3) of Regulation (EC) No 834/2007 for the purpose of importing organic products into the EU (OJ L 465, 29.12.2021)

Figure 3 The organic logo.

The European Union organic logo gives a coherent visual identity to organic products produced in the EU. This makes it easier for consumers to identify organic products and helps farmers to market them across the entire EU. The organic logo can only be used on products that have been certified as organic by an authorised control agency or body. This means that they have fulfilled strict conditions on how they must be produced, processed, transported and stored. The logo can only be used on products when they contain at least 95% organic ingredients and additionally, respect further strict conditions for the remaining 5%. The same ingredient cannot be present in organic and non-organic form.

Next to the EU organic logo, a code number of the control body must be displayed as well as the place where the agricultural raw materials composing the product have been farmed **[26]**.

HYGIENE MANUALS FOR MILK AND DAIRY PRODUCTS

Regulation (EC) No **852/2004** **[4]** of the European Parliament and of the Council on the hygiene of foodstuffs of 29 April 2004, Chapter 1, Article 1 paragraph 1. states that "the guides to good practice are a valuable instrument to aid food business operators at all levels of the food chain with compliance with food hygiene rules and with the application of the HACCP principles;" and Chapter III, Article 8 paragraph 4. requires that, "The

Member States shall forward to the Commission national guides complying with the requirements of paragraph 3. The Commission shall set up and run a registration system for such guides and make it available to the Member States" **[21]**.

Table 7 Hygiene manuals for milk and dairy products.

Co.	Original Title	Title in English	Contact point	Product	Register of national guides to good hygiene practice
AT	Leitlinie über mikrobiologische Kriterien für Milch und Milchprodukt	Guideline for microbiological criteria for Milk and dairy products	www.verbrauchergesundheit.gv.at	dairy products, milk	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=741**
AT	Leitlinie für bäuerliche Milchverarbeitungsbetriebe	Guideline for milk and dairy products on farms	www.verbrauchergesundheit.gv.at	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=728**
AT	Leitlinie für Milchverarbeitung auf Almen	Guideline for the milk production and dairy products on farms in the mountain	www.bmg.gv.at	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=729**
BE	G-034 Guide d'autocontrôle pour la production et la vente de produits laitiers à la ferme Autocontrolegids voor de productie en verkoop van zuivelproducten op het landbouwbedrijf	Guide for self-checking for the production and sale of dairy products on the farm	www.bcz-cbl.be/ http://www.favv-afsca.fgov.be/autocontrole-fr/guides/distribution/g034/	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=769**
BE	G-002 Guide système d'autocontrôle industrie laitière Gids autocontrolesysteem zuivelindustrie	Guide for self-checking in the milk industry	www.bcz-cbl.be/	dairy products, milk	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=744**
CH	Leitlinie für die gute Verfahrenspraxis bei der Milchgewinnung und -verarbeitung in Sömmerungsbetrieben	Guideline for the milk production and dairy products on alpine farms	Schweizerischer Alpwirtschaftlicher Verband SAV Seilerstrasse 4 3001 Bern	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=786**
CZ	Pravidla správné hygienické a výrobní praxe – Mléko a mléčné výrobky; Czech Technical Standard – ČSN 56 9601	Guidelines for Good Hygiene and Manufacturing Practice - Milk and Milk Products; Czech Technical Standard – ČSN 56 9601	www.mze.cz	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=809**
DK	Branchekode for egenkontrol: Mejerier	Hygiene and own checks, code of practice for production of milk	www.foedevarestyrelsen.dk	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=911**
ES	Guía de Análisis de Peligros y Puntos de Control Crítico en leches UHT y leches pasterizadas	Hazard Analysis and Critical Control Points Guide in UHT and pasteurized milk	http://www.aecosan.msssi.gob.es http://www.aecosan.msssi.gob.es/AECOSAN/docs/documentos/seguridad_alimentaria/gestion_riesgos/guia_leche_final.pdf	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1456**

FR	Guides de bonnes pratiques hygiéniques: Fabrication de produits laitiers et fromages fermiers	Guide to Good Hygiene Practice: Milk products and artisanal cheese	www.journal-officiel.gouv.fr	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1032**
FR	Guides de bonnes pratiques hygiéniques: Détaillant en produits laitiers	Guide to Good Hygiene Practice: Retailers of dairy products	www.journal-officiel.gouv.fr	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1043**
FR	Collecte lait cru et fabrication de produits laitiers	Association de la transformation laitière française	http://www.ladocumentationfrancaise.fr	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1075**
IT	Manuale volontario di corretta prassi igienica per le aziende del settore lattiero-caseario	Voluntary Handbook of Good Hygiene Practice for the milk/cheese sector	www.ministerosalute.it	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1173**
IT	Manuale di corretta prassi igienica per le produzioni lattiero-casearie artigianali e d'alpeggio	Guide to Good Hygiene Practice for the artisanal/on-farm production of milk and milk products	www.ministerosalute.it	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1185**
IT	Manuale di corretta prassi igienica per il formaggio Gorgonzola	Good hygiene practice for gorgonzola chees	Assolatte.Soria@assolatte.it	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1174**
LT	Tiesiogiai iš ūkių parduodamų pieno produktu geros higienos praktikos taisyklės	Good hygiene practice guide for primary diary production and direct supply	www.sam.lt	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1239**
LV	Labas higiēnas un ražošanas prakses vadlīnijas piena pārstrādes uzņēmumiem	Guidelines of good hygiene and production practice for milk processing establishments	www.zm.gov.lv/?sadala=1062	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1261**
NO	Retningslinjer for småskala mjølkeforedling	Guide to small-scale artisan cheese and dairy products	www.norskgardsost.no	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1507**
NO	Retningslinje for hygiene i meieriindustrien	Guide to hygiene in the dairy industries	www.tine.no	dairy products, milk	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1510**
PL	Zasady procesu produkcyjnego i higieny w zatwierdzonej urzędowo serowarni farmerskiej i rzemieślniczej	Rules of the manufacturing process and hygiene for officialy approved farmer and artisan cheese factory	https://sery.wrotapodlasia.pl	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1462**
PL	Poradnik dobrych praktyk higienicznych wytwarzania serów i innych produktów mleczarskich w farmerskim i rzemieślniczym przetwórstwie mleka	Guide for Good Hygiene Practices in farmhouse and artisan cheese and other dairy production	www.efrwp.p	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1463**

PT	Manual de procedimentos relativos às medidas nacionais de aplicação da flexibilidade em matéria de higiene alimentar	Manual of procedures for national measures for the application of flexibility in food hygiene	http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?actualmenu=63931&generico=62393&cboui=62393	meat (in general), dairy products, eggs and egg products, fishery products, honey	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1485**
PT	Código de boas práticas de higiene indústria de leite e produtos lácteos	Guide to Good Hygiene Practice for dairy and milk industry	www.anilact.com http://www.anilact.pt/documentos/Publicacao_06.pdf	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1326**
SE	Fäbodnäringens branschriktlinjer, Bilaga 6	The mountain pasture association's guide, Annex 6	http://www.eldrimner.com/om-eldrimner/31887.kontakta_oss.html http://www.livsmedelsverket.se/globalassets/produktion-handel-kontroll/branschriktlinjer/fabodnaringens-branschriktlinjer-bilaga-6---riskbedomning-och-kritiska-styrpunkter-vid-framstallning-av-fabodprodukter.pdf	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1452**
SE	Fäbodnäringens branschriktlinjer till god hygienpraxis vid fäbodar vid tillverkning av mjölkprodukter med traditionella metoder	The mountain pasture association's guide to good hygiene practice in chalets in the manufacturing of milk products using traditional methods	http://www.eldrimner.com/om-eldrimner/31887.kontakta_oss.html http://www.livsmedelsverket.se/globalassets/produktion-handel-kontroll/branschriktlinjer/fabodnaringens-branschriktlinjer-till-god-hygienpraxis-vid-fabodar-vid-tillverkning-av-mjolkprodukter-med-traditionella-metoder	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1387**
SE	Branschriktlinjer för kontroll av den obehandlade mjölkens kvalitet	Guide for control of raw milk hygienic quality	http://www.lrf.se/om-lrf/kontakta-oss/branschavdelningar/lrf-mjolk/ http://www.livsmedelsverket.se/globalassets/produktion-handel-kontroll/branschriktlinjer/mjolkens-kvalitet---kontroll-av-den-obehandlade-mjolkens-kvalitet.pdf	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1363**
SE	Branschriktlinjer för hygienisk praxis vid hantverksmässig produktion av ost och andra mjölkprodukter - Guidestart	Guide for hygienic practice in small scale production of cheese and other milk products - Guide start	http://www.eldrimner.com/om-eldrimner/31887.kontakta_oss.html http://www.livsmedelsverket.se/globalassets/produktion-handel-kontroll/branschriktlinjer/ost---guide-till-god-hygiensk-praxis-	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1361**

			vid-hantverksmassig-tillverkning-av-ost-och-andra-mjolkprodukter. pdf		
SE	Branschriktlinjer för hygienisk praxis vid hantverksmässig produktion av ost och andra mjölkprodukter - Grundförutsättningar och arbetsrutiner	Guide for hygienic practice in small scale production of cheese and other milk products - GHP and work routines	http://www.eldrimner.com/om-eldrimner/31887.kontakta_oss.html http://www.livsmedelsverket.se/globalassets/produktion-handel-kontroll/branschriktlinjer/ost---grundforutsattningar-och-arbetsrutiner-vid-hantverksmassig-tillverkning-av-ost-och-andra-mjolkprodukter.pdf	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1450**
SE	Branschriktlinjer för hygienisk praxis vid hantverksmässig produktion av ost och andra mjölkprodukter - Arbetsblad	Guide for hygienic practice in small scale production of cheese and other milk products - Work sheets	http://www.eldrimner.com/om-eldrimner/31887.kontakta_oss.html http://www.livsmedelsverket.se/produktion-handel--kontroll/branschriktlinjer2/arbetsblad-ost/	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1451**
SE	Branschriktlinjer för hygienisk intransport av obehandlad mjölk från gård	Guide for hygienic transport of raw milk from farm	http://www.lrf.se/om-lrf/kontakta-oss/branschavdelningar/lrf-mjolk/ http://www.livsmedelsverket.se/globalassets/produktion-handel-kontroll/branschriktlinjer/mjolk-fran-gard---hygienisk-intransport-av-obehandlad-mjolk-fran-gard.pdf	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1364**
SK	Hygienická príručka na zásadách HACCP pre výrobu ovčieho hrudkového syra v salašných podmienkach	Guide to Good Hygiene Practice on principles of the HACCP for production of sheep cheese in the farm conditions (salaš)	-	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1415**
SK	Hygienická príručka na zásadách HACCP pre výrobu a predaj výrobkov z ovčieho mlieka v salašníckych podmienkach - Ovčí hrudkový syr na priamu spotrebu, ovčí údený syr, žinčica, bačovská bryndza, parenice, korbáčiky - časť 2	Hygienic guide on HACCP principles for production and distribution of sheep milk products in mountain conditions - Ovčí hrudkový syr na priamu spotrebu, ovčí údený syr, žinčica, bačovská bryndza, parenice, korbáčiky - part 2	-	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1419**
SK	Hygienická príručka na zásadách HACCP pre spracovanie ovčieho mlieka a výrobu tradičných výrobkov z ovčieho	Hygienic guide on HACCP principles for processing of sheep milk and manufacturing of traditional products from sheep milk in the chalet	-	dairy products	**https://webgate.ec.europa.eu/dyna/hygienelegislation/details.cfm?id=1418**

mlieka v podmienkach salaša - Spracovanie mlieka, Výroba ovčieho hrudkového syra pre bryndziarne, Výroba tradičných špecialít a to –„Ovčí hrudkový syr – salašnícky“, „Ovčí salašnícky údený syr”, „Žinčica“, Predaj tradičných špecialít	conditions - Spracovanie mlieka, Výroba ovčieho hrudkového syra pre bryndziarne, Výroba tradičných špecialít a to – „Ovčí hrudkový syr – salašnícky“, „Ovčí salašnícky údený syr”, „Žinčica“, Predaj tradičných špecialít	

***CODEX ALIMENTARIUS* STANDARD FOR MILK AND DAIRY PRODUCTS**

The Codex Alimentarius Commission, established in 1963 by the Food and Agriculture Organization of the United Nations (FAO) and WHO, develops harmonized international food standards, guidelines and codes of practice to protect the health of consumers and ensure fair trade practices in the food trade **[22]**. Standards for milk products are present in Table 8, standards for cheese products are present in Table 9 and Table 10.

Table 8 Standards for milk products.

Code	Name	Register
CXS 281-1971	**Standard for Evaporated Milks** This Standard applies to evaporated milks, intended for direct consumption or further processing, in conformity.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B281-1971%252FCXS_281e.pdf**
CXS 282-1971	**Standard for Sweetened Condensed Milks** This Standard applies to sweetened condensed milks, intended for direct consumption or further processing, in conformity.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B282-1971%252FCXS_282e.pdf**
CXS 280-1973	**Standard for Milkfat Products** This Standard applies to Anhydrous Milkfat, Milkfat, Anhydrous Butter oil, Butter oil and Ghee, which are intended for further processing or culinary use.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B280-1973%252FCXS_280e.pdf**
CXS 288-1976	**Standard for Cream and Prepared Creams** This Standard applies to cream and prepared creams for direct consumption or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B288-1976%252FCXS_288e.pdf**
CXS 289-1995	**Standard for Whey Powders** This Standard applies to Whey Powder and Acid Whey Powder, intended for direct consumption or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B289-1995%252FCXS_289e.pdf**
CXS 290-1995	**Standard for Edible Casein Products** This Standard applies to edible acid casein, edible rennet casein and edible caseinate, intended for direct consumption or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B290-1995%252FCXS_290e.pdf**
CXS 206-1999	**General Standard for the Use of Dairy Terms** This General Standard applies to the use of dairy terms in relation to foods to be	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B206-1999%252FCXS_206e.pdf**

	offered to the consumer or for further processing.	
CXS 207-1999	**Standard for Milk Powders and Cream Powder** This Standard applies to milk powders and cream powder, intended for direct consumption or further processing, in conformity with the description in Section 2 of this Standard.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B207-1999%252FCXS_207e.pdf**
CXS 243-2003	**Standard for Fermented Milks** This standard applies to fermented milks, that is Fermented Milk including, Heat Treated Fermented Milks.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B243-2003%252FCXS_243e.pdf**
CXS 250-2006	**Standard for a Blend of Evaporated Skimmed Milk and Vegetable Fat** This Standard applies to a blend of evaporated skimmed milk and vegetable fat, also known as a blend of unsweetened condensed skimmed milk and vegetable fat, which is intended for direct consumption, or further processing	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B250-2006%252FCXS_250e.pdf**
CXS 251-2006	**Standard for a Blend of Skimmed Milk and Vegetable Fat in Powdered Form** This Standard applies to a blend of skimmed milk and vegetable fat in powdered form, intended for direct consumption, or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B251-2006%252FCXS_251e.pdf**
CXS 252-2006	**Standard for a Blend of Sweetened Condensed Skimmed Milk and Vegetable Fat** This Standard applies to a blend of sweetened condensed skimmed milk and vegetable fat, intended for direct consumption, or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B252-2006%252FCXS_252e.pdf**
CXS 253-2006	**Standard for Dairy Fat Spreads** This Standard applies to dairy fat spreads intended for use as spreads for direct consumption, or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B253-2006%252FCXS_253e.pdf**
CXS 279-1971	**Standard for Butter** This Standard applies to butter intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B279-1971%252FCXS_279e.pdf**
CXS 331-2017	**Standard for Dairy Permeate Powders** This Standard applies to dairy permeate powders, intended for further processing and/or as ingredient in other foods.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B331-2017%252FCXS_331e.pdf**

Table 9 Horizontal cheese standards.

Code	Name	Register
CXS 208-1999	**Group Standard for Cheeses in Brine** This Standard applies to Cheeses in Brine, intended for direct consumption or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B208-1999%252FCXS_208e.pdf**
CXS 221-2001	**Group Standard for Unripened Cheese including Fresh Cheese** This Standard applies to unripened cheese including fresh cheese, intended for direct consumption or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B221-2001%252FCXS_221e.pdf**
CXS 278-1978	**Standard for Extra Hard Grating Cheese**	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B278-1978%252FCXS_278e.pdf**
CXS 284-1971	**Standard for Whey Cheeses** This Standard applies to all products intended for direct consumption or further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B284-1971%252FCXS_284e.pdf**

Table 10 Individual cheese standards.

Code	Name	Register
CXS 262-2006	**Standard for Mozzarella** This Standard applies to Mozzarella intended for direct consumption or for further processing,	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B263-1966%252FCXS_263e.pdf**
CXS 263-2006	**Standard for Cheddar** This Standard applies to Cheddar intended for direct consumption or for further processing	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B263-1966%252FCXS_263e.pdf**
CXS 264-2006	**Standard for Danbo** This Standard applies to Danbo intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B264-1966%252FCXS_264e.pdf**
CXS 265-2006	**Standard for Edam** This Standard applies to Edam intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B265-1966%252FCXS_265e.pdf**
CXS 266-2006	**Standard for Gouda** This Standard applies to Gouda intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B266-1966%252FCXS_266e.pdf**
CXS 267-2006	**Standard for Havarti** This Standard applies to Havarti intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B267-1966%252FCXS_267e.pdf**
CXS 268-2006	**Standard for Samsø** This Standard applies to Samsø intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FSta**

		ndards%252FCXS%2B268-1966%252FCXS_268e.pdf
CXS 269-2006	**Standard for Emmental** This Standard applies to Emmental intended for direct consumption or for further processing	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B269-1967%252FCXS_269e.pdf**
CXS 270-2006	**Standard for Tilsiter** This Standard applies to Tilsiter intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B270-1968%252FCXS_270e.pdf**
CXS 271-2006	**Standard for Saint-Paulin** This Standard applies to Saint-Paulin intended for direct consumption or for further processing	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B271-1968%252FCXS_271e.pdf**
CXS 272-2006	**Standard for Provolone** This Standard applies to Provolone intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B272-1968%252FCXS_272e.pdf**
CXS 273-2006	**Standard for Cottage Cheese** This Standard applies to Cottage Cheese intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B273-1968%252FCXS_273e.pdf**
CXS 274-2006	**Standard for Coulommiers** This Standard applies to Coulommiers intended for direct consumption or for further processing	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B274-1969%252FCXS_274e.pdf**
CXS 275-2006	**Standard for Cream Cheese** This Standard applies to Cream Cheese intended for direct consumption or for further processing	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B275-1973%252FCXS_275e.pdf**
CXS 276-2006	**Standard for Camembert** This Standard applies to Camembert intended for direct consumption or for further processing	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B276-1973%252FCXS_276e.pdf**
CXS 277-2006	**Standard for Brie** This Standard applies to Brie intended for direct consumption or for further processing.	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B277-1973%252FCXS_277e.pdf**
CXS 276-2006	**Standard for Extra Hard Grating Cheese**	**https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B278-1978%252FCXS_278e.pdf**

ISO STANDARDS FOR MILK AND MILK PRODUCTS

Standards are the distilled wisdom of people with expertise in their subject matter and who know the needs of the organizations they represent – people such as manufacturers, sellers, buyers, customers, trade associations, users or regulators **[23]**. The actual list of ISO standard for dairy sector is present in Table 11.

Table 11 Milk and milk products in general.

ICS	FIELD	Register	
67.100.01	**Milk and milk products in general**	https://www.iso.org/ics/67.100.01/x/	
STANDARD AND/OR PROJECT (62)		**STAGE**	**TC**
ISO 707:2008 Milk and milk products — Guidance on sampling		90.93	**ISO/TC 34/SC 5**
ISO 3889:2006 Milk and milk products — Specification of Mojonnier-type fat extraction flasks		90.93	**ISO/TC 34/SC 5**
ISO 3890-1:2009 Milk and milk products — Determination of residues of organochlorine compounds (pesticides) — Part 1: General considerations and extraction methods		90.93	**ISO/TC 34/SC 5**
ISO 3890-2:2009 Milk and milk products — Determination of residues of organochlorine compounds (pesticides) — Part 2: Test methods for crude extract purification and confirmation		90.93	**ISO/TC 34/SC 5**
ISO 5538:2004 Milk and milk products — Sampling — Inspection by attributes		90.60	**ISO/TC 34/SC 5**
ISO 5738:2004 Milk and milk products — Determination of copper content — Photometric method (Reference method)		90.93	**ISO/TC 34/SC 5**
ISO 6611:2004 Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 degrees C		90.93	**ISO/TC 34/SC 5**
ISO 6732:2010 Milk and milk products — Determination of iron content — Spectrometric method (Reference method)		90.93	**ISO/TC 34/SC 5**
ISO/TS 6733:2006 Milk and milk products — Determination of lead content — Graphite furnace atomic absorption spectrometric method		90.93	**ISO/TC 34/SC 5**
ISO 7208:2008 Skimmed milk, whey and buttermilk — Determination of fat content — Gravimetric method (Reference method)		90.20	**ISO/TC 34/SC 5**
ISO 8260:2008 Milk and milk products — Determination of organochlorine pesticides and polychlorobiphenyls — Method using capillary gas-liquid chromatography with electron-capture detection		90.20	**ISO/TC 34/SC 5**
ISO 8262-1:2005 Milk products and milk-based foods — Determination of fat content by the Weibull-Berntrop gravimetric method (Reference method) — Part 1: Infant foods		90.60	**ISO/TC 34/SC 5**
ISO 8262-2:2005 Milk products and milk-based foods — Determination of fat content by the Weibull-Berntrop gravimetric method (Reference method) — Part 2: Edible ices and ice-mixes		90.60	**ISO/TC 34/SC 5**
ISO 8262-3:2005 Milk products and milk-based foods — Determination of fat content by the Weibull-Berntrop gravimetric method (Reference method) — Part 3: Special cases		90.60	**ISO/TC 34/SC 5**
ISO 8870:2006 Milk and milk-based products — Detection of thermonuclease produced by coagulase-positive staphylococci		90.93	**ISO/TC 34/SC 5**
ISO 9231:2008 Milk and milk products — Determination of the benzoic and sorbic acid contents		90.20	**ISO/TC 34/SC 5**
ISO 10932:2010 Milk and milk products — Determination of the minimal inhibitory concentration (MIC) of antibiotics applicable to bifidobacteria and non-enterococcal lactic acid bacteria (LAB)		90.92	**ISO/TC 34/SC 5**
ISO/TS 11059:2009 Milk and milk products — Method for the enumeration of Pseudomonas spp.		90.92	**ISO/TC 34/SC 5**

ISO 11813:2010 Milk and milk products — Determination of zinc content — Flame atomic absorption spectrometric method	90.60	**ISO/TC 34/SC 5**
ISO 11866-1:2005 Milk and milk products — Enumeration of presumptive Escherichia coli — Part 1: Most probable number technique using 4-methylumbelliferyl-beta-D-glucuronide (MUG)	90.93	**ISO/TC 34/SC 5**
ISO 11866-2:2005 Milk and milk products — Enumeration of presumptive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using membranes	90.93	**ISO/TC 34/SC 5**
ISO 11870:2009 Milk and milk products — Determination of fat content — General guidance on the use of butyrometric methods	90.93	**ISO/TC 34/SC 5**
ISO 12078:2006 Anhydrous milk fat — Determination of sterol composition by gas liquid chromatography (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 13082:2011 Milk and milk products — Determination of the lipase activity of pregastric lipase preparation	90.93	**ISO/TC 34/SC 5**
ISO 14156:2001 Milk and milk products — Extraction methods for lipids and liposoluble compounds	90.60	**ISO/TC 34/SC 5**
ISO 14156:2001/AMD 1:2007 Milk and milk products — Extraction methods for lipids and liposoluble compounds — Amendment 1	60.60	**ISO/TC 34/SC 5**
ISO 14673-1:2004 Milk and milk products — Determination of nitrate and nitrite contents — Part 1: Method using cadmium reduction and spectrometry	90.93	**ISO/TC 34/SC 5**
ISO 14673-2:2004 Milk and milk products — Determination of nitrate and nitrite contents — Part 2: Method using segmented flow analysis (Routine method)	90.93	**ISO/TC 34/SC 5**
ISO 14673-3:2004 Milk and milk products — Determination of nitrate and nitrite contents — Part 3: Method using cadmium reduction and flow injection analysis with in-line dialysis (Routine method)	90.93	**ISO/TC 34/SC 5**
ISO 14891:2002 Milk and milk products — Determination of nitrogen content — Routine method using combustion according to the Dumas principle	90.93	**ISO/TC 34/SC 5**
ISO 15163:2012 Milk and milk products — Calf rennet and adult bovine rennet — Determination by chromatography of chymosin and bovine pepsin contents	90.93	**ISO/TC 34/SC 5**
ISO/TS 15495:2010 Milk, milk products and infant formulae — Guidelines for the quantitative determination of melamine and cyanuric acid by LC-MS/MS	90.93	**ISO/TC 34/SC 5**
ISO 17129:2006 Milk powder — Determination of soy and pea proteins using capillary electrophoresis in the presence of sodium dodecyl sulfate (SDS-CE) — Screening method	90.60	**ISO/TC 34/SC 5**
ISO/TS 17193:2011 Milk — Determination of the lactoperoxidase activity — Photometric method (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 17792:2006 Milk, milk products and mesophilic starter cultures — Enumeration of citrate-fermenting lactic acid bacteria — Colony-count technique at 25 degrees C	90.93	**ISO/TC 34/SC 5**
ISO 18252:2006 Anhydrous milk fat — Determination of sterol composition by gas liquid chromatography (Routine method)	90.93	**ISO/TC 34/SC 5**
ISO 18329:2004 Milk and milk products — Determination of furosine content — Ion-pair reverse-phase high-performance liquid chromatography method	90.93	**ISO/TC 34/SC 5**
ISO 18330:2003 Milk and milk products — Guidelines for the standardized description of immunoassays or receptor assays for the detection of antimicrobial residues	90.60	**ISO/TC 34/SC 5**

ISO/TS 19046-1:2017 Cheese — Determination of propionic acid level by chromatography — Part 1: Method by gas chromatography	90.93	**ISO/TC 34/SC 5**
ISO/TS 19046-2:2017 Cheese — Determination of propionic acid level by chromatography — Part 2: Method by ion exchange chromatography	90.93	**ISO/TC 34/SC 5**
ISO 19662:2018 Milk — Determination of fat content — Acido-butyrometric (Gerber method)	60.60	**ISO/TC 34/SC 5**
ISO 20541:2008 Milk and milk products — Determination of nitrate content — Method by enzymatic reduction and molecular-absorption spectrometry after Griess reaction	90.20	**ISO/TC 34/SC 5**
ISO 21187:2021 Milk — Quantitative determination of microbiological quality — Guidance for establishing and verifying a conversion relationship between results of an alternative method and anchor method results	60.60	**ISO/TC 34/SC 5**
ISO 21543:2020 Milk and milk products — Guidelines for the application of near infrared spectrometry	60.60	**ISO/TC 34/SC 5**
ISO 22184:2021 Milk and milk products — Determination of the sugar contents — High performance anion exchange chromatography with pulsed amperometric detection method (HPAEC-PAD)	60.60	**ISO/TC 34/SC 5**
ISO 22186:2020 Milk and milk products — Determination of nitrofurazone	60.60	**ISO/TC 34/SC 5**
ISO 22662:2007 Milk and milk products — Determination of lactose content by high-performance liquid chromatography (Reference method)	90.92	**ISO/TC 34/SC 5**
ISO 22935-1:2009 Milk and milk products — Sensory analysis — Part 1: General guidance for the recruitment, selection, training and monitoring of assessors	90.92	**ISO/TC 34/SC 5**
ISO/DIS 22935-1 Milk and milk products — Sensory analysis — Part 1: General guidance for the recruitment, selection, training and monitoring of assessors	40.20	**ISO/TC 34/SC 5**
ISO 22935-2:2009 Milk and milk products — Sensory analysis — Part 2: Recommended methods for sensory evaluation	90.92	**ISO/TC 34/SC 5**
ISO/DIS 22935-2 Milk and milk products — Sensory analysis — Part 2: Recommended methods for sensory evaluation	40.20	**ISO/TC 34/SC 5**
ISO 22935-3:2009 Milk and milk products — Sensory analysis — Part 3: Guidance on a method for evaluation of compliance with product specifications for sensory properties by scoring	90.92	**ISO/TC 34/SC 5**
ISO/DIS 22935-3 Milk and milk products — Sensory analysis — Part 3: Guidance on a method for evaluation of compliance with product specifications for sensory properties by scoring	40.20	**ISO/TC 34/SC 5**
ISO 22964:2017 Microbiology of the food chain — Horizontal method for the detection of Cronobacter spp.	90.20	**ISO/TC 34/SC 9**
ISO 23058:2006 Milk and milk products — Ovine and caprine rennets — Determination of total milk-clotting activity	90.93	**ISO/TC 34/SC 5**
ISO 23293:2020 Milk-based infant formula powders — Quantification of whey protein content by sodium dodecyl sulfate-capillary gel electrophoresis (SDS-CGE)	60.60	**ISO/TC 34/SC 5**
ISO/TS 23758:2021 Guidelines for the validation of qualitative screening methods for the detection of residues of veterinary drugs in milk and milk products	60.60	**ISO/TC 34/SC 5**
ISO 26323:2009	90.93	**ISO/TC 34/SC 5**

Milk products — Determination of the acidification activity of dairy cultures by continuous pH measurement (CpH)		
ISO/TS 26844:2006 Milk and milk products — Determination of antimicrobial residues — Tube diffusion test	90.93	**ISO/TC 34/SC 5**
ISO 27105:2016 Milk and cheese — Determination of hen's egg white lysozyme content by high performance liquid chromatography	90.60	**ISO/TC 34/SC 5**
ISO 29981:2010 Milk products — Enumeration of presumptive bifidobacteria — Colony count technique at 37 degrees C	90.92	**ISO/TC 34/SC 5**
ISO/CD 29981 Milk products — Enumeration of bifidobacteria — Colony count technique at 37 °C	30.60	**ISO/TC 34/SC 5**
ICS	**FIELD**	**Register**
67.100.10	**Milk and processed milk products Including dried milk, condensed milk and evaporated milk**	https://www.iso.org/ics/67.100.10/x/
STANDARD AND/OR PROJECT (80)	**STAGE**	**TC**
ISO 1211:2010 Milk — Determination of fat content — Gravimetric method (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 1736:2008 Dried milk and dried milk products — Determination of fat content — Gravimetric method (Reference method)	90.20	**ISO/TC 34/SC 5**
ISO 1737:2008 Evaporated milk and sweetened condensed milk — Determination of fat content — Gravimetric method (Reference method)	90.20	**ISO/TC 34/SC 5**
ISO 1740:2004 Milkfat products and butter — Determination of fat acidity (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 2446:2008 Milk — Determination of fat content	95.60	**ISO/TC 34/SC 5**
ISO 2911:2004 Sweetened condensed milk — Determination of sucrose content — Polarimetric method	90.93	**ISO/TC 34/SC 5**
ISO 3356:2009 Milk — Determination of alkaline phosphatase	90.60	**ISO/TC 34/SC 5**
ISO 3976:2006 Milk fat — Determination of peroxide value	90.93	**ISO/TC 34/SC 5**
ISO/DIS 4214 Milk and milk products — Determination of amino acids in infant formula and other dairy products	40.60	**ISO/TC 34/SC 5**
ISO 5536:2009 Milk fat products — Determination of water content — Karl Fischer method	90.93	**ISO/TC 34/SC 5**
ISO 5537:2004 Dried milk — Determination of moisture content (Reference method)	90.92	**ISO/TC 34/SC 5**
ISO 5764:2009 Milk — Determination of freezing point — Thermistor cryoscope method (Reference method)	90.60	**ISO/TC 34/SC 5**
ISO 5765-1:2002 Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 1: Enzymatic method utilizing the glucose moiety of the lactose	90.93	**ISO/TC 34/SC 5**
ISO 5765-2:2002 Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 2: Enzymatic method utilizing the galactose moiety of the lactose	90.93	**ISO/TC 34/SC 5**
ISO 6091:2010 Dried milk — Determination of titratable acidity (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 6092:1980 Dried milk — Determination of titratable acidity (Routine method)	90.93	**ISO/TC 34/SC 5**
ISO 6731:2010 Milk, cream and evaporated milk — Determination of total solids content (Reference method)	90.93	**ISO/TC 34/SC 5**

ISO 6734:2010 Sweetened condensed milk — Determination of total solids content (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 8069:2005 Dried milk — Determination of content of lactic acid and lactates	90.60	**ISO/TC 34/SC 5**
ISO 8070:2007 Milk and milk products — Determination of calcium, sodium, potassium and magnesium contents — Atomic absorption spectrometric method	90.93	**ISO/TC 34/SC 5**
ISO 8156:2005 Dried milk and dried milk products — Determination of insolubility index	90.93	**ISO/TC 34/SC 5**
ISO 8196-1:2009 Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 1: Analytical attributes of alternative methods	90.93	**ISO/TC 34/SC 5**
ISO 8196-2:2009 Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 2: Calibration and quality control in the dairy laboratory	90.93	**ISO/TC 34/SC 5**
ISO 8196-3:2022 Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 3: Protocol for the evaluation and validation of alternative quantitative methods of milk analysis	60.60	**ISO/TC 34/SC 5**
ISO 8967:2005 Dried milk and dried milk products — Determination of bulk density	90.60	**ISO/TC 34/SC 5**
ISO 8968-1:2014 Milk and milk products — Determination of nitrogen content — Part 1: Kjeldahl principle and crude protein calculation	90.60	**ISO/TC 34/SC 5**
ISO 8968-3:2004 Milk — Determination of nitrogen content — Part 3: Block-digestion method (Semi-micro rapid routine method)	90.60	**ISO/TC 34/SC 5**
ISO 8968-3:2004/COR 1:2011 Milk — Determination of nitrogen content — Part 3: Block-digestion method (Semi-micro rapid routine method) — Technical Corrigendum 1	60.60	**ISO/TC 34/SC 5**
ISO 8968-4:2016 Milk and milk products — Determination of nitrogen content — Part 4: Determination of protein and non-protein nitrogen content and true protein content calculation (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 9622:2013 Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometry	90.93	**ISO/TC 34/SC 5**
ISO 9874:2006 Milk — Determination of total phosphorus content — Method using molecular absorption spectrometry	90.93	**ISO/TC 34/SC 5**
ISO 9874:2006/COR 1:2007 Milk — Determination of total phosphorus content — Method using molecular absorption spectrometry — Technical Corrigendum 1	60.60	**ISO/TC 34/SC 5**
ISO/TS 9941:2005 Milk and canned evaporated milk — Determination of tin content — Spectrometric method	90.20	**ISO/TC 34/SC 5**
ISO 11285:2004 Milk — Determination of lactulose content — Enzymatic method	90.93	**ISO/TC 34/SC 5**
ISO 11814:2002 Dried milk — Assessment of heat treatment intensity — Method using high-performance liquid chromatography	90.93	**ISO/TC 34/SC 5**
ISO 11815:2007 Milk — Determination of total milk-clotting activity of bovine rennets	90.93	**ISO/TC 34/SC 5**
ISO 11816-1:2013 Milk and milk products — Determination of alkaline phosphatase activity — Part 1: Fluorimetric method for milk and milk-based drinks	90.60	**ISO/TC 34/SC 5**
ISO 11865:2009 Instant whole milk powder — Determination of white flecks number	90.93	**ISO/TC 34/SC 5**
ISO 11868:2007	90.93	**ISO/TC 34/SC 5**

Heat-treated milk — Determination of lactulose content — Method using high-performance liquid chromatography		
ISO 12080-1:2009 Dried skimmed milk — Determination of vitamin A content — Part 1: Colorimetric method	90.93	**ISO/TC 34/SC 5**
ISO 12080-2:2009 Dried skimmed milk — Determination of vitamin A content — Part 2: Method using high-performance liquid chromatography	90.93	**ISO/TC 34/SC 5**
ISO 12081:2010 Milk — Determination of calcium content — Titrimetric method	90.93	**ISO/TC 34/SC 5**
ISO 13366-1:2008 Milk — Enumeration of somatic cells — Part 1: Microscopic method (Reference method)	90.60	**ISO/TC 34/SC 5**
ISO 13366-1:2008/COR 1:2009 Milk — Enumeration of somatic cells — Part 1: Microscopic method (Reference method) — Technical Corrigendum 1	60.60	**ISO/TC 34/SC 5**
ISO 13366-2:2006 Milk — Enumeration of somatic cells — Part 2: Guidance on the operation of fluoro-opto-electronic counters	90.60	**ISO/TC 34/SC 5**
ISO 13875:2005 Liquid milk — Determination of acid-soluble beta-lactoglobulin content — Reverse-phase HPLC method	90.93	**ISO/TC 34/SC 5**
ISO 14377:2002 Canned evaporated milk — Determination of tin content — Method using graphite furnace atomic absorption spectrometry	90.93	**ISO/TC 34/SC 5**
ISO 14378:2009 Milk and dried milk — Determination of iodide content — Method using high-performance liquid chromatography	90.93	**ISO/TC 34/SC 5**
ISO 14501:2021 Milk and milk powder — Determination of aflatoxin M1 content — Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography	60.60	**ISO/TC 34/SC 5**
ISO 14637:2004 Milk — Determination of urea content — Enzymatic method using difference in pH (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 14674:2005 Milk and milk powder — Determination of aflatoxin M1 content — Clean-up by immunoaffinity chromatography and determination by thin-layer chromatography	90.93	**ISO/TC 34/SC 5**
ISO 14675:2003 Milk and milk products — Guidelines for a standardized description of competitive enzyme immunoassays — Determination of aflatoxin M1 content	90.60	**ISO/TC 34/SC 5**
ISO 14892:2002 Dried skimmed milk — Determination of vitamin D content using high-performance liquid chromatography	90.93	**ISO/TC 34/SC 5**
ISO 15151:2018 Milk, milk products, infant formula and adult nutritionals — Determination of minerals and trace elements — Inductively coupled plasma atomic emission spectrometry (ICP-AES) method	60.60	**ISO/TC 34/SC 5**
ISO 15174:2012 Milk and milk products — Microbial coagulants — Determination of total milk-clotting activity	90.93	**ISO/TC 34/SC 5**
ISO 15322:2005 Dried milk and dried milk products — Determination of their behaviour in hot coffee (Coffee test)	90.93	**ISO/TC 34/SC 5**
ISO 15323:2002 Dried milk protein products — Determination of nitrogen solubility index	90.93	**ISO/TC 34/SC 5**
ISO 15884:2002 Milk fat — Preparation of fatty acid methyl esters	90.93	**ISO/TC 34/SC 5**
ISO 15885:2002 Milk fat — Determination of the fatty acid composition by gas-liquid chromatography	90.60	**ISO/TC 34/SC 5**

ISO 16297:2020 Milk — Bacterial count — Protocol for the evaluation of alternative methods	60.60	**ISO/TC 34/SC 5**
ISO 16958:2015 Milk, milk products, infant formula and adult nutritionals — Determination of fatty acids composition — Capillary gas chromatographic method	90.92	**ISO/TC 34/SC 5**
ISO/DIS 16958 Milk, milk products, infant formula and adult nutritionals — Determination of fatty acids composition — Capillary gas chromatographic method	40.99	**ISO/TC 34/SC 5**
ISO 17678:2019 Milk and milk products — Determination of milk fat purity by gas chromatographic analysis of triglycerides	60.60	**ISO/TC 34/SC 5**
ISO/TS 17758:2014 Instant dried milk — Determination of the dispersibility and wettability	90.93	**ISO/TC 34/SC 5**
ISO 17997-1:2004 Milk — Determination of casein-nitrogen content — Part 1: Indirect method (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 17997-2:2004 Milk — Determination of casein-nitrogen content — Part 2: Direct method	90.93	**ISO/TC 34/SC 5**
ISO 19344:2015 Milk and milk products — Starter cultures, probiotics and fermented products — Quantification of lactic acid bacteria by flow cytometry	90.93	**ISO/TC 34/SC 5**
ISO 20128:2006 Milk products — Enumeration of presumptive Lactobacillus acidophilus on a selective medium — Colony-count technique at 37 degrees C	90.93	**ISO/TC 34/SC 5**
ISO 21422:2018 Milk, milk products, infant formula and adult nutritionals — Determination of chloride — Potentiometric titration method	60.60	**ISO/TC 34/SC 5**
ISO 21424:2018 Milk, milk products, infant formula and adult nutritionals — Determination of minerals and trace elements — Inductively coupled plasma mass spectrometry (ICP-MS) method	60.60	**ISO/TC 34/SC 5**
ISO/TS 22113:2012 Milk and milk products — Determination of the titratable acidity of milk fat	90.60	**ISO/TC 34/SC 5**
ISO 22160:2007 Milk and milk-based drinks — Determination of alkaline phosphatase activity — Enzymatic photo-activated system (EPAS) method	90.93	**ISO/TC 34/SC 5**
ISO 23065:2009 Milk fat from enriched dairy products — Determination of omega-3 and omega-6 fatty acid content by gas-liquid chromatography	90.93	**ISO/TC 34/SC 5**
ISO 23291:2020 Milk and milk products — Guidelines for the application of in-line and on-line infrared spectrometry	60.60	**ISO/TC 34/SC 5**
ISO 23305:2020 Fortified milk powders, infant formula and adult nutritionals — Determination of total biotin by liquid chromatography coupled with immunoaffinity column clean-up extraction	60.60	**ISO/TC 34**
ISO/DIS 23318 Milk, dried milk products and cream — Determination of fat content — Gravimetric method	40.60	**ISO/TC 34/SC 5**
ISO 23970:2021 Milk, milk products and infant formula — Determination of melamine and cyanuric acid by liquid chromatography and tandem mass spectrometry (LC-MS/MS)	60.60	**ISO/TC 34/SC 5**
ISO 26462:2010 Milk — Determination of lactose content — Enzymatic method using difference in pH	90.93	**ISO/TC 34/SC 5**
ISO 27205:2010 Fermented milk products — Bacterial starter cultures — Standard of identity	90.93	**ISO/TC 34/SC 5**
ISO/TS 27265:2009 Dried milk — Enumeration of the specially thermoresistant spores of thermophilic bacteria	90.92	**ISO/TC 34/SC 5**
ICS	**FIELD**	**Register**

67.100.20	Butter	https://www.iso.org/ics/67.100.20/x/	
STANDARD AND/OR PROJECT (12)		**STAGE**	**TC**
ISO 1738:2004 Butter — Determination of salt content		90.93	**ISO/TC 34/SC 5**
ISO 1739:2006 Butter — Determination of the refractive index of the fat (Reference method)		90.93	**ISO/TC 34/SC 5**
ISO 3727-1:2001 Butter — Determination of moisture, non-fat solids and fat contents — Part 1: Determination of moisture content (Reference method)		90.93	**ISO/TC 34/SC 5**
ISO 3727-2:2001 Butter — Determination of moisture, non-fat solids and fat contents — Part 2: Determination of non-fat solids content (Reference method)		90.93	**ISO/TC 34/SC 5**
ISO 3727-3:2003 Butter — Determination of moisture, non-fat solids and fat contents — Part 3: Calculation of fat content		90.93	**ISO/TC 34/SC 5**
ISO 7238:2004 Butter — Determination of pH of the serum — Potentiometric method		90.93	**ISO/TC 34/SC 5**
ISO 8851-1:2004 Butter — Determination of moisture, non-fat solids and fat contents (Routine methods) — Part 1: Determination of moisture content		90.93	**ISO/TC 34/SC 5**
ISO 8851-2:2004 Butter — Determination of moisture, non-fat solids and fat contents (Routine methods) — Part 2: Determination of non-fat solids content		90.93	**ISO/TC 34/SC 5**
ISO 8851-3:2004 Butter — Determination of moisture, non-fat solids and fat contents (Routine methods) — Part 3: Calculation of fat content		90.93	**ISO/TC 34/SC 5**
ISO 15648:2004 Butter — Determination of salt content — Potentiometric method		90.60	**ISO/TC 34/SC 5**
ISO 16305:2005 Butter — Determination of firmness		90.60	**ISO/TC 34/SC 5**
ISO 17189:2003 Butter, edible oil emulsions and spreadable fats — Determination of fat content (Reference method)		90.60	**ISO/TC 34/SC 5**
ICS	**FIELD**	**Register**	
67.100.30	**Cheese** **Including cottage cheese, whey cheese**	**https://www.iso.org/ics/67.100.30/x/**	
STANDARD AND/OR PROJECT (24)		**STAGE**	**TC**
ISO 1740:2004 Milkfat products and butter — Determination of fat acidity (Reference method)		90.93	**ISO/TC 34/SC 5**
ISO 1854:2008 Whey cheese — Determination of fat content — Gravimetric method (Reference method)		90.20	**ISO/TC 34/SC 5**
ISO 2920:2004 Whey cheese — Determination of dry matter (Reference method)		90.93	**ISO/TC 34/SC 5**
ISO 2962:2010 Cheese and processed cheese products — Determination of total phosphorus content — Molecular absorption spectrometric method		90.93	**ISO/TC 34/SC 5**
ISO/TS 2963:2006 Cheese and processed cheese products — Determination of citric acid content — Enzymatic method		90.93	**ISO/TC 34/SC 5**
ISO 3432:2008 Cheese — Determination of fat content — Butyrometer for Van Gulik method		90.93	**ISO/TC 34/SC 5**
ISO 3433:2008 Cheese — Determination of fat content — Van Gulik method		90.93	**ISO/TC 34/SC 5**
ISO 5534:2004 Cheese and processed cheese — Determination of the total solids content (Reference method)		90.60	**ISO/TC 34/SC 5**
ISO 5534:2004/COR 1:2013 Cheese and processed cheese — Determination of the total solids content (Reference method) — Technical Corrigendum 1		60.60	**ISO/TC 34/SC 5**

ISO 5765-1:2002 Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 1: Enzymatic method utilizing the glucose moiety of the lactose	90.93	**ISO/TC 34/SC 5**
ISO 5765-2:2002 Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 2: Enzymatic method utilizing the galactose moiety of the lactose	90.93	**ISO/TC 34/SC 5**
ISO 5943:2006 Cheese and processed cheese products — Determination of chloride content — Potentiometric titration method	90.93	**ISO/TC 34/SC 5**
ISO 9233-1:2018 Cheese, cheese rind and processed cheese — Determination of natamycin content — Part 1: Molecular absorption spectrometric method for cheese rind	60.60	**ISO/TC 34/SC 5**
ISO 9233-2:2018 Cheese, cheese rind and processed cheese — Determination of natamycin content — Part 2: High-performance liquid chromatographic method for cheese, cheese rind and processed cheese	60.60	**ISO/TC 34/SC 5**
ISO 11816-2:2016 Milk and milk products — Determination of alkaline phosphatase activity — Part 2: Fluorimetric method for cheese	90.60	**ISO/TC 34/SC 5**
ISO 12082:2006 Processed cheese and processed cheese products — Calculation of the content of added citrate emulsifying agents and acidifiers/pH-controlling agents, expressed as citric acid	90.60	**ISO/TC 34/SC 5**
ISO/TS 17996:2006 Cheese — Determination of rheological properties by uniaxial compression at constant displacement rate	90.60	**ISO/TC 34/SC 5**
ISO/TS 18083:2013 Processed cheese products — Calculation of content of added phosphate expressed as phosphorus	90.93	**ISO/TC 34/SC 5**
ISO/TS 19046-1:2017 Cheese — Determination of propionic acid level by chromatography — Part 1: Method by gas chromatography	90.93	**ISO/TC 34/SC 5**
ISO/TS 19046-2:2017 Cheese — Determination of propionic acid level by chromatography — Part 2: Method by ion exchange chromatography	90.93	**ISO/TC 34/SC 5**
ISO 23319:2022 Cheese and processed cheese products, caseins and caseinates — Determination of fat content — Gravimetric method	60.60	**ISO/TC 34/SC 5**
ISO 24223:2021 Cheese — Guidance on sample preparation for physical and chemical testing	60.60	**ISO/TC 34/SC 5**
ISO/TS 27106:2009 Cheese — Determination of nisin A content by LC-MS and LC-MS-MS	90.93	**ISO/TC 34/SC 5**
ISO 27871:2011 Cheese and processed cheese — Determination of the nitrogenous fractions	90.93	**ISO/TC 34/SC 5**
ICS **FIELD**	**Register**	
67.100.40 **Ice cream and ice confectionery Including sorbets**	**https://www.iso.org/ics/67.100.40/x/**	
STANDARD AND/OR PROJECT (4)	**STAGE**	**TC**
ISO 3728:2004 Ice-cream and milk ice — Determination of total solids content (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 5765-1:2002 Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 1: Enzymatic method utilizing the glucose moiety of the lactose	90.93	**ISO/TC 34/SC 5**
ISO 5765-2:2002 Dried milk, dried ice-mixes and processed cheese — Determination of lactose content — Part 2: Enzymatic method utilizing the galactose moiety of the lactose	90.93	**ISO/TC 34/SC 5**
ISO 7328:2008 Milk-based edible ices and ice mixes — Determination of fat content — Gravimetric method (Reference method)	90.20	**ISO/TC 34/SC 5**
ICS **FIELD**	**Register**	

67.100.99 Other milk products	https://www.iso.org/ics/67.100.99/x/	
STANDARD AND/OR PROJECT (16)	**STAGE**	**TC**
ISO 5547:2008 Caseins — Determination of free acidity (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 5548:2004 Caseins and caseinates — Determination of lactose content — Photometric method	90.60	**ISO/TC 34/SC 5**
ISO 5550:2006 Caseins and caseinates — Determination of moisture content (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 5739:2003 Caseins and caseinates — Determination of contents of scorched particles and of extraneous matter	90.93	**ISO/TC 34/SC 5**
ISO 6731:2010 Milk, cream and evaporated milk — Determination of total solids content (Reference method)	90.93	**ISO/TC 34/SC 5**
ISO 7889:2003 Yogurt — Enumeration of characteristic microorganisms — Colony-count technique at 37 degrees C	90.92	**ISO/TC 34/SC 5**
ISO/CD 7889 Yogurt — Enumeration of characteristic microorganisms — Colony-count technique	30.60	**ISO/TC 34/SC 5**
ISO 8381:2008 Milk-based infant foods — Determination of fat content — Gravimetric method (Reference method)	90.20	**ISO/TC 34/SC 5**
ISO/TS 11869:2012 Fermented milks — Determination of titratable acidity — Potentiometric method	90.93	**ISO/TC 34/SC 5**
ISO 12779:2011 Lactose — Determination of water content — Karl Fischer method	90.93	**ISO/TC 34/SC 5**
ISO 13580:2005 Yogurt — Determination of total solids content (Reference method)	90.60	**ISO/TC 34/SC 5**
ISO 19660:2018 Cream — Determination of fat content — Acido-butyrometric method	60.60	**ISO/TC 34/SC 5**

CONCLUSION

The legal regulations of the European Union, hygiene manuals, ISO standards, and *Codex Alimentarius* standards create a framework that regulates the production of milk and dairy products, payment systems for primary producers, contains rules for their safety, and quality, labeling, control, and consumer health protection. These rules apply at all stages of the food chain, in primary production, production, and various forms of sale, including import and export. The main objectives of the legislation include consumer protection, consumer information, free movement of milk and dairy products within the EU, regulation of imports and exports of these products, and establishing a quality system for specific products such as protected label products or organic food.

REFERENCES

1. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health. OJ L 84, 31.3.2016, p. 1–208.
2. Commission Delegated Regulation (EU) 2020/692 of 30 January 2020 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for entry into the Union, and the movement and handling after entry of consignments of certain animals, germinal products and products of animal origin. OJ L 174, 3.6.2020, p. 379–520.
3. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.
4. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.
5. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.
6. Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001,

(EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation)Text with EEA relevance. OJ L 95, 7.4.2017, p. 1–142.

7. Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls. OJ L 131, 17.5.2019, p. 51–100.
8. Commission Implementing Regulation (EU) 2021/405 of 24 March 2021 laying down the lists of third countries or regions thereof authorised for the entry into the Union of certain animals and goods intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council. OJ L 114, 31.3.2021, p. 118–150.
9. Commission Implementing Regulation (EU) 2020/2235 of 16 December 2020 laying down rules for the application of Regulations (EU) 2016/429 and (EU) 2017/625 of the European Parliament and of the Council as regards model animal health certificates, model official certificates and model animal health/official certificates, for the entry into the Union and movements within the Union of consignments of certain categories of animals and goods, official certification regarding such certificates and repealing Regulation (EC) No 599/2004, Implementing Regulations (EU) No 636/2014 and (EU) 2019/628, Directive 98/68/EC and Decisions 2000/572/EC, 2003/779/EC and 2007/240/EC. OJ L 442, 30.12.2020, p. 1–409.
10. Commission Implementing Regulation (EU) 2019/1014 of 12 June 2019 to lay down detailed rules on minimum requirements for border control posts, including inspection centres, and for the format, categories and abbreviations to use for listing border control posts and control points. OJ L 165, 21.6.2019, p. 10–22.
11. Commission Implementing Regulation (EU) 2019/2130 of 25 November 2019 establishing detailed rules on the operations to be carried out during and after documentary checks, identity checks and physical checks on animals and goods subject to official controls at border control posts. OJ L 321, 12.12.2019, p. 128–138.
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The use of vapor condensation cavitation to increase the activity of milk of lime in sugar beet production

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ABSTRACT

The paper presents research on increasing the activity of milk of lime in beet sugar production using vapor condensation cavitation. The work aimed to develop a rational way of activating milk of lime using the effects of vapor condensation cavitation and its hardware design, substantiating the optimal values of the process parameters. It was established that to increase the activity of milk of lime at a steam potential of 0.18 MPa, an optimal consumption of water vapor using vapor condensation cavitation is required, which is 1 .75 – 2.0% to the weight of the suspension. This is ensured by the action of the combined CaO particles with the bulk of the steam, due to which the Their additional solution is due to the t-plot, which occurs through the boundary layer from the bubbles. As a result, a pre-saturated, water-lime suspension is created, in which the It is the number of dissolved calcium ions. It has been proven that the water vapour-treated suspension is 1.5 times lower It expands and has a volume of sediment of the solid phase in the medium that is 10% larger than that of processed suspension. Such a study is indirect evidence of the increase in the dispersal of these systems after Her husband's work. By increasing the activity of milk of lime, it is possible to increase the effect of cleaning juices at various stages of the technological process and reduce the consumption of limestone for the production of granulated sugar.

Keywords: water-lime suspension, milk of lime, lime, vapour condensation cavitation, activity.

INTRODUCTION

One of the most important processes of beet sugar production, which largely determines the efficiency of the use of raw materials, fuel-energy materials, and material resources, as well as the final results of the plant, is the purification of the diffusion juice with the use of lime and saturated gas **[1]**. The existing technology for the purification of diffusion juice and concentrated sugar-containing solutions, and its hardware design, in principle, have not changed much during the last decades **[2]**. The lack of necessary theoretical developments and experimental data inhibits the development of known and the development of new technological processes **[3]**. Therefore, the main direction of increasing the effectiveness of lime-carbonic acid cleaning of sugar-containing solutions is the disclosure of its unused reserves and their implementation in practice.

The scientific problem of choosing a rational direction for improving the technology of lime-carbonic acid purification of solutions, which ensures the production of granulated sugar in conditions of changes in the quality of raw materials, is very relevant and has an important national economic significance, especially in the conditions of a market economy. The problem of reducing limestone consumption, which is used to obtain a water-lime suspension for cleaning solutions from non-sugars, is also relevant **[4]**. One of the ways to solve it is to increase the activity of this suspension to make fuller use of the adsorption capacity of calcium carbonate particles with a simultaneous increase in the filtration properties of saturated sediments **[5]**. Therefore, the paper conducted a study and proposed a physicochemical method of increasing the reactivity of the water-lime suspension and calcium carbonate particles.

Scientific hypothesis

In work **[6]**, we presented the effects of vapour condensation and hydrodynamic cavitation on the transformation of associated and complex compounds of non-sugars of diffusion and beet juices with the

formation of substances with increased reactivity. Furthermore, in work **[7]**, the effectiveness of the simultaneous application of the effects of vapour condensation cavitation and a calcium-containing reagent for the treatment of diffusion juice before preliminary defecation was theoretically substantiated and experimentally confirmed, which contributes to the formation of sediment of preliminary defecation juice with minimal hydrophilicity and saturation sediments with high filtration capacity. Therefore, there is an assumption that the applicant applying the vapour-condensation cavitation effect to the milk of lime can lead to its activation, which affects the purification of the diffusion juice from non-sugars. Thus, by increasing the activation of milk of lime, it is possible to reduce its total amount for cleaning, which will lead to a decrease in the cost price and the cost of the finished product - granulated sugar.

MATERIAL AND METHODOLOGY

Samples

The research was carried out using limestone from the Polupaniv deposit of the Ternopil region, which was crushed in laboratory conditions and diluted with water to a density of 1.14 g/cm^3.

Chemicals

0.1 n. hydrochloric acid (HCl, hydrochloric acid, manufactured by the private enterprise "Khimreaktivy", Ivano-Frankivsk, Ukraine).

Instruments

Portions of the water-lime suspension were treated with steam on a steam condensing unit, which is shown in Figure 1.

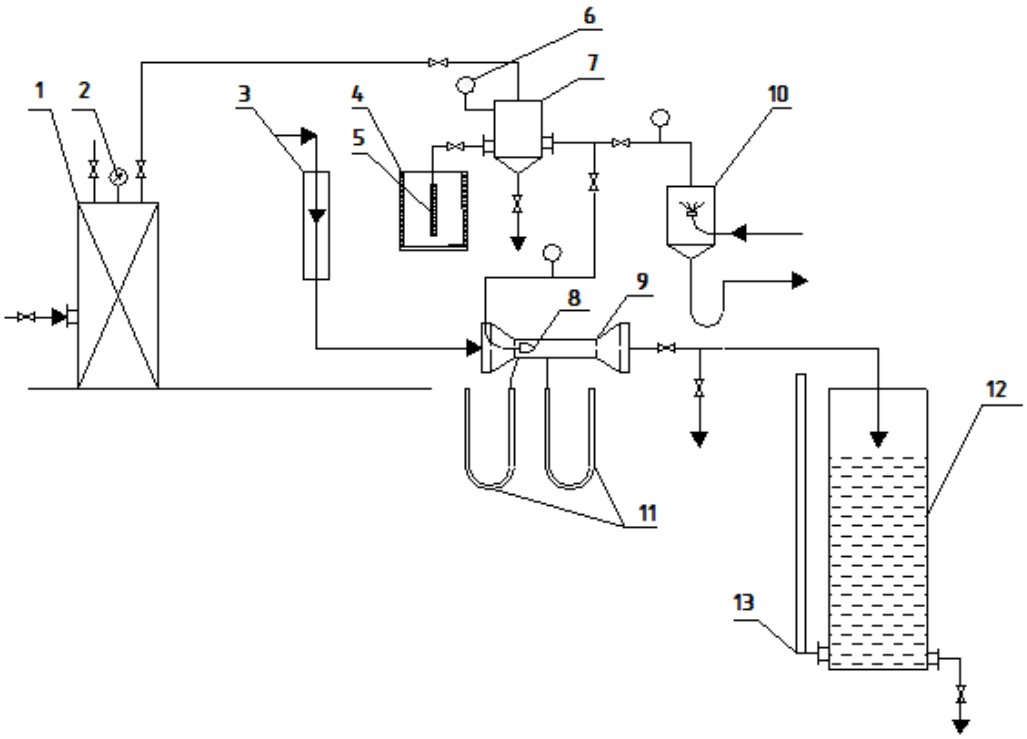

Figure 1 Diagram of a laboratory steam condensing unit. Note: 1 – steam boiler" 2, 6 – manometers; 3 – rotameter; 4 – thermostat capacity; 5 – bubbler; 7 – droplet catcher; 8 – nozzle; 9 – transparent section of the vapor condensation device; 10 – steam contact device; 11 – water diffmanometers; 12 – collection; 13 – measuring glass.

The steam-condensing cavitation device 9 consists of a working area into which a pipeline for supplying steam with a nozzle 8 attached to its end is inserted. It consists of confusion, a transparent cylindrical channel with a diameter of 25.4 mm, and a diffuser. With the help of pipelines, this device is connected to communication through the rotameter 3 with the water collector 12 and to the steam boiler 1 through the droplet separator 7. The steam pressure in front of the nozzle is controlled by the manometer 6. During the experiments, the hydrodynamics of the flow of the vapour-liquid mixture was visually observed.

The steam pressure in front of the nozzle was measured with a standard manometer MO 1 227 (manufactured by the Spetsavtomatika Ukrainy company, Kharkiv, Ukraine).

The pressure of the liquid in front of the nozzle Rs, the pressure of the vapor-water mixture, and the pressure at the exit from the working area What did you measure with the help of the DSP-160-M1 digital meter (manufactured by Spetsavtomatyka Ukrainy, Kharkiv, Ukraine).

The steam torch length was measured with a ruler's help 1 ± 10^{-3} m.

The rate of stratification of the water-lime suspension was determined using a ruler and a stopwatch.

The temperature was controlled with the help of the TLS-2 thermometer (manufactured by the Sklopryladplant, Kyiv, Ukraine).

Crushing of limestone was carried out using a DM 4x3 hammer crusher (producer LLC "Progress Industrial Equipment Plant", Cherkasy, Ukraine).

Time intervals were determined and monitored with a stopwatch with an accuracy of 1 s.

The density of the water-lime suspension was determined with an AZP-1 hydrometer (manufactured by the Sklopryladplant, Kyiv, Ukraine).

The sediment volume was measured using a measuring cylinder 1-100-1 (manufactured by the Sklopryladplant, Kyiv, Ukraine).

The specific electrical conductivity of the water-lime suspension was determined using a laboratory pH-meter/conductometer XS PC 50 VioLab Complete Kit, which consists of a pH-electrode type 201T and a conductometric cell type 2301T (manufactured by XS Instruments, Carpi, Italy).

Laboratory Methods

Determination of the activity of lime in the milk of lime was carried out by the method **[8]**, which is based on the titration of the weight of milk of lime with hydrochloric acid. To do this, it is necessary to filter milk of lime in the amount of 500 cm^3, and take a weight of milk of lime in the amount of 50 – 60 g, after which it is titrated with 0.1 n. hydrochloric acid and the activity of lime in the milk of lime are determined as a percentage ratio of the concentration of lime in the milk of lime to the solubility of lime in it.

The cost of steam at the exit from the nozzle was calculated based on the thermal balance.

The velocity of the liquid flow was calculated analytically from the dependence:

$$V = Q/S \quad (1)$$

Where:

Q – single environmental costs, determined using a meter; S – the cross-sectional area at the point of velocity determination.

Description of the Experiment

Sample preparation: the water-lime suspension was obtained by preliminary crushing of limestone with a hammer crusher followed by mixing with water until the suspension density was 1.14 g/cm^3.

Number of samples analyzed: eight samples of water-lime suspension were used to study the influence of suspension activity on the steam potential and the amount of steam for processing.

Number of repeated analyses: all the experiments were conducted three times.

Number of experiment replication: all the experiments were replicated three times.

Design of the experiment: according to the created laboratory installation (see Figure 1) with the help of the meter, the non-observable constant well, water consumption through the working transparent part of the steam-condensing cavitation device. By regulating the flow of steam with a fan, they chose the lifetime of the steam torch, which they looked at in the transparent part, and photographed the pattern of the flow of steam mixtures Next, they switched on the supply of steam to the thermostatic container 5, filled with water lime suspension, in which the bar is placed. They supplied the steam for a certain time and determined the thermal balance. spend a couple Analogously, you determined the consumption of steam for a steam-powered apparatus 10. Recorded indicators and diffmanometers, the height of the liquid column in collector 12, the temperature of the water at the entrance and exit from the transparent working area of the cavitation device, and the temperature of the juice before and after its processing in the vapor contact apparatus, into which the juice was fed under pressure through a centrifugal nozzle, were recorded.

Statistical Analysis

All data are expressed as the mean standard deviation of three parallel experiments and indicate significance at $p < 0.05$. The data obtained during the research were subjected to statistical analysis using Excel and STATISTICA 13 programs (Dell, StatSoft).

RESULTS AND DISCUSSION

The water-lime suspension, traditionally called "lime milk" **[9]** by sugar makers, is shown in Figure 2 and is the main chemical reagent for cleaning semi-products of sugar beet production. Depending on the conditions of its production in factory conditions, such a suspension can have different quantitative and qualitative compositions **[10]**. The quantitative composition is characterized by density, which depends on the ratio of calcium hydroxide and water content. A density of 1.18 – 1.19 g/cm^3 is considered normal, but due to the high content of impurities (unslaked CaO and recalcitrant) and the low dispersion of $Ca(OH)_2$ particles, which leads to rapid delamination of the suspension factories use a lime suspension with a density of 1,12 – 1.14 g/cm^3. And this means that for the consumption of 2.5% of CaO to the mass of beets, an additional 2.6% of water, which must be evaporated, is introduced into the technological flow.

Figure 2 Water-lime suspension.

The qualitative composition of the water-lime suspension is characterized by activity. The activity of milk of lime is the ability of calcium hydroxide to quickly and completely react with non-sugars during preliminary and main defecation and with carbon dioxide at saturation. It is quantitatively evaluated as a percentage of the content of active CaO to its total content **[11]**, **[26]**. The consumption of milk or lime for cleaning the diffusion juice also depends on the amount of activity.

At temperatures close to the dissociation temperature of $CaCO_3$ (about 1000 °C), highly dispersed and highly active lime is obtained, which can be extinguished even with cold water in a few minutes. As the firing temperature increases, lime recrystallization processes take place, after which the sintering process begins. The latter interferes with the access of water to CaO particles during quenching, and lime, remaining chemically free, becomes inactive for reaction with water **[12]**, **[27]**.

At the same time as calcium recrystallization and sintering, the processes of its high-temperature process there are mods with admixtures and the formation of complex compounds: silicates, aluminates, and calcium oxide ferrites of various modifications. Films of these compounds slag the parts of the plaster, as well as prevent access to water. But if these films are destroyed by rubbing or damage, they are not active. It is transformed into action **[13]**, **[28]**.

In the 80s of the 20th centuries, VNDICP was developed and implemented in some sugar factories' methods of hydrodynamic cavitation activation of aqueous suspension **[14]**, **[29]**. The increase in its reaction capacity under these conditions is explained by the destruction of aggregates in suspension by cumulative micro streams into colloidal parts. But by using such a method in industrial conditions, significant abrasive wear of activator parts was found, which prevented their wide distribution **[15]**. We conducted a series of experiments to study the possibility of activation of water-lime suspension by using the effects of vapor condensation cavitation.

To compare the influence of the steam temperature on the effectiveness of the activation of the water-lime suspension With such processing, the temperature difference before and after processing was the same in all cases, which is composed of 5 °C and according to the method described above, the content of active calcium and its activity were determined. The obtained data are presented in Figure 3, which testify that the activity of calcium carbonate increases with the increase in temperature of the steam, but after the temperature of the steam is higher than 0.18 MPa, the increase in lime activity decreases.

Dependence of the activity of calcium carbonate milk on different consumption of steam with a pressure of 0.18 MPa gives the possibility to create, that for effective processing you have optimized the loss of water vapor is 1.7 – 2.0% of the mass of the suspension.

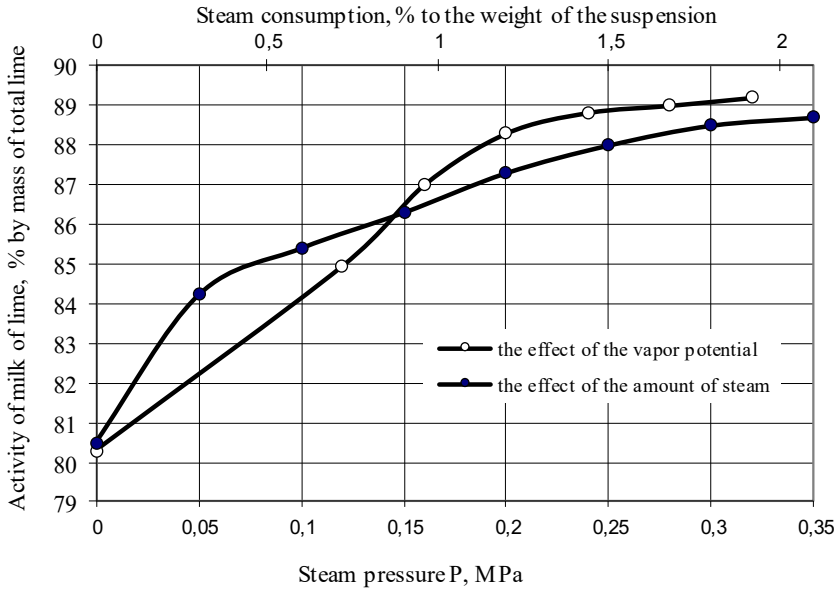

Figure 3 Change in the activity of milk of lime during its treatment with steam.

The obtained results can be explained, taking into account such assumptions. First of all, milky milk is a single suspension that appears with Both of them are the alkaline solution of calcium hydrogen oxide **[16]**. It also contains inorganic $Ca(OH)_2$ in the form of agglomerates that form over time in mixers for "ripening" milk of lime as a result of coagulation of soluble colloidal particles and the so-called hexaquark complexes of calcium hydroxide **[17]**. As a result of the collapse of the left bubbles in the water-lime suspension, part of the bubbles coalesce with the formation of cumulant streams, which destroy aggregates and complexes of $Ca(OH)_2$ **[18]**, **[30]**. When crushed particles come into contact with bubbles, which burst while maintaining symmetry, their additional dissolution occurs due to the heat perceived through the boundary layer from the bubbles. As a result, we get a supersaturated hydro line suspension, in which the amount of dissolved calcium ions increases **[19]**, **[31]**. The destruction of the constituent parts of the solid phase of the aqueous suspension is subject to increasing the dispersion and increasing the volume of the solid phase after processing Not a couple. The rate of dissolution and the volume of sedimentation of the solid phase of the aqueous suspension to We followed the method **[20]**, **[32]**. Data for the study of the rate of expansion and the volume of sediment of the solid phase of the suspension before and after steam processing is shown in the Table. 1.

Table 1 Comparative data of the speed of stratification and the volume of sediment of water-lime suspension before and after treatment with vapor condensation cavitation.

№	Before processing		After processing		
	delamination speed, mm/min	sediment volume, %	the amount of steam, % to the mass of the suspension	delamination speed, mm/min	sediment volume, %
1	2.5 ±0.05	72 ±0.7	1.7	1.7 ±0.04	81 ±0.8
2	2.5 ±0.05	75 ±0.7	1.8	1.6 ±0.04	80 ±0.8
3	2.5 ±0.05	71 ±0.7	1.9	1.4 ±0.04	82 ±0.8
Average	2.5 ±0.05	73 ±0.7	1.8	1.6 ±0.04	81 ±0.8

Table 1 testifies that the suspension is 1.5 times more processed with water vapor It expands more slowly and has a volume of sediment of the solid phase in the medium by 10% white better than unprocessed suspension. Such a phenomenon is indirect evidence of an increase in the dispersibility of the system after its steam treatment.

Secondly, in the aqueous suspension, there are parts of undissolved CaO, which are externally covered with a layer of insoluble $Ca(OH)_2$ particles. At the contact of such a part with a cumulative current, there is its destruction and further dissolution by calcium hydroxide. Liberation from the protective layer of CaO particles is accompanied by their extinguishing, which increases calcium ions' content in an aqueous suspension **[21]**, **[35]**. This was confirmed by measuring the electrical conductivity of the various numbers before steam suspension treatment was 7.9×10^{-3} sim/sm, and after treatment – 8.1×10^{-3} sim/sm.

At this stage of research, it is difficult to conclude whether the increase in specific electrical conductivity depends only on the increase in the solubility of lime in lime water, or whether it is the sum of the increase in the solubility of lime and the increase in the degree of electrolytic dissociation of hydroxy calcium ($CaOH^+$) with the formation of an additional amount of Ca^{2+} ions in the solution. In any case, increased solubility will positively affect the coagulation of substances of colloidal dispersion and the degree of decomposition of non-sugars in the diffusion juice **[22]**, **[33]**.

In addition to the expected decrease in the consumption of active milk and water- calcium suspension on the previous defecation will contribute to complete coagulation of substances of colloidal dispersity with the formation of poorly hydrated sediment and, as a result, an increase in the sedimentation and filtration properties of the juice and saturation **[23]**, **[34]**. An increase in the dispersion of calcium hydroxide particles contributes to the formation of highly dispersed calcium carbonate particles during the first saturation, which will increase the overall cleaning effect **[24]**.

At the same time, in work **[25]** it was established that the duration of the relaxation of the hydrodynamic cavity activated by applying the effects of It takes 20 – 30 minutes to prepare the milk. Therefore, we also studied the influence of the lime milk storage duration lime milk storage activated by vapour condensation cavitation on the change in its activity. The experiments showed that after the initial activity of the milk, after the activity of 89.4% CaO, the latter does not change for almost 15 minutes, and after 30 minutes it becomes smaller by 3 – 4% CaO. This means that it is advisable to carry out such activation before dosing milk of lime for preliminary and main defecation.

In this way, we can state that under the influence of the effects of vapour condensation cavitation, the activity of the vapour increases. of fresh milk up to 10% due to the destruction of calcium hydroxide particles and hexoaquacomplexes and the "advance" of parts of CaO, which contributes to an increase in the solution of active calcium ions (+2), necessary for interaction with the non-sugars of the diffusion juice during its lime-carbon dioxide purification.

CONCLUSION

It was established that to increase the activity of milk of lime at a steam potential of 0.18 MPa, an optimal consumption of water vapor with the use of vapor condensation cavitation in the amount of 1.75 – 2 is required. 0% to the weight of the suspension. This is ensured by the action of suspended CaO particles with steam bubbles, due to which their additional dissolution occurs due to the heat that the second is due to the boundary layer from the bubbles. As a result of this, a re-saturated single-calcium suspension is formed, in which the It is the number of dissolved calcium ions. It has been proven that the water vapour-treated suspension is 1.5 times lower It expands and has a volume of sediment of the solid phase in the medium that is 10% larger than that of processed suspension. Such a study is indirect evidence of an increase in the dispersity of the system after its steam treatment. As a result of the experiments, it was proved that due to the application of the effects of steam condensation cavitation, the activity of lime milk increases by 8 – 10%, which contributes to the temporary increase in the solution of active Ca^{2+} ions, which are necessary for interaction with the non-sugars of the diffusion juice during its lime-carbonation purification and simultaneously increasing the dispersion of calcium hydroxide particles. By increasing the activity of milk of lime, it is possible to increase the effect of juice purification at various stages of the technological process and reduce the consumption of limestone for the production of granulated sugar.

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The effect of astaxanthin and lycopene on the content of fatty acids in the yolks of chicken eggs under different storage regimes

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ABSTRACT
The level of consumers' satisfaction with the quality of edible chicken eggs is determined, in particular, by the attractive appearance of the yolks and their content of biologically active substances that have functional properties. Such compounds include carotenoids astaxanthin and lycopene, which can be deposited in the yolks, provide their pigmentation, and as powerful antioxidants, affect the stability of the fatty acid composition of lipids during egg storage. The aim This study aimed mine the effect of supplements of oil extracts of astaxanthin (10, 20, and 30 mg/kg of feed) or lycopene (20, 40, and 60 mg/kg of feed) on the Dion of young hens on the fatty acid composition of the yolks during eggs storage in temperature conditions 4 ±0.5 °C and 12 ±0.5 °C for 30 days. The experiment used 45 High-Line W36 crossbred laying hens at 24 weeks of age. It was found that the storage temperature of eggs (4 ±0.5 °C and 12 ±0.5 °C) equally affected the fatty acid composition of lipids of egg yolks obtained from laying hens fed lycopene supplements in doses of 20, 40, and 60 mg/kg or astaxanthin in doses of 10, 20 and 30 mg/kg of feed for 30 days. Doses of lycopene from 20 to 60 mg/kg or astaxanthin from 10 to 30 mg/kg in the diet of laying hens contributed to a decrease in egg yolks at both storage temperatures of ω6 PUFA particles: Eicosatetraenoic and 6.9, 12-okadekatrienic acids until their complete disappearance. The addition of astaxanthin to the diet of laying hens reduced and stabilized the ratio of ω3/ω6 PUFA in yolks during egg storage to a greater extent than the addition of lycopene. Storage of lycopene or astaxanthin-enriched edible chicken eggs at 4 ±0.5 °C and 12 ±0.5 °C for 30 days can be used to correct the fatty acid profile of yolk lipids.

Keywords: astaxanthin, lycopene, egg yolks, fatty acids, storage

INTRODUCTION
Chicken eggs are foodstuffs containing essential nutrients and biologically active substances easily digested by humans **[25]**. Such biologically active compounds include carotenoids **[39]** and fatty acids contained in egg yolks **[44]**. One of the criteria determining consumer demand for chicken eggs is the intensity of pigmentation of the yolk. To achieve an attractive color of chicken egg yolks, manufacturers use natural carotenoids that do not possess provitamin activity in animals and humans, but can be deposited in egg yolks, in particular, astaxanthin **[11], [38]** and lycopene **[2], [32]**. Recent studies have shown that carotenoids to some extent, can affect the content and ratio of individual fatty acids in chicken egg yolks **[33]**. Particularly relevant are studies on the development of methods to reduce the ratio of ω6/ω3 fatty acids in the lipid structure of egg yolks **[41]** to the optimal level, which should be in the range of 2:1 − 4:1. It has been proven that ω3 fatty acids play an important role in the body as components of phospholipids that form the structures of cell membranes. In particular, the axenic acid content is high in the retina, brain, and semen **[24]**. In addition to the structural role in cell membranes, fatty acids, which are ω3 and ω6, provide the body with energy and are precursors of eicosanoids, which, as signaling molecules, perform functions in the cardiovascular, respiratory, immune, and endocrine systems **[3]**.

The effect of antioxidants such as astaxanthin and lycopene on the fatty acid profile of chicken egg yolks is also one of the important criteria for assessing their suitability for storage at different temperatures. It is known that the processes of lipid peroxidation that occur in egg yolks during storage **[22]** adversely affect the sensory characteristics of eggs because they impair their taste **[16], [20]**. However, studies on the effect of egg storage on the fatty acid profile of yolks enriched with astaxanthin or lycopene are insufficient in the available literature.

They are mainly devoted to the study of the effectiveness of combining oils with carotenoids of various origins in the diets of laying hens to modify the fatty acid composition of egg yolks **[14]**, **[15]**, **[28]**. Therefore, our study aimed to investigate the effect of astaxanthin and lycopene on the fatty acid content of yolks under different storage regimes of eggs.

Scientific Hypothesis. It was assumed that the enrichment of the diet of laying hens with lycopene or astaxanthin would affect the fatty acid composition of egg yolks depending on the temperature regime of their storage. The ω6/ω3 ratio of polyunsaturated fatty acids in chicken egg yolks undergoes particular changes that depend on non doses of lycopene or astaxanthin in chicken feed during storage regardless of temperature conditions.

MATERIAL AND METHODOLOGY

Samples

All eggs from each group of laying hens were selected for the study from 25 to 31, from 55 to 61, and 85 to 91 days of the experiment.

Chemicals

Supelco 37 Component FAME Mix certified reference material, *Trace*CERT®, in dichloromethane (varied conc.), ampule of 1 mL.
Sodium hydroxide, chemically pure for analysis (Spain).
Sodium chloride, pure for analysis (Germany).
n-Hexan (purity GC 98%), Merck (Germany).
Methanol (HPLC grade), Lot: 1419984, Fisher Scientific UK.
Chloroform for chromatography, Merck (Germany).

Animals and Biological Material

45 laying hens of the High-line W36 cross at 23 weeks were used for the experiment. Laying hens on the principle of groups of analogues were divided into 3 groups of 15 heads in each and kept in cage batteries of 5 heads in each cage. The experiment lasted for 90 days (Table 1). As a source of astaxanthin used 10% oil extract was obtained from the biomass of the alga *Haematococcus Pluvialis* (ALGAE Technologies, Israel). As a source of lycopene, laying hens were fed with a 6% oil extract of lycopene derived from tomatoes (LycoRed, Israel). Laying hens were fed with complete feed, the composition of which is given by **[40]**. Experimental diets were prepared for 4 days, and the feed mixture was mixed and stored in airtight food plastic containers. Watering of laying hens was carried out at will with cup drinkers. Daylight was 16 hours, light − 30 lux, darkness − 8 hours. The air temperature in the room for keeping laying hens was at the level of 21 – 23 °C; relative humidity was in the range of 60 – 62%.

Table 1 Scheme of the experiment.

Group	Diet		
	1 – 30 day	**31 – 60 day**	**61 – 90 day**
Control	Basic diet[1] + 0.33 g/kg of refined sunflower oil	Basic diet[2] + 0.66 g/kg refined sunflower oil	Basic diet[3] + 1.0 g/kg refined sunflower oil
Lycopene diet	Basic diet[1] + 20 mg/kg lycopene (LP20)	Basic diet[2] + 40 mg/kg lycopene (LP40)	Basic diet[3] + 60 mg/kg lycopene (LP60)
Astaxanthin diet	Basic diet[1] + 10 mg/kg astaxanthin (AST10)	Basic diet[2] + 20 mg/kg astaxanthin (AST20)	Basic diet[3] + 30 mg/kg astaxanthin (AST30)

Note: In the basic diet, the same superscripts [1, and 2, 3] show one new content of the refined solar system in the diet.

Instruments

Fatty acid methyl esters were analyzed on a Trace GC Ultra gas chromatograph (USA) using a flame ionization detector (FID) and a 100 m long high-polar capillary column (Supelco, USA). Chromatography conditions: column temperature 140 – 240 °C, detector temperature 260 °C. The sample was added to the chromatography using an automatic dispenser (TriPlus autosampler) in 1 μL. The duration of one analysis on the device was 65 minutes. The fatty acid peaks of egg yolk lipids were identified by comparing them with the time of the release of the peaks of the standard sample Supelco 37 Component FAME Mix, which includes 37 names of fatty acids.

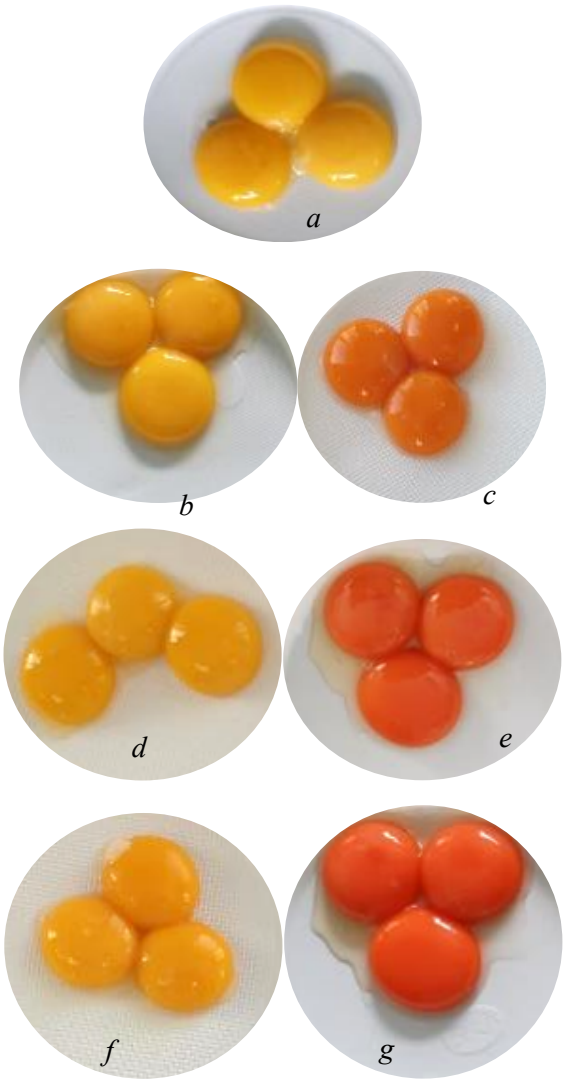

Figure 1 Color of egg yolks of chickens of control group (a), LP20 (b), AST10 (c), LP40 (d), AST20 (e), LP60 (f), AST30 (g).

Laboratory Methods

Lipids were extracted from chicken egg yolks according to the method **[45]**. This was followed by hydrolysis and methylation of fatty acids of chicken egg yolk lipids according to ISO 12966-2:2017.

Description of the Experiment

After weighing and sorting, 9 freshly laid eggs were selected from each group of laying hens to determine the fatty acid composition of the yolks. The remaining eggs from each group of laying hens were divided into two batches and stored: the first batch was stored at a temperature of 4 ±0.5 °C and relative humidity of 80 − 85%, and the second batch − at a temperature of 12 ±0.5 °C and relative humidity 70 − 75% for 30 days. At the end of the storage period, 9 eggs were taken from each group of laying hens, and the fatty acid content of the yolks was examined.

Sample preparation: The proportion of fatty acids in the lipids of chicken egg yolks was calculated by internal normalization, determining their content in percent. The study was performed in 3 parallels. The following fatty acids were determined in chicken egg yolks: dodecanoic (12:0), tetradecanoic (14:0), myristoleic (14:1), pentadecanoic (15:0), hexadecanoic (16:0), trans-3-hexadecene (16:1), heptadecane (17:0), cis-10-heptadecenoic (17:1), octadecane (18:0), oleic (18:1n9c), 9.12 octadecadienoic (18:2n6c), linolenic (18:3n3), 6,9,12-ocadecatriene (18:3n6), eicosan (20:0), cis-11-eicosene (20:1), cis-11,14-eicosadienoic (20:2n6), eicosatetraenoic (20:3n6), arachidonic (20:4n6), 5,8,11,14, 17-eicosapentaenoic (20:5n3), docosan (22:0), 4,7,10,13,16,19-docosahexaenoic (22:6n3).

Statistical analysis

The data obtained in the study were analyzed statistically using the ANOVA program. The normality of data distribution was confirmed using the program R-3.6.3 for Windows **[36]**. The difference between the values in the groups was determined using the Tukey test. The difference was considered significant at $p < 0.05$ (considering the Bonferroni correction).

RESULTS AND DISCUSSION

Storage of chicken eggs for 30 days at a temperature of 4 ±0.5 °C and 12 ±0.5 °C did not affect the ratio in the yolks of saturated fatty acids such as dodecanoic, pentadecanoic, heptadecanoic, eicosenoic, and docosanic. Still, it increased ($p < 0.05$) the proportion of tetradecanoic acid on the background of a decrease ($p < 0.05$) in the content of hexadecanoic and octadecanoic acids compared with freshly laid eggs. Among monounsaturated fatty acids under the above temperature storage conditions of eggs, only the proportion of myristoleic acid in the yolks did not change, while the content of trans-3-hexadecenoic, cis-9-octadecenoic, and cis-11-eicosenoic acids increased ($p < 0.05$) compared with freshly laid eggs. The proportion of cis-10-heptadecenoic acid decreased ($p < 0.05$) in the yolks only when storing eggs at 4 ±0.5 °C compared with freshly laid eggs (Table 2).

Table 2 The content of fatty acids in the yolks of chicken eggs under different storage regimes (% of the total fatty acid content) (control group).

Acid	Egg storage mode			SEM[1]	*p*-value
	Fresh laid	4 ±0.5 °C	12 ±0.5 °C		
Dodecane, 12:0	0.02	0.01	0.01	0.002	0.729
Myristic, 14:0	0.26	0.30*	0.28*	0.006	0.031
Myristoleic, 14:1	0.07	0.06	0.06	0.002	0.729
Pentadecanoic, 15:0	0.06	0.05	0.06	0.002	0.729
Palmitic, 16:0	28.59	27.25*	27.25*	0.155	<0.001
Palmitoleic, 16:1	2.34	2.61*	2.47*,**	0.054	0.024
Heptadecanoic, 17:0	0.19	0.18	0.18	0.002	0.729
Cis-10-heptadecenoic, 17:1	0.05	0.03*	0.04	0.004	0.064
Stearic, 18:0	12.58	9.94*	10.15*	0427	<0.001
Oleic, 18:1n9c	34.92	37.79*	37.29*,**	0.465	<0.001
Linoleic, 18:2n6c	15.90	17.27*	16.99*,**	0.220	<0.001
Gamma-linolenic, 18:3n6	0.01	0.01	0.01	0.002	1.000
Linolenic, 18:3n3	0.45	0.22*	0.22*	0.040	<0.001
Arachic, 20:0	0.14	0.16	0.15	0.004	0.256
Cis-11-eicosenoic, 20:1	0.24	0.47*	0.45*,**	0.038	<0.001
Cis-11,14-eicosadienoic, 20:2n6	0.17	0.15*	0.14*	0.004	0.050
Eicosatetraenoic, 20:3n6	0.22	0.20*	0.20*	0.004	0.009
Arachidonic, 20:4n6	2.70	2.76	2.67**	0.029	0.944
5,8,11,14,17-eicosapentaenoic, 20:5n3	ND	ND	ND	ND	ND
Behenic, 22:0	0.06	0.04	0.04	0.003	0.047
4,7,10,13,16,19-docosahexaenoic, 22:6n3	1.04	0.94*	0.94*	0.018	0.003
Σ SFA	41.90	38.44*	38.53*	0.574	<0.001
Σ UFA	58.10	62.52*	61.09*,**	0.700	<0.001
Σ MUFA	37.61	40.96*	40.31*,**	0.541	0.011
Σ PUFA	20.49	21.56	21.16	0.178	<0.001
Σ ω3 PUFA	1.49	1.16*	1.15*	0.056	<0.001
Σ ω6 PUFA	19.00	20.39*	20.00*	0.222	<0.001
ω3/ω6 PUFA	12.76	17.57*	17.34*	0.789	<0.001

Note: * – $p < 0.05$ compared with freshly laid eggs, ** − $p < 0.05$ compared with data for storage of eggs at 4 ±0.5 °C using Tukey test with Bonferroni correction; SEM[1] − standard error of the mean (n = 9).

Among the polyunsaturated fatty acids, which belong to ω 3, there was a decrease ($p < 0.05$) in the content of linolenic and 4,7,10,13,16,19-docosahexaenoic in yolks compared to freshly laid eggs, and 5, 8,11,14,17-eicosapentaenoic acid was not detected at all in the yolks of freshly laid eggs, and different storage temperatures.

Storage of chicken eggs at temperatures of 4 ±0.5 °C and 12 ±0.5 °C for 30 days did not affect the ratio in the yolks of 6,9,12-octadecatrienoic and 5,8,11,14-eicosatetraenoic acids but contributed to the redistribution of other ω6 PUFA compared to freshly laid eggs. This was expressed in an increase ($p < 0.05$) in the proportion of linoleic

and a decrease (p <0.05) in the level of cis-11,14-eicosadienoic and cis-8,11,14-eicosatrienoic acids in the yolks compared to freshly laid eggs. Such changes in the storage process of edible eggs caused a decrease in FA SFA due to an increase (p <0.05) Σ UFA in the yolks compared to freshly laid eggs. Under these conditions, Σ UFA in chicken egg yolks was mainly increased (p <0.05) due to the proportion of monounsaturated fatty acids, while Σ PUFA remained stable at both storage temperatures of chicken eggs. Among polyunsaturated fatty acids in chicken egg yolks during storage decreased (p <0.05) Σ ω3 PUFA relative to Σ ω6 PUFA, which in turn increased (p <0.05) the ratio ω3/ω6 PUFA.

Table 3 The effect of lycopene at a dose of 20 mg/kg of feed on the fatty acid content in chicken egg yolks under different storage regimes (% of the total fatty acid content).

Acid	Egg storage mode			SEM[1]	*p*-value
	Freshly laid	4 ±0.5 ºC	12 ±0.5 ºC		
Lauric, 12:0	0.02	0.01	0.01	0.002	0.729
Myristic, 14:0	0.23	0.29*	0.28*	0.010	<0.001
Myristoleic, 14:1	0.04	0.06*	0.05*	0.004	0.050
Pentadecanoic, 15:0	0.07	0.05	0.06	0.003	0.171
Palmitic, 16:0	26.70	27.67*	27.43*	0.162	0.009
Palmitoleic, 16:1	2.06	2.28*	2.25*	0.037	<0.001
Heptadecanoic, 17:0	0.19	0.18	0.17*	0.005	<0. 001
Cis-10-heptadecenoic, 17:1	0.07	0.05*	0.05*	0.004	0.008
Stearic, 18:0	11.84	11.23*	11.23*	0.105	<0.001
Oleic, 18:1n9c	34.68	36.75*	37.01*	0.389	<0.001
Linoleic, 18:2n6c	16.26	15.63*	15.53*,**	0.123	<0.001
6,9,12-octadecatrienoic, 18:3n6	0.02	0.16*	0.15*	0.023	<0.001
Linolenic, 18:3n3	0.20	0.40*	0.39*	0.033	<0.001
Arachidic, 20:0	0.16	0.02*	0.02*	0.023	<0.001
Cis-11-eicosenoic, 20:1	0.45	0.39*	0.39*	0.010	<0.001
Cis-11,14-eicosadienoic, 20:2n6	0.17	0.07*	0.06*	0.017	<0.001
Eicosatetraenoic, 20:3n6	0.26	0.24	0.22	0.007	0.181
Arachidonic, 20:4n6	4.66	3.26*	3.53*	0.236	0.010
5,8,11,14,17-eicosapentaenoic, 20:5n3	1.86	1.21*	1.14*	0.115	<0.001
Behenic, 22:0	0.09	0.07*	0.06*	0.004	0.005
4,7,10,13,16,19-docosahexaenoic, 22:6n3	ND	ND	ND	ND	ND
Σ SFA	39.29	39.54	39.26	0.081	0.359
Σ UFA	60.71	60.49	60.78	0.075	0.272
Σ MUFA	37.29	39.53*	39.75*,**	0.410	0.045
Σ PUFA	23.42	20.96*	21.03*	0.415	0.127
Σ ω3 PUFA	2.06	1.61*	1.53*	0.083	<0.001
Σ ω6 PUFA	21.36	19.35*	19.50*	0.336	<0.001
ω3/ω6 PUFA	10.37	12.00*	12.72	0.356	<0.001

Note: * – p <0.05 compared with freshly laid eggs, ** − p <0.05 compared with data for storage of eggs in conditions of 4 ±0.5 °C using Tukey test with Bonferroni correction; SEM[1] – standard error of the mean (n = 9).

Feeding of laying hens with the addition of lycopene at a dose of 20 mg/kg of feed significantly affected the fatty acid composition of the yolks at different temperatures of egg storage. The content of saturated fatty acids, such as dodecanoic and pentadecanoic, did not change compared to freshly laid eggs' data (Table 3). The proportions of tetradecanoic and hexadecanoic acids increased (p <0.05) due to a decrease (p <0.05) in the particles of octadecanoic, eicosanoic, and behenic acids in the structure of yolk lipids compared to freshly laid eggs. As for the content of heptadecanoic acid, its level probably decreased (p <0.05) in chicken egg yolks only when stored at 12 ±0.5 °C for 30 days compared to freshly laid eggs. Storage of eggs obtained from laying hens treated with lycopene at a dose of 20 mg/kg of feed, caused almost the same redistribution of monounsaturated acids in the structure of yolk lipids in both modes of storage. It was found that the content of myristoleic, trans-3-hexadecenoic, and cis-9-octadecenoic acids increased (p <0.05), and the proportion of cis-10-heptadecenoic and cis-11-eicosenoic acids decreased (p <0.05) in the yolks compared to freshly laid eggs.

Among ω3 PUFA, which were detected in egg yolks with the use of lycopene laying hens at a dose of 20 mg/kg of feed, the content of 9,12,15-octadecanoic acid increased (p <0.05) against a background of decreasing (p <0.05) level 5, 8,11,14,17-eicosapentaenoic acid at both storage temperatures compared to freshly laid eggs. The peak of

4,7,10,13,16,19-docosahexaenoic acid was not detected on the chromatograms of the yolks of freshly laid eggs and during their storage at different temperatures. The addition of lycopene at a dose of 20 mg/kg of feed for laying hens did not affect the content of only cis-8,11,14-eicosatrienoic acid in egg yolks during storage, while the proportion of the remaining ω 6 PUFA was redistributed as follows: content 9, 12-octadecadienoic, cis-11,14-eicosadienoic and 5,8,11,14-eicosatetraenoic acids decreased (p <0.05), and the level of 6,9,12-octadecanoic acid increased (p <0.05) compared to freshly laid eggs. Increasing the dose of lycopene to 40 mg/kg of feed for laying hens did not affect the ratio of saturated acids such as dodecanoic and pentadecanoic. In contrast, the proportions of tetradecanoic, heptadecanoic, eicosanoic, and behenic acids increased (p <0.05), and the proportion of hexadecane p <0.05) in the yolks at both temperatures of egg storage. Among the monounsaturated fatty acids that are part of the structure of yolk lipids, there was a decrease (p <0.05) only the proportion of cis-11-eicosenoic acid. In contrast, the proportion of trans-3-hexadecenoic, cis-10-heptadecenoic, and cis-9-octadecenoic acids increased (p <0.05) compared with freshly laid eggs (Table 4).

Table 4 The effect of lycopene at a dose of 40 mg/kg of feed on the fatty acid content in chicken egg yolks for different storage modes (% of the total fatty acid content).

Acid	Egg storage mode			SEM[1]	*p*-value
	Freshly laid	4 ±0.5 ºC	12 ±0.5 ºC		
Lauric, 12:0	0.02	0.01	0.01	0.002	0.125
Myristic, 14:0	0.31	0.39*	0.38*	0.013	<0.001
Myristoleic, 14:1	0.03	0.02	0.03	0.002	0.178
Pentadecanoic, 15:0	0.02	0.01	0.02	0.002	0.178
Palmitic, 16:0	26.48	21.48*	21.30*	0.852	<0.001
Palmitoleic, 16:1	2.09	2.93*	2.93*	0.140	<0.001
Heptadecanoic, 17:0	0.15	0.20*	0.19*	0.008	<0.001
Cis-10-heptadecenoic, 17:1	0.01	0.07*	0.06*	0.009	<0.001
Stearic, 18:0	12.19	10.28*	10.13*	0.344	<0.001
Oleic, 18:1n9c	36.46	40.64*	40.24*	0.668	<0.001
Linoleic, 18:2n6c	17.29	19.67*	19.63*	0.396	<0.001
Gamma-linolenic, 18:3n6	ND	ND	ND	ND	-
Linolenic, 18:3n3	0.50	0.73*	0.71*	0.037	<0.001
Arachidica, 20:0	0.15	0.18*	0.18*	0.006	0.011
Cis-11-eicosenoic, 20:1	0.09	0.06*	0.05*	0.006	<0.001
Cis-11,14-eicosadienoic, 20:2n6	0.16	0.11*	0.10*	0.010	<0.001
Eicosatetraenoic, 20:3n6	ND	ND	ND	ND	-
Arachidonic, 20:4n6	2.20	2.50*	2.42*	0.051	0.001
5,8,11,14,17-eicosapentaenoic, 20:5n3	0.27	0.24*	0.24*	0.006	0.045
Behenic, 22:0	0.21	0.26*	0.25*	0.009	0.002
4,7,10,13,16,19-docosahexaenoic, 22:6n3	1.37	1.13	1.13	0.053	0.138
Σ SFA	39.53	32.81*	32.47*	1.159	<0.001
Σ UFA	60.47	68.08*	67.53*	1.230	<0.001
Σ MUFA	38.69	43.72*	43.31*	0.809	<0.001
Σ PUFA	21.79	24.36*	24.14*	0.424	<0.001
Σ ω3 PUFA	2.14	2.09	2.08	0.040	0.837
Σ ω6 PUFA	19.65	22.27*	22.15*	0.432	<0.001
ω3/ω6 PUFA	9.24	10.64	10.67	0.280	0.025

Note: * – p <0.05 compared with freshly laid eggs, ** − p <0.05 compared with data for storage of eggs in conditions of 4 ±0.5 ºC using Tukey test with Bonferroni correction; SEM[1] – standard error of the mean (n = 9).

Feeding laying hens with lycopene at a dose of 40 mg/kg of feed changed the ratio in the yolks of eggs of polyunsaturated fatty acids belonging to ω 3, in particular, increased (p <0.05) the proportion of Linolenic, but decreased (p <0.05) the content of 5,8,11,14,17-eicosapentaenoic acid compared to freshly laid eggs. At the same time, 6,9,12-octadecatrienoic acid disappeared from chicken egg yolks, and 4,7,10,13,16,19-docosahexaenoic acid appeared which did not depend on the temperature of their storage. Lycopene at a dose of 40 mg/kg in the diet of laying hens changed the ratio Σ SFA in favour (p <0.05) Σ UFA in lipids of egg yolks. This was due to both an increase (p <0.05) in the particles of Σ MUFA and Σ PUFA in the lipids of chicken egg yolks. It should be noted that the increase (p <0.05) in the level of Σ PUFA, in this case, was characterized by an increase (p <0.05) in the proportion of Σ ω6 PUFA in both storage regimes, but it did not affect the ratio ω3/ω6 PUFA in

chicken egg yolks. A further increase in the dose of lycopene to 60 mg/kg in the diet of laying hens contributed to an increase (p <0.05) in the content of most saturated fatty acids in yolks in both egg storage regimes, except for dodecanoic and heptadecanoic, the level of which did not change and tetradecanoic acid, decreased (p <0.05) compared with freshly laid eggs (Table 5). This dose of lycopene did not affect the level of such monounsaturated fatty acids as myristoleic and cis-10-heptadecenoic with a simultaneous decrease (p <0.05) in the proportion of trans-3-hexadecenoic and increase (p <0.05) cis-11-eicosenoic acids in yolks in both storage modes compared to freshly laid eggs. Among ω3 fatty acids in the yolks of eggs of laying hens fed lycopene at a dose of 60 mg/kg of feed, a decrease (p <0.05) in the proportion of 5,8,11,14,17-eicosapentaenoic and 4,7,10,13,16,19-docosahexaenoic on the background of increasing (p <0.05) share of linolenicoic acid in both storage modes compared to freshly laid eggs. Under such conditions, a redistribution of ω 6 fatty acid particles in chicken egg yolks was detected, which was characterized by the disappearance of 6,9,12-ocadecatrienic and cis-8,11,14-eicosatric acid peaks on the chromatograms and a decrease (p <0.05) in the 5.8 particles, 11,14-eicosatetraenoic with a simultaneous increase (p <0.05) in the level of Linoleic and cis-11,14-eicosadienoic acids in both storage modes compared to freshly laid eggs. The use of lycopene at a dose of 60 mg/kg of feed for laying hens thus contributed to an increase (p <0.05) Σ SFA relative to Σ UFA in egg yolks in both storage modes. The decrease (p <0.05) in the proportion of Σ UFA in chicken egg yolks under the influence of lycopene, in this case, occurred only due to Σ MUFA. The increase (p <0.05) in the proportion of U PUFA in yolks was observed in both storage regimes of chicken eggs, and it was due to an increase (p <0.05) in the content of Σ ω6 PUFA, which ultimately led to an increase (p <0.05) in the coefficient ω3/ω6 PUFA in the structure of lipids.

Table 5 The effect of lycopene at a dose of 60 mg/kg of feed on the content of fatty acids in chicken egg yolks under different storage regimes (% of the total fatty acid content).

Acid	Egg storage mode			SEM[1]	*p*-value
	Freshly laid	4 ±0.5 ºC	12 ±0.5 ºC		
Lauric, 12:0	0.02	0.01	0.01	0.001	0.078
Myristic, 14:0	0.33	0.23*	0.23*	0.017	<0.001
Myristoleic, 14:1	0.03	0.02	0.02	0.002	0.125
Pentadecanoic, 15:0	0.02	0.05*	0.04*	0.005	<0.001
Palmitic, 16:0	26.40	28.23*	28.25*	0.311	<0.001
Palmitoleic, 16:1	2.77	2.11*	2.11*	0.110	<0.001
Heptadecanoic, 17:0	0.11	0.13	0.13	0.004	0.078
Cis-10-heptadecenoic, 17:1	0.02	0.02	0.01	0.002	0.729
Stearic, 18:0	10.90	11.70*	11.70*	0.136	<0.001
Oleic, 18:1n9c	38.80	36.41*	36.48*	0.395	<0.001
Linoleic, 18:2n6c	12.82	14.36*	14.31*	0.255	<0.001
Gamma-linolenic, 18:3n6	ND	ND	ND	ND	-
Linolenic, 18:3n3	0.35	0.41*	0.41*	0.010	<0.001
Arachidic, 20:0	0.12	0.14*	0.14*	0.005	0.049
Cis-11-eicosenoic, 20:1	0.02	0.06*	0.06*	0.007	<0.001
Cis-11,14-eicosadienoic, 20:2n6	0.12	0.13*	0.13*	0.002	0.031
Eicosatetraenoic, 20:3n6	ND	ND	ND	ND	-
Arachidonic, 20:4n6	5.12	4.39*	4.39*	0.124	<0.001
5,8,11,14,17-eicosapentaenoic, 20:5n3	0.17	0.10*	0.09*	0.025	<0.001
Behenic, 22:0	0.16	0.20*	0.19*	0.007	<0.001
4,7,10,13,16,19-docosahexaenoic, 22:6n3	1.68	1.28*	1.25*	0.071	<0.001
Σ SFA	38.05	40.68*	40.69*	0.443	<0.001
Σ UFA	61.95	59.32*	59.31*	0.443	<0.001
Σ MUFA	41.63	38.64*	38.67*	0.498	<0.001
Σ PUFA	20.33	20.67*	20.63*	0.065	0.023
Σ ω3 PUFA	2.27	1.84*	1.81*	0.076	<0.001
Σ ω6 PUFA	18.05	18.88*	18.82*	0.139	<0.001
ω3/ω6 PUFA	7.95	10.28*	10.40*	0.405	<0.001

Note: * – p <0.05 compared with freshly laid eggs, ** − p <0.05 compared with data for storage of eggs in conditions of 4 ±0.5 ºC using Tukey test with Bonferroni correction; SEM[1] – standard error of the mean (n = 9).

Feeding laying hens with astaxanthin at a dose of 10 mg/kg of feed did not affect the content of saturated fatty acids such as dodecanoic and pentadecanoic, but reduced (p <0.05) the proportion of octadecanoic, eicosanoic, and behenic acids on the background of increasing (p <0.05) acid in the yolks in both storage modes compared to freshly laid eggs. As for heptadecanoic acid, its level increased (p <0.05) in chicken egg yolks only when stored at 4 ±0.5 °C compared to freshly laid eggs (Table 6). Astaxanthin supplementation in the diet of laying hens at the above dose increased (p <0.05) the proportion of all monounsaturated fatty acids, except for cis-10-heptadecenoic, the level of which was stable in the yolks in both egg storage regimes.

The use of astaxanthin at a dose of 10 mg/kg of feed for laying hens contributed to changes in the ratio of individual ω3 PUFA, namely an increase (p <0.05) in the proportion of Linolenicoic acid. In contrast, the level of 5,8,11,14, 17-eicosapentaenoic and 4,7,10,13,16,19-docosahexaenoic acids decreased (p <0.05) in yolks under both storage regimes compared to freshly laid eggs. An even greater effect of astaxanthin at the above dose was found on the level of ω 6 PUFA, which was characterized by the disappearance on the chromatogram of the peak of 6,9,12-octadecatrienoic acid with a simultaneous decrease (p <0.05) in the proportion of cis-11,14-eicosadienoic, cis-8, 11,14-eicosatrienoic, and 5,8,11,14-eicosatetraenoic acids in the yolks under both storage modes compared to freshly laid eggs. Thus, astaxanthin at a dose of 10 mg/kg of feed for laying hens contributed to a decrease (p <0.05) in the proportion of Σ SFA in favour of Σ UFA. This was due to an increase in the proportion of Σ MUFA in the lipid structure of chicken egg yolks. Despite the low content of astaxanthin in the diet of laying hens, it also contributed to a decrease (p <0.05) in the proportion of Σ PUFA, which is due to a decrease (p <0.05) in both part ω3 PUFA and Σ ω6 PUFA in chicken egg yolks during storage in both temperature regimes. As a result of this redistribution of unsaturated fatty acids in chicken egg yolks under the influence of astaxanthin increased (p <0.05) the coefficient ω3/ω6 PUFA compared to freshly laid eggs.

Table 6 The effect of astaxanthin at a dose of 10 mg/kg of feed on the content of fatty acids in chicken egg yolks under different storage regimes (% of the total fatty acid content).

Acid	Egg storage mode			SEM[1]	p-value
	Freshly laid	4 ±0.5 ºC	12 ±0.5 ºC		
Lauric, 12:0	0.02	0.03	0.02	0.002	0.179
Myristic, 14:0	0.23	0.29*	0.29*	0.010	<0.001
Myristoleic, 14:1	0.03	0.06*	0.06*	0.005	<0.001
Pentadecanoic, 15:0	0.07	0.07	0.06	0.002	0.729
Palmitic, 16:0	27.00	27.50*	27.53*	0.089	<0.001
Palmitoleic, 16:1	2.03	2.13*	2.13*	0.017	<0.001
Heptadecanoic, 17:0	0.20	0.22*	0.21	0.004	<0.001
Cis-10-heptadecenoic, 17:1	0.06	0.06	0.06	0.002	1.000
Stearic, 18:0	12.88	10.64*	10.61*	0.380	<0.001
Oleic, 18:1n9c	32.57	35.40*	35.39*	0.471	<0.001
Linoleic, 18:2n6c	16.67	17.96*	17.98*	0.219	<0.001
Gamma-linolenic, 18:3n6	ND	ND	ND	ND	-
Linolenic, 18:3n3	0.47	0.49*	0.49*	0.005	0.011
Arachidic, 20:0	0.05	0.01*	0.01*	0.006	<0.001
Cis-11-eicosenoic, 20:1	0.42	0.50*	0.50*	0.014	<0.001
Cis-11,14-eicosadienoic, 20:2n6	0.26	0.15*	0.15*	0.019	<0.001
Eicosatetraenoic, 20:3n6	0.29	0.27*	0.27*	0.004	0.079
Arachidonic, 20:4n6	4.79	2.84*	2.84*	0.325	<0.001
5,8,11,14,17-eicosapentaenoic, 20:5n3	0.21	0.18*	0.18*	0.006	<0.001
Behenic, 22:0	0.09	0.06*	0.06*	0.005	<0.001
4,7,10,13,16,19-docosahexaenoic, 22:6n3	1.69	1.17*	1.17*	0.086	<0.001
Σ SFA	40.52	38.81*	38.79*	0.289	<0.001
Σ UFA	59.48	61.19*	61.21*	0.289	<0.001
Σ MUFA	35.11	38.14*	38.13*	0.505	<0.001
Σ PUFA	24.37	23.22*	23.08*	0.215	<0.001
Σ ω3 PUFA	2.36	1.84*	1.84*	0.087	<0.001
Σ ω6 PUFA	22.01	21.21*	21.23*	0.139	0.002
ω3/ω6 PUFA	9.31	11.53*	11.52*	0.370	<0.001

Note: * – p <0.05 compared with freshly laid eggs, ** − p <0.05 compared with data for storage of eggs in conditions of 4 ±0.5 °C using Tukey test with Bonferroni correction; SEM[1] – standard error of the mean (n = 9).

Increasing the dose of astaxanthin in the diet of laying hens to 20 mg/kg did not affect the ratio in the yolks of saturated fatty acids such as dodecanoic and pentadecanoic. However, it increased (p <0.05) the proportion of hexadecanoic, heptadecanoic, and eicosanoic, and behenic acids in the background 0.05) particles of tetradecanoic and octadecanoic acids in both modes of storage of eggs compared with freshly laid (Table 7). Enrichment of the diet of laying hens with astaxanthin in the specified dose did not affect the content of such monounsaturated fatty acids as cis-9-myristic and cis-10-heptadecenoic but increased (p <0.05) the level of palmitoleic, oleic, and cis-11-nicotine in both modes of storage of eggs compared with freshly laid. The content of individual ω3 PUFA in egg yolks was affected by astaxanthin at a dose of 20 mg/kg, depending on the storage temperature. Thus, the content of Linolenicoic acid in the yolks increased (p <0.05) at a temperature of 4 ±0.5 °C, but at a temperature of 12 ± 0.5 °C, it decreased (p <0.05) compared to freshly laid eggs. The proportion of 5,8,11,14,17-eicosapentaenoic acid in chicken egg yolks under the influence of astaxanthin in the above dose decreased (p <0.05), while the level of 4,7,10,13,16,19-docosahexaenoic acid the additive was not affected. Even more, pronounced changes in the composition of ω6 PUFA were found in chicken egg yolks during storage at different temperatures under the influence of astaxanthin. They were characterized by the disappearance on the chromatogram of the peaks of 6,9,12-octadecatrienoic and cis-8,11,14-eicosatrienoic acids in the yolks of both freshly laid eggs and their storage at different temperatures. At the same time, against the background of a decrease (p <0.05) in the content of linoleic increase (p <0.05), the level of cis-11,14-eicosadienoic fatty acids in egg yolks in both modes of their storage compared to freshly laid. In this case, the ratio of Σ SFA and Σ UFA remained stable in the yolks of chicken eggs in both modes of storage. However, the use of astaxanthin in laying hens at a dose of 20 mg/kg of feed caused a redistribution in the structure of the UFA particles Σ PUFA and Σ MUFA in favour (p <0.05) of the latter. The proportion of Σ PUFA decreased (p <0.05) due to a decrease (p <0.05) in the level of Σ ω 6 PUFA in the lipid structure of chicken egg yolks, although the ratio ω3 / ω6 PUFA remained unchanged.

Table 7 The effect of astaxanthin at a dose of 20 mg/kg of feed on the content of fatty acids in chicken egg yolks under different storage regimes (% of the total fatty acid content).

Acid	Egg storage mode			SEM[1]	p-value
	Freshly laid	4 ±0.5 °C	12 ±0.5 °C		
Lauric, 12:0	**0.02**	**0.02**	0.02	0.003	0.070
Myristic, 14:0	0.30	0.27*	0.27*	0.007	<0.001
Myristoleic, 14:1	0.03	0.05	0.05	0.003	0.072
Pentadecanoic, 15:0	0.03	0.02	0.02	0.002	0.125
Palmitic, 16:0	26.23	28.37*	28.14*	0.357	<0.001
Palmitoleic, 16:1	2.11	2.60*	2.59*	0.084	0.000
Heptadecanoic, 17:0	0.15	0.18*	0.18*	0.006	<0.001
Cis-10-heptadecenoic, 17:1	0.02	0.02	0.02	0.001	0.629
Stearic, 18:0	12.23	10.12*	10.21*,**	0.347	<0.001
Oleic, 18:1n9c	36.80	38.01*	38.21*	0.265	0.043
Linoleic, 18:2n6c	17.28	15.32*	15.49*	0.334	<0.001
Gamma-linolenic, 18:3n6	ND	ND	ND	ND	-
Linolenic, 18:3n3	0.51	0.53*	0.50*,**	0.006	0.005
Arachidic, 20:0	0.15	0.21*	0.20*	0.009	<0.001
Cis-11-eicosenoic, 20:1	0.09	0.21*	0.19*	0.019	<0.001
Cis-11,14-eicosadienoic, 20:2n6	0.16	0.19*	0.18*,**	0.005	<0.001
Eicosatetraenoic, 20:3n6	ND	ND	ND	ND	-
Arachidonic, 20:4n6	2.21	2.26*	2.21**	0.012	0.002
5,8,11,14,17-eicosapentaenoic, 20:5n3	0.27	0.22*	0.20*,**	0.011	<0.001
Behenic, 22:0	0.21	0.25*	0.22**	0.007	0.007
4,7,10,13,16,19-docosahexaenoic, 22:6n3	1.21	1.20	1.11	0.042	0.200
Σ SFA	39.32	39.42	39.25	0.098	0.834
Σ UFA	60.68	60.58	60.75	0.098	0.834
Σ MUFA	39.05	40.88*	41.06*	0.352	0.004
Σ PUFA	21.63	19.70*	19.68*	0348	0.003
Σ ω3 PUFA	1.99	1.94	1.81	0.049	0.347
Σ ω6 PUFA	19.64	17.76*	17.88*	0.326	0.002
ω3/ω6 PUFA	9.95	9.19	9.90	0.235	0.389

Note: * – p <0.05 compared with freshly laid eggs, ** − p <0.05 compared with data for storage of eggs in conditions of 4 ±0.5 ºC using Tukey test with Bonferroni correction; SEM[1] – standard error of the mean (n = 9).

Feeding laying hens with astaxanthin at a dose of 30 mg/kg of feed had an even stronger effect on the fatty acid profile of yolk lipids, both freshly laid and at different egg storage regimes compared with doses of 10 and 20 mg/kg (Table 8). It was found that this increase in astaxanthin content in the diet of laying hens caused the disappearance on the chromatogram of the peaks of two saturated fatty acids: dodecanoic and tetradecanoic, and reduced (p <0.05) the proportion of hexadecanoic and octadecanoic acids but did not affect the content of pentadic acids in the yolks under different storage modes of eggs compared to freshly laid. Among the monounsaturated acids of chicken egg yolks was also detected under the influence of astaxanthin disappearance on the chromatogram of the peak of myristoleic acid and decrease (p <0.05) the level of cis-11-eicosenoic acid on the background of increasing (p <0.05) content of palmitoleic, cis-10-heptadecenoic and cis-9-octadecenoic acids in both modes of storage of eggs compared with freshly laid. Changes in the level of ω3 PUFA in egg yolks of laying hens treated with astaxanthin at a dose of 30 mg/kg were characterized only by a decrease (p <0.05) in the proportion of 5,8,11,14,17-eicosapentaenoic acid, while the content of other acids changed significantly did not give in.

Table 8 The effect of astaxanthin at a dose of 30 mg/kg of feed on the fatty acid content in chicken egg yolks for different storage modes (% of the total fatty acid content).

Acid	Egg storage mode			SEM[1]	p-value
	Freshly laid	4 ±0.5 ºC	12 ± 0.5 ºC		
Lauric, 12:0	**0.01**	ND	ND	0.004	-
Myristic, 14:0	0.30	ND	ND	0.051	-
Myristoleic, 14:1	ND	ND	ND	ND	-
Pentadecanoic, 15:0	0.03	0.02	0.01	0.003	0.068
Palmitic, 16:0	26.49	24.31*	24.31*	0.365	<0.001
Palmitoleic, 16:1	2.09	3.28*	3.28*	0.200	<0.001
Heptadecanoic, 17:0	0.15	0.16	0.16	0.004	0.369
Cis-10-heptadecenoic, 17:1	0.02	0.05*	0.06*	0.007	<0.001
Stearic, 18:0	12.19	8.57*	8.57*	0.613	<0.001
Oleic, 18:1n9c	36.47	42.19*	42.07*	0.946	<0.001
Linoleic, 18:2n6c	17.29	15.49*	15.75*	0.312	0.013
Gamma-linolenic, 18:3n6	ND	ND	ND	ND	-
Linolenic, 18:3n3	0.51	0.47	0.46	0.008	<0.001
Arachidic, 20:0	0.15	0.17	0.16	0.003	0.086
Cis-11-eicosenoic, 20:1	0.08	0.02*	0.02*,**	0.010	<0.001
Cis-11,14-eicosadienoic, 20:2n6	0.16	0.17	0.16	0.002	0.178
Eicosatetraenoic, 20:3n6	ND	ND	ND	ND	-
Arachidonic, 20:4n6	2.21	3.17*	3.09*	0.155	<0.001
5,8,11,14,17-eicosapentaenoic, 20:5n3	0.27	0.21*	0.20*	0.054	<0.001
Behenic, 22:0	0.21	0.22	0.21	0.003	0.236
4,7,10,13,16,19-docosahexaenoic, 22:6n3	1.35	1.51	1.50	0.039	0.400
Σ SFA	39.53	33.44*	33.42*	1.025	<0.001
Σ UFA	60.47	66.56*	66.58*	1.025	<0.001
Σ MUFA	38.69	45.55*	45.42*	1.135	0.001
Σ PUFA	21.78	21.02	21.16	0.195	0.258
Σ ω3 PUFA	2.13	2.19	2.16	0.051	0.907
Σ ω6 PUFA	19.65	18.83	19.00	0.187	0.163
ω3/ω6 PUFA	9.35	8.60	8.82	0.243	0.491

Note: * – p <0.05 compared with freshly laid eggs, ** − p <0.05 compared with data for storage of eggs in conditions of 4 ±0.5 ºC using Tukey test with Bonferroni correction; SEM[1] – standard error of the mean (n = 9).

Astaxanthin at a dose of 30 mg/kg of feed had a more pronounced effect on the level of ω6 PUFA in egg yolks, which was characterized by the disappearance on the chromatogram of the peaks of 6,9,12-ocadecatrienic and cis-8,11,14-eicosatrienoic acids, both fresh and eggs that were stored at both temperatures. At the same time, a decrease (p <0.05) in the proportion of linoleic acid and an increase (p <0.05) in the proportion of 5,8,11,14-eicosatetraenoic acids in chicken egg yolks were observed under both storage regimes compared to freshly laid eggs. Such changes in the ratio of individual fatty acids in chicken egg yolks, in turn, caused a decrease (p <0.05)

in the proportion of Σ UFA in favour of Σ SFA. The reason for such changes in balance was an increase (p <0.05) in the proportion of U MUFA in the lipid structure of chicken egg yolks in both storage regimes compared to freshly laid eggs. It should also be noted that the feeding of laying hens astaxanthin at a dose of 30 mg/kg of feed did not affect the balance of Σ PUFA, in particular, Σ ω3 PUFA and Σ ω6 PUFA in the yolks at different egg storage regimes.

Our research results show that the storage temperatures of chicken eggs 4 ±0.5 °C and 12 ±0.5 °C, do not differ significantly in terms of the effect on the ratio of fatty acids in the yolks. Similar results were obtained in a study by **[13]**, who did not show a significant difference in the ratio of α-linolenic acid, arachidonic and docosahexaenoic acids in the yolks for egg storage from 4 °C to 20 °C for 6 weeks.

It is known that during the storage of edible eggs in the yolks, there are changes characteristic of the oxidation of lipids and certain fatty acids and, consequently, a decrease in their absolute content **[29]**. Lipid oxidation is a process that affects the stability of egg yolk during storage. This can change the quality of table eggs and lead to a deterioration in taste, aroma, and color and the formation of toxic substances in eggs **[37], [43]**. One of the factors that characterize the intensity of lipid oxidation is the level of malonic dialdehyde in egg yolks **[34]**. The concentration of malonic dialdehyde in egg yolks increased after storage at 4 °C for 60 and 90 days. Enrichment of chicken egg yolks ω 3 and ω6 with fatty acids also stimulated the formation of malonic dialdehyde when stored at 4 °C for 30 and 60 days **[37]**, which also indicates the process of lipid oxidation in yolks. The same fact was confirmed in studies by **[8]**, **[10]**, who reported a decrease in the total content of ω 3 fatty acids in the yolks of eggs of laying hens fed fish oil or olive leaves, after 60 days of storage at 4 °C.

This destruction of fatty acids in the yolks in our experiment occurred during the storage of eggs for 30 days at 4 ±0.5 °C and 12 ±0.5 °C. It contributed to a decrease in the proportion of FA SFA mainly due to the two main saturated fatty acids − hexadecanoic and octadecane. Nevertheless, the proportion of Σ UFA in egg yolks increased mainly due to Σ MUFA, which to some extent normalized the ratio of Σ SFA to Σ MUFA, which became desirable for food lipids and reached the limit of 1:1 **[29]**. As can be seen from the table. 2, among Σ UFA during storage of table eggs, there is a destruction of ω3 PUFA, while the share of ω6 PUFA against this background increases slightly, which increases the shift ω3/ω6 PUFA by almost 5 units in both modes of egg storage. However, 5,8,11,14,17-eicosapentaenoic acid in the yolks of freshly laid eggs of laying hens of the control group was not detected, which is probably due to the low intensity of its de novo synthesis from precursors in the body, as well as its oxidation.

Prevention of auto-oxidation of fatty acids in chicken egg yolks can be achieved by adding antioxidants to chicken feed, such as vitamin E **[7]**, sources of natural flavonoids **[19]**, and carotenoids **[21]**.

In our experiment, feeding laying hens with lycopene supplements significantly affected egg yolks' fatty acid profile during both storage regimes. At the same time, most of the fatty acids that were part of the structure of egg yolk lipids changed. Given the fact that all fatty acids of chicken egg yolk, which were stored at different temperatures, could be exposed only to exogenous influences, we can only talk about changing their ratio from the standpoint of resistance to oxidation at different doses of lycopene in the diet of laying hens, and hence in the eggs themselves **[39]**. Thus, feeding laying hens a supplement of lycopene at a dose of 20 mg/kg of feed helped to improve the preservation in egg yolks of one of the main saturated acids − hexadecanoic, but this did not apply to octadecanoic acid, the proportion of which did not affect the balance Σ SFA under their storage. The proportion of Σ MUFA increased, and Σ PUFA decreased in egg yolks due to Σ ω3 PUFA and Σ ω6 PUFA.

The use of lycopene at a dose of 40 mg/kg of feed for laying hens prevented the oxidation of the main saturated fatty acids of egg yolks, as well as individual ω3 PUFA and ω6 PUFA, but at a dose of 60 mg/kg ultimately contributed to a shift in the ratio of ω3 PUFA/ω6 PUFA in favour of the latter. With increasing lycopene dose to 40 and 60 mg/kg of feed, changes in the profile of fatty acids in chicken egg yolks during both storage regimes intensified, which was expressed in the disappearance of peaks of two ω6 PUFA − Gamma-linolenic and cis-8,11,14-eicosatriene, which are not the main, while the proportion of Linoleic, which is the main ω6 PUFA in the yolks, increased. This may be due to both the effect of lycopene on the activity of the synthesis of these fatty acids in the body **[4]** and the antioxidant protection process against their destruction during storage **[5]**.

As shown in the **[31]**, the inclusion of antioxidants in the diet of laying hens, including sources of lycopene (dried tomatoes, red peppers) may affect the oxidative stability of egg yolk lipids during storage. The diet with flaxseed (4.5%) in combination with sweet red pepper and a mixture of tomatoes (2%) caused higher oxidative stability of lipids than the diet with flaxseed. Stating eggs reduced the yolks' antioxidant activity but did not reduce the stability of lipids to oxidation. In another study **[1]** it was shown that the concentrations of lycopene, β-carotene, lutein, and vitamin A in serum and egg yolk increased against the background of reduced concentrations of malonic dialdehyde when used in the diet of laying hens tomato powder at a dose of 5 and 10 g/kg of feed.

In general, as can be seen from the data obtained in our experiment, a clear pattern of the effect of lycopene dose on the fatty acid profile of chicken egg yolks during storage is not observed. Still, changes in the ratio of fatty acids in both saturated and unsaturated series almost did not differ at both temperatures.

The use of astaxanthin in the diet of laying hens is due to its ability to color the yolks and its antioxidant properties. It is known that the mechanism of action of astaxanthin is its ability to absorb free radicals by autooxidation, followed by degradation of this carotenoid **[17], [26]**. In addition, astaxanthin *H. Pluvialis* increases the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and total antioxidant capacity against the background of reduced levels of malonic dialdehyde in blood plasma, liver of laying hens, and egg yolk **[18]**.

The use in our experiment in feeding laying hens astaxanthin *H. Pluvialis* dose-dependently affected the fatty acid profile in the lipids of egg yolks, both freshly laid and stored at different temperatures (Tables 5 − 7). Among the saturated fatty acids of the yolks during egg storage, there was a redistribution as follows: the proportion of hexadecanoic acid increased in the structure of egg yolk lipids only until the dose of astaxanthin 20 mg/kg of feed, and at 30 mg/kg its value probably decreased, while the share of the second of the main saturated acids of egg yolks − octadecane was reduced for all doses of astaxanthin in the diet of laying hens. During the same period, the main monounsaturated acid of chicken egg yolks oleic showed an increase in the proportion in the structure of lipids. In egg yolks during storage, the addition of astaxanthin at a dose of 10 mg/kg in the diet of laying hens increased the proportion of basic ω6 PUFA linoleic. In contrast, in higher doses, there was a decrease in its share in the structure of yolk lipids. In addition, a dose of astaxanthin 10 mg/kg caused the disappearance of the peak on the yolk chromatogram of only one ω6 PUFA gamma-linolenic. In comparison, a dose of astaxanthin 20 mg/kg in the diet of laying hens also disappeared eicosatetraenoic. A dose of 30 mg/kg of diet caused the disappearance of three more short-chain acids, dodecanoic and tetradecanoic, and myristoleic during egg storage. These changes in the fatty acid composition of yolks in both egg storage regimes may be due to lipolysis and lipid oxidation **[43]**. During egg storage, lipids can be hydrolyzed by endogenous enzymes in the yolk with the release of free fatty acids, and the process of lipid peroxidation can promote the formation and accumulation of hydroperoxides and secondary oxidation products.

Our data on the fluctuations of Σ MUFA in yolks during egg storage using lycopene or astaxanthin supplements in the diet of laying hens are consistent with the data of **[35]** on the distribution of fatty acids in yolks enriched with ω3 PUFA when stored under 4 °C.

The disappearance and redistribution of several saturated and unsaturated fatty acids in the yolks of freshly laid chicken eggs in our experiment may indicate the effect of different doses of astaxanthin on desaturase Δ6 activity in chicken tissues, as well as a complex mechanism of competition for the same enzyme essential polyunsaturated fats carbon chain extensions **[12], [27]**. In turn, the disappearance of certain saturated fatty acids in the yolks under the influence of different doses of astaxanthin during the storage of chicken eggs requires further research. It is difficult to compare our research results with other scientists' data because there is very little such information in the literature **[23]**. Most researchers have used sources of various oils to modify the fatty acid composition of egg yolk lipids **[9], [30], [42]**.

As can be seen from our research results, storage of edible chicken eggs enriched with lycopene or astaxanthin **[39]**, at both temperatures, helps to achieve a ratio of Σ SFA to Σ MUFA within 1:1, which corresponds to physiological parameters for humans **[29]**.

Our research results show that the storage of edible eggs obtained from laying hens that were not fed carotenoid supplements helps to increase the ratio of ω3/ω6 PUFA from 1:12.76 to 1:17.57, 17.34 depending on the temperature, which is not desirable for consumers' health. At the same time, the feeding of laying hens to the addition of lycopene at a dose of 60 mg/kg of feed can provide this ratio in freshly laid eggs at 1:7.95, and during the storage period − 1:10.28, 1:10.40 (Tables 2 − 4). Even better in this regard is the addition of astaxanthin in the diet of laying hens: starting with a dose of 20 mg/kg of feed, you can achieve a ratio of ω3/ω6 PUFA in the yolks of freshly laid eggs at 1:9.95 y, and at a dose of 30 mg/kg, respectively 1:9.35. It is important to maintain this ratio for both modes of egg storage (Tables 5 – 7). The researchers agree that the optimal ratio of ω3/ω6 PUFA should not exceed 1:2 − 1:4. This is because a nutritional imbalance in polyunsaturated fatty acids (excess ω6 and deficiency ω3) is a major cause of many chronic diseases, including cardiovascular disease, cancer, inflammatory diseases, autoimmune diseases, and many physiological disorders in humans **[6]**.

CONCLUSION

Temperature storage regimes of 4 ±0.5 °C and 12 ±0.5 °C eggs equally affect the fatty acid composition of egg yolks obtained from hens fed supplements of lycopene at doses of 30, 40, and 60 mg/kg or astaxanthin at doses of 10, 20, and 30 mg/kg of compound feed for 30 days, compared to freshly laid eggs. An increasing dose of lycopene from 20 to 60 mg/kg or astaxanthin from 10 to 30 mg/kg in the diet of laying hens in the yolks of freshly laid eggs, as well as at both temperatures of their storage decreases the proportion of ω6 PUFA: cis-8,11, 14-eicosatrienoic, and 6,9,12-ocadecatrienic acids until their complete disappearance. The addition of astaxanthin to the diet of laying hens is characterized by a much stronger effect on the ratio of saturated and unsaturated fatty acids in chicken egg yolks during storage than the addition of lycopene. Feeding astaxanthin to laying hens reduces and stabilizes the ω3/ω6 PUFA ratio in yolks during egg storage to a greater extent than adding lycopene to the diet of laying hens. The obtained research results can be the basis for the choice of storage regime of carotenoid-enriched edible chicken eggs, taking into account the correction of the fatty acid profile of yolk lipids.

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Characteristics of protein, lipid, and carbohydrate metabolism of fish of the Kremenchuk Reservoir in the prespawning period

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ABSTRACT

The paper presents the results of scientific research aimed at studying the peculiarities of metabolism in the body of seven species of mature fish in the Kremenchuk reservoir in the pre-spawning period under ecological conditions that differ from existing ones according to the Dnipro Reservoir Rules of Operation. Somewhat increased levels of total protein accumulation were found during this period in the muscles of zander, perch, and gibel carp. More statistically significant differences between the content of total protein in the liver and muscles were found in other fish species, in particular in roach it was 51.2%, in bream – 57.8%, in European flounder, and zope – 40.6%. Slightly elevated total lipids were found in the muscles of these fish. Thus, in the muscles of silver bream, it was 12.07 mg/g of raw weight, and in the muscles of gibel carp – 18.5 mg/g, while in the muscles of all other studied species of fish, this figure was in the range of 6.7 to 8.71 mg/g of raw weight. The glycogen content in the muscles of different objects of the Kremenchuk reservoir in the pre-spawning period was different. Its highest content was found in the gibel carp muscle, which reached 74 mg/g of raw weight. Significantly lower (2.7 times) was the level of glycogen accumulation in zander muscles and 3.2 times – in roach muscles. In the muscles of bream, European perch and silver bream found close, relatively low levels of glycogen, which was in the range of 10 – 13 mg/g of raw mass, and the lowest level of its accumulation was recorded in the muscles of the zope (only 4.9 mg/g). The glycogen content of the liver of all studied fish species significantly exceeded that recorded in their muscles.

Keywords: reservoir, fish, liver, white skeletal muscle, proteins, lipids, glycogen

INTRODUCTION

After wintering under certain temperature conditions and the presence of forage organisms for these fish species, the body's energy potential is gradually restored and prepared for one of the most important periods-spawning. This preparation is carried out in the so-called pre-spawning period, which is characterized by specific conditions of metabolism and the functional state of the organism. It is known that in this period, there is a differentiation and then trophoplasmic growth of oocytes and spermatogonia **[1]**, **[42]**.

In most fish species, the pre-spawning period is divided into two subperiods. The first subperiod is mainly associated with the differentiation of sexual products and is characterized as the protoplasmic growth of oocytes (period of low growth). The second subperiod (period of high growth) is mainly associated with the trophoplasmic growth of oocytes. According to these subperiods, adult fish's physiological and biochemical characteristics change **[2]**, **[41]**. During the first subperiod, there is the most significant decrease in lipids in the body of fish, and during the second subperiod-intensification of protein growth **[3]**, **[43]**.

During the pre-spawning period, the fish liver activates the processes of protein synthesis associated with the differentiation and growth of generative tissue. Plastic material for forming sexual products of fish is food components, as well as reserves that are concentrated in the body due to feeding in the previous year **[4]**, **[5]**.

In fish that feed before spawning, partially restored energy resources ensure the maturation of sexual products and the spawning process itself **[6]**.

However, it should be noted that the effectiveness of the pre-spawning period for the maturation of sexual products and preparation of the body for spawning is largely determined by the level and temperature of the reservoir.

It is known that the role of the Kremenchuk Reservoir is related to the need to maintain different water levels throughout the year. Thus, in particular, according to the Rules of Operation of the Dnipro Reservoirs, the capacity of this reservoir before floods is reduced by lowering the water level by 3.0 – 5.0 m. That is, only during this period, the maximum area of flooding and the useful volume of the reservoir is observed. However, the pre-spawning period of many fish species occurs somewhat earlier **[7]**.

Meanwhile, the analysis of the decadal dynamics of the level regime of the Kremenchuk reservoir shows that in 2020 there was No. spring operation, as there were not enough flood waters, we recorded sufficient water filling in March-May, which did not reach the level of NSH only by 20 – 70 cm. A similar monthly water regime of the Kremenchuk Reservoir was also registered from 2017 to 2019 only with insignificant minimum and maximum values (±10 – 20 cm), which did not harm the ecological situation of the reservoir and the development of biota in it. The established level regime in the reservoir during this period was maintained at a sufficiently high level, recommended by the Interdepartmental Commission because of the low floods in the Dnipro due to global warming.

The established level and temperature regime of water can significantly impact the development of biota and, accordingly, the processes of preparation of the organism, especially mature fish, for spawning.

Despite the urgency of this problem, in the literature, we have not found relevant information concerning the study of metabolic processes in the body of mature individuals of different species of fish of the Kremenchuk reservoir in the pre-spawning period under the existing hydrological regime and complex effects of natural and anthropogenic factors.

With this in mind, our research aimed to assess the physiological status of mature fish of the Kremenchuk Reservoir with different types of nutrition in terms of metabolism in the pre-spawning period under existing environmental conditions recommended by the Interdepartmental Commission and under the influence of anthropogenic factors.

Scientific Hypothesis

We studied how changes in the content of glycogen, proteins, and lipids occur in the body of freshwater fish of the Kremenchuk Reservoir. The difference in indicators shows a change in the intensity and direction of their metabolic processes.

MATERIAL AND METHODOLOGY

Research fishing was carried out in the spring of 2021 in the pre-spawning period during monitoring studies of the middle part of the Kremenchuk Reservoir, one of the six largest reservoirs in the cascade on the Dnipro River in Poltava, Kirovohrad, and Cherkasy regions of Ukraine. Located between Kaniv and Kamyanka reservoirs and is formed by the dam of Kremenchuk HPP.

Samples

White skeletal muscles and liver of mature bream (*Abramis brama*), roach (*Rutilus rutilus*), white bream (*Blicca bjoerkna*), zander (*Sander lucioperca*), European perch (*Perca fluviatil*e), zope (*Ballerus ballerus*) and gibel carp (*Carassius gibelio*) were selected as biological material for in-house processing. The collected material determined the total content of proteins, lipids, and glycogen (Figures 1, 2, 3, 4, 5, 6, 7).

Figure 1 Bream (*Abramis brama*).

Figure 2 White bream (*Blicca bjoerkna*).

Figure 3 Gibel carp (*Carassius gibelio*).

Figure 4 Zope (*Ballerus ballerus*).

Figure 5 European perch (*Perca fluviatilus*).

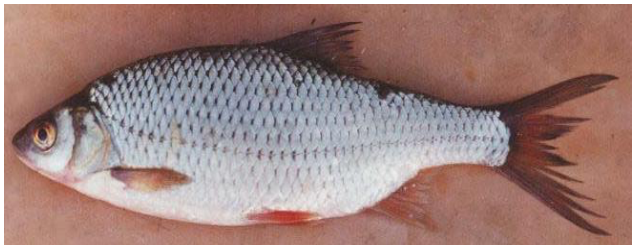

Figure 6 Roach (*Rutilus rutilus*).

Figure 7 Zander (*Sander lucioperca*).

Chemicals

Potassium hydroxide (KOH), anthrone ($C_{14}H_{10}O$), concentrated sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), potassium sodium tartaric acid ($KNaC_4H4O_6 \times 4H_2O$), copper sulfate ($CuSO_4$), Folin–Ciocalteu reagent (FC), vanillin reagent (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Animals and Biological Material

In total, 35 specimens were processed – mature bream (*Abramis brama*), roach (*Rutilus rutilus*), white bream (*Blicca bjoerkna*), zander (*Sander lucioperca*), European perch (*Perca fluviatil*e), zope (*Ballerus ballerus*) and gibel carp (*Carassius gibelio*) caught from the Kremenchuk reservoir.

Instruments

Set of grids with a mesh step from 30 to 100 mm (producer «CrayFish» Limited Liability Company, Finland).

Electronic laboratory scales (TBE-0.15-0.001-a-2, producer «Inter-Synthesis» Limited Liability Company, Ukraine).

Spectrophotometer Unico 280 UV/VIS (producer: ALTALAB Limited Liability Company, Ukraine).

Laboratory Methods

The content of total proteins in tissue samples was determined by the method of Lowry et al. (1951) **[8]**, and the content of total lipids was determined using a phosphorovaniline reagent **[9]**. The anthrone method determined glycogen content in fish tissues **[10]**.

Description of the Experiment

Sample preparation: When determining the chemical composition in the organs and tissues of fish from the reservoir were selected 5 specimens of fish of each species.

Number of samples analyzed: 70 samples of tissues and organs (liver, white muscles) were taken from the fish caught from the reservoir to determine the number of proteins, lipids, and carbohydrates.

Number of repeated analyzes: The research in the Kremenchuk Reservoir was executed one time.

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

Design of the experiment: The content of total proteins in tissue samples was determined by the method of Lowry et al (1951) **[8]**. Briefly, 0.1 g of tissue and organ was hydrolyzed for 1 hour in 10 mL of 10% NaOH at a temperature of 60 °C. To 0.1 mL of the hydrolysate was added 10 mL of solution No. 3, and staining was carried out for 15 minutes. Then, the sample added 1.0 mL of Folin's reagent diluted 1:1 with distilled water. The staining was carried out for 30 minutes. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 720 nm against control. The amount of protein was set according to the calibration schedule. Solution No. 3 was prepared from solutions No. 1 and No. 2 in a ratio of 9:1. Solution No. 1 was prepared based on 0.1 n NaOH with the addition of 20 g Na_2CO_3 and 0.5 g of potassium and sodium tartaric acid. Solution No. 2 contained 1 g $CuSO_4$ per 1 liter of distilled water.

The content of total lipids was determined using a phosphorovaniline reagent. Briefly, 100 mg of tissue was hydrolyzed in 1.5 mL of concentrated sulfuric acid for 15 minutes. About 0.1 mL of the hydrolysate was added with 3 mL of vanillin reagent (10 mmol L^{-1} of vanillin and 11.5 mmol L^{-1} of phosphoric acid). The solution was stained for 40 min. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 530 nm against control. The amount of lipid was set according to the calibration schedule.

The content of glycogen was determined by the anthrone method. Briefly, 0.1 g of tissue was hydrolyzed for 1 hour in 3 mL of 30% KOH at a temperature of 100 °C, 0.9 mL of distilled water and 3 mL of 0.2% anthrone were added to 0.1 mL of the hydrolysate. Then the sample was boiled at 100 °C for 10 minutes. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 620 nm against control. The amount of glycogen was established according to the calibration graph.

Statistic analysis

The statistical evaluation of the results of the content of total proteins, lipids, and carbohydrates was carried out 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.

RESULTS AND DISCUSSION

Given that the metabolism of fish depends on environmental factors **[2]**, in our opinion, it is appropriate to present information that characterizes the dynamics of the level and temperature regime of the Kremenchuk Reservoir in the spring of 2021 (pre-spawning period) **[11]**, **[12]**.

Based on the analysis of materials submitted by the Poltava Fish Protection Patrol, in the spring of 2021, in the pre-spawning and spawning periods, relatively high and stable indicators characterized the water level in the Kremenchuk Reservoir. Thus, in March, the water level fluctuated between 78.7 – 79.5 m, and in April - within was 79.5 – 80.8 m. Thus, in March, the water level in the reservoir was lower than NSH (81 m) by 2.3 – 1.5 m, and in April – 1.5 – 0.2 m.

The average daily water temperature of the Kremenchuk Reservoir in the pre-spawning and spawning periods fluctuated within: in March – within 3 – 5 ℃, in April – within 6 – 9 ℃, in May – 9 – 19 ℃.

One of the integral indicators that determine the physiological status of fish is metabolism, which is based on indicators of metabolic processes associated with the biosynthesis of proteins, lipids, carbohydrates, and other organic compounds that support the body in different seasons and its adaptation to changed ecological conditions of existence **[13]**, **[14]**, **[15]**.

In addition, metabolic indicators are a kind of biomarkers that characterize not only the physiological status of fish but also the water quality and ecological status of water bodies they inhabit **[16]**, **[17]**, **[18]**.

During the study period, the content of total proteins, lipids, and glycogen was determined in the liver – and organs characterized by its multifunctional activity, including protein-synthesizing, lipid-forming, glycogen-storing, and other functions, as well as white skeletal muscle **[19]**, **[20]**.

Protein: In the pre-spawning period, intensive processes of protein synthesis associated with the differentiation and growth of generative tissue occur. Reserve substances formed in the body in the previous period of feeding and components of food consumed by fish in the pre-spawning period are plastic materials for the formation of sexual products.

The main source of energy for protein synthesis in the pre-spawning period are fat reserves, which accumulate in significant quantities in the body, and food energy. During the period of energy mobilization, the absolute and

relative amount of fat in the fish body is usually reduced. This process is especially intense in the early stages of gonadal development, as the processes of differentiation of sexual products are more energy-intensive than the growth of fish. In this case, a certain part of lipids is used not in energy but in plastic metabolism, participating in the processes of egg yolk formation. However, our research has shown that in the pre-spawning period, the total protein content in the white skeletal muscles of mature fish of the Kremenchuk Reservoir was relatively uniform and high. Somewhat increased levels of total protein accumulation were found during this period in the muscles of zander, perch, and gibel carp (Table 1).

Table 1 The total protein content in the organs and tissues of zander, perch, and gibel carp in the Kremenchuk reservoir in the pre-spawning period of the annual cycle of 2021 (M ±m, mg/g of raw tissue mass, n = 5).

Fish species	Protein	
	Muscles	Liver
Zander	187.53 ±20.52	208.30 ±8.75
Perch	175.1 9 ±10.53	209.60 ±4631
Gibel carp	172.36 ±5.58	154.86 ±11.02

Note: M is the simple arithmetic mean, and m is the error of the arithmetic mean.

Approximately the same and relatively high level of total protein in the muscles of fish with different types of food may indicate its low cost of accumulated reserves during the feeding period last year during the winter, on the one hand, as well as optimal environmental conditions and availability in the aquatic environment feed resources, which contributed to the high intensity of protein-synthesizing function of the fish liver in this period from another.

Evidence of the liver's high activity in protein biosynthesis in the pre-spawning period is its higher total content, which is found in the liver compared to muscle. It should be noted that only a slight excess (11%) of total protein in zander liver and 19.6% in perch liver was found in the pre-spawning period, compared to its content in muscle.

More statistically significant differences between the content of total protein in the liver and muscles were found in other fish species, in particular in roach it was 51.2%, in bream – 57.8%, in European flounder and zope – 40.6% (Table 2).

Table 2 The total protein content in the organs and tissues of roach, bream, European flounder, and zope of the Kremenchuk reservoir in the pre-spawning period of the annual cycle of 2021 (M ±m, mg/g of raw mass of tissue, n = 5).

Fish species	Protein	
	Muscles	Liver
Roach	159.63 ±20.09	241.33 ±17.75
Bream	145.31 ±12.51	229.28 ±11.66
Silver bream	159.06 ±7.69	225.46 ±10.54
Zope	157.21 ±11.59	222.29 ±11.28

Note: M is the simple arithmetic mean, and m is the error of the arithmetic mean.

Only in the liver of gibel carp the content of total protein was lower by 11.3% compared to muscle, which, in our opinion, is due to its possible activity in search of food at elevated water temperatures, as well as with its use in the maturation of sexual products.

The increased content of total protein in the fish's liver may be due to the need to use it in the processes of generative metabolism during the trophoplasmic growth of oocytes, as well as to ensure the spawning process.

Other researchers have found that the protein content in the meat of bighead carp of the Kremenchuk Reservoir averages from 16% to 18.4%, and in common carp meat from 16% to 18.8% (spring catch) **[21], [22], [23].**

Lipids: In addition, in the spring, the increase in water temperature is accompanied by an increase in the intensity of metabolic processes, which causes a significant increase in organs and tissues of total lipids, which are the basis for all intracellular membranes **[24]**, **[25]**, **[26]**.

The need for fish for lipids is met by synthesizing them in the body, as well as due to lipids, which are part of the natural and artificial feed base **[27]**, **[28]**, **[29]**.

With increasing water temperature in the reservoir, there is an intensive development of natural fodder base, which contributes to increased food consumption and, accordingly, the use of its components in the biosynthesis of organic matter **[30]**, **[31]**.

In this respect, the results of studies are noteworthy, which found that during pre-spawning feeding, the content of total lipids in the white skeletal muscles of mature fish of the Kremenchuk reservoir was at about the same level, except for gibel carp and silver bream. Slightly elevated total lipids were found in the muscles of these fish (Table 3). Thus, in the muscles of silver bream, it was 12.07 mg/g of raw weight, and in the muscles of gibel carp – 18.5 mg/g, while in the muscles of all other studied species of fish, this figure was in the range of 6.7 to 8.71 mg/g of raw weight.

Table 3 The content of total lipids in the organs and tissues of the studied fish of the Kremenchuk reservoir in the pre-spawning period of the annual cycle of 2021 (M ± m, mg/g of raw mass of tissue, n = 5).

Fish species	Lipids	
	Muscles	Liver
Zander	6.73 ±1.10	37.26 ±3.89
European perch	8.67 ±1.03	38.42 ±6.56
Gibel carp	18.50 ±2.18	40.46±3.95
Roach	8.71 ±1.05	50.41 ±7.01
Bream	8.41±1.91	40.00±3.84
Silver bream	12.07 ±2.16	50.11 ±10.53
Zope	6.89 ±1.03	38.16 ±2.70

Note: M is the simple arithmetic mean, and m is the error of the arithmetic mean.

Approximately the same content of total lipids in the muscles of the studied fish species of Kremenchuk Reservoir in the pre-spawning period may indicate the maximum accumulation of energy resources needed to ensure the functional activity of these tissues in the pre-spawning period and during spawning.

According to other researchers, the content of lipids in the meat of bighead carp of the Kremenchuk Reservoir averages from 4% to 6.6%, and in common carp meat from 3% to 4.6% (spring catch) **[21]**.

It is noteworthy that the liver's total lipids content in the pre-spawning period is higher than in the fish's muscles. Studies have shown that the total lipid content in the liver of gibel carp, European perch, bream, zope, and zander was about the same, relatively high level, which is 4 – 5 times higher than the values recorded in the muscles of these fish species.

The content of total lipids in the liver of roach and silver bream was slightly higher, which exceeded the average of other fish species by about 20 – 25%.

The significant level of accumulation of total lipids in the liver of fish in the pre-spawning period is primarily due to the high functional activity of the liver in the biosynthesis of lipids from digested food components and their deposition to energy protoplasmic growth of oocytes at the final stage of their development and spawning process.

According to the literature, a higher initial level of lipids (at the beginning of the pre-spawning period) provides a more intensive process of fish maturation and greater spawning. The author believes that the initial level of fat reserves in fish at the beginning of the pre-spawning period may be one of the important indicators of the readiness of fish for their maturation and spawning. The higher the fat content of fish, the faster it matures and therefore reaches a higher maturity.

In addition, fat reserves largely provide energy synthesis of generative tissue. In gamete formation, lipids partially pass to the gonads and are included in the yolk of oocytes as a nutrient, and after hatching and fertilization of eggs, they act as the main source of endogenous food during embryo development. Therefore, the level of fat reserves in the organs and tissues of fish and the intensity of their use in the maturation of the gonads largely depends on the effectiveness of the spawning process of fish. That is, indicators of the dynamics of lipid content in the organs and tissues of fish can be indicators of fish readiness for spawning.

Thus, in the body of the studied fish species, intensive fat accumulation in the pre-spawning period is established, as these accumulated reserve substances after wintering are spent on the energy supply of sexual production processes at the final stage of their development and spawning.

The results coincide with the data of other researchers, who proved that at the beginning of the pre-spawning period (III-IV stages of maturity of sexual products) in the organs and tissues of fish, there is an increase in protein and lipids **[40]**.

In the pre-spawning period, when the process of gonadal formation takes place, the level of lipids in the muscles of the Kremenchuk Reservoir was quite high. Immediately before spawning (March), bream liver is maximally saturated with protein and the maximum amount of lipids

At the end of the pre-spawning period, the total content of proteins and lipids decreases, which is due to the use of reserve substances in the process of trophoplasmic growth of oocytes and spermatocytes

Temperature conditions largely determine the rate of lipid formation in the fish liver. As the water temperature decreases, the lipid content in the liver decreases sharply, and it is replaced by glycogen, which in warm water reaches 18 – 20%, and at high temperatures decreases to 2 – 3%.

Glycogen: Glycogen plays an important role in the accumulation of energy in fish under anaerobic conditions due to its easy mobility, high degree of recovery, and ability to release energy. This is its specificity as a source of energy for fish. The main stores of glycogen are concentrated mainly in the muscles, but the most mobile "depot" of glycogen is in the liver of fish **[32]**, **[33]**, **[34]**.

Muscle glycogen is mainly used as an energy source for fish movement, so its changes are related to seasonal physiological rhythms. Muscle glycogen stores are most abundant during fish wintering when food intake is sharply reduced. Used glycogen stores in muscles are constantly replenished not only due to their reserves in the liver but also as a result of its intensive biosynthesis from intermediate products of the breakdown of proteins and lipids, which are mobilized from muscle tissue and the liver itself as a result of processes gluconeogenesis **[35]**, **[39]**.

It should be noted that the use of lipids and glycogen in energy metabolism in fish is closely related. Thus, in fish glycogen can be synthesized from the products of fat metabolism, and lipids – from carbohydrate metabolism. The blood glucose level regulates the intensity of lipid mobilization from fat depots.

During the pre-spawning season, carbohydrate parameters usually increase by 1.5 – 2 times, due to the intensive nutrition of fish and an increase in the overall level of energy metabolism (Table 4).

Table 4 Glycogen content in the organs and tissues of the studied fish of the Kremenchuk Reservoir in the pre-spawning period of the annual cycle of 2021 (M ±m, mg/g of raw tissue mass, n = 5).

Fish species	Glycogen	
	Muscles	Liver
Zander	27.34 ±8.43	138.32 ±24.97
European perch	13.35 ±2.85	27.99 ±4.24
Gibel carp	73.96 ±11.41	254.59 ±8.86
Roach	23.03 ±2.41	69.45 ±6.48
Bream	17.03 ±2.31	130.31 ±14.19
Silver bream	10.59 ±1.58	49.06 ±8.41
Zope	4.90 ±1.09	31.16 ±10.81

Note: M is the simple arithmetic mean, and m is the error of the arithmetic mean.

The glycogen content in the muscles of different objects of the Kremenchuk reservoir in the pre-spawning period was different. Its highest content was found in the gibel carp muscle, which reached 74 mg/g of raw weight. Significantly lower (2.7 times) was the level of glycogen accumulation in zander muscles (Table 2) and 3.2 times – in roach muscles. In the muscles of bream, European perch and silver bream found close, relatively low levels of glycogen, which was in the range of 10 – 13 mg/g of raw mass, and the lowest level of its accumulation was recorded in the muscles of the zope (only 4.9 mg/g).

The glycogen content of the liver of all studied fish species significantly exceeded that recorded in their muscles. The highest level of glycogen accumulation was found in the liver of gibel carp, which reached 270 mg/g of raw weight. The content of glycogen in the liver of zander and bream was significantly lower (1.8 – 2 times), respectively, compared to its maximum accumulation in the liver of gilber carp. Glycogen was three times lower in the liver of roach, European perch, silver bream, and zope, but they were significantly higher than those found in the muscles of these fish species.

Different glycogen content in the muscles and liver of different species of fish in the pre-spawning period, apparently, due to different needs of the body in its supply of energy during spawning, and may be related to the physiological processes occurring in fish in the pre-spawning period, and to some extent can be determined by environmental conditions that characterize the functional activity of the organism as a whole.

The dynamics of carbohydrate metabolism in fish are determined by the easy mobilization and recovery of carbohydrate reserves and the ability to release large amounts of energy in a short time.

There are two glycogen peaks in the muscles and liver of demersal fish. The first maximum is observed at the end of the feeding period when glycogen accumulates simultaneously with lipids. Then, during the winter, it is consumed, and its content increases again before spawning due to biosynthesis from the breakdown products of protein-lipid muscle complexes. In muscle, glycogen serves as a backup energy source for muscle function **[36]**.

In the liver, glycogen is a carbohydrate reserve from which glucose is formed when certain enzymes are involved. Some carbohydrates in fish are converted into fats and stored in the liver and muscles. If necessary, glycogen is easily converted into glucose, which can participate in fish's energy supply of metabolic processes **[37]**.

Glycogen levels in muscle, especially in the liver, are thought to be indicators of the body's physiological condition **[38]**.

CONCLUSION

Analysis of the results of field and experimental studies shows that in the pre-spawning period under existing conditions in white skeletal muscle, the total protein content of all studied species of mature fish of the Kremenchuk Reservoir was relatively high (from 145 to 187 mg/g raw weight). Approximately the same and relatively high level of total protein in the muscles of fish with different types of food may indicate its low cost of accumulated reserves during the feeding period last year during the winter, on the one hand, as well as optimal environmental conditions and availability in the aquatic environment feed resources, which contributed to the high intensity of protein-synthesizing function of the fish liver in this period from another. Evidence of the liver's high activity in protein biosynthesis in the pre-spawning period is its higher total content, which is found in the liver compared to muscle. It should be noted that only a slight excess (11%) of total protein in zander liver and 19.6% in perch liver was found in the pre-spawning period, compared to its content in muscle. More statistically significant differences between the content of total protein in the liver and muscles were found in other fish species, in particular in roach it was 51.2%, in bream – 57.8%, in European flounder, and zope –40.6%. In the pre-spawning period, approximately the same content of total lipids was found in the muscles of most species of fish studied. Its content in gibel carp and silver bream muscles was slightly higher. Thus, in the muscles of silver bream, it was 12.07 mg/g of raw weight, and in the muscles of gibel carp – 18.5 mg/g, while in the muscles of all other studied species of fish, this figure was in the range of 6.7 to 8.71 mg/g of raw weight. The content of total lipids in the liver of all studied fish species was significantly higher than in the muscles, which may indicate high activity of lipid-forming function of the liver in the presence in the aquatic environment of sufficient components necessary for lipid biosynthesis. The glycogen content in the muscles of different objects of the Kremenchuk reservoir in the pre-spawning period was different. Its highest content was found in the gibel carp muscle, which reached 74 mg/g of raw weight. Significantly lower (2.7 times) was the level of glycogen accumulation in zander muscles and 3.2 times – in roach muscles. In the muscles of bream, European perch and silver bream found close, relatively low levels of glycogen, which was in the range of 10 – 13 mg/g of raw mass, and the lowest level of its accumulation was recorded in the muscles of the zope (only 4.9 mg/g). The glycogen content of the liver of all fish species was higher than that of muscle. The highest glycogen content was recorded in the liver of gibrl carp, zander, and bream, less – in other fish species. The high content of glycogen in the organs and tissues of fish indicates a high level of glycogen-storing function of the liver and its role in ensuring the energy supply of fish in spawning.

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Conflict of Interest:

The authors declare No. conflict of interest.

Ethical Statement:

Following the protocols, No. 3/2021, No. 5/2021, and No. 7/2021 at the meeting of the Ethics Commission of the Faculty of Animal Husbandry and Aquatic Bioresources of the National University of Life and Environmental Sciences of Ukraine during the experimental catches signed Acts No. 1/3, 2/5 and 1/1 ie in the process of catching (all norms of the current legislation of Ukraine according to **DSTU 2284:2010** are observed).

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A model for increasing the business activity of personal subsidiary farms based on small-scale poultry meat production

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ABSTRACT

The basis of this article is the study of such a form of farming in rural areas as personal subsidiary farms (PSF). The importance of private farming is actualized both in matters of a social nature in rural areas and issues of sustainable development of entire sectors of the economy. The article clarifies the main socio-economic functions of individual subsidiary farms. The basics of motivation and goal setting for entrepreneurship are considered. And in this regard, a model is given for increasing the business activity of personal subsidiary farms based on small-scale poultry meat production. The model is described both from the point of view of the mechanisms of interaction of participants and from the organisation's point of view. The financial mechanisms of this model and its features are also given. Many economists consider PSF the most massive, and economically stable; one might even say the surviving producer of agricultural products sustainably. This phenomenon lies in the economic nature of PSF. In these conditions, personal subsidiary farms are additional for those who are engaged in hired work. For the majority, this is about 3 million people who are considered "self-employed", the only source of income. Of particular interest is the financial model of this project, which was developed by the project's authors and tested for three years. This model allows you to reduce the price of finished products and keep it 15% below the market. The project showed that personal subsidiary farms without special conditions could not transform massively into individual entrepreneurs or peasant farms. To do this, the state needs to organize prototypes of such operators on the ground, which will begin to perform all intermediary functions to improve the business environment of each rural locality.

Keywords: Personal subsidiary farms, small-scale production, business activity, motivation for entrepreneurial activity, economic model of interaction

INTRODUCTION

Today, the Republic of Kazakhstan is looking for mechanisms through which it is possible to solve the socio-economic problems of the rural population, the main of which are: increasing the incomes of rural residents, increasing productive employment of the population, reducing the unemployment rate, both in rural areas and in the Republic as a whole. In Kazakhstan, the activities of private subsidiary farms are regulated by the Land Code of the Republic of Kazakhstan **[1]** and the Tax Code of the Republic of Kazakhstan **[2]**. In today's realities, private farms have become significant producers of both agricultural products in general and livestock products.

Table 1 shows that in the households of the population in 2019, up to 79% of dairy cattle, up to 65% of horses, up to 69% of sheep and goats and up to 16% of poultry are produced in the structure of production of all agricultural products.

This is because in 2019 more than 45% of the population of the republic or 7,733.8 thousand people out of 18,157.3 thousand people live in rural areas. Of the almost 4 million economically active rural population, about 500 thousand people are employed in agricultural production (according to the Statistics Committee of the Ministry of National Economy of the Republic of Kazakhstan).

Table 1 Statistics of livestock activity of agricultural enterprises and households of the population of the Republic of Kazakhstan in 2019, million tenges.

Type of activity	All categories of farms		Including					
			agricultural enterprises		Individual entrepreneur and peasant (farm) farms		households of the population	
	million tenge	%	million tenge	as a % of the total	million tenge	as a % of the total	million tenge	as a % of the total
Breeding of dairy cattle	928 885,6	100%	48 447,7	5%	141 988,7	15%	738 449,3	79%
Breeding of other cattle and buffaloes	627 938,3	100%	78 657,7	13%	148 333,8	24%	400 946,7	64%
Breeding of horses and other equine animals	211 114,3	100%	7 821,6	4%	65 486,6	31%	137 806,0	65%
Breeding of camels and other animals of the camel family	18 660,7	100%	1 411,6	8%	4 049,9	22%	13 199,1	71%
Sheep and goat breeding	217 378,6	100%	5 065,2	2%	62 282,3	29%	150 031,0	69%
Pig breeding	64 884,1	100%	22 049,9	34%	5 369,3	8%	37 464,9	58%
Breeding of poultry	238 489,4	100%	198 471,5	83%	688,0	0%	39 329,9	16%
Breeding of other animal species	12 145,7	100%	790,5	7%	2 374,0	20%	8 981,2	74%

Their average salary at the end of 2018 was 91,084 tenge, which is 1.7 times lower than the average in the whole country and is the lowest among all industries. Many economists consider personal subsidiary farms the most massive and economically stable; one might even say the surviving producer of agricultural products sustainably. This phenomenon lies in the economic nature of individual subsidiary farms.

According to the definition of many scientists, "personal subsidiary farming is a form of non-entrepreneurial activity for the production and processing of agricultural products carried out by the personal labour of a citizen and his family members to meet personal needs on a plot of land provided or acquired for personal subsidiary farming" **[3, 4, 5, 6]**. Other economists expand the importance of private farms and define them as "the main forms of economic activity of the rural population for the production of agricultural products to meet the needs of the population in food and to act as a means of solving problems to ensure food security of the country and the preservation and development of rural areas, rural lifestyle, national life and cultural heritage" **[7, 8, 9]**. The business activity of personal subsidiary farms as an economical category is also reflected in the scientific works of scientists **[10, 11, 12, 13, 14]**.

When considering the business activity of personal subsidiary farms, it combines the concepts of the business activity of an individual as an owner and the business activity of an enterprise as an economy, on the one hand. On the other hand, a personal subsidiary farm is not an individual but cannot be attributed to a full-fledged enterprise. Also, the rural aspect of this issue further complicates the understanding and definition of the concept of business activity in private households.

Personal subsidiary farms are additional for those engaged in wage labour, and for the majority, it is about 3 million people who are considered "self-employed", in fact, the only source of income. The average per capita income of villagers from private households is only 20.1 thousand tenges per month.

Such figures tell us that such a phenomenon as personal subsidiary farms should be studied, and interaction mechanisms with them should be built. This will lead to an increase in the income of the villagers.

Scientific Hypothesis

The study of the model of increasing the business activity of personal subsidiary farms based on small-scale poultry meat production will establish interaction mechanisms to increase villagers' incomes.

MATERIAL AND METHODOLOGY

Samples

A questionnaire survey, the method of questioning, was used to solve research tasks. A questionnaire survey was used to obtain the primary data. The object of the study was personal subsidiary farms focused on breeding broiler chickens in the Karaganda region (Republic of Kazakhstan).

Instruments

Questionnaire survey

Laboratory Methods

Our evaluation material was questionnaires and the respondents' answers to the questionnaire. The questionnaire contained 51 questions, to which the respondents answered numerically, verbally, or by supplementing the answers. The questions were open to respondents.

Description of the Experiment

Sample preparation: The sorting method studied and processed data from questionnaires filled out by personal subsidiary farms. Cumulative totals, interval, and percentage range in individual response classes

Number of samples analyzed: The study covered 70 respondents, including 70% of women and 30% of men. Most of the respondents were aged from 17 to 59 years (89%), that is, people of the "young" and "middle" age categories according to the WHO age classification.

Design of the experiment: In writing this article, depending on the nature of the tasks being solved, the following research methods were used: abstract-logical; monographic; economic-statistical; computational-constructive; graphic. Software products of general and special purpose were used in the course of the work: By the monographic method, all the main directions are studied and presented in this work: introduction, literary review, main results; Questionnaires were compiled by an experimental method to survey residents of the owners of private households; The graphical method was used to build diagrams, visualize models, functional relationships and mechanisms of interaction of model participants, as well as to visualize large amounts of statistical data; A financial model of the project was developed using the design calculation method; The abstract-logical method was also used to describe the basic ideas of the model, to put forward hypotheses, substantiate the relevance and novelty of this issue; The information and empirical base of the study was made up of official data stat.gov.kz, Statistics Committee of the Ministry of National Economy of the Republic of Kazakhstan, Report on the research work "Development of a model for the effective functioning of personal subsidiary farms on the example of poultry meat production", personal observations of the authors.

Statistical Analysis

Multiple correspondence analysis was used to visualize the data obtained from the questionnaire survey. Statistical significance was determined based on the significance of the p-value. The statistical program R studio (vs. 1.3.959) was used for data processing. Multiple correspondence analysis (MCA) is an extension of simple correspondence analysis to summarize and visualize a data table containing more than two categorical variables. It can also be understood as a generalization of the main components' analysis when the analyzed variables are qualitative instead of quantitative.

RESULTS AND DISCUSSION

Let's consider and define, in our opinion, the main socio-economic functions of private households in the Republic of Kazakhstan:

1) production and processing of agricultural products in small volumes for self-sufficiency,
2) increasing the income of rural families and their material security by selling surplus products,
3) solving the problems of rural employment related to the seasonal nature of the main work,
4) reduction of social aggravation and unemployment through the possibility of self-employment and independence from the employer,
5) increase in productive employment,
6) solving the problems of food security of the population,
7) introduction into circulation of unsuitable and hard-to-reach land plots,
8) labour education and professional orientation of rural youth,
9) care and support of older generations,
10) formation of the qualities of business activity,
11) consumption of fresh, high-quality, and environmentally friendly products,
12) preservation of rural areas, landscape, and biodiversity of species,
13) preservation of the identity and traditions of folk culture.

Thus, personal subsidiary farms are not enterprises and therefore do not pursue the goal of making a profit from their activities; their goal is only to close the need for food and significant savings on them. Another purpose of

running a private farm is to obtain surplus agricultural products for the sale and purchase of material assets, housing, children's studies, etc. On the other hand, the owners of private farms, if necessary, cover all their losses on maintaining a subsidiary farm and selling products from their salaries, which they receive at their main job. It is this way of personal subsidiary farms formed for decades that is most stable today. Today there is a division of villagers into those who have a main job and those who do not have it, i.e., self-employed.

For the first category, the management of private households for their owners is not the main, but auxiliary activity and is carried out in their free time from their main work. Knowing about the seasonal nature of the main activity of rural residents, the management of private households is a good help in the total income of the villagers.

For the second category, the management of private farming is the main activity, and the sale of private farming products is the main source of livelihood.

This classification of private farms gives rise to the further development of their self-employed owners into individual entrepreneurs or into their transformation into peasant farms. The basis for such a transformation can be the development of the business activity of the rural population. Scientists, economists, sociologists, and psychologists emphasize that business activity can be developed only through the creation of conditions. Let's consider the process of motivation for the development of personal subsidiary farms. This process is influenced by two driving forces: needs and opportunities, each of which has internal and external sources. The main internal need of a person is the need for self-realization, the realization of spiritual values and personal growth, as well as communication. The external need of a person is the receipt of monetary and material values, and the closure of the needs for food, housing, and security. The second driving force- capabilities are also divided into internal capabilities and external conditions.

The internal capabilities of a person consist of his housing and living conditions, material and living conditions, professional qualifications and knowledge, social skills and abilities, cultural and national traditions, mental attitudes, and health.

External conditions are the environment in which a person lives and works, in which the target settings are implemented, and are also divided into natural-climatic, geographical, socio-economic, and legal. And it is through the external conditions created that we could create an environment in which the owner of a private farm will make a decision and come to conscious entrepreneurship.

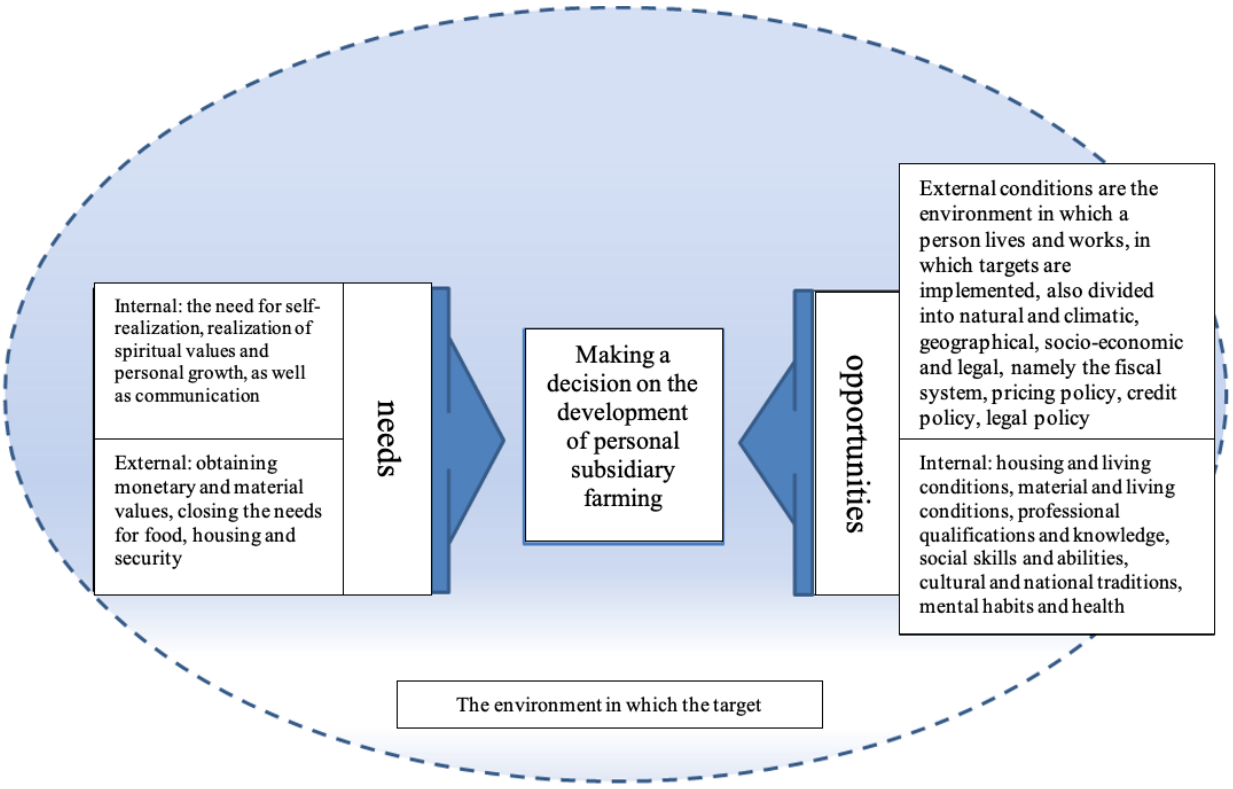

Figure 1 A model of the decision-making process for the development of Personal Subsidiary Farms.

Based on this model, we can say that it is possible to develop a system and a mechanism for influencing the development of the business activity of private households. In our opinion, the main directions of such support should be:

1) Development of infrastructure for entrepreneurship
2) Creation of economic conditions for entrepreneurship
3) Formation of social conditions and maintenance incentives for further growth of microbusinesses in rural areas.

All these areas are being successfully implemented within the framework of the pilot and long-term programs for developing the agro-industrial complex of Kazakhstan. Let's consider the survey results of the owners of private households **[15]** for their willingness to engage in entrepreneurial activity. The study covered 70 respondents: 70% were women, and 30% were men. Most of the respondents were aged from 17 to 59 years (89%), that is, people of the "young" and "middle" age categories according to the WHO age classification (Figure 2).

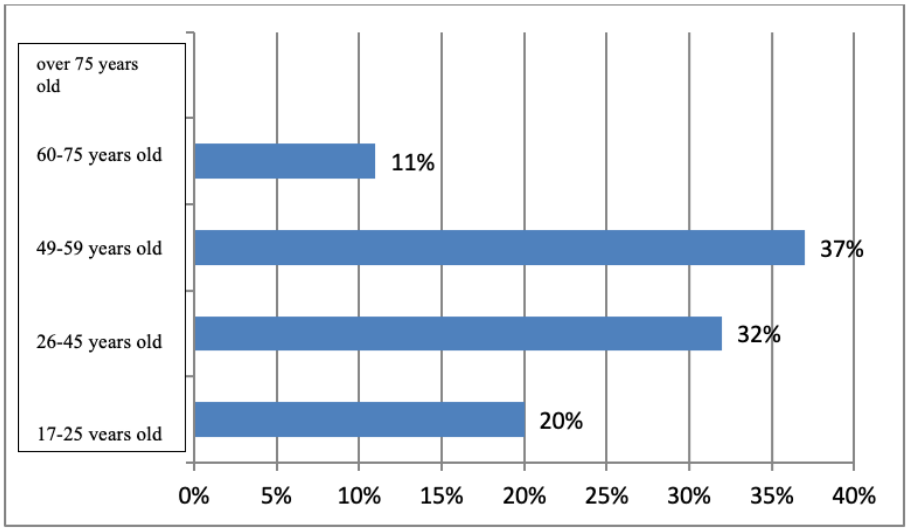

Figure 2 Characteristics of respondents by age, %.

Even though most of the respondents belong to the economically active age, 33% of them are unemployed (Figure 3).

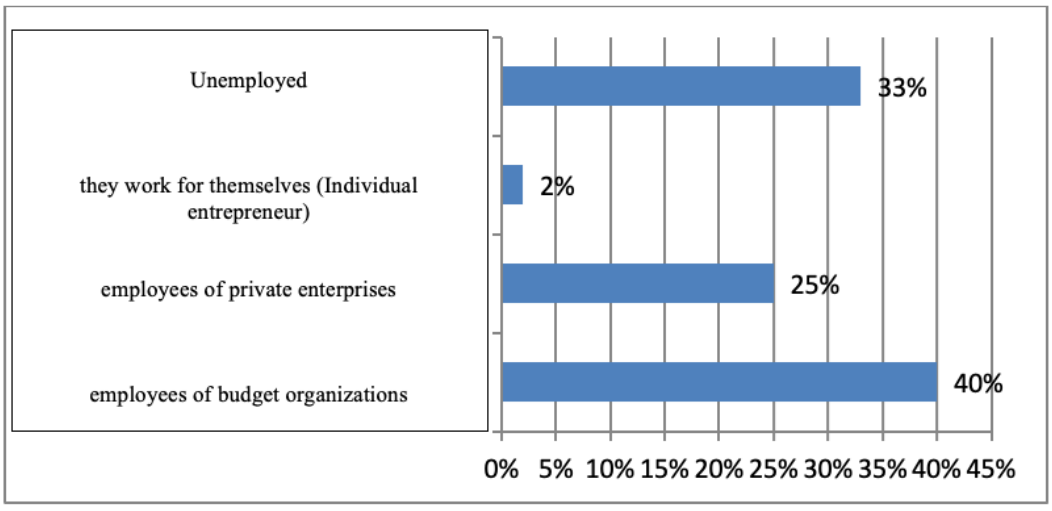

Figure 3 Characteristics of respondents by place of work, %.

Thus, the percentage of employed rural residents, according to the study, was 67%. Education level: 12% have higher education, 38% have specialized secondary education, 50% have secondary education.

The information obtained through the questionnaire about the positive factors contributing to the development of business in rural areas allows us to rank them as follows:

* Low competition in the production of natural products, availability of free niches for this business (35%),
* Proximity to Nur-Sultan (31%),
* Availability of sufficient land resources for the cultivation of livestock and plant products (20%),
* Availability of state programs of preferential lending to support businesses in rural areas (14%).

If we divide private households into three categories, depending on the degree of their participation in the sale of manufactured products, then we can distinguish "non-commodity" (produce products only for their own consumption, and sell it only with the occasional appearance of surpluses), "low-commodity" (produce products for their own consumption and the sale of surpluses), "high-commodity" (produce products primarily for sale and partly for their own consumption). As the study shows, this village is mostly represented by "non-commodity" and "low-commodity" private households. So, for example, only 25% are engaged in the sale of milk and dairy products, and 14% of the surveyed owners of private farms are engaged in the sale of meat. Most respondents cultivate poultry and plant products for personal consumption. These facts indicate the low entrepreneurial activity of the population.

To the question: "Have you tried to start your own business?" – 80% of respondents answered in the negative. At the same time, 64% of those who did start their own business tried to master the sphere of trade and socio-cultural services (shops, cafes, baths, etc.), 7% tried to grow vegetables, 7% – milk and dairy products, 22% – cattle fattening.

To the question: "What problems did you encounter when starting a business?" - respondents noted the following problems: lack of initial capital; no labour resources; limited access to bank products (credit, leasing, etc.); high cost of feed (the study showed that 93% of villagers buy feed and only 7% use their own harvested feed); lack of awareness and legal literacy in doing business.

As the results of the survey show, the owners of private farms need a wide range of educational and consulting services for startups and business development in rural areas. So, to the question: "Have you taken training courses on entrepreneurship and starting a business?" – 90% of the villagers answered in the negative. And to the question: "Would you like to develop a business in rural areas, if so, which one?" – 45% of respondents answered in the affirmative, giving greater preference to business development in the field of trade and socio-cultural services (shops, cafes, baths, etc.) (73%), fattening livestock (10%), growing vegetables (10%), milk production and dairy products (7%).

The analysis of the results of the survey of citizens engaged in private farming shows that there is a certain dependence of the level of development of private farming on the level of income of citizens. So, to the greatest extent, private households are developed among rural residents whose monthly incomes are above 80 thousand tenges. To expand small-scale production in private farms, additional financial investments are needed, which can be made if they are available. In general, there is a low level of income of the population in the studied region. Thus, 23% of villagers had income below 50 thousand tenge, 40% in the range from 50 to 80 thousand tenge, 22% above 80 thousand tenge and 15% of respondents had no income (Figure 4).

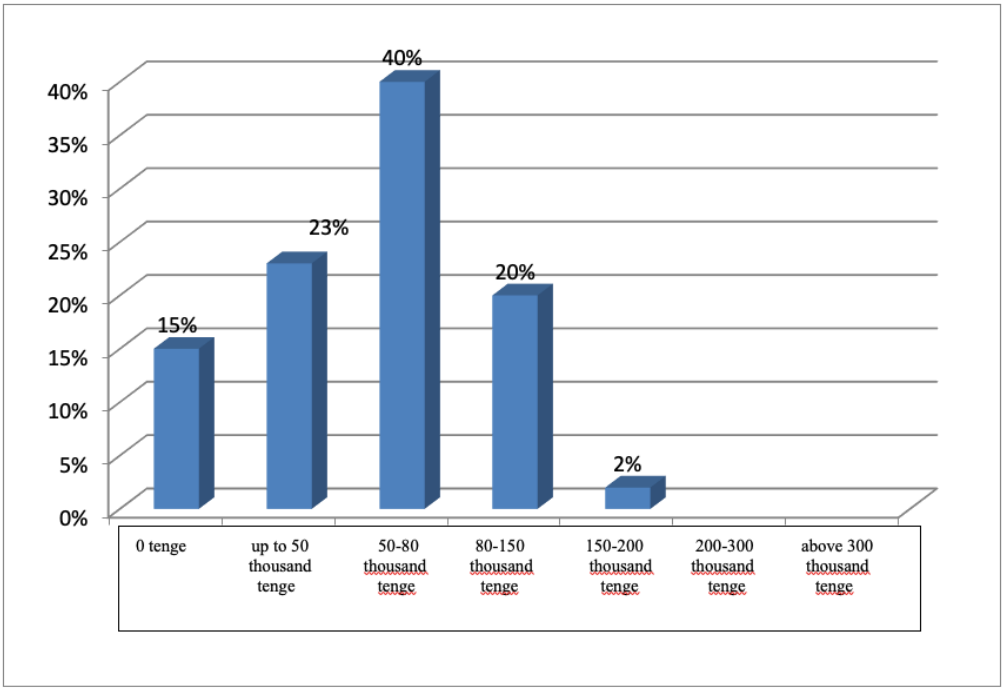

Figure 4 Respondents' income level, thousand tenges.

Low income, lack of initial capital, lack of availability of banking products, and bureaucratic "delays", according to respondents, are among the main factors (22%) hindering the development of business in rural areas. However,

the most significant deterrent factor is the underdeveloped infrastructure (there is frequent disconnection of water, electricity, and Internet), this was indicated by the majority of respondents (51%), Lack of awareness and legal literacy in doing business (17%) and the lack of a stable sales market (10%) they are also the main constraining factors of business development according to the villagers.

Based on the data of the survey, in this article, we propose a model for increasing the business activity of private households on the example of small-scale poultry meat production on farmsteads. (This model is undergoing three-year testing within the framework of grant funding from the Ministry of Education and Science of the Republic of Kazakhstan.)

The essence of this model is to create a platform and a mechanism that will directly connect stakeholders (Figure 4). And there are four of them in our model.

The first part is the private households (within the framework of the approbation, the private households of the village of Akhmetaul of the Nurinsky district of the Karaganda region were selected) who want:

* Increase your income,
* Open your own business in pilot mode,
* Get help and advice from scientists,
* Try a new kind of business,
* To enter the city market with your products.

The second group of participants are urban residents, and investors (within the framework of the project, these are employees and teachers of KazATU named after S. Seifullin, Nur-Sultan), they pursue the following goals:

* Get verified suppliers of fresh, environmentally friendly products,
* Save on marketing tricks and inflated prices of intermediaries,
* Get fresh ecological poultry meat products,
* See how products are grown for them,
* Support the villagers.

The third group of participants - suppliers of material and technical means (Astana-Kus LLP - chicken supplier, Alix LLP-feed supplier, Veterinary Pharmacy No. 1, Nur-Sultan) For this group, it is important:

* New partners for the sale of their products, expansion of sales markets,
* Fulfilment of contractual obligations by partners.

The fourth group is the University (Kazakh Agrotechnical University named after S.Seifullin, Nur-Sultan) For this group it is important:

* Testing of the latest scientific achievements in technology,
* Approbation of the mechanisms of interaction between the parties of the model,
* Obtaining a prototype of a model for the development of personal subsidiary farms business activity.

The main link of this model is the OPERATOR who performs all the key roles of this model:

* Selects personal subsidiary farms for work,
* Looking for interested MTS suppliers,
* Looking for interested urban residents-investors,
* Selects scientific consultants for scientific support of the project,
* Enters contracts with all parties to the project,
* Is the main guarantor of the fulfilment of the terms of contracts,
* Organizes transportation of finished products and means of production to places,
* Does all calculations for the model and for the project as a whole,
* Organizes training and transfer of knowledge and technology in general,
* Monitors the fulfilment of obligations of all project participants,
* Conducts financial collection of investments and purchases materials and means of production for all parties to the project.

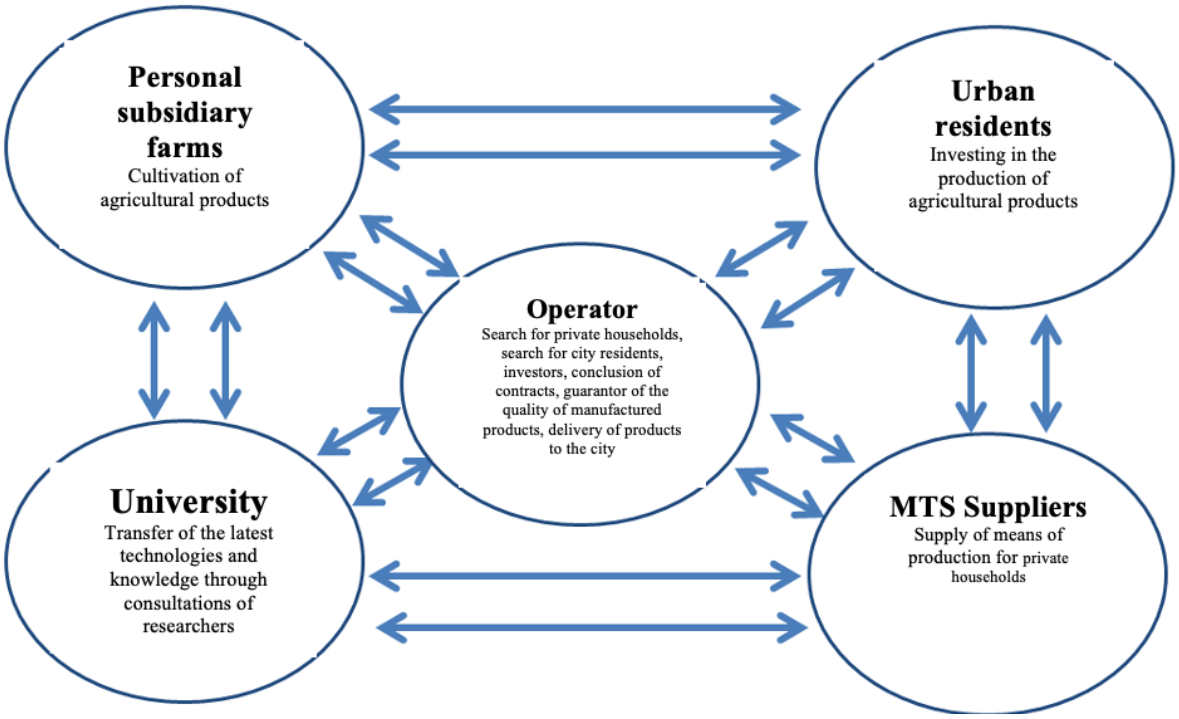

Figure 5 Mechanisms of interaction of the parties in the creation model.

The greatest interest in this model is caused by the financial model and the financial mechanism of interaction between the parties.

The financial model calculation methodology

To begin with, we will determine the total amount of funding:

1. Determine the volume of livestock, which should be no less than the break-even point:

$$Po = Pu + Px + PCattle\ deaths \quad (1)$$

Where:

Po – total livestock, head; Pu – the livestock that will go to the investor is 60% of the Pu; Px – the livestock that will remain as a payment for the work and expenses of the private farm is 30% of the Po; P – the expected case of a bird is 10% of the Po, if the case is less, then the proportion of Pu increases, if the case is more than planned, then the proportion of Px decreases.

2. We determine the upcoming costs:

$$Etc = Ey + TCy + Efeed + TCfeed + Evet + TCvet + Eslaughter + TCprod + Eserv + Eexpenses \quad (2)$$

Where:

Etc – total costs, units; Ey – costs for young animals; TCy – transportation costs for the delivery of young animals; Efeed – feed costs; TCfeed – transportation costs for the delivery of feed; Evet – costs of veterinary drugs; TCvet – transportation costs for the delivery of veterinary medicines; Eslaughter – the cost of slaughtering a grown bird; TCprod – transportation costs for the delivery of finished products to the city; Eserv – costs for the services of specialists; Eexpenses – other costs.

3. We plan the optimal productivity of poultry, it depends on the cross of broilers (in our case, Cobb-500 and Arbor Aikres) and to a greater extent on the conditions of keeping on farmsteads, we settled on the value of 2.2 – 2.3 kg in slaughter weight.

4. We consider the planned price of meat for urban residents.

$$C = Etc/Pi^{*}\ weight\ gain \quad (3)$$

Where:
C – the price of one kilogram of meat for investment calculations.

As shown by 3 years of testing, the price of poultry meat is on average 15% lower than the market price of farm poultry sold in the city of Nur-Sultan.

5. We determine the amount of financing that city residents will invest in the project

$$Fi = \sum Pi*C \quad (4)$$

Where:
Fi – the volume of planned financing by the i-th investor, units; Pi – the volume of purchased products by the i-th investor, kg; C – estimated price of one kilogram of meat.

Practice shows that the amount of financing by one city investor corresponds to an average of 22 – 23 kg of meat. The main mechanism of financial interaction is built on a contractual basis between the participants of the model and is an integral part of the general mechanism of interaction.

The present linear (take-make-waste) model of economy representing as well with textile and clothing industry has a slight chance of effectively adopting sustainable development principles. In recent decades, clothing production has significantly changed and has grown into a Fast fashion trend characterized by mass production of clothes, low prices, and their short life cycle. It is essential to support sustainability, circularity, and resource efficiency of materials, processes, and overall business operations in this sector. The paper deals with the issue of the negative environmental, social, and economic impact of the clothing industry on society. To better understand the situation on the market paper analyses and evaluates consumer behaviour in the clothing industry through the results of a questionnaire survey. Draws attention to the negatives of the linear economy model and proposes solutions to mitigate the negative impact of the clothing industry on the environment and society through education, stricter legislation, simplification of the certification process, support, and promotion of organic production, and highlighting the need to move from linear to the circular economy. Mitigating the negative impact of the clothing industry is essential to achieving sustainable living conditions **[16]**. Consumption is among the key determinants of milk production and profitability. The main purpose of this paper is to present the level of and changes in milk and dairy products consumption in the EU from 2004 – 2018. Due to changing consumer preferences, the average consumption of milk and milk products in EU countries is on an increase. In turn, Poland witnessed a growth in the consumption of milk for ripening and processed cheese and yoghurt. From 2004 – 2017, per capita consumption of ice cream, cheese and powdered milk followed a downward trend. To examine changes in the consumption of milk and milk products, a forecast was prepared which shows that in 2018 – 2022, Poland will experience an increase in the average monthly consumption of milk, ice cream and cheese. On the other hand, the EU will report growth in consumption of fresh dairy products, butter, cheese, skim milk and powdered milk, and a decrease in casein consumption **[17]**. There has been increasing awareness of the benefits of healthy and organic food products as more knowledge has been gained on their effects on health, environment, social convenience, and sustainable development. Acquiring insight into consumer attitudes is essential for the industry to grow. Compared with the rest of the world, the Kurdistan region of Iraq is still in the early stages of understanding the importance of healthy and organic food products. The study's aim was to investigate the attitudes of Kurdish consumers concerning healthy and organic food. I administered an online survey to 452 respondents, and their responses were analysed by using descriptive statistics and performing correlation, linear regression, and factor analysis. The findings indicated that health concerns were the main reason for healthy and organic food consumption. I also found that quality and taste were important factors in purchasing decisions and that consumers were willing to pay a premium price if these foods were available. However, there was a general lack of concern about food production's effects on the environment and animal welfare. This research provides new insight into the attitudes of Kurdish consumers in Iraq towards healthy and organic food. This population has not been covered before, which will add to the literature on this subject **[18]**. The present paper reviews and analyzes existing models, providing an intact point-of-view by integrating key elements into a bigger framework. Key determinants of general food choice are identified and categorized, including food-internal factors (sensory and perceptual features), food-external factors (information, social environment, physical environment), personal-state factors (biological features and physiological needs, psychological components, habits and experiences), cognitive factors (knowledge and skills, attitude, liking and preference, anticipated consequences, and personal identity), as well as sociocultural factors (culture, economic variables, political elements). Moreover, possible directions of influence among the factors towards final food choice were discussed. The need for multidisciplinary

impulses across the research field with the support of empirical data is crucial for understanding factors influencing food choice and enriching existing conceptual models. The framework proposed here would serve as a roadmap for facilitating communications and collaborations between research fields structurally and systematically **[19]**. These include strong global competition and continuous changes in consumer perceptions about food safety, animal welfare and environmental protection. The loss of consumer confidence and trust in the quality and safety of poultry meat and poultry products will remain a major challenge. Many human foodborne bacterial infections are linked to poultry. Control and elimination of these organisms present a great challenge. The development of antibiotic-resistant bacteria will also be a continuous public health hazard. The future concept of animal health will cover not only the absence of disease in birds but also the relationship between the health of animals and their welfare. It will also consider social, economic and ethical considerations and support the achievement of a high level of environmental protection. The emergence and re-emergence of infectious turkey diseases will remain an important non-ending challenge **[20]**. Current lifestyles always pose increasing time pressure that can result in unhealthy diets. Our study addresses the role high-quality plant-based convenience foods can play in promoting healthier consumption. While convenience foods are often associated with poor nutritional values, the spread of healthy convenience foods could respond to the needs of new lifestyles and promote better food choices. The study is based on a multi-component model of the Theory of Planned Behaviour that has made it possible to verify how control factors such as cooking skills, product availability, budget, time pressure, and interest in healthy eating can affect the consumption precooked plant-based foods. The results of Structural Equation Models applied to a sample representative of the Italian population (600 individuals) highlight a consistent group of consumers (almost 70%) that consider plant-based convenience foods as a useful means to improve their diet. For this cluster, market availability, interest in healthy eating, and time pressure are the control factors that significantly influence behaviour. The advancements in knowledge that this research produces are translated into guidelines for producers, retailers, and policymakers that, in synergy, might encourage consumers to replace unhealthy foods with healthy ones **[21]**. The global human community is facing an increasingly urgent dilemma: How do we improve living standards while lessening our environmental impact? This special issue presents recent contributions from psychological and interdisciplinary research on sustainable consumption. To situate these articles in a broader context, we first establish the necessity of improving sustainable consumption and discuss some foundational psychological work addressing this issue. Second, we outline how sustainability can be addressed at various stages, from production and marketing to consumption and waste. Third, we stress the need to broaden the focus on individual consumption to include collective action. Fourth, we discuss several critiques of past research on sustainable consumption. Finally, we highlight the importance of interdisciplinary research in supporting sustainable development. These themes are addressed in greater depth by each contribution to the special issue **[22]**. Reward systems are being described with a new conceptual approach where liking—the pleasure derived from eating a given food—and wanting—motivational value, desire, or craving—can be seen as the significant forces guiding eating behaviour. Our work shows that pleasure (liking), desire (wanting), and the interaction between them influence good predictors of food choice and intake. Reward responses to food are closely linked to food choice, inducing caloric overconsumption. Based on the responses to a self-administered questionnaire measuring liking and wanting attitudes, we found three different segments: ' Reward lovers,' 'Half Epicurious,' and 'Non-indulgents'. Their behaviour when choosing food is quite different. Results show differential effects on caloric consumption depending on segments. Introducing more food choices that try to balance their content is a win-win strategy for consumers, companies, and society **[23]**. Global food systems are currently challenged by unsustainable and unhealthy consumption and production practices. Food labelling provides information on key characteristics of food items, thereby potentially driving more sustainable food choices or demands. This review explores how consumers value three elements of sustainable diets: Comparing consumer response to nutrition information on food labels against environmental and social responsibility information. Six databases were systematically searched for studies examining consumer choice/preference/evaluation of nutrition against environmental and social responsibility attributes on food labels. Studies were quality assessed against domain-based criteria and reported using PRISMA guidelines. Thirty articles with 19,040 participants met inclusion criteria. Study quality was mixed, with samples biased towards highly-educated females. Environmental and social responsibility attributes were preferred to nutrition attributes in 17 studies (11 environmental and six social), compared to nine where nutrition attributes were valued more highly. Three studies found that a combination of attributes was valued more highly than either attribute in isolation. One study found no significant preference. The most preferred attribute was organic labelling, with a health inference likely. Consumers generally have a positive view of environmental and social responsibility food labelling schemes. Combination labelling has potential, with a mix of sustainable diet attributes appearing well-received **[24]**. This study investigated stakeholders' perceptions of animal welfare issues in the Chinese transport and slaughter industry using utility scores and adaptive conjoint analysis. An initial workshop for experts in this

field identified key concerns; these were then included in a questionnaire distributed electronically to stakeholders. Stakeholders, particularly those with higher levels of education, were most concerned about the absence of pre-slaughter stunning and failure to maintain unconsciousness throughout the slaughter process. For all livestock species, electrical stunning was considered the best method of stunning and blunt trauma the worst; for cattle and sheep, stunning using a penetrating captive bolt was considered preferable to the use of a percussive captive bolt. Other important concerns were journey quality and livestock workers' experience and attitudes. Heat stress and closed-sided vehicles were of greater concern than cold stress. Loading facilities and journey length were considered of intermediate importance, while lairage and methods for catching chickens were of the least concern **[25]**. Mortality of broilers during transport and lairage before slaughter represents an economic loss to the poultry industry and a welfare issue that needs to be addressed. In Canada, broilers can be transported long distances and exposed to environmental factors, such as cold temperatures, affecting the percentage of dead-on-arrivals or DOAs. Slaughter plant records for loads transported over 19 months in 2009 – 2010 were examined to identify factors affecting mortality risk (% DOA) during transportation from the rearing barn to the slaughter plant. Information from 2007 loads was analysed using a multilevel linear model. Most of the variation in the mortality risk occurred at the load level rather than at the producer or barn level. There were significant effects of bird sex, age and weight, catching team, journey duration and holding barn duration on mortality risk. The following environmental risk factors increased mortality risk: cold temperatures during the journey and in the holding barn, soft crate stocking density during journeys at cold temperatures and increased trailer temperature when in the holding barn **[26]**. This study aimed to identify welfare problems occurring during the consecutive stages of commercial broiler transportation and to identify risk factors associated with the identified welfare problems. Commercial Belgian transports (n = 81) were assessed in spring (n = 14), summer (n = 33), autumn (n = 10), and winter (n = 24), and potential risk factors were recorded by the observer. Animal-based welfare indicators were scored before the start of the pre-slaughter phase and after the catching, transport and lairage, and slaughter stages to assess the impact of each stage. The most frequently observed welfare impairments were vent and thigh lesions, panting, wing fractures, and bruising on wings and breasts. Our results show that the impact of the pre-slaughter phase on broiler welfare is multifaceted. The overall pre-slaughter phase resulted in a mean weight decrease of 5.3%, a prevalence of 1.4% in leg bruising, and 3.7% in breast or wing bruising. Wing fractures occurred mainly during the catching stage: Prevalence increased from 0.1% to 1.9% (p = 0.003). A welfare comparison before and after transportation and lairage revealed that plumage had become more soiled (p = 0.003), body temperature decreased by 0.7 °C (p <0.001), huddling prevalence increased by 0.5% (p = 0.008), prevalence of birds with splayed legs increased by 0.08% (p = 0.008), prevalence of supine birds decreased by 0.05% (p = 0.003), and 0.1% fewer birds with wings stuck in the crates (p = 0.010) were observed. Risk factor analyses revealed that carefully choosing the catching crew, minimising thermal stress, reducing the duration of transportation, and worker training are promising actions that may improve broiler welfare during the pre-slaughter phase **[27]**. This study will review the environmental implications of dynamic policy objectives and instruments outlined in the European Union 7th Framework Programme (EU-FP7) Project DYNAmic policy MIXes for absolute decoupling of EU resource use from economic growth (DYNAMIX) to address reductions in food consumption, food waste and a change in waste handling systems. The environmental implications of protein intake, food waste reduction, food waste management and donations are addressed using a life cycle approach to find the greenhouse gas (GHG) emissions, land use and water consumption. Data are provided from the Statistics Division of the Food and Agriculture Organization (FAOSTAT) food balance sheets for the European Union (EU) with the base year of 2010 and life cycle inventory (LCI) data from a meta-study of available GHG, land use and water consumption data for major food products. The implications are reviewed using several scenarios for the years 2030 and 2050, assuming policy instruments are fully effective. Results indicate that reductions in animal-based protein consumption significantly reduce environmental impacts, followed after that by reductions in food waste (assuming this also reduces food consumption). Despite the positive implications the policy mixes may have for targets for decoupling, they are not enough to meet GHG emissions targets for the EU outlined in the DYNAMIX project. However, land and water use have no significant change compared to 2010 levels **[28]**. Growing population and increased demand for food, inefficient resource use and distribution, environmental impacts, and high rates of food wasted at all stages of the food system are all calling for the transition towards more sustainable practices. In this article, we apply the concept of circular economy to the case of a sustainable food system. Furthermore, we explore the transition towards a circular food system through the lens of socio-technical transition theory towards sustainability. We discuss challenges and potential solutions for the production stage (focusing on nutrient flow), the consumption stage (focusing on meat consumption), and food waste and surplus management and prevention **[29]**. Food consumption outside the home is a growing phenomenon rapidly gaining importance in terms of its impact on both consumers and the food system. This paper presents an innovative tool for measuring the sustainability of food intended for public consumption in organisations such as

schools, hospitals and workplaces. Drawing on an in-depth review of the food sustainability literature, the FOODSCALE method quantifies eleven sustainability categories that cover thirty-six food sustainability indicators. Several characteristics distinguish the FOODSCALE method from other food sustainability assessment tools. First, it covers the three dimensions of sustainability – society, economy, and environment – treating these as interdependent and coexisting. Secondly, it considers the entire food system, thus incorporating aspects of production, distribution, procurement, consumption and waste disposal. Cross-cutting health and human agency themes complement the eleven specified categories to present a holistic assessment of food sustainability. The tool helps identify good practice and areas for improvement and points toward specific measures for increasing food sustainability. Following a detailed discussion of the tool, the paper presents the results of a comparative study of eight cases across five organisations in the Republic of Ireland. Results show significant differences in sustainability performance across cases and within organisations. The role of key decision-makers in organisations and possible intervention points are highlighted in the discussion **[30]**. In the context of " 2014-European Year against Food Waste " and the EU project FUSIONS, a study has been conducted in a first attempt to define, describe and quantify food losses and waste from harvest to retail in various food supply chains in France. The present communication focused on meat of the Gallus species, i.e. meat of chicken and culled laying hens. In the present study, food losses were defined as products discarded from human consumption for sanitary reasons: mortality between harvesting and stunning and condemnation at the slaughterhouse. Food waste was defined as any part of the animal which is edible or could, after processing, be eaten by humans yet used for other purposes, such as pet food. The study drew in diagrams of the different technical tracks from the live animal to the end product, with the various associated by-products coming out along the slaughter and processing lines and valorisation. Determinants for food losses and waste were either technical, such as technical characteristics of processing tools, economic, such as the market demand side, regulatory or organisational, such as shelf management at retail concerning products' expiry dates. Quantifying food losses and waste is difficult due to the personal character of business data. Issuing from the representation of the different slaughter and process steps, a calculation sheet has been implemented to estimate the share of food losses and waste according to various hypotheses, such as e.g. percentage of carcasses devoted to cutting or percentage of giblets valued for human consumption. The stages of marketing and retailing remained, however poorly documented. This preliminary study needs to be discussed with a larger professional audience and challenged by further research on this topic to increase public attention **[31]**. The average mortality for end-of-lay hens dead on arrival (DOA) was 0.27 per cent (median 0.15 per cent) in a survey of 13.3 million hens transported in 2009. A statistical data model indicated main risk factors for DOA to be slaughter plant, distance travelled and external air temperature, with longer journeys and low external air temperatures increasing the risk. Other highly significant risk factors (p <0.001) related to the condition of the birds on the farm, where an increased risk of DOA was positively associated with poor feather cover, lower body weight, cumulative mortality of the flock and poor health (indicated by a high proportion of the load rejected at the plant for traumatic injury and disease state). However, the data indicate that taking risk factors into consideration makes it possible to transport hens up to 960 km with low losses in temperate conditions. Mean levels of on-farm mortality, during the laying period, for a total of 1486 flocks were significantly lower in cages (5.39 per cent) than in barns (8.55 per cent), free-range (9.52 per cent) or organic flocks (8.68 per cent) according to producer records a median of seven days before depopulation, with considerable variation between flocks in all systems **[32]**. Standardised data on husbandry were recorded for a flock of birds in one house on each of 150 broiler farms in the UK during the 4 d before slaughter.

2. For each flock, the incidence of birds found dead on arrival (DoAs) and the Meat Hygiene Service carcase rejection records were recorded at the slaughterhouse.

3. The mean percentage of birds in each flock found DoA was 0.12% (range 0 – 0.64%), and the mean percentage of Total Carcase Rejects (TCRs) for each flock was 1.23% (range 0.07 – 5.51%). 4. A general linear model was developed to examine factors associated with flock percentage DoAs. Assuming a linear relationship, all other factors remaining the same, a one percentage point (PP) increase in small/emaciated birds will result in a 0.155 PP increase in DoAs and a 1 PP increase in wheat in diet four will result in a 0.003 PP decrease. An increase by one in the total number of vaccines administered will cause a 0.029 PP decrease in DoAs, a 1 g increase in live weight at slaughter will be associated with a 0.000043 PP increase, and a 1 PP increase in mortality on the farm would be associated with a 0.000044 PP increase. A 1 PP increase in Ross birds decreases DoAs by 0.0004 PPS: there is also a seasonal effect **[33]**. Food waste in the global food supply chain is reviewed about the prospects for feeding a population of nine billion by 2050. Different definitions of food waste concerning the complexities of food supply chains (FSCs)are discussed. An international literature review found a dearth of data on food waste, and estimates varied widely; those for post-harvest grain losses in developing countries might be overestimated. As much of the post-harvest loss data for developing countries was collected over 30 years ago, current global losses cannot be quantified. A significant gap exists in understanding the food waste implications of the rapid

development of 'BRIC' economies. The limited data suggest that losses are much higher in developing countries at the immediate post-harvest stages and higher for perishable foods across industrialised and developing economies. For affluent economies, post-consumer food waste accounts for the greatest overall losses. To supplement the incomplete picture and to gain a forward view, interviews were conducted with international FSC experts. The analyses highlighted the scale of the problem, the scope for improved system efficiencies and the challenges of affecting behavioural change to reduce post-consumer waste in affluent populations **[34]**. This observational study was conducted to identify the cause of death and load level factors associated with mortality in 1 090 733 Manitoba broiler chickens transported to slaughter in spring and early summer. Death loss in transit was 0.346% and accounted for 19% of the total carcass condemnation. The death loss pattern was bimodal, with a low death loss in 180 of 198 shipments. Cumulative death loss during the growing phase of production was consistently associated with increased transport mortalities in load level models and when comparing high death loss with low death loss truckloads. The high ambient temperature at the time of slaughter and the loading density of the truck were the major factors associated with exceptional death loss **[35]**. This observational study was conducted to identify the cause of death and load level factors associated with mortality in 1 090 733 Manitoba broiler chickens transported to slaughter in spring and early summer. Death loss in transit was 0.346% and accounted for 19% of the total carcass condemnation. The death loss pattern was bimodal, with a low death loss in 180 of 198 shipments. Cumulative death loss during the growing phase of production was consistently associated with increased transport mortalities in load level models and when comparing high death loss with low death loss truckloads. The high ambient temperature at the time of slaughter and loading density of the truck were the major factors associated with exceptional death loss **[36]**. The incidence of dead on arrival (DOA) birds were surveyed over 33 broilers, 11 turkeys, and 19 spent hen abattoirs representing the majority (around 70%) of the Italian poultry slaughter plants. Data were recorded monthly during a 4-yr period (August 2001 to July 2005), considering a total of 1266 million chicken broilers, 118 million turkeys, and 54 million spent hens, representing 67.7, 84.0, and 28.4% of the national production, respectively. The overall average incidence of DOA was found to be 0.35, 0.38, and 1.22% in broilers, turkeys, and spent hens, respectively. The season significantly ($p <$ or = 0.01) influenced the mortality of all considered poultry categories, with the higher incidence being observed during the summer (0.47, 0.52, and 1.62% for broilers, turkeys, and spent layers, respectively). The incidence of DOA broilers was lower in small slaughter plants compared with medium and large slaughter plants (0.28 vs 0.38 and 0.35%, $p <$ or = 0.01). The data obtained in this study might be used for establishing limit values of DOA as a welfare indicator during the preslaughter time of birds, including catching, loading, transportation, and lairage **[37]**. A field trial was conducted to compare the manual catching of broilers with a mechanical catching method. Both methods were compared concerning the incidence of bruises and dead on arrival, stress parameters, and meat quality. Also, the dynamics of corticosterone, glucose, and lactate were investigated on the day broilers were killed. The broilers originated from 8 commercial broiler farms; visits were made on the day of catching during the spring and autumn of 2001. Broilers of one house were caught manually, and those of the second house was caught mechanically. Plasma samples were taken before catching started, 30 min after the start of catching, 30 min before the end of catching, and at exsanguination of broilers from the first- and last-loaded transport vehicles. Postmortem measurements of pH, temperature, and water-holding capacity were made. Mechanical catching was associated with higher DOA percentages than manual catching in spring, although the difference was not significant in autumn. The catching method did not influence the percentage of bruises or meat quality. Moreover, corticosterone levels indicated that both methods induced the same amount of stress. The dynamics of corticosterone, glucose, and lactate levels showed a similar pattern. Plasma levels increased at the start of catching and increased during transport, shackling, and stunning. However, during catching itself, no large changes were observed. Our findings indicated that attempts to reduce stress in broilers during the last day of life could better be focused on factors other than catching **[38]**. The catching of broilers is the first stage in the transfer of birds to the slaughterhouse. The catching process entails a high risk of stress, injury, and bird death. Associated injury and mortality rates have important implications not only for animal welfare but also for the economics of the procedure. Catching machines are advantageous for labour costs and standards and may also reduce damage to the birds. In the present investigation, a sweeper-type catching machine was compared with manual catching under commercial conditions. Data were collected during 43 mechanical and 40 manual catching events evenly distributed over one year. Dead-on-arrival rates were recorded, and 108 068 mechanically caught and 87 916 manually caught birds were examined for injuries on the shackles at the processing plant. Injury rates of all types were significantly reduced after mechanical catching. This improvement was biggest concerning leg injuries. There was no significant difference in the number of dead-on-arrivals except during the spring period when there were higher losses of birds caught mechanically; this was thought to be attributable to climatic conditions. The loading of the transport containers with equal numbers of birds and the initial familiarisation period of the catching team with the machine is potentially problematic factors with potential for improvement. The catching machine

investigated here, with its lower risk of injury to broilers than commercial manual catching, has the potential to limit the impairment of bird welfare during catching **[39]**. Animals may be subjected to various stressors during transport, which may compromise their health, welfare, and meat quality. In the chain of operations between a farm and a slaughterhouse, animal transport is probably the most stressful and injurious stage. Data on mortality is commonly collected at slaughterhouses as a retrospective indicator of animal welfare during transport. Ten-year prevalence of mortality of all the species and categories of animals (cattle, pigs, goats, sheep, poultry, rabbits and ostriches) regularly scheduled for slaughter in the Czech slaughterhouses was assessed as dead on arrival after road transport from 2010 to 2019. Among livestock, the highest mortality was found in pigs (0.065%), statistically higher compared to cattle (0.027%) and sheep (0.015%). In animals shipped in containers (rabbits, broiler chickens, end-of-lay hens, turkeys, geese and ducks), the highest prevalence was found in laying hens (0.507%), statistically higher compared to broiler chickens (0.425%) and rabbits (0.199%). The lowest prevalence was observed in geese (0.003%). There was a trend for decreasing death losses of pigs in recent years, and losses in broiler chickens and ducks increased. The results indicate that the current transport conditions should be re-evaluated for poultry. Emphasis should be put on the assessment of animal fitness before transport. This is especially important for animals such as dairy cows, sows, and laying hens at the end of their production cycle. They were more likely to die during the journey **[36]**. Groups of 15 broilers aged 32 to 33 d were exposed to an air stream regulated to -5, -10, or -15 °C. Birds were placed into a typical transport drawer. Following baseline observations, the drawer was placed into a test chamber where cold air was drawn past the birds for three h. Three replications were conducted at each temperature. The birds adjusted their position within the drawer based on the drawer's temperature distribution. Compared to the baseline period, exposing the birds to a cold air stream caused them to avoid the front plane ($p = 0.003$), which was the coldest area within the drawer. The birds did not adjust their usage of the middle ($p = 0.308$) and rear ($p = 0.640$) planes because these were the warmer areas within the drawer. The total amount of space the birds occupied within the drawer did not decrease when exposed to the test chamber ($p = 0.669$). The core body temperature (CBT) did not vary and was within the known normal range during the normal ($p = 0.528$), pre-chamber ($p = 0.060$), and post-chamber ($p = 0.285$) periods. The CBT of the birds significantly decreased during the in-chamber period ($p < 0.001$) and then increased during the lairage period ($p < 0.001$). The shrink loss ($p = 0.981$) and amount of time to resume feed consumption ($p = 0.357$) were not affected by exposing the birds to temperatures of -5 °C and colder. Exposing birds to temperatures of -5°C and colder hurt the CBT of the birds. However, the birds demonstrated behaviours which mitigated the negative effect that cold exposure could have on their CBT **[40]**.

CONCLUSION

Personal subsidiary farms, not enterprises, have become the primary and most widespread forms of economic activity of the rural population of the republic. They are focused on producing agricultural products to meet the needs of the people in food. The business activity of personal subsidiary farms acts as a means of solving the tasks of ensuring the food security of the republic and the preservation and development of rural areas, rural lifestyle, national life and cultural heritage in general. It is necessary to create the conditions for orienting private households to entrepreneurial activity. And as an example, the article cites the current model of increasing the business activity of personal subsidiary farms developed by the authors, which is based on small-scale poultry meat production. Following it, the project's financial model is presented in the article. The main driving force of this model is the OPERATOR. This project team conducts all the project coordination activities, providing the project's main participants to engage in their core activities. Of particular interest is the financial model of this project, which was developed by the project's authors and tested for three years. This model allows you to reduce the price of finished products and keep it 15% below the market. The project showed that personal subsidiary farms without special conditions could not transform massively into individual entrepreneurs or peasant farms. To do this, the state needs to organise prototypes of such operators on the ground, which will begin to perform all intermediary functions to improve the business environment of each rural locality.

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The study of "muscle eye" in bulls of Ukrainian black-spotted dairy-meat breed as a factor in improving the properties of meat products

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ABSTRACT
The impact of age, live weight, and growth rate of the bulls of Ukrainian breeds on the area of "muscle eye" (cross-section of *m. longissimus dorsi* when the carcass is divided into front and rear between the 12th and 13th ribs) was studied. The correlation between the size of the "muscle eye" and the carcass's characteristics and the meat's qualitative indicators was also determined. The research was conducted on the bulls of Ukrainian black-and-white dairy (UBWDB) and Ukrainian meat (UMB) breeds. Living animals "muscle eye" area was determined with the ultrasonic analyser Emperor 860, after slaughter. It was found that UMB bulls have the area of "muscle eye" twice as big as their UBWDB peers. The "muscle eye" area increases when growing the cattle to 400 – 450 kg. In the future, it will be practically independent of the age and weight of the animals and remains stable. An increase in the average daily gains within the breed leads to an increase in the "muscle eye" area. The area of "muscle eye" has a weak negative connection (r = -0.193) with meat tenderness and dry matter content (r = -0.345) and a positive one with slaughter weight (r = 0.614) and slaughter yield (r = 0.653). Of the three parameters (length, depth, and area) of "muscle eye", the greatest impact on the technological properties of meat has depth. Its increase has a negative connection with meat tenderness (r = -0.810) and moisture (r = -0.474), but it has a positive impact on the moisture retention capacity (r = 0.338) and weight of weighed portion after heat treatment. The obtained results can be used to clarify the optimal growing parameters of the bulls of Ukrainian black-and-white dairy and meat breeds for meat and determine the optimal age and live weight of the cattle slaughter.

Keywords: beef, live weight, carcass weight, meat boiling, "muscle eye"

INTRODUCTION
The share of the valuable cuts of the beef carcass impacts on the final cost of products. Direct determination of their yield in each animal has technological difficulties and is not used during lifetime assessment **[1]**. One of the features that are directly related to the yield of the valuable cuts of the carcass is the area of the "muscle eye" of *m. longissimus dorsi* is used due to the connection with the consumer and nutritional characteristics of the beef products **[2]**, **[3]**. The area of the "muscle eye" varies depending on the breed and race of the animal **[2]** as well as the intramuscular fat content **[4]**. The area of the "muscle eye", measured by ultrasound in young cattle, is rather closely (r = 0.80) correlated with the weight of the muscle tissue in the carcass, including the highest grade (r = 0.69) **[5]**. The data concerning the area of "muscle eye" of *m. longissimus dorsi*, obtained by ultrasound, are used **[5]**, **[6]** to predict the obtained beef amount and belonging to a certain grade. The heritability ratio of the "muscle eye" area is 0.45 **[7]**. Among the carcasses of the same weight and fat content, an increase in the area of this muscle in the split indicates an increase in the yield of the cuts. The larger the "muscle eye" area, the greater the weight of steaks that have the highest cost during a retail sale **[8]**.

In Ukraine, the principal amount of beef is obtained from the slaughter of dairy cattle and an insignificant one - from the slaughter of specialized meat animals. The most common of the dairy breeds are the Ukrainian black-and-white dairy breed. Due to their biological properties, these animals can be intensively raised for meat up to

a live weight of 500 – 600 kg. A significant role in solving the beef deficit problem should be played by the cattle of the Ukrainian meat breed **[9]**, **[10]**. This is a promising local breed characterized by large sizes and rapid growth. The data of the area of "muscle eye" of *m. longissimus dorsi* in these breed animals and its connection with the quantitative and qualitative characteristics of the beef is not enough yet.

Scientific Hypothesis

The "muscle eye" area (cross-section of *m. longissimus dorsi* when the carcass is divided into front and rear between the 12th and 13th ribs) is directly related to the yield of the valuable cuts of the carcass and can be estimated during the lifetime. Therefore, it was decided to study the impact of age, live weight, and growth rate of the bulls of Ukrainian breeds on the area of the "muscle eye" as well as to determine the correlation between the size of the "muscle eye" and the characteristics of the carcass and the qualitative indicators of the meat for determining the value of the carcass.

MATERIAL AND METHODOLOGY

Sample

The research objects were the bulls of Ukrainian black-and-white dairy (UBWDB) and Ukrainian meat (UMB) breeds.

Animals and Biological Material

The research was conducted on the bulls of Ukrainian black-and-white dairy (UBWDB) and Ukrainian meat (UMB) breeds.

Instruments

Ultrasonic device (Emperor 860, Vinno 6, China).
Warner-Bratzler device (Lab Logistic Group GmbH, Germany).
Electronic analytical scale (KERN ABS 120-4, SE "Khimtex", Ukraine).
Laboratory ruler (ElizLabs 68933, Ukraine)
Measuring tape (Schweikin, Ukraine).

Laboratory Methods

The area of "muscle eye" was calculated by formula (1):

$$S = a \times b \times 0.8 \quad (1)$$

Where:
S – is the area of "muscle eye", cm^2; a – is the length of "muscle eye", cm; b – is the depth of "muscle eye", cm; 0.8 – is the ratio.

The carcass weight, the length of thigh and carcass, and the thigh circumference **[11]**. The meat tenderness, moisture retention capacity, and boiling were assessed during the heat treatment.

The carcass front (l_1) length was measured from the front point of the aitch-bone (at the cutting) to the middle of the front edge of the first rib. The thigh length (l_2) was measured from the highest point of the hocks to the extreme front point of the aitch bone at the cutting. The carcass length ($l_1 + l_2$) was measured by the sum of the length of the carcass front and the thigh length. The thigh circumference (b - b) was measured in the plane deviated by 60 degrees from the measurement line of the thigh length and perpendicular to this line. According to these indicators, the fleshing index (ratio of carcass weight to its length – K_1) and the thigh fullness ratio (ratio of thigh circumference to its length – K_2) were calculated.

The raw meat tenderness was determined with the help of the Warner-Bratzler device by the effort required to cut the meat sample of 0.15 kg **[8]**. The meat samples 25 mm thick were extracted from *m. longissimus dorsi* in the area of the 12th – 13th ribs. The samples were cut from the fat and bones. The force acting on the knife was calculated by the formula (2):

$$P_2 = \frac{v \times t \times l_1}{l_2} \quad (2)$$

Where:
P_2 – is the knife force at the cutting end (kg); v – is the fraction eruption rate (g.sec.); t – is the cutting time calculated on the electronic stopwatch; l_1 – is the distance from the center of the receiving box to the axis; l_2 – is the distance from the knife to the axis.

The bound water content determined the moisture retention capacity of meat to the meat weight by the percentage. The bound moisture content was determined by a "press method" by the amount of water released from the meat under the action of light pressing and absorbed into the filter paper, forming a wet spot. The spot area size depends on the capacity of the meat to hold water. The better the moisture retention capacity, the smaller the wet spot. The total area of the spot (S_2), which is formed under the pressed meat and the released moisture absorbed by the filter paper (S_1), was determined with the help of the planimeter. The area of the wet spot (A) was determined by the difference between the total area of the spot (S_2) and the area of the occupied meat (S_1). An integral rectangular sample weighing 0.15 kg was cut from *m. longissimus dorsi* to determine the residual meat weight after the heat treatment (boiling). The samples were weighed on technical scales with an accuracy of 0.01 g, then placed in a pan with 4 – 5 liters and poured with 2 – 3 liters of cold distilled water. The pan was placed on the stove. Water was brought to a boil and boiled for 1.5 hours. Then the sample was removed from the water, cooled to 20 °C, and weighed. The meat boiling was determined by formula (3):

$$Sm = \frac{Cm \times 100}{Rm} \tag{3}$$

Where:
Sm – is the meat shrinkage, %; *Cm* – is the weight of boiled meat, g; *Rm* – is the weight of the raw sample, g.

The bound water content in meat was determined by formulas (4, 5):

$$B = \frac{(W - 8.4 \times S) \times 100}{M} \tag{4}$$

$$B_1 = \frac{(W - 8.4 \times S) \times 100}{W} \tag{5}$$

Where:
B – bound moisture content to meat weight, %; B_1 – bound moisture content to total moisture content, %; *W* – moisture content in weighed portion, mg; *S* – an area of a wet spot, cm^2; M – weighed portion of meat, mg.

Description of the Experiment

Sample preparation:

The research was conducted on the bulls of Ukrainian black-and-white dairy (UBWDB) and Ukrainian meat (UMB) breeds (Figure 2). The impact of age, live weight, and growth rate of the bulls of Ukrainian breeds on the area of the "muscle eye" were studied, as well as the correlation between the size of the "muscle eye" and the characteristics of the carcass and the qualitative indicators of the meat were determined for determining the value of the carcass. Cattle were bred in the village of Kalynivka, Cherkasy region.

Figure 1 Ukrainian meat breeds.

Number of samples analyzed: 27 samples of Ukrainian black-and-white dairy breed (UBWDB) and 12 samples of Ukrainian meat breed (UMB) were analyzed to determine the area of "muscular eye" of the bulls depending on the slaughter age. 36 samples of UBWDB and UMB were analyzed to determine the area of "muscle eye" of the bulls, depending on live weight before slaughter. 27 samples of UBWDB and 10 samples of UMB were analyzed to determine the area of the "muscle eye" of the bulls, depending on the growth rate.

Number of repeated analyses: 3.

Number of experiment replication: 2.

Design of the experiment: UBWDB bulls were held in groups of 25 heads from birth to 4 months. They consumed 547 kg of whole milk during this period and 182 kg of skim milk. Further completion of growing of the animals was carried out at the feedlot. During the period from birth to 20 and 22 months of age, each bull consumed 31.486 and 36.120 MJ of metabolizable energy, respectively. The feed consumption was as follows: coarse 12.3 and 12.4%, juicy 14.3 and 14.3, green 27.8 and 28.4, concentrated 18.4 and 18.8%. The bull slaughter was carried out in a slaughterhouse (Kalynivka village).

The bulls of the Ukrainian meat breed were held with their mothers on suction from birth to ex-communication. From the age of 14 days, they were additionally fed with the concentrated feed. At 8 months, the animals were tested for their productivity, which lasted until they reached 22 months of age. During the period from 8 to 20 months and from 8 to 22 months, each bull consumed the feed with an energy value of metabolizable energy of 24.697 and 26.243 MJ, respectively. The animal slaughter was carried out at the Cherkasy meat-packing plant.

The area of the "muscle eye" was determined during the animal life using the ultrasonic investigation (ultrasound) and after slaughter. The animals were fixed in the slaughter machine. The hair was cut to a hair length of max 1.5 cm in the study area. In order to provide the maximum sensor and skin contact, vegetable oil was applied to the measurement site before scanning. The temperature of the oil applied to the skin was above 20 °C. Under temperatures below 8 °C, the oil containers were heated in a water bath. The device was installed between the 12^{th} and 13^{th} ribs of the animals to measure the area of the "muscle eye".

The live weight of each animal was determined within ±7 days from the scanning date. The animals were slaughtered within 24 – 48 hours after the ultrasound. After slaughtering on the cross-section of *m. longissimus dorsi*, where the carcass is divided into front and rear between the 12^{th} and 13^{th} ribs, the length and depth of the "muscle eye" were measured using the ruler according to the scheme shown in Figure 2.

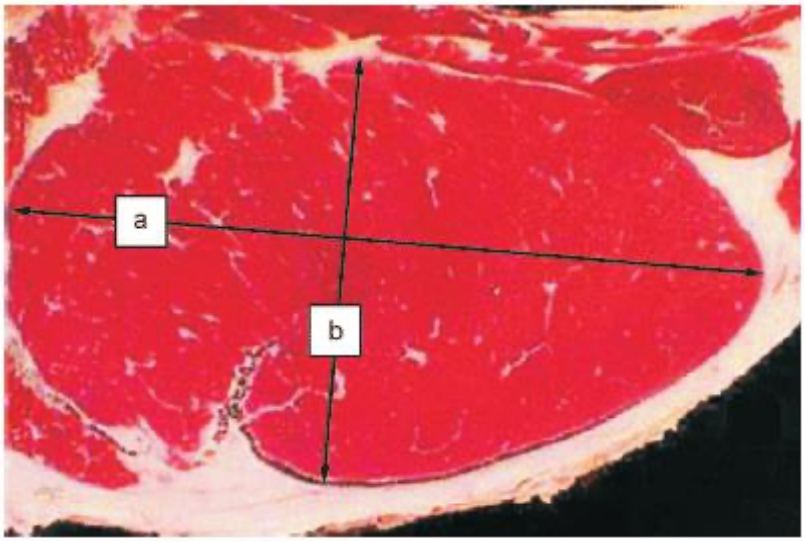

Figure 2 Length (a) and depth (b) of "muscle eye".

Statistical Analysis

The statistical analysis of the results was carried out according to common methods. The arithmetic means with the statistical error, and the reliability criteria were determined. Pearson's correlation coefficient was determined when the correlation analysis was carried out. The statistical analysis data were produced by Microsoft excel and Statistica 15. 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. Linear programming problems were solved using the MS Excel spreadsheet processor "Search for a solution" setting (Excel Solver).

RESULTS AND DISCUSSION

The "muscle eye" area significantly depends on the breed and direction of animal productivity. This feature in the animals of the Ukrainian meat breed is approximately 2 times greater (p <0.001) than in the peers of the Ukrainian black-and-white dairy breed 1).

Table 1 Area of "muscle eye" of bulls, depending on slaughter age, m^2.

Slaughter age, months	UBWDB		UMB	
	n	M ±m	n	M ±m
20	11	0.006529 ±4.07	6	0.01354 ±7.6***
22	16	0.00686 ±4.38	6	0.01335 ±6.9***

Note: ***) p <0.001.

A long-term purpose selection forms this feature of the Ukrainian meat breed. By the area of "muscle eye", the bulls of Ukrainian meat breed significantly predominate the animals of other breeds, not only the dairy productivity. Thus, the average area of "muscle eye" in 30-month-old bulls of the Korean breed (Hanvoo) is 87.4 cm^2 **[6]**. The increase in the slaughter age from 20 to 22 months did not practically impact the bulls' area of "muscle eye", both Ukrainian black-and-white dairy and Ukrainian meat breeds. This result suggested that the transverse growth of *m. longissimus dorsi* is suspended for a certain ontogenesis period, so further animal growth does not impact the area of the "muscle eye". This assumption is confirmed by analyzing the area of the "muscle eye", depending on the live weight of the animals before slaughter (Table 2).

Table 2 Area of "muscle eye" of bulls, depending on live weight before slaughter, m^2.

Live weight, kg	UBWDB		UMB	
	n	M ±m	n	M ±m
350 to 400	12	0.005529 ±2.31	-	-
401 to 450	15	0.0073 ±3.41**	2	0.01342 ±7.10
451 to 500	5	0.00843 ±4.74**	3	0.01372 ±6.82
more than 500	4	0.0072 ±6.78	7	0.013269 ±6.41

Note: **) p <0.01 compared to the animals with a live weight of 350 to 400 kg.

The area of "muscle eye" of UBWDB bulls slaughtered with a live weight of 401 – 450 kg was 32% more than the area of "muscle eye" of the animals with a live weight of 350 – 400 kg. Subsequently, when the live weight of the animals of this breed is increased, the increased tendency of the area of the "muscle eye" was not sufficiently pronounced. Thus, *m. longissimus dorsi* of UBWDB bulls is significantly increased in diameter until they reach a live weight of 400 – 450 kg, after which its growth slows down.

In UMB animals slaughtered with a live weight of 401 kg or more, the difference in the area of "muscle eye was not found. It also confirms the previous conclusion about the suspension of transverse growth of *m. longissimus dorsi*, after reaching a live weight of 400 – 450 kg by the bulls.

The results between the ultrasonic investigation of the live animals and the determination of the "muscle eye" area on the carcass were used to verify the assessment accuracy of the "muscle eye" area. According to ICAR recommendations **[12]**, the difference between the scanning results and the average carcass assessment should be minimal, and the correlation coefficients between them should be at least 0.8. The study results of UBWDB bulls with different live weights show that the ultrasound's lifetime assessment of the area of the "muscle eye" is a reliable criterion with high recurrence after slaughter. According to our data, the average difference between the ultrasound prediction and post-slaughter assessment is 0.053 m^2 at 22 months and 0.058 m^2 at 20 months. A close correlation characterizes both determination methods. Thus, the correlation coefficients are 0.99 for the live weight of the bulls from 401 to 450 kg and 0.92 from 451 to 500.

The area of the "muscle eye" of *m. longissimus dorsi* depends on the growth rate of the animals from birth to slaughter (Table 3).

Thus, the area of the "muscle eye" of UBWDB and UMB bulls increases with increasing the average daily gains of live weight from birth to slaughter. An increase in the growth rate of UBWDB animals by 0.15 – 0.20 kg contributes to an increase in the area of the "muscle eye" by 35% (p <0.01).

A similar tendency was found for UMB. With the average daily gain of more than 0.1 kg, the bulls of this breed had the area of the "muscle eye" greater by 9%. Thus, an increase in the growth rate of young animals, which improves the protein deposition in the muscle tissue, is the main way to impact on the area of the "muscle eye" within the breed without using the selection methods.

Table 3 Area of "muscle eye" of bulls, depending on the growth rate, m^2.

Average daily gain, g	UBWDB		UMB	
	n	M ±m	n	M ±m
up to 550	9	0.00571 ±4.17	-	-
551 – 600	10	0.00688 ±5.27	-	-
651 – 700	8	0.00771 ±4.53**	-	-
701 – 800	-	-	5	0.012969 ±7.24
801 – 900	-	-	5	0.014119 ±8.00

Note: **) p <0.01 compared to a gain of up to 0.55 kg.

The area of the "muscle eye" of *m. longissimus dorsi* is positively correlated with the slaughter weight and yield. Its increase contributes to increased carcass weight ratio to its length, indicating its best musculature development (Table 4).

The positive correlations between the "muscle eye" area and the pre-slaughter live weight and carcass weight are also typical for other breeds. In particular, such results were found in Hanvoo bulls **[13]**. The positive correlation of "muscle eye" with the slaughter weight (of carcass) can be explained by the fact that *m. longissimus dorsi* presents the two most valuable cuts of the carcass: lumbar and dorsal, which are the significant part of the muscle tissue in the carcass.

The "muscle eye" area is weakly correlated with the length of the carcass and thigh, and its increase partially contributes to a decrease in meat tenderness **[14]**. Its intramuscular fat content significantly affects meat tenderness **[15]**. Optimal distribution in the muscle tissue improves this valuable technological property.

Among the carcass characteristics, the increase in the "muscle eye" area in the section indicates only an increase in the cut yield. It does not predict the assessment of the beef quality in the meat animals **[16]**, **[17]**. The "the muscle eye" area does not correlate with the moisture retention capacity. This indicates that its increase does not lead to changes in the beef properties to hold moisture, depending on the quality of many meat products produced from these raw materials: taste, aroma, juiciness, hardness, and quality of sausage products **[18]**, **[19]**. These beef properties can be improved by increasing the level of the intramuscular fat to the optimal values. The beef of older animals contains more intramuscular fat **[20]**, **[21]**. The carcasses with lower fat content are obtained from the bulls than oxen **[22]**, **[23]**, **[24]**.

Table 4 Correlation coefficients (r) between measurements of "muscle eye" of *m. longissimus dorsi* and quantitative and qualitative characteristics of carcasses (n = 12).

Carcass feature	"Muscle eye" features		
	length	depth	area
Slaughter weight	0.229	0.168	0.614
Slaughter yield	0.058	0.140	0.653
Moisture retention capacity of meat	0.167	0.338	-0.037
Meat boiling	0.047	0.451	0.000
Moisture content in meat	0.273	-0.474	0.094
Dry matter content in meat	-0.221	0.109	-0.345
Meat tenderness	-0.226	-0.810	-0.193
Cutting time	0.365	0.216	0.056
Carcass length ($l_1 + l_2$)	-0.169	-0.422	0.197
Thigh length (l_2)	0.167	0.348	0.220
Thigh circumference (b - b)	0.306	0.094	0.094
Meat spot (S_1)	-0.291	-0.151	0.04
Wet spot (S_2)	-0.333	-0.168	0.052
General spot (A)	0.211	-0.110	0.345
Bound moisture content to weight of the weighed portion of meat (M)	0.332	0.146	-0.235
Bound moisture content to general moisture (D)	-0.307	-0.173	-0.018
The ratio of slaughter weight (of carcass) to its length (K_1) Fleshing index	0.563	-0.038	0.659
The ratio of thigh circumference to its length (K_2) Thigh fullness ratio	-0.235	0.596	-0.354

Since the area of the "muscle eye is positively correlated with the quantitative characteristics of the beef, but not with qualitative ones, and the intramuscular fat content is, on the contrary, then in many countries, the area of the "muscle eye" is complemented by marbling in the assessment methods of the cattle **[25]**, **[26]**. Thus, beef marbling is included in its quality determination system according to the system of USDA **[7]**, EUROP, IMCA, and **[27]**, **[28]**. Unlike the area of the "muscle eye", other indicators, in particular its depth, have a more significant impact on the technological properties of meat. It was found that an increase in the depth of *m. longissimus dorsi* on the cross-section of UMB bulls leads to a rise in the meat hardness, decrease in its moisture, an increase in the moisture-retaining properties, and reduce in the weight loss during the heat treatment. The main reason for these properties of meat can be an increase in the diameter of the muscle fibres, due to which the depth of the "muscle eye" increases **[29]**, **[30]**, **[31]**. Recently, the demand for lean and biologically complete beef has been growing. To obtain such meat in the required amount in Ukraine, it is advisable to attach great importance to the animals of the Ukrainian meat breed **[32]**, **[33]**, **[34]**. Its bulls at the age of 20 and 22 months have only 0.6 and 0.5% internal fat content. The market requirements are more consistent with the Ukrainian meat breed, which responds to satisfactory feeding with the rapid growth of the muscle tissue and the late formation of the fat. The biological feature of this cattle is that its weight gain up to 20 – 22 months of age is mainly due to the accumulation of muscle tissue and moderate fat content. The beef of these cattle should be considered lean, and it is in great demand **[35]**, **[36]**, **[37]**. As a result, it has a high moisture retention capacity and low weight losses during the cooking procedure.

CONCLUSION

The area of the "muscle eye" of *m. longissimus dorsi* intensively increases in the bulls to a live weight of 400 – 450 kg, it is significantly influenced by the average daily gains. By the area of "muscle age" the animals of Ukrainian meat breed are twice as large as their peers of Ukrainian black-and-white dairy breeds. Quantitative signs of the carcass can be predicted by the area of the "muscle eye", the qualitative characteristics of the beef are very weak. A number of the technological properties of meat, in particular tenderness, moisture, moisture retention capacity, and weight loss during the heat treatment, are affected by the depth of *m. longissimus dorsi* on the cross section.

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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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The effect of vibration massage on the salting process of ostrich meat

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ABSTRACT
Existing massagers are characterized by relatively high energy consumption during operation, the structure's metal consumption, and the drive mechanism's complexity. Therefore, the search for effective implementation schemes of mixing operations and uniform structure formation of viscous and elastic-plastic raw materials, particularly minced meat, subject to increased contact interaction while minimizing the force on the products, is relevant to the conducted research. The purpose of this work is to substantiate the technological preparation modes of the given minced meat with the use of a developed vibrating massager, as well as to determine the kinematic parameters of the oscillation system and graphic-analytical analysis of their change. The experimental model of the vibrating massager with an eccentric drive mechanism, a measuring evaluation base of rheological characteristics of the minced ostrich meat, and kinematic parameters of the vibrating drive of the massager amplitude-frequency and speed characteristics were developed to carry out the specified tasks. High technological results were obtained using the forced eccentric drive of the massager, characterized by a minimum mass of the oscillation masses of the parts compared to traditional unbalanced vibrators, which reduce 2 – 2.5 times the energy consumption to drive the vibrating massager under study. The practical value of the conducted work includes the use of the eccentric forced vibrating exciter for obtaining the force control over the minced meat to be formed, which reduces the oscillation masses of the drive and minimizes the energy consumption for the process, accordingly; it has the simplest structure among the mechanical vibrators, significantly reduces the dynamic loads on the supporting units of the vibrator as well as provides a sufficiently high contact interaction for both the vibration impact and the processing intensity in general.

Keywords: meat massage, vibrating massagers, mixing, oscillating system, oscillation amplitude

INTRODUCTION

The meat processing industry remains a high-priority and strategic one for Ukraine. The globalization strengthening and Ukraine's integration into the world community make new demands on the meat processing industry to be developed: compliance with the international standards of quality, environmental friendliness, and safety; transition to an innovative industry development model, and active implementation of modern resource-saving production technologies based on the integrated use of raw materials, etc. **[1]**, **[2]**. The tendency to search for and develop innovative technological solutions to produce products characterized by a high level of quality, environmental friendliness, and biosafety, as well as their functional orientation, has been prevailing in the modern food industry. Today, ensuring the completeness and balance of nutrition is one of the urgent nutrition problems of the population **[3]**, **[4]**.

The production of high-quality non-traditional raw materials, which can be used in combination with beef and veal to produce meat products and expansion of this kind of products is an important task. The African ostrich meat represents an example of non-traditional meat which can be imported or produced domestically. This kind of meat can be accepted or not accepted by consumers **[5]**.

One of the priority tasks facing the meat processing industry is to implement resource-saving technologies and produce quality products with high consumer properties. These requirements are most fully satisfied by the group of ham products.

Meat delicacies, which traditionally occupy a significant share in the diet of the Ukrainian population majority, are a technological form for ham products to be created **[6]**. The use of enzyme technologies largely determines the development success of many modern economy industries, including the food industry. The application scope

of enzyme preparations has been significantly expanded due to the creation and implementation of innovative technologies. Today, the enzymes help improve product quality and safety, increase the efficiency of the technological processes, and reduce production costs and the anthropogenic impact on the environment **[44]**.

In meat production technology, the quality of the final product primarily depends on the used technology, raw materials, and technical equipment.

At present, salty meat products have a high demand among the population. The meat salting process includes the meat processing with table salt or its solution and subsequent maintaining for a period sufficient for uniform salt distribution and internal physicochemical processes of the meat maturation, which provide the finished product with the intended taste qualities. There are three classic methods of meat salting: dry, wet, and combined. The dry method involves the application of salting mixtures on the meat surface; preferably, it is widely used when salting shredded raw materials. The wet salting method consists of the meat immersion in the solution of salting substances - brine or its distribution inside the product by injection. The combined approach combines the dry and wet methods of salting. According to classical technology, the meat salting process is divided into long-term and short-term: the long-term process consists of several days to several weeks, and it is preferably used when producing salted and smoked products, dried sausages of some varieties; the short-term process consists of a few hours, it is mainly used when making the boiled sausages.

To obtain high-quality meat products, it is essential to provide the maximum uniform filtration saturation of the meat piece throughout its weight, exposure for further diffusion distribution of salting ingredients, and biochemical transformations. The efficiency of the filtration saturation depends on the pore and capillary sizes as well as the driving force of the process - the pressure difference in the brine accumulation area **[7]**. Salting raw meat materials is one of the most complex and technologically significant processes in producing meat products. Properly selected methods, equipment, and parameters of technological processes guarantee the receipt of the products characterized by high organoleptic indicators, output, and stability during storage **[8]**.

The modern production technologies of meat products involve various acceleration methods of meat salting, including meat massage, the temperature increase of the brines, and injection of the meat pieces. One of the universal salting methods of the meat raw materials is the intramuscular injection of the brine solution and subsequent massage or tumbling to ensure the uniform distribution of the brine in the muscle tissue. To intensify these processes when producing the meat products after the technological operation of the meat injection, the use of vacuum and vibration impact during the massage of the meat raw materials is becoming more common, which justifies the research's relevance.

Domestic and foreign researchers, particularly, have shown that the proteolytic enzyme preparations of animal, vegetable, and microbial origin and own enzymes of the meat can affect the proteins and simultaneously increase the functional and technological properties of the raw materials as well as improve the organoleptic and structural-mechanical performance of the finished products **[9]**. An outstanding contribution to the development of the vibration technologies for implementing the mixing processes and uniform distribution of the components of the meat raw materials in the working volume, which is relevant to the technology under study, was made by well-known domestic and foreign scientists **[10]**. To increase the energy potential of the executive bodies of mixing devices, it is advisable to use the auger and blade mixing elements **[11]**. The Leningrad Institute of Technology has developed the vibrating mixer with combined blade executive bodies **[12]**, providing the counter-movement of materials in the working area for "plastic" products; it allows to create the continuous mode of the product processing, to delay the mass of the meat raw materials at the beginning of the mixer operation to improve the homogenization process or vice versa - to accelerate the environment movement, which indicates the technological flexibility of the equipment. Such design execution is inherent in the blade vibrating mixers of DVS – N type, which is characterized by the processing efficiency and quite complex design execution. The vibrating tray mixer for the elastic-plastic meat raw materials **[11]** is characterized by high productivity at small power consumption and structure simplicity. Improving the mixing efficiency is achieved due to the intense oscillations of the blades relative to the hinges with a frequency proportional to the stiffness of the latter ones and the own weight of the mixing elements. Displacement of the tray center of gravity towards its free-oscillation end provides the largest oscillation amplitude at minimal external energy consumption. However, the processing efficiency of the complex structured systems, particularly the minced meat, is insufficient. When mixing such product masses, the high efficiency is inherent to the drum-type devices, which are also equipped with additional mixture activators for increasing the device productivity, improving the quality of the finished mixture, and preventing it from sticking to the executive bodies of the equipment **[11]**. However, in such devices, a circulating material flow movement in one direction increases the processing duration. To create the multi-directional flows of the technological loading in the modified vibrating mixer **[11]**, the inserts are freely placed in the container and made in the form of a spring, one half of which has the left winding direction, and the other half has the right winding direction, are provided instead of a cylindrical roller. These problems can be ignored when using the vibration

impact on the mixing process of the meat raw materials; it can significantly increase the device productivity, reduce energy consumption, and improve the product quality. At the same time, in some cases, the vibration can only intensify the main process. In other cases the vibration can cause specific vibration effects, namely, increasing the contact interaction, ignoring the "dead zones" when processing, as well as uniform energy saturation of the technological masses in the working area **[12]**.

The purpose of this work is to substantiate the technological preparation modes of the given minced meat with the use of a developed vibrating massager, as well as to determine the kinematic parameters of the oscillation system and graphic-analytical analysis of their change. The following tasks must be solved to achieve this purpose:

- to substantiate the required technological parameters of the meat raw materials of the ostrich according to the developed technology;
- to obtain the dependences of movement trajectory, angular and linear speeds of the executive bodies of the developed oscillation system;
- to make the graphic-analytical substantiation of the studied process modes of the vibration mixing of the minced meat according to determined kinematic parameters.

Scientific hypothesis

The conceptual hypothesis of this work consists of the creation of the separate processing stages of the local compression and stretching areas of the meat raw materials at micromechanical impact with required structure formation within the technological environment when implementation, which is followed by the creation of the fluidized layer of the technological mass, increase of the contact surface planes of its particles, prevention of the segregation of the product layers under the action of these power factors, which operate separately or in combination, ensuring the implementation of the above technological effects.

MATERIAL AND METHODOLOGY

Samples

The meat products of the control and studied samples were researched with the use of the following meat raw materials: African ostrich meat, according to TU U 15.1-2497315254-001:2006 **[3]**, functional supplement "Poltermyshung Rot Superior" LLC "TD Lagos", made in Germany – according to the product specification, enzyme preparation of plant origin – papain, LLC "Alex", made in China – according to the product specification, table salt according to DSTU 3583 **[4]**, white sugar according to DSTU 4623 **[5]**, sodium nitrite according to GOST 32781 (2014) **[6]**.

Chemicals

Sodium hydroxide, NaOH (grade A, analytical grade, (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Methyl red, $C_{15}H_{15}N_3O_2$ (grade A, analytical grade, (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Sulfuric acid, H2SO4 (grade A, chemically pure, (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Petroleum ether, $H_3C\text{-}O\text{-}CH_3$ (excise, analytical grade, (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Animals and Biological Material

For studies, used ostrich thigh and leg meat were obtained after slaughtering the bird at 12 months (supplier farm "Agro-Soyuz" of Dnipropetrovsk region, 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

Determination of parameters characterizing the chemical composition of ham was carried out according to established appropriate standard methods: the mass fraction of moisture by drying the product sample down to a fixed weight at a temperature of 100 – 105 °C according to DSTU ISO 1442:2005 **[13]**; the mass fraction of the

total fat content was determined by the Soxhlet method, which consists in the extraction of fat from the dry mass of the sample with a solvent, based on determining the change in the sample's weight after fat extraction with a solvent by DSTU 8380:2015 **[14]**; the mass fraction of protein by determining the total nitrogen by the Kjeldahl method. Cinefaction of samples was performed on the Velp Scientifica DK6 series (Italy) with a vacuum pump (JP). Distillation was made on a steam distillation device Velp Scientifica UDK 129 (Italy), GOST 25011-2017 **[15]**; the mass fraction of ash by weight method, after mineralization of the product's sample weight in a muffle furnace at a temperature of 500 – 600 °C according to DSTU ISO 936:2008 **[16]**. Determination of ultimate shear stress was carried out by measuring shear characteristics at small deformations, which are subsequently used to evaluate the product's strength, tenderness, and consistency **[17]**. Study of active acidity by determination of pH - by the potentiometric method according to DSTU ISO 2917 – 2001 **[18]**.

The temperature of the studied samples was measured using a digital needle thermometer TH310 Milwaukee.

Laboratory technical scales AXIS BDM 3. were used to weigh the samples.

Description of the Experiment

Sample preparation: Ham samples were used for research, made according to three different formulations of injection brines. The study was conducted in the laboratory conditions of the department of technology of meat, fish, and marine products, National University of Life and Environmental Sciences of Ukraine.

Number of samples analyzed: Three types of ham were used in the study: control – ham with the classical introduction of brine for pickling, and two samples of ham based on a functional additive and the addition of papain.

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 analyzes.

Design of the experiment:

Ostrich meat was portioned into pieces, which were injected with brine (30, 40, and 50% by weight of raw material) at a temperature of 0 – 4 °C for 12 hours.

Recipes of brines were presented in the following sequence:

- classic brine for pickling;
- brine based on a functional additive;
- brine based on a functional additive with the introduction of papain.

Then the ham was massaged at 8 rpm for 20 minutes and subjected to heat treatment - cooking at t = 80 °C ±2 °C until the temperature in the center of the product was 72 – 75 °C. The ham samples were cooled to a temperature in the product layer of 8 °C and stored at a temperature ranging from 0 to +4 °C.

Statistical Analysis

The statistical evaluation of the results was carried out 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.

RESULTS AND DISCUSSION

Products with different nutritional characteristics are produced from the same type of meat raw material by changing the regimes and conditions of raw material preparation, influencing the tissue structure, and changing thermal processes. The peculiarity of the ham products production technology is that at the stage of ham formation, it is salted, injected with 30, 40, and 50% brine at t = 0 – 4 °C. The results of the organoleptic evaluation showed that the ham with the addition of the enzyme papain, after the proposed process of vibration massage, had better taste indicators: the appearance of the ham was rounded, with a dry, clean surface without damage, dense and elastic consistency and was noticeably different from the control sample of ham without the use of vibration massage, with greater juiciness and tenderness, without extraneous smells and tastes. The results of studies on the chemical composition of ham made from ostrich meat are shown in Table 1.

Table 1 Characteristics of the chemical composition of ham.

Sample of ham	Mass fraction, %			
	moisture content	protein	fat	mineral substances
Control sample	67.34 ±2.21	26.17 ±0.42	1.63 ±0.28	1.05 ±0.04
Ham after salting	69.85 ±2.37	27.64 ±0.66	1.55 ±0.88	1.02 ±0.06

Note: (n = 5, $p \leq 0.05$).

It should be noted that the protein content in the control sample is 26.17%, in the experimental sample 27.64% - due to the introduction of a functional additive and papain enzyme, which positively affects the taste properties of the developed ham products and makes them juicier with using an improved massaging process.

During the maturation of the meat raw materials enriched with functional ingredients, one of the main problems is accelerating the diffusion of brine components and their uniform distribution by mass of the meat raw materials. The vibration massage has been used in the developed drum-type device to solve this problem. A series of similar experiments on processing raw materials of plant and animal origin without vibration massage is described in the works of I. Palamarchuk **[19]**, **[20]**, M. Mushtruk **[21]**, **[22]**, V. Palamarchuk **[23]**, **[24],** and others **[25]**, **[26]**, which will negatively affect the quality and organoleptic indicators of the final product.

The studied vibrating device is an oscillation system with two freedom degrees (Figure 1), which is actuated by the drive that provides the shaft, equipped with the eccentric axis of rotation, with the constant rotation torque. Similar technological schemes of equipment without vibration effects are used in the production of various products in the food industry **[27]**, **[28]**, **[29]**. Still, the use of such equipment without conditions determines its performance, which can lead to an increase in material costs during production.

The executive body absorbs the energy from the vibrating drive of the device and acts directly on the products being processed. This mechanism in the vibrating massager is the actual working container. The object being processed consists of the meat products in the form of the individual pieces, salt solution, and other ingredients provided by the technology. The main disadvantages of this model are the increase of dynamic loads on the bearing assemblies and the need to disassemble the mechanism to perform adjustment operations, which are not significant enough compared to the disadvantages of the pronated system **[30]**, **[31]**, **[32]**. When operating the device, the engine torque is converted into the vibrating motion of the working container, which transmits the oscillations to the environment being processed; and the inertial, elastic, and dissipative properties of the technological load influence the movement of the working body and indirectly on the elements of the vibrating drive, in particular, on the rotation of the drive shaft of the vibrating exciter.

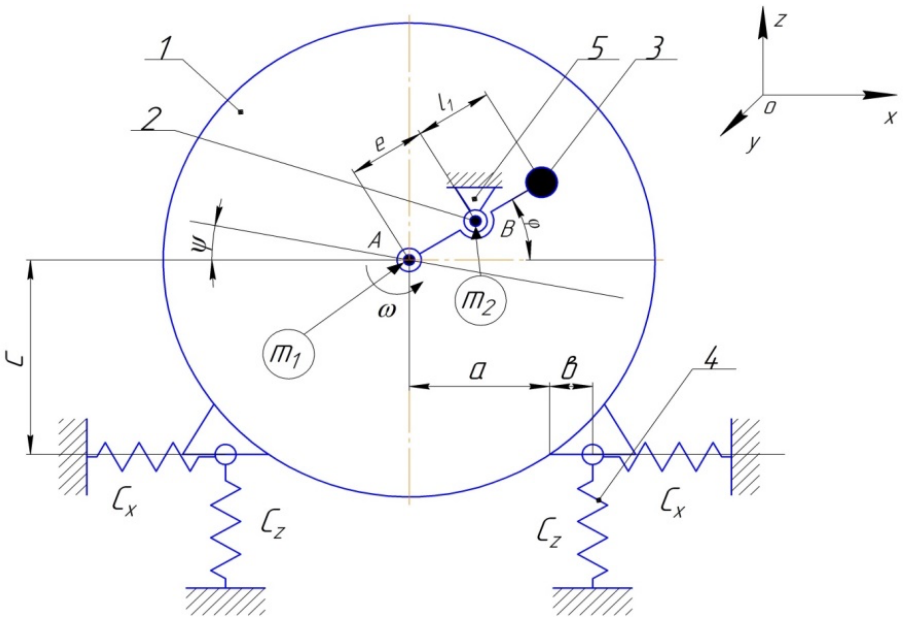

Figure 1 Calculation scheme of the developed vibrating massager. Note: 1 – drum-type working container; 2 – eccentric drive shaft; 3 – counterweight; 4 – elastic elements of the container; 5 – support bearing assembly; e – eccentricity of the drive shaft; l1 – counterweight ordinates; m_1, m_2 – main mass of vibrating device; φ, ψ – freedom degrees of the device; C_x, C_z – stiffness components of elastic elements relative to axes O_x, O_z; ω – angular rotation speed of drive shaft.

It is assumed that the moment of resistance forces between the eccentric and container 1 is proportional to the angular rotation speed of the eccentric. For the generalized coordinates, we take the rotation angle of the eccentric φ and the rotation angle of the container ψ. To make the motion equations of the vibrating device, we will use the Lagrange equation of the second kind:

$$\begin{aligned} A\ddot{\varphi} + B\ddot{\varphi} &= M - \beta\dot{\varphi} + f(\varphi,\psi) \\ B\ddot{\varphi} + D\ddot{\varphi} &= \beta\dot{\varphi} + g(\varphi,\psi) \end{aligned} \quad (1)$$

Where: $A = m_1 l_1^2 + m_2 e^2 + M_K e^2 + J_1 + J_2\ B = M_K e h_C;\ D = J_K + M_K h_C^2$

Where:
$M_к$ – the mass of the working container; M – drive moment; J_K – container moment of inertia; h_C – vertical component of container center of mass; $f(\varphi,\psi) = Q_\varphi^K$, $g(\varphi,\psi) = Q_\psi^K$ – conservative components of generalized forces of the system under study.

The above conservative components of the generalized forces of this oscillation system have the form:

$$\begin{aligned} Q_\varphi^K = \frac{\partial \Pi}{\partial \phi} = &\left(m_1 g l_1 + m_{ct} g \frac{l_1}{l_2} - m_e g e + M_K g h_C \right) \sin\varphi - \\ &- 2c_x \left(e\sin\varphi + a\cos\psi - c\sin\psi + b\cos\psi \right) e\cos\varphi - \\ &- 2c_y \left(a\sin\psi - a\cos\varphi + c\cos\psi \right) e\sin\varphi \end{aligned} \tag{2}$$

$$\begin{aligned} Q_\psi^K = -\frac{\partial \Pi}{\partial \phi} = &-M_K g h_C \sin\psi + 2c_x \left(e\sin\varphi + a\cos\psi - c\sin\psi + b\cos\psi \right) \cdot \\ &\cdot \left(a\sin\psi - a\cos\psi + b\sin\psi \right) + 2c_y \left(a\sin\psi - e\cos\varphi + c\cos\psi \right) \cdot \left(a\sin\psi - a\cos\psi \right) \end{aligned}$$

As a result of the mathematical transformations when using the Lagrange formula of the motion equation of t.

The vibrating platform (Figure 1) takes the form:

$$\begin{aligned} \mathrm{x} &= \frac{\mathrm{Pg}\cos\omega\mathrm{t}}{2\mathrm{G}_2(\omega^2 - \omega_0^2)}(2\cos\omega\mathrm{t} - \mathrm{e}^{\omega_0 \mathrm{t}} - \mathrm{e}^{-\omega_0 \mathrm{t}}) + \frac{1}{2\omega_0^2}(2 - \mathrm{e}^{\omega_0 \mathrm{t}} - \mathrm{e}^{-\omega_0 \mathrm{t}}) \\ \mathrm{z} &= \frac{\mathrm{Pg}\cos\omega_0\mathrm{t}}{\omega_0 \mathrm{G}_2(\omega^2 - \omega_0^2)} + \left(\frac{\mathrm{G}_2}{\mathrm{C}_\mathrm{z}} - \frac{\mathrm{G}_1}{\omega_0^2}\right)\cos\omega_0\mathrm{t} + \frac{\mathrm{G}_1}{\omega_0^2} + \frac{\mathrm{Pg}\sin\omega\mathrm{t}}{\mathrm{G}_2(\omega^2 - \omega_0^2)} \end{aligned} \tag{3}$$

Where: $P = (m_1 + m_2)\ g$ – *the* mass of moving parts of vibrating drive;

Where:
$G_2 = m_2 g$; $G_1 = m_1 g$; ω and ω_0 – respectively forced and free speed; t – processing time; C_z – stiffness of elastic elements in the direction of the z-axis.

The first three terms of this equation describe the own system oscillations, the fourth term describes the forced oscillations.

The working oscillation amplitude of the executive bodies of the vibrating massager is:

$$\mathrm{A_P} = \frac{\mathrm{Pg}}{\mathrm{G}_2(\omega^2 - \omega_0^2)} \tag{4}$$

The equation of motion for the working drum at the steady operation mode of the vibrating massager is:

$$x = \frac{2Pg\cos 2\omega t}{G^2\left(\omega^2 + \omega_0^2\right)} \tag{5}$$

$$x = \frac{2Pg\sin \omega t}{G^2\left(\omega^2 - \omega_0^2\right)} \tag{6}$$

Then the speed components of the working container take the form:

$$\vartheta_x = \dot{x} = \frac{2Pg\omega\sin 2\omega t}{G^2\left(\omega^2 - \omega_0^2\right)} \tag{7}$$

$$\vartheta_z = \dot{z} = \frac{2Pg\omega\cos 2\omega t}{G^2\left(\omega^2 - \omega_0^2\right)} \tag{8}$$

The absolute speed value of the working container is:

$$\vartheta_B = \sqrt{\vartheta_x^2 + \vartheta_x^2} = \frac{Pg\omega}{G^2\left(\omega^2 + \omega_0^2\right)}\sqrt{\cos^2 \omega t - 4\sin^2 2\omega t} \tag{9}$$

The oscillation amplitude of the working container is:

$$A_p = \frac{Pg}{G^2\left(\omega^2 - \omega_0^2\right)} = \frac{m^1 e\omega^2}{m^2\left(\omega^2 - \omega_0^2\right)} \tag{10}$$

Using the formula (10), we construct the amplitude-frequency characteristics of the oscillation system under study. In this case, we vary the ratio of m_1/m_2 (Figure 2) for a fixed value of the eccentricity *e* of the drive shaft; as well as the amplitude-frequency characteristics of the container of the vibrating massager when changing the eccentricity e at a fixed ratio m_1/m_2 (Figure 3). In the scientific works **[33]**, **[34]**, **[35]** by the authors, only calculations of the amplitude-frequency characteristics of similar systems were carried out without constructing graphical dependencies, which does not provide an opportunity. In the works of the following authors **[36]**, **[37]**, **[38]**, the variation of the mass ratio at a fixed value of the eccentricity of the drive shaft was not taken into account. The amplitude-frequency characteristics of the container were determined only at one fixed mass ratio, which can lead to the equipment entering unregulated modes of operation.

The value of the angular speed ω=28 rad/s can be noted in the resonant processing mode, which is characterized by the difficulty of maintaining the operating amplitude and frequency under conditions of low-frequency oscillations of the container with the mass of the meat raw materials being processed. In scientific manuscripts **[39]**, **[40]**, **[41]**, various ranges of angular velocities in the range from 10 to 20 rad/s were studied, where the resonant mode of processing is also noted. In my opinion, this is true in the fast-operating modes, the equipment will simply fail, and the technological production process will be disrupted. Further to the value of ω = 45 – 50 rad/s, there is a transient mode, after which there is a resonant mode. The latter is characterized by the constancy of the operating amplitude, which contributes to the reduction of dynamic loads on the support nodes, due to which it is possible to increase the service life of the equipment and reduce the costs of electron carriers, which will lead to a decrease in the cost of the final product. In scientific works **[42]**, **[43]**, the authors investigated speed ranges from 55 to 60 rad/s during salting of pork and chicken, where transient processing modes are indicated without specifying constant amplitudes. Still, such characteristics of the work process will contribute to a significant increase in the workload on workers' organs of the machine, which can lead to a violation of the technological process — for example, abnormal salting of raw materials or mechanical damage to the finished product.

For statistical optimization of the received dependences of the kinematic characteristics of the Vibro massage, we note the following parameters: the amplitude of oscillations A of the working container, which affects the penetration of mechanical vibrations deep into the stuffing mass; frequency of oscillations ω, which determines the intensity of vibrations; the eccentricity of the drive shaft e, which determines the amount of dynamic impact due to contact interaction within the technological mass of the product; the ratio of rotating and oscillating m_1/m_2, which determines the metal capacity and, accordingly, the energy capacity of the machine's vibration exciter. Variations of these parameters are accepted within the following limits: e = 1 – 5 mm, ω = 0 – 120 rad/s, angle of rotation of the drive shaft φ = 0 – 3600, m_1/m_2 = 4 – 13 .

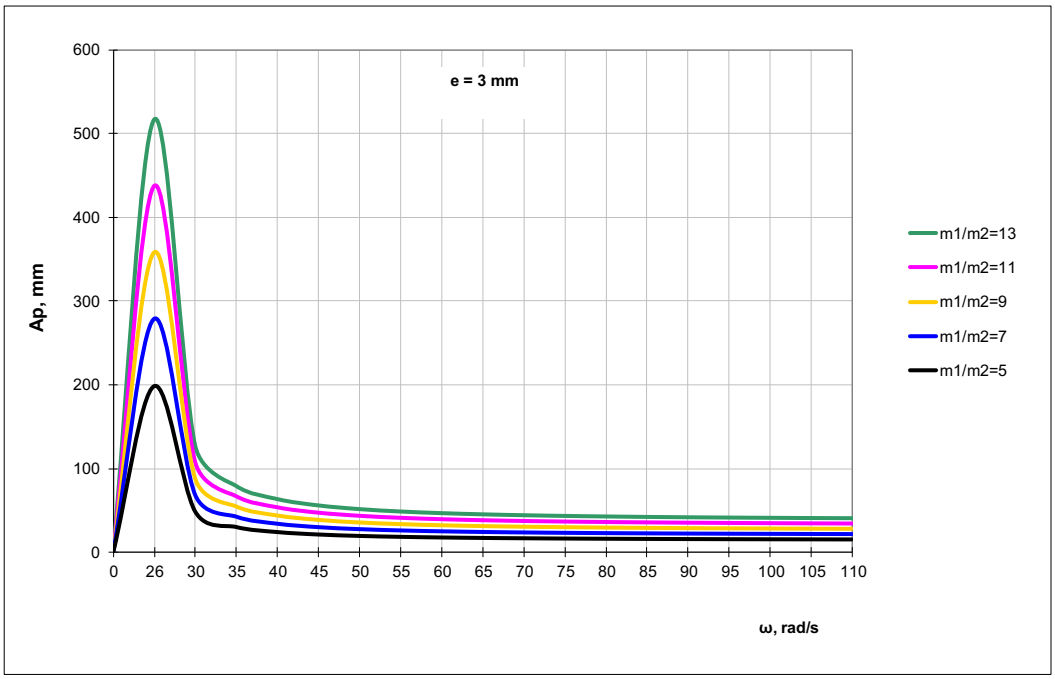

Figure 2 Amplitude-frequency characteristics of oscillation system under study when changing ratio m_1/m_2 and constant value of eccentricity e.

Using the formulas (5) and (6), we construct the trajectories of horizontal (Figure 4) and vertical (Figure 5) motions of the center of mass of the working container of the oscillation system under study. In this case, we vary the ratio of m_1/m_2 at a constant value of eccentricity e = 2 mm.

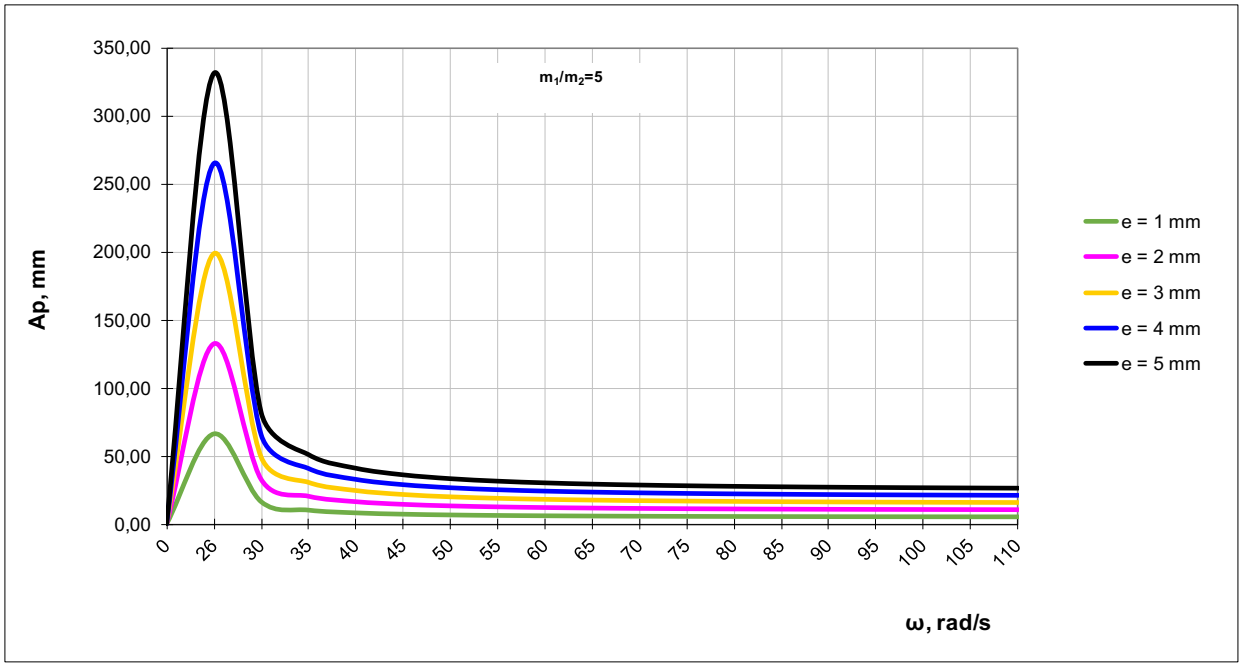

Figure 3 Amplitude-frequency characteristics of oscillation system under study when changing the value of eccentricity e and constant ratio m_1/m_2.

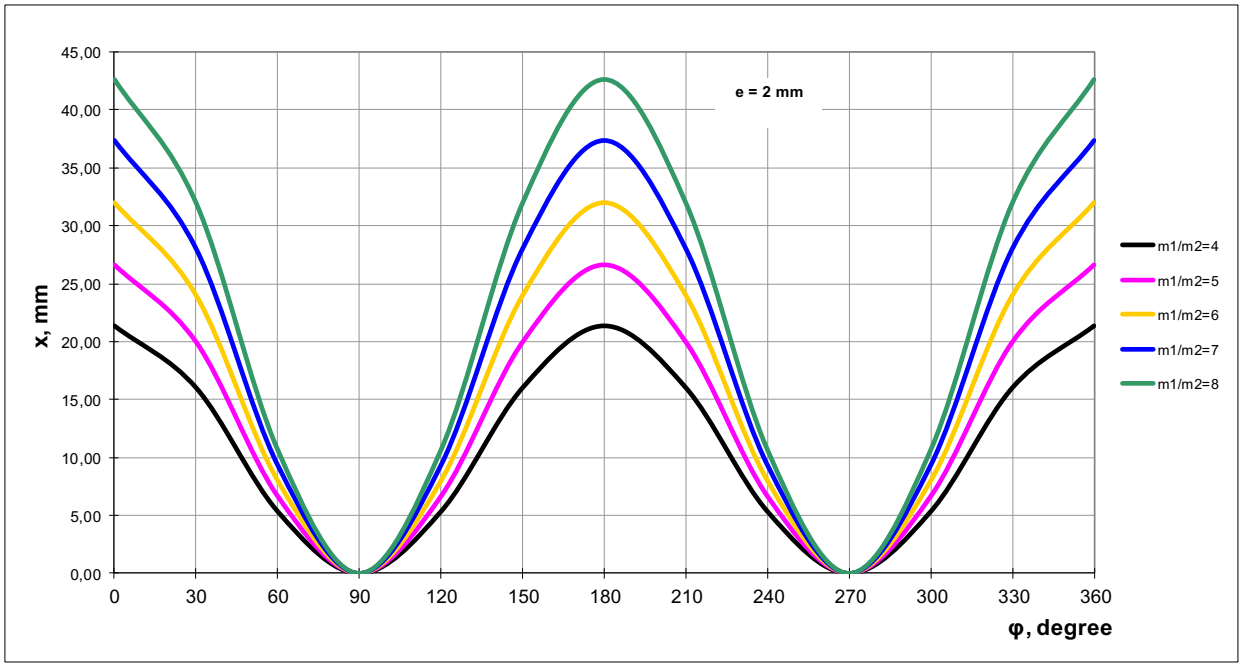

Figure 4 Trajectories of horizontal motion of the center of mass of working container of oscillation system under study when changing ratio of m_1/m_2 and constant value of eccentricity e = 2 mm.

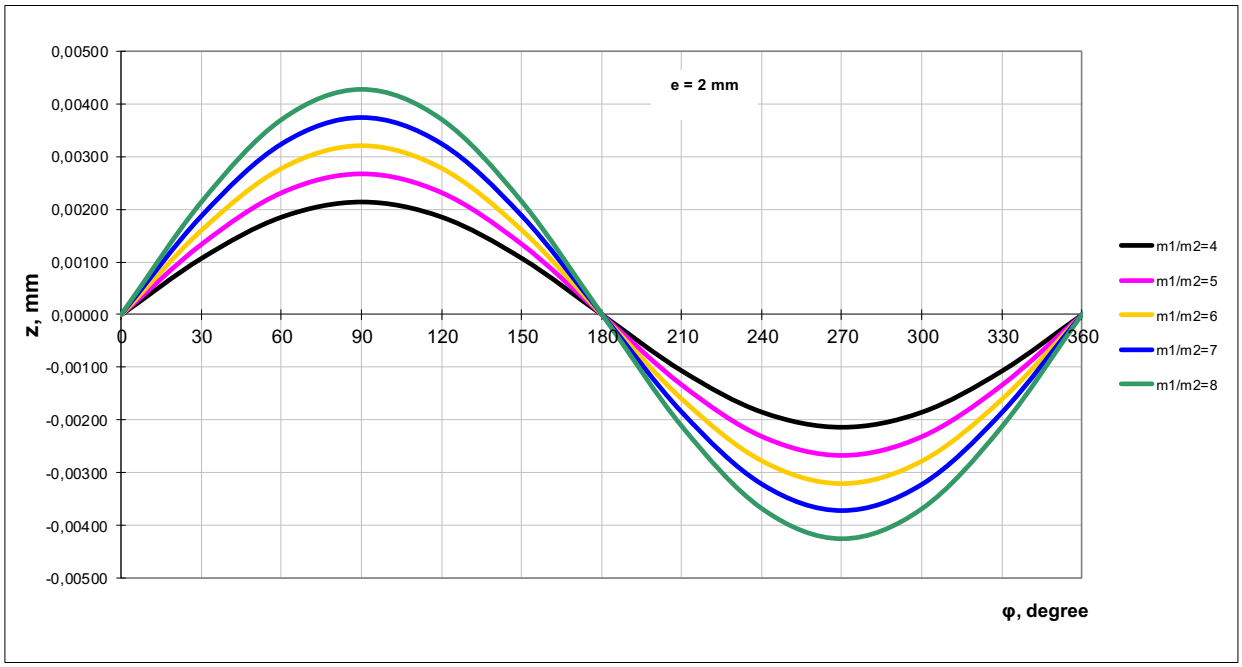

Figure 5 Trajectories of vertical motion of the center of mass of working container of oscillation system under study when changing ratio of m_1/m_2 and constant value of eccentricity e = 2 mm.

Using the formulas (7) and (8), we construct the speed characteristics of the oscillation system under study. In this case, we vary the ratio of m_1/m_2 (Figure 6) and assume a constant value of eccentricity e = 2 mm.

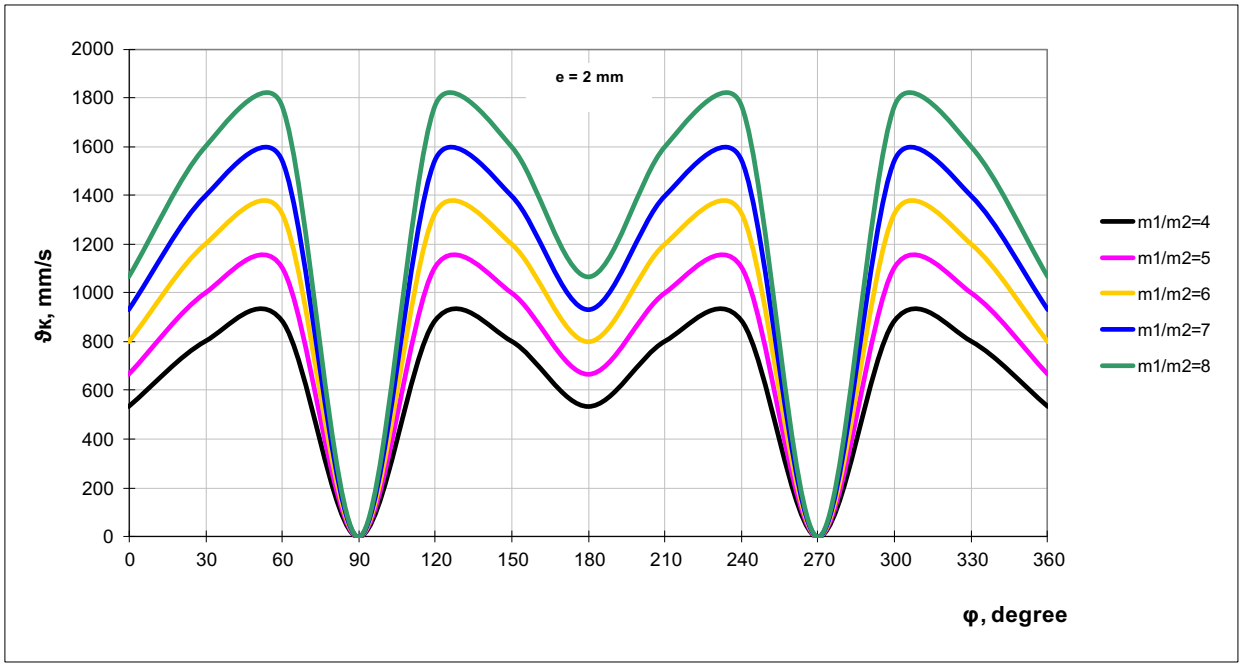

Figure 6 Speed characteristics of the center of mass of working container of oscillation system under study when changing ratio of m_1/m_2 and constant value of eccentricity e = 2 mm.

The dependences of the kinematic characteristics of the oscillation system under study (Figures 2 – 6) indicate that at angular speed ω = 50 rad/s, there is a stabilization of the processing operation mode of the meat products at the value of the oscillation amplitude A = 6.5 mm. Therefore, in a further graphic-analytical study, we believe that the operating parameters of the vibrating massager are $\omega = \omega_p = 50$ rad/s and $A = A_p = 6.5$ mm.

Based on the research results during the development of technology, a brine containing papain enzyme was used for whole-muscle products from ostrich meat. As the main raw material, ostrich thigh meat, obtained after slaughtering the bird at 12 months, was chosen. The enzyme papain was added to the composition of the multicomponent brine in the amount of 0.07% of the mass of the brine, which corresponded to 0.015% of the mass of unsalted raw materials at 30% injection. The composition of syringe brine is shown in Table 2.

Table 2 Composition of injection brine, kg.

Name component	Content of salting ingredients (per 100 kg of brine)			
	control	Brine No 1	Brine No 2	brine No 3
Water, kg	91.2	90.9	92	91.93
Salt, kg	7.5	7.5	4.7	4.5
Sodium nitrite (in the form of 2.5% solution), kg	0.3	0.3	-	-
Sugar, kg	1	1.	-	-
Phosphates, kg	-	0.3	-	-
Papain enzyme	-	-	-	0.07
Functional supplement, kg	-	-	3,5	3,5
Total:	100	100	100	100

Thus, the penetration of saline active substances in the physical and chemical composition of meat tissues and their interaction with proteins changes the product, determining the main properties of salted meat (swelling, consistency, viscosity, etc.). Changes in meat proteins during salting are accompanied by an increase in bound moisture in the product, the product at further heat-treated retains moisture better and rises product output.

As a result of the research, the salt content in the finished product in the control sample of ham is 3.45% due to the loss of moisture during traditional mixing. The experimental sample is 3.3% due to the improved massaging process. Similar series of experimental studies are described in scientific papers **[45]**, **[46]**, **[47]**, where cattle and pig meat were used as raw materials. Still, the scientific teams carried out the salting process without using the massaging process, therefore, in our opinion, the salt content in such samples may be overestimated, which in turn can negatively affect the organoleptic indicators of finished products.

CONCLUSION

1. The value of the angular speed of the drive shaft in the range of $\omega_p = 10 – 28$ rad/s corresponds to the resonant operation mode of the container with the technological masses, which creates difficulties in controlling the operating mode sustainability and increases the load on the executive bodies of the device.
2. If the value of the drive shaft's angular speed exceeds $\omega_p = 45 – 50$ rad/s, there is a stabilization of the oscillation amplitude for almost all ratios m_1/m_2, which increases the durability of the support units of the drive.
3. The effective parameters for the operating mode of the container of the vibrating massager include the following: the vibration amplitude $A_p = 6.5$ mm, the eccentricity of the drive shaft e = 2 mm, and the mass ratio of the container and the kinematic vibrator $m_1/m_2 = 5$.
4. Under the selected regime parameters of the Vibro massage process, introducing the functional additive and enzyme papain with a content of 27.64% in the experimental sample improves the taste properties of the developed ham products, particularly, juiciness.

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The effect of technological parameters on functional, technological and physicochemical indicators of horse meat minces with added chicken combs

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ABSTRACT

This study aimed to determine the effect of technological parameters of the production of horse meat minces with the addition of protein-oil emulsion from chicken combs on the functional, technological and physicochemical indicators. Chicken combs were pre-treated with bacterial concentrate to improve their properties. Experimental approach: The ultimate shear stress and technological indicators – water holding capacity and oil holding capacity – were determined to set the optimal time for cutting raw materials. Physicochemical analyses of the meat minces were conducted. Results and conclusions: The research results have shown that the cutting time significantly affects the meat minces' rheological, functional and technological indicators. The optimum mixing time for meat minces is 6 min. Adding a protein-oil emulsion from biotechnologically processed chicken combs, cottonseed oil, and water into the minced horse meat does not significantly affect the nutritional value. Adding 15 – 20% protein-oil emulsion (POE) is recommended to get minced meat with optimal rheological parameters. Novelty and scientific contribution: The research results allow the rational use of poultry by-products.

Keywords: meat mince, chicken comb, horse meat, protein-oil emulsion, bacterial concentrate

INTRODUCTION

Industrial poultry breeding all over the world is gaining momentum. Complexes of various capacities appear in many countries, using modern technologies and highly productive crosses **[1]**, **[2]**, **[3]**. Today, most poultry processing enterprises face the problem of maximum and rational use of poultry by-products rich in proteins **[4]**, **[5]**, **[6]**. The stomach, legs, and combs are by-products obtained during the primary processing of poultry and are used to a limited extent in modern production. Various chicken by-products have been reported to contain significant amounts of proteins, enzymes, and lipids **[7]**. Their constituent connective tissue is a source of fodder protein and food protein **[5]**. Therefore, it is important to expand the use of collagen-containing poultry by-products. One of the fundamental ways to improve the properties of food-grade raw materials is their preliminary biotechnological processing **[8]**, **[9]**. In the process of directed biomodification, not only optimal functional and technological indicators of raw materials are formed, but also the biological value increases **[10]**. In technological practice, it is necessary to adjust the functional properties of meat systems when using different types of meat — the structure of the minced meat changes when the meat ingredients are combined. The interaction between individual structural elements is determined by the chemical composition, biochemical parameters, temperature, dispersion, and technological factors **[11]**. The muscle and connective tissue proteins are minced meat's main structural units. The quantitative content of protein in the system, its qualitative composition, and processing conditions predetermine the degree of stability of the resulting meat systems and affect the structural and mechanical properties **[12]**, **[13]**, **[14]**. It is known that horsemeat has increased rigidity and is also a source of acid radicals (pH 5.65–5.95), which disrupts the acid-base balance in the human body, shifting it to the acidic side.

[15]. To improve the structural and mechanical characteristics of minced meat products based on horse meat, it is proposed to use a protein-oil emulsion from chicken combs pre-treated with a bacterial concentrate. Chicken combs contain more salt-soluble proteins, which can affect the hydrophilicity of the protein fraction, which causes a higher pH than in horse meat. Thus, combining various raw materials in the product allows for optimal-quality meat systems **[16]**, **[17]**, **[18]**, **[19]**.

The objective of this study was to determine the effect of technological parameters of the production of horse meat minces with the addition of the protein-oil emulsion from chicken combs on the functional, technological and physicochemical indicators.

Scientific Hypothesis

The scientific hypothesis of this work is the experimental determination of the effect of technological production parameters of the horse meat minces with the addition of the protein-oil emulsion from chicken combs on the formation of the functional, technological and physicochemical indicators.

MATERIAL AND METHODOLOGY

Samples

Horse meat, produced at the Arkalyk (Petropavlovsk, Republic of Kazakhstan) farm, was used chilled to make minced meat. The proximate composition of the horsemeat was as follows (%): protein – 19.5, fat – 9.9, moisture – 69.6, and ash – 1.0. To prepare the protein-oil emulsion (POE), chicken combs and cottonseed oil were used. The combs were separated from the heads of 42-day old broiler chicken immediately after the poultry slaughter at Ardager in Semey (Republic of Kazakhstan). The resulting combs were cooled at 2 ±2 °C and transported chilled to the laboratory to obtain the POE. The cottonseed oil (Altyn may, Bagdaulet-1, Republic of Kazakhstan) was purchased locally and stored at room temperature until the POE was obtained.

Chemicals

All chemicals were purchased from the company Alfa-lab (Semey, Republic of Kazakhstan) and had the quality of analytical purity.

Animals and Biological Material

For biotechnological treatment, we used the bacterial concentrate Bifilakt-AD (Experimental Biofactory, Uglich, Russian Federation), which includes the following types of bacteria: *Lactococcus lactis subsp. Diacetilactis, Streptococcus thermophiles, Lactobacillus acidophilus, Bifidobacterium bifidum, B. longum*, and *B. teenis*.

Instruments

The grinder (Fimar 32/RS Unger, Italy), cutter (CFS/GEA Cutmaster), devices DK6, UDK129 (VELP SCIENTIFICA, Italy), centrifuge (Orbita, Russian Federation), consistometer (KF40, Brookfield), structometer ST-2 (Quality Laboratory, Russian Federation) were used in this research.

Laboratory Methods

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 **[20]**.

– moisture was determined by drying the sample in a metal bottle at a temperature of 105 °C to constant weight **[21]**.

– Total fat was determined via the Soxhlet method **[22]**.

– The ash content was determined via the dry ashing method.

The water-holding capacity (WHC) was determined by the gravimetric method proposed by Zhang et al. **[23]**.

The oil-holding capacity (OHC) was determined by the method described by Salavatulina **[24]**.

The meat minces' ultimate shear stress (USS) was determined on the consistometer (KF40, Brookfield).

The adhesion was determined on the structometer ST-2 (Quality Laboratory, Russian Federation) by measuring the force of separation of a specially selected plate from the test specimen. The measure of adhesion is the amount of peel force per unit contact surface **[25]**.

Description of the Experiment

Sample preparation: To increase the biological value of the combs, they were pre-treated with a bacterial concentrate Bifilakt-AD (Experimental Biofactory, Uglich, Russian Federation).

Before processing, the bacterial concentrate was activated in pre-sterilized skim milk at 37 ±1 °C for 5 – 6 h until the acidity reached 80 °T. Chicken combs were passed through a grinder (Fimar 32/RS Unger, Italy) fitted with a plate with 3-mm diameter holes, and activated sourdough was added to them in 20% of the raw material weight. After mixing, the combs were kept in a thermostat at 37 ±1 °C for 3 – 4 h until the raw material was tenderized.

After biotechnological treatment, chicken combs were finely ground in a cutter (CFS/GEA Cutmaster) with water and cottonseed oil in a ratio of 75:10:15, respectively, to obtain the POE. During the preparation of test samples of the minces, the POE was added to the chopped horse meat in an amount of 10 to 25% (Figure 1).

10% POE 15% POE 20% POE 25% POE control

Figure 1 Samples of minced horse meat.

Number of samples analyzed: 5 batches of samples were made for research: a control sample of minced horse meat, 4 test samples with the addition of 10, 15, 20 and 25% POE to minced horse meat.
Number of repeated analyses: each of analyse was measured three times.
Number of experiment replication: analyses were carried out in five replicates.
Design of the experiment: The change in the chopped horse meat's functional, technological, and physicochemical indicators was studied.

The WHC was determined by the gravimetric method. In centrifuge tubes (Orbita, Russian Federation), samples were centrifuged at 70 × g for 15 min at 4 °C. Then the samples were heated at 75 °C and weighed after separating the supernatant. After ageing at 4 °C for 24 hours, the samples were centrifuged again, the resulting supernatant was separated, and samples were weighed again. Water-holding capacity (%) was calculated by Equation 1:

$$WHC\ (\%) = [(m2 - m)/(m1 - m)] \times 100\% \quad (1)$$

Where:
m – the mass of the sample in g; m1 is the mass of the sample after heating and decanting the supernatant in g; m2 – the mass of the sample after centrifugation and removal of the resulting supernatant in g.

The OHC was determined as a difference between the oil mass fraction in the mince and the quantity of oil, separated by heat treatment on the water bath.

The meat minces' ultimate shear stress (USS) was determined on the consistometer (KF40, Brookfield). The container for the product was filled with the test sample, the surface was levelled with a spatula, and its level was set relative to the zero division of the instrument scale. The depth of immersion of the cone in the product (mm) was determined by a scale, setting, and selecting a certain weight.
The USS (Pa) was calculated by Equation 2:

$$USS = K \times M/h2 \quad (2)$$

Where:
K is the cone constant, for $\alpha = 60°$, K = 2.1 m/kg; M is the cone's mass with a rod and additional weight in kg; h is immersion depth of the cone, m.

The adhesion was determined on the structurometer ST-2 (Quality Laboratory, Russian Federation) by measuring the force of separation of a specially selected plate from the test specimen. The measure of adhesion is the amount of peel force per unit contact surface **[18]**.

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 offered by Assaad et al. **[26]**.

RESULTS AND DISCUSSION

The meat minces have a plastic-viscous structure characterized by a set of properties: the ultimate shear stress **[13]**, **[27]**. The consistency of the meat minces directly depends on the moisture and fat content and the degree of grinding **[28]**, **[29]**. The ultimate shear stress is most sensitive to changes in technological and mechanical factors. Therefore, this indicator is used to evaluate the minced meat during manufacture. Pelenko et al. **[30]** determined the effect of processing time of the grinding, forming, or mixing on the properties of minced meat. Kabulov et al. **[31]** detected the effect of combined processes on the quality of minced meat. The amount and quality of protein in the food system and processing conditions affect the degree of stability of the resulting meat systems and structural and mechanical properties **[32]**. The resulting product's nutritional value, processing, sensory and rheological characteristics vary depending on the added ingredients, water, and fat content **[12]**, **[33]**. Kumar et al. **[34]** noted that plant oils in the composition of meat products significantly affect the technological properties of meat emulsion. It is important to set rheological indicators. Rivas and Sherman **[35]** noted that water-soluble meat proteins exhibited important viscoelastic properties. Water-soluble proteins demonstrate strong adhesion adsorbed on the surface of contacting oil droplets when oils are added to the meat system. The results of determining the USS of the test samples of the minces with the addition of the POE are shown in Figure 2. Based on experimental data. The mixing process is divided into 4 periods. In the 1st period, particles are crumpled, moisture transitions from a free to a more bound state, and the structure of the minced meat is strengthened. With further mixing (2nd period), the temperature of the minced meat increases. The meat system is aerated, and the constituent particles of the minced meat are emulsified **[36]**. In the third mixing period, further strengthening of the structure occurs, and the USS decreases (Figure 2). At the same time, the fat droplets merge with the protein fraction, strengthening the structure; more persistently retaining moisture. The fourth period is characterized by a decrease in the values of the USS, which is associated with the loosening of the minced meat.

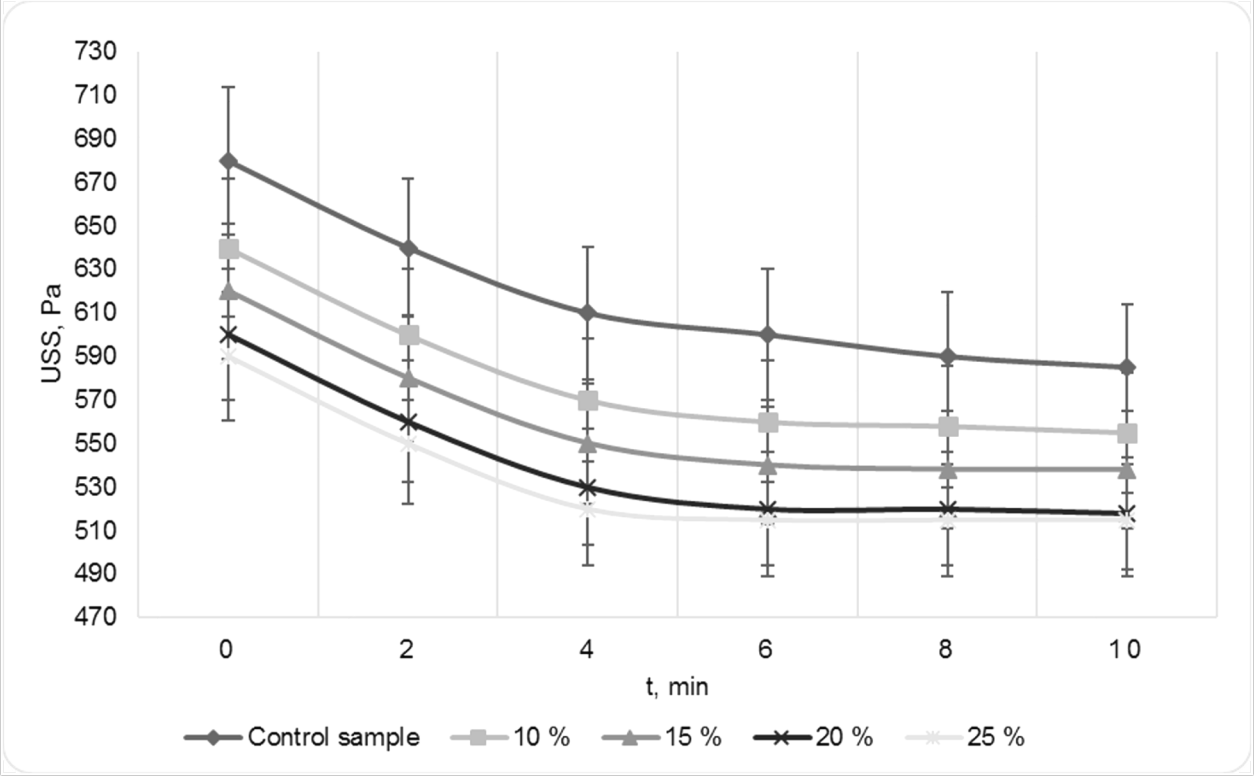

Figure 2 The dependence of the USS of the meat minces on the duration of cutting at different amounts of the POE.

The addition of the POE to the meat minces in an amount of 10 to 25% affects the decrease of USS (Figure 2). The addition of the POE to the meat minces in an amount of 10 to 25% affects the decrease of USS (Figure 2). The USS is significantly ($p \leq 0.05$) reduced up to 6 minutes of mixing, a monolithic structure is formed, and further mixing leads to a slight decrease of the USS. The control sample of the meat minces, consisting of horse meat, showed higher USS values compared to all test samples of the meat minces.

Adhesion is of great importance in forming a monolithic structure of minced meat. This indicator is characterized by the force of interaction between the surfaces of the interacting structural material and the product during

separation. The stickiness of raw meat is caused by the accumulation of salt-soluble proteins on the surface of the meat **[25]**.

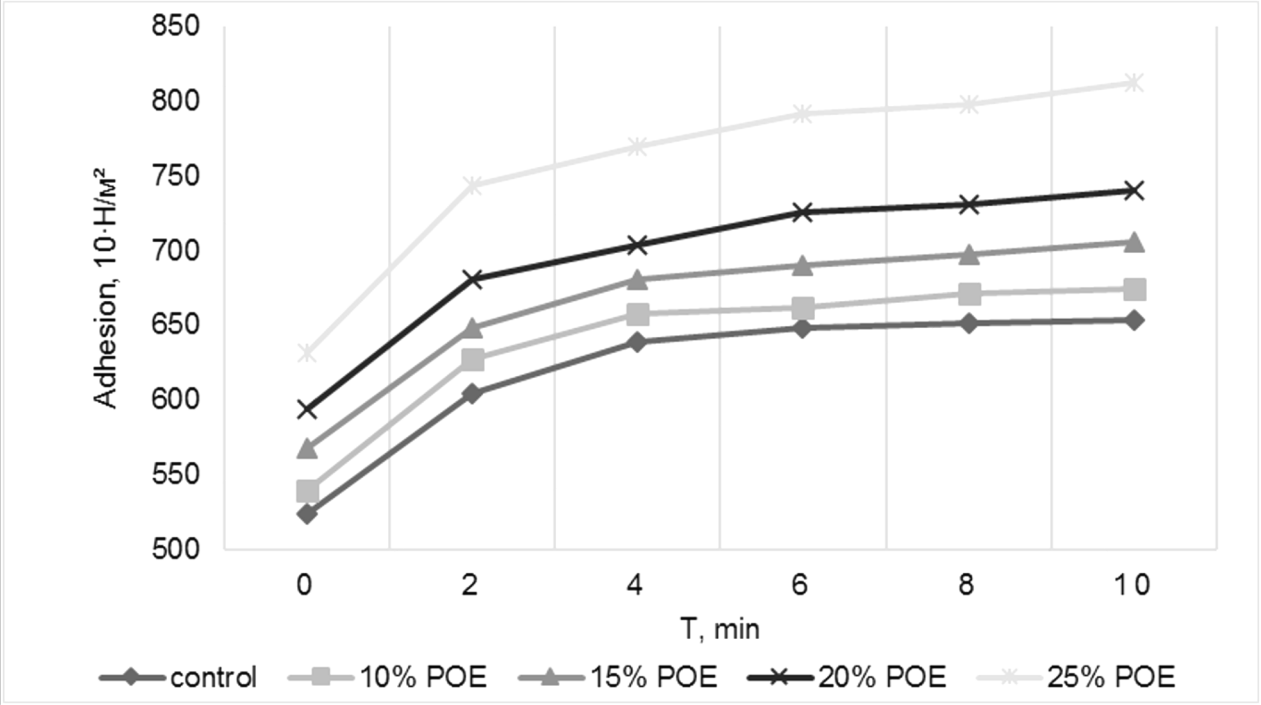

Figure 3 The adhesion of the meat minces is dependent on the duration of cutting at different amounts of the POE.

Figure 3 shows the results relative to the adhesion of the meat minces. Adhesion increases with an increase in the amount of the POE in the formulation. It was also noted that after 6 – 8 minutes of minced meat cutting, adhesion increases insignificantly in all samples. The lowest adhesion values were noted for the control sample, regardless of the cutting time.

Results of rheological studies have shown that an increase in the amount of the POE in the composition of the meat minces leads to a significant decrease in its strength characteristics, an improvement in plasticity, and an increase in stickiness and adhesion.

One of the most important technological properties of meat and minced meat is its WHC **[37]**. Many authors noted that the higher this indicator, the less moisture loss during heat treatment, and the higher the yield of finished products and their sensory characteristics: tenderness, juiciness, and taste **[38]**, **[39]**, **[40]**, **[41]**, **[42]**.

The dependence of the WHC of the test meat minces on the duration of cutting for different amounts of the POE is shown in Figure 4. The results show that the optimal value of the WHC for the test samples of meat minces varied from 90.5 to 93.9 ($p \leq 0.05$) with cutting for 6 min.

The results of Danilov et al. **[9]** indicated improvement of functional and technological properties of heat-treated horse mince with an increasing amount of the paste made from the fermented rumen. The authors noted that defibrillation of modified collagen fibres improves the functional and technological properties **[9]**, **[43]**, **[44], [45]**.

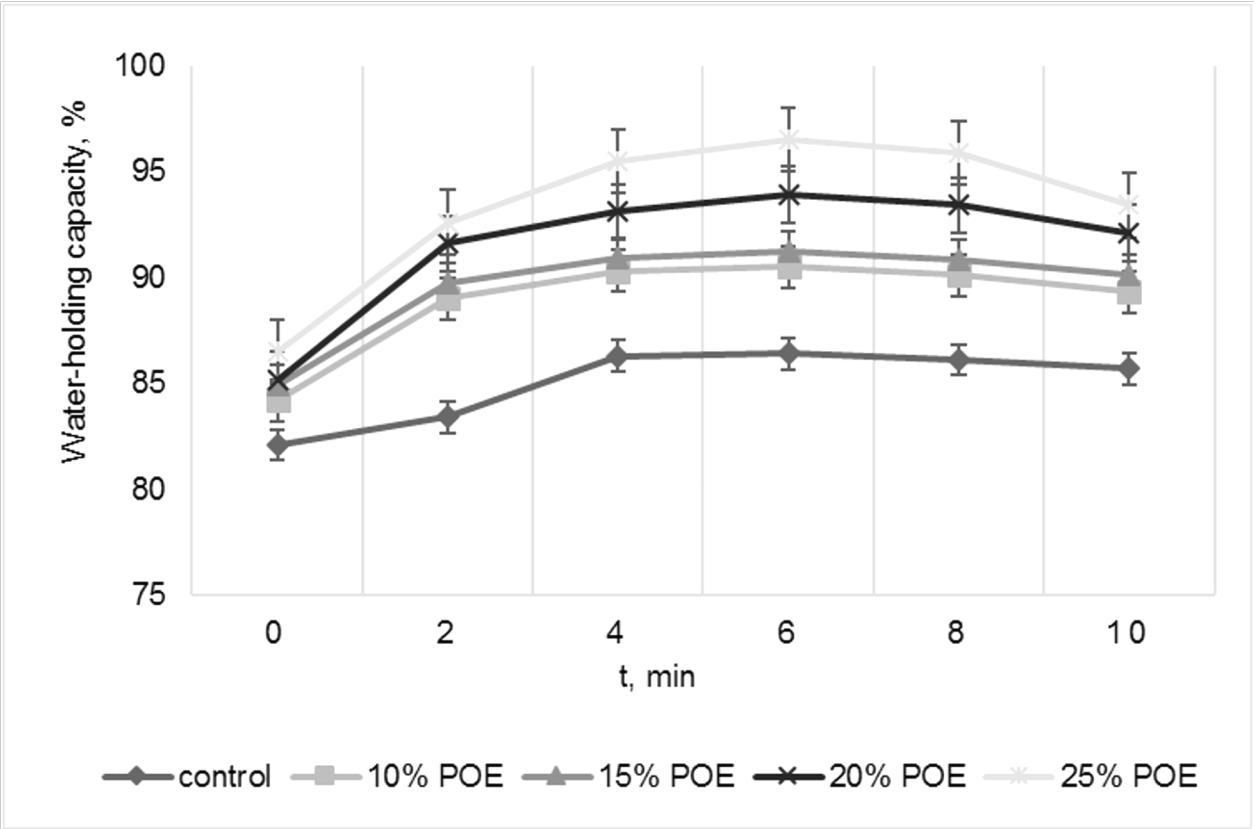

Figure 4 The dependence of the WHC of the meat minces on the duration of cutting at different amounts of the POE.

With an increase in the POE content in the meat minces, there is an increase in the WHC. This can be explained by a decrease in the amount of a well-connected protein-oil-water system in the meat minces, which is stable during heat treatment. The results of determining OHC, presented in Figure 5, showed that adding more than 20% of the POE into the minced meat contributes to a slight decrease in OHC. The same dependence was noted for the cutting time – the optimal interval is from 4 to 6 min. Further cutting leads to local overheating of the minced meat and a decrease in the ability of horse meat proteins to bind the added oil. Gombozhapova et al. **[46]** explain the decrease in moisture-binding and water-retaining capacity with a long time of massaging by partial denaturation of proteins. Kotliar et al. **[47]** determined an increase in water retention capacity of the meat paste sample with added protein-fat emulsion on a vegetable oils basis.

The results of the physicochemical analyses are presented in Table 1. The results show that with an increase in the POE content, the protein content in the meat minces decreases, but POE remains at a high level – 16.3% ($p \leq 0.05$) even in the sample with the addition of 25%. The fat content in the meat minces increased from 10.1 to 15.1% ($p \leq 0.05$). With increasing POE content, the moisture content changed insignificantly in samples with the added 15, 20, and 25% POE. The approximate composition of the POE can explain this: protein – 9.65%, fat – 15.1%, moisture – 74.26%, and ash – 0.99. The given POE indices differ from the horse meat indices, and therefore the replacement of a part of the horse meat in the minced meat with POE leads to a decrease in protein. The sample of pate with the addition of gizzard and 15.36% olive oil was found to be similar in protein content **[48]**.

The research results showed that the rheological and functional-technological indicators of the meat mince change during the cutting process. The amount of POE injected was also affected.

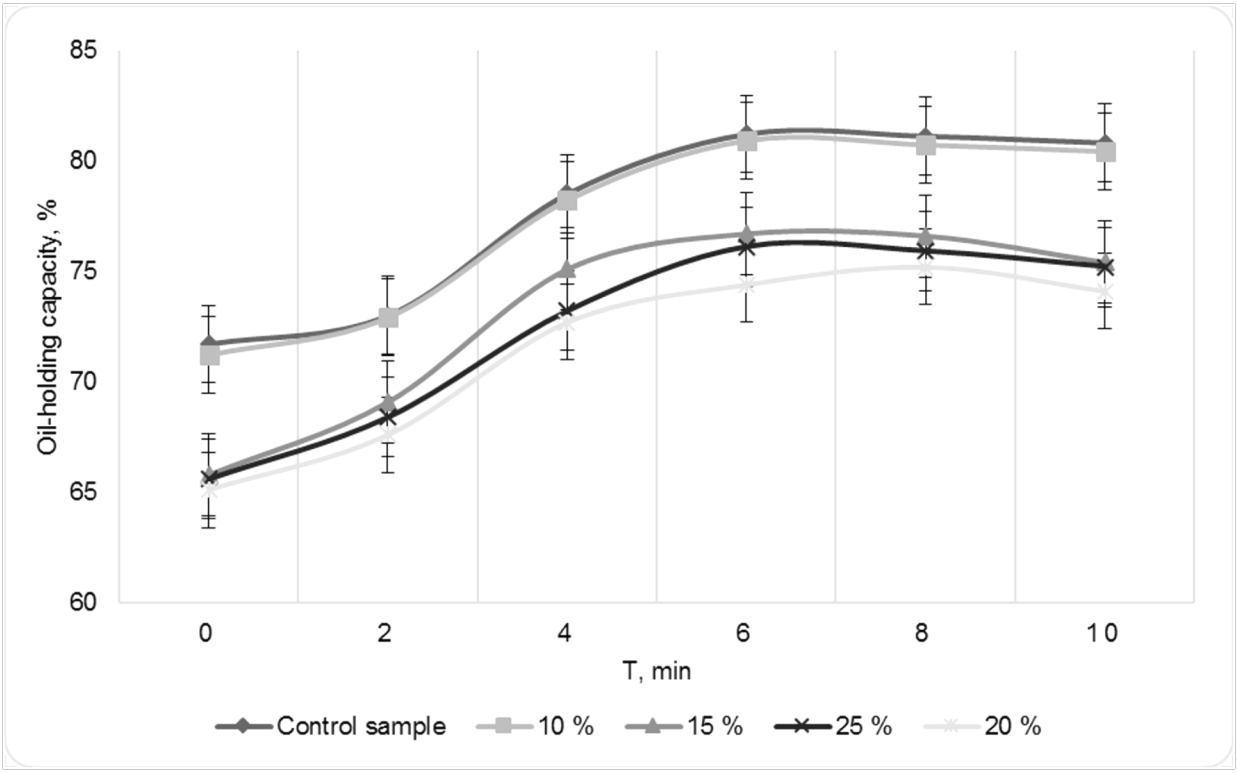

Figure 5 The dependence of the OHC of the meat minces on the duration of cutting at different amounts of the POE.

Table 1 Physicochemical indicators of the meat mince with the addition of the POE.

Indicators	10% POE	15% POE	20% POE	25% POE	Control
Moisture	68 ±0.0872^{b}	67 ±0.0812^{c}	66.8 ±0.112^{c}	66.5 ±0.086^{c}	69.1 ±0.333^{a}
Protein	17.9 ±0.132^{b}	17.4 ±0.129^{b}	16.8 ±0.0843^{c}	16.3 ±0.086^{c}	18.4 ±0.14^{a}
Fat	11.6 ±0.0493^{c}	14 ±0.0927^{b}	14.8 ±0.0922^{a}	15.1 ±0.0374^{a}	10.1 ±0.203^{d}
Ash	1.81 ±0.0102^{b}	1.78 ±0.0202^{b}	1.55 ±0.0224^{c}	1.43 ±0.0152^{d}	2.43 ±0.0129^{a}

Note: Values are means ±SEM, n = 5 per treatment group. Means in a row without a common superscript letter differ (p <0.05) as analysed by one-way ANOVA and the TUKEY.

CONCLUSION

The research results have shown that cutting time significantly affects meat minces' rheological, functional, and technological indicators. The optimum mixing time for meat minces is 6 min. Adding a protein-oil emulsion obtained from biotechnologically processed chicken combs, cottonseed oil, and water into the minced horse meat does not significantly affect the product's nutritional value but, at the same time, allows the rational use of poultry by-products. To obtain minced meat with optimal rheological parameters, adding 15 – 20% POE is recommended.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

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

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The production of wine vinegar using different types of acetic acid bacteria

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ABSTRACT

This work aimed to study the properties of acetic fermentation bacteria during the acetic fermentation of wine. Attention was focused on the ability of the bacteria to metabolize selected organic substances and their suitability for wine vinegar production. For the production of wine vinegar, white wine of the variety Veltliner Green was used. Three variants were established for this experiment. The first variant was fermented with *Gluconobacter oxydans*, the second with *Acetobacter aceti,* and the third variant of vinegar production was carried out by spontaneous fermentation. During the vinegar fermentation, samples were taken at regular 24-hour intervals and subsequently analyzed. The alcohol, acetic, malic, and tartaric acid contents were monitored. The results showed that all variants showed a strong acetic and malic acid increase. Bacteria *Acetobacter aceti* produced the most acetic acid within nine days (25 $g.L^{-1}$). This bacterium also produced the most lactic acid (18 $g.L^{-1}$). Tartaric acid was also produced in all three variants, but not to the same extent as the previous two organic acids. *Acetobacter aceti* was found to metabolize ethanol more rapidly than *Gluconobacter oxydans*.

Keywords: vinegar fermentation, wine vinegar, *Acetobacter aceti*, *Gluconobacter oxydans*, spontaneous fermentation

INTRODUCTION

The earliest mentions of vinegar can be found in the Old and New Testaments. It is believed that since people have known spirits, they have also known vinegar. As early as the fourteenth century, there are references to artisanal vinegar production and the establishment of vinegar makers' guilds. Many types of fruit were used, and they were left to ripen on the tree. The harvested fruit was transferred to uncovered pots, which were left to ferment spontaneously. The resulting fermented liquid was placed in barrels left to ferment spontaneously **[1]**. Vinegar fermentation bacteria are widely used in the biotechnology and food industries. Most strains of vinegar bacteria are used in the food industry to produce important organic acids. In addition to acetic acid, these include gluconic, glucuronic, and propionic acids **[2]**. In biotechnology, vinegar bacteria are mainly used to produce ascorbic acid (vitamin C) or cellulose. The primary acetic acid producers are strictly aerobic bacteria of the genus *Acetobacter*. In addition to ethanol oxidation, these bacteria can oxidize aliphatic alcohols, and some species oxidize carbohydrates. This is a two-stage process of substrate oxidation. First, ethyl alcohol is oxidized to acetaldehyde by the enzyme alcohol dehydrogenase, and in the second stage, acetaldehyde is further oxidized to acetic acid by the enzyme acetaldehyde dehydrogenase **[3]**. The genus *Acetobacter* of the family *Acetobacteraceae* is an aerobic bacterium with a pronounced ability to oxidize ethanol to acetic acid. For this reason, this genus is exclusively used in vinegar fermentation. Adversely, these bacteria are found in the acetation of wine or beer or as an undesirable contaminant in yeast production. Bacteria of the genus *Acetobacter* have a strictly aerobic metabolism so that even a short interruption of the oxygen supply leads to their death in the presence of ethanol. When the ethanol concentration drops, the bacteria oxidize the resulting acetic acid to carbon dioxide and water (so-called over-fermentation) **[4]**. Researchers **[5]** determined values of 0.26 to 0.41 $mg.L^{-1}$ of volatile acids expressed as acetic acid in white Slovak wines of the 2015 – 2017 vintages. These were faultless wines. The legal limit for the content of volatile acids in wine within the EU is above 1 $mg.L^{-1}$ per wine category. Wine with a volatile acid content above 1.4 $mg.L^{-1}$ is already considered diseased. However, it can be used for

the production of wine vinegar. Some strains can grow in solutions of up to 24% vol. ethanol. These microorganisms can also grow at low pH values. These agents are also capable of spoiling must and can cause strong acidification, and 'must disease' in sweet musts, characterized by an unpleasant smell and taste and producing acetaldehyde with polyphenols that cause a milky, colloidal precipitate **[6]**. The genus *Gluconobacter* is characterized by its ability to incompletely oxidize a wide range of carbohydrates and alcohols, with the resulting metabolites, such as aldehydes, ketones, and organic acids, almost entirely excreted into the environment. The enzyme dehydrogenase catalyzes these reactions. These organisms can grow at low pH values and in environments with high concentrations of sugars. Members of this genus are used in modern fermentation processes such as L-sorbose (synthesis of vitamin C) and 6-amino-L-sorbose (synthesis of the antidiabetic drug Miglitol). These species produce other important metabolites: dihydroxyacetone, gluconates, and ketogluconates **[7]**. The most widely used method for vinegar production is the so-called submerged method, which can use either a fed, a continuous, or a discontinuous process. In this method, the bacteria are dispersed throughout the dilution volume. This method produces vinegar in a device known as an acetator. This equipment depends on the correct oxygen content since a short interruption in aeration will kill the bacteria. These vessels are equipped with an agitator and with refrigeration and aeration equipment. As a rule, the dilution contains ethanol at a concentration of 11 – 12% vol. and is enriched with additional nutrients such as glucose, urea, glycerol, yeast, casein, phosphates, magnesium salts, potassium salts, and many trace elements. The process is completed by reducing the ethanol to 0.3% vol. The production cycle time is 48 to 72 hours. This method has a high yield, with daily increments of acetic acid up to 4%. The resulting product must be subsequently purified and filtered due to the high turbidity of the bacterial origin **[8]**. Other methods of producing vinegar from wine by bacteria are the traditional surface Orleans method and the generator method using current and carriers **[9]**. Wine vinegar is one of the most used vinegar in our country. It is formed by natural transformation from wine when exposed to air. Its quality depends on the quality of the wine. The wine used to make vinegar may be of poor quality or even poorly oaked, but it must not be defective or diseased. Bitter wines, wines with a mousy taste, and wines affected by lactic fermentation are unsuitable. The bitter or musty flavour is not removed during vinegar-making but is accentuated. The lactic acid is usually converted into butyric acid, rendering the vinegar inedible. Vinegar from red and white wines can be made separately or in mixtures. However, white wine vinegar must never be mixed with red wine vinegar. The wine used to make vinegar should contain at least 8% alcohol. The wine is usually between 10 and 12% alcohol **[10]**. For higher alcohol contents, it is recommended that the wine be diluted with water before vinegar-making to give an alcohol content of 8% to 9%. In the original recipes, the vinegar should be aged for a long time in barrels. Modern methods try to speed up the process, but this is done at the expense of quality, as the vinegar loses its characteristic aroma. Wine vinegar can be the basis for other vinegar types **[11]**. A necessary condition for the successful course of vinegar fermentation in the production of wine vinegar from the wine of the genus *Acetobacter* is a temperature above 18 °C, a low value of free SO_2 below. 10 $mg.L^{-1}$ and access of oxygen to the wine, e.g. in the form of air (20%) **[12]**. Each genus of vinegar fermentation bacteria has its specific metabolism. Differences in metabolism are even found in different strains of these bacteria. The most significant differences in the resulting metabolites between species are found in the production of higher fatty acids, furan compounds, enol derivatives, and some esters. The significant changes in acetic fermentation products also depend on the bioconversion of acetic acid from ethanol. This is primarily a function of the composition of the wine itself and the fermentation temperatures, aeration intensities, etc. **[13]**.

Scientific Hypothesis

Although several genera of acetic fermentation bacteria are capable of complete acetic fermentation, some of these genera are more suitable for this fermentation because they produce more organic acids and, in some cases, more rapidly. This study looks at which strain is more suitable for wine vinegar production.

MATERIALS AND METHODOLOGY

Samples

A total of three variants were investigated. The first variant was fermented with *Gluconobacter oxydans*, the second with *Acetobacter aceti* and the third variant of vinegar production was carried out by spontaneous fermentation.

Chemicals

Wine: For this experiment, a specific wine had already been produced in 2018. Spontaneous fermentation was used in producing this wine by the reductive method. No malolactic fermentation took place in this wine, of which 50 litres were produced. After the alcoholic fermentation, the must, which was no longer fermenting, was statically racked off, and filtration was not used. Only minimal amounts of sulphur preparations were applied to the wine

during production. Therefore, the wine was a 2018 vintage of the Green Veltliner variety. This wine was characterized by low free and bound sulphur (total sulphur: 24 mg.L^{-1}).

Distilled water

Animals and Biological Material

Gluconobacter oxydans CCM 3618.

Acetobacter aceti CCM 3620.

Instruments

WineScan analyser (Foss, Denmark), microbiological incubators (Liebherr, Germany), compressors (Tetra, Germany).

Laboratory Methods

Spectroscopy: To measure the basic analytical parameters of wine and subsequent vinegar, a WineScan analyzer (Foss, Denmark) was used. This method is based on Fourier transform infrared spectroscopy combined with the partial least squares method. Sampling was carried out with an autosampler, using approximately 50 mL of sample for duplicate measurements, including a pre-wash system.

Description of the Experiment

Sample preparation: The wine was sterilized, and the alcohol level was adjusted before the individual bacteria were inoculated.

Number of samples analyzed: The total number of samples analysed was 81.

Number of repeated analyses: Each sample was analyzed three times, and the result is the average of these measurements.

Number of experiment replications: Each of the three variants was performed in three repetitions. Thus, nine mini-acetators were produced.

Design of the experiment: In vinegar production, three variants were chosen. Among the acetic fermentation bacteria, one representative of acetic fermentation bacteria from the genus *Gluconobacter* and one from the genus *Acetobacter* were selected for this research experiment. The first species was *Gluconobacter oxydans* CCM 3618, and the second was *Acetobacter aceti* CCM 3620. These bacterial species came from the Czech Collection of Microorganisms of the Faculty of Science of Masaryk University in Brno. In the third variant, vinegar fermentation was spontaneous. The wine of the variants inoculated with specific strains of acetic fermentation bacteria was sterilised at 70 °C for 120 minutes before inoculation. Subsequently, it was diluted to 8% alcohol to ensure a smooth vinegar fermentation **[11]**. Each of the different variants was run in three repetitions. Thus, nine 5L mini-acetators were produced by us and oxygenated by a compressor (Tetra, Germany). The acetators were placed in special microbiological incubators (Liebherr, Germany), and each variant had its incubator to avoid cross-contamination. Fermentation was carried out over nine days, with samples taken from each acetator every 24 hours during fermentation. Samples were collected with a glass pipette into 50 mL Eppendorf® mini centrifuge tubes (Sigma–Aldrich, Germany). After collection, all samples were placed in a freezer at -25 °C. After fermentation, all samples were thawed and subsequently analyzed on a WineScan analyzer (Foss, Denmark).

Statistical Analysis

The experiment used statistical analysis of variance (one-way ANOVA), which showed statistically significant differences between the variants. Statistical results are significant at $p = 0.05$. Subsequently, a post-hoc test (Tukey HSD test) was performed for each variant. All statistical evaluation is available from the author.

RESULTS AND DISCUSSION

Monitoring the Acetic Acid Content

According to Table 1, we can observe that *Acetobacter aceti* could produce the most acetic acid of all the variants during fermentation. This bacterium produced up to 25 g.L^{-1} of acetic acid in nine days. The other variants did not achieve such high values. *Gluconobacter oxydans* was able to produce only 15.14 g.L^{-1}. In the variant with spontaneous fermentation, we measured 16.96 g.L^{-1} acetic acid on the last day of fermentation. In Figure 1, we can see that *Acetobacter aceti* produced the most acetic acid and that this bacterium started fermentation earlier than the other variants. The acetic acid production of *Gluconobacter oxydans* was not as intense compared to the previous representative of acetic fermentation. The same applies to the variant with spontaneous fermentation.

Table 1 Changing values of acetic acid (expressed in $g.L^{-1}$) during acetic fermentation.

Acetic acid	*Acetobacter aceti*	*Gluconobacter oxydans*	Spontaneous fermentation
Day No. 1	0.21 ±0.01	0.20 ±0.00	0.20 ±0.00
Day No. 2	4.13 ±0.44	1.64 ±1.22	0. 67 ±0.13
Day No. 3	8.35 ±1.03	2.73 ±1.84	2.84 ±1.97
Day No. 4	10.67 ±1.50	3. 54 ±2.00	3.43 ±2.26
Day No. 5	13.36 ±2.67	5.88 ±2.29	4. 74 ±3.08
Day No. 6	16.06 ±1.53	9.49 ±2.18	6. 76 ±3.75
Day No. 7	17.91 ±2.19	12.08 ±1.77	8.68 ±4.22
Day No. 8	18.65 ±1.38	12. 98 ±0.64	10.79 ±3.09
Day No. 9	24.75 ±3.53	15.14 ±0.70	16.96 ±5.07

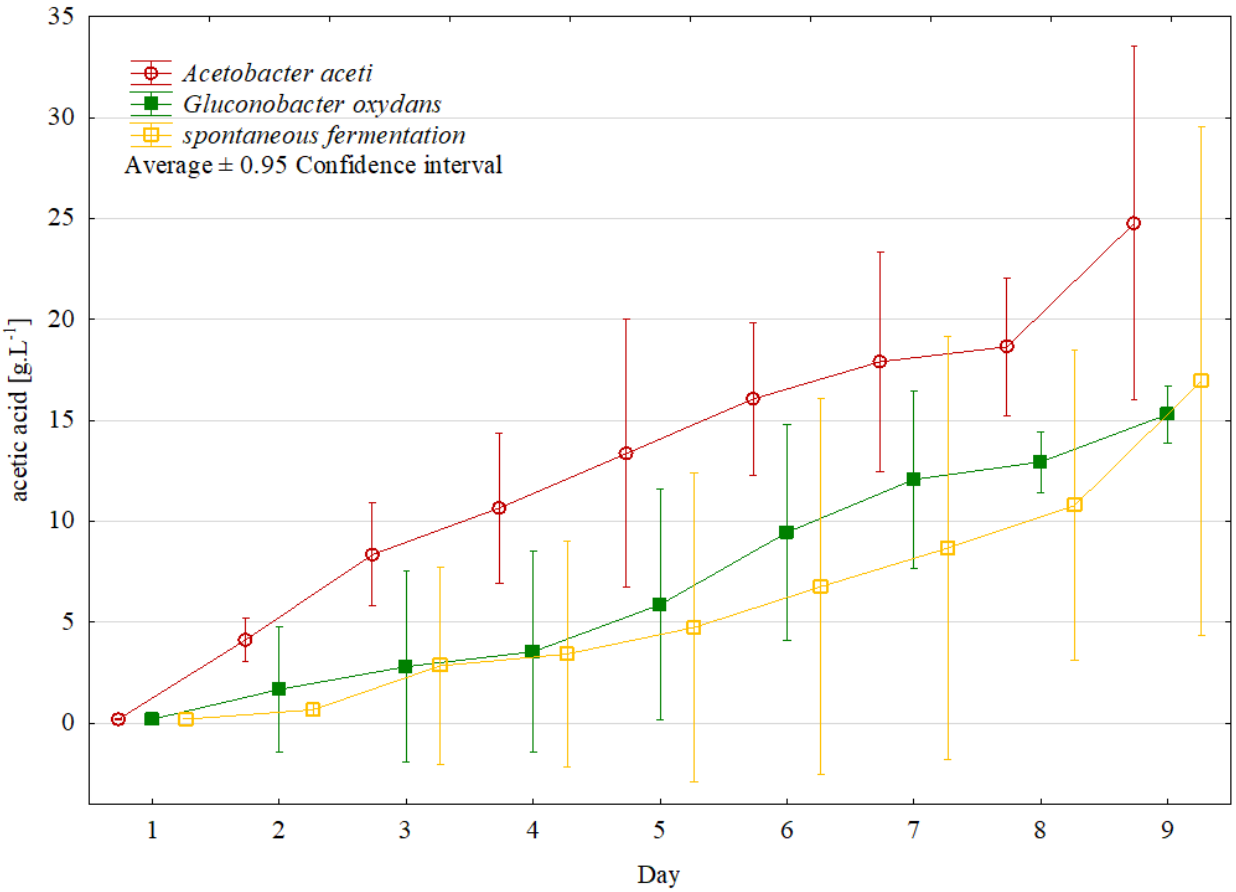

Figure 1 Statistical expression of changing acetic acid values (expressed in $g.L^{-1}$) during acetic fermentation.

Monitoring Malic Acid Content

Table 2 shows the changing values of malic acid during acetic fermentation. The bacterium *Acetobacter aceti* was capable of the highest malic acid production. Similarly to the previous result, *Acetobacter aceti* produced most of this organic acid of all the variants during acetic fermentation. This bacterium produced up to 18.53 $g.L^{-1}$ of acetic acid over nine days. The other variants did not reach such high values. Similar values were measured for the other two variants. *Gluconobacter oxydans* was able to produce only 13.70 $g.L^{-1}$. For the spontaneous fermentation variant, we measured only a slightly lower malic acid value of 13.47 $g.L^{-1}$ on the last day of fermentation. According to Figure 2, Acetobacter aceti again produced the most malic acid, and this bacterium started fermentation at the earliest of the three variants. Compared with the other variants, the production of this organic acid was already much more intense from the first day of fermentation, and higher values were measured.

Table 2 Changing values of malic acid (expressed in $g.L^{-1}$) during acetic fermentation.

Malic acid	*Acetobacter aceti*	*Gluconobacter oxydans*	Spontaneous fermentation
Day No. 1	1.57 ±0.6	1.53 ±0.06	1.50 ±0.00
Day No. 2	2.77 ±0.91	1.60 ±0.20	2.00 ±0.44
Day No. 3	4.93 ±1.55	2.17 ±0.32	3.13 ±1.18
Day No. 4	6.40 ±0.96	2.97 ±0.85	3.53 ±1.27
Day No. 5	7.73 ±1.25	4.20 ±0.82	4.63 ±1.54
Day No. 6	9.93 ±1.98	6.03 ±0.40	5.90 ±1.93
Day No. 7	12.54 ±2.17	7.97 ±0.35	7.10 ±2.09
Day No. 8	14.67 ±3.48	10.97 ±2.91	8.30 ±0.85
Day No. 9	18.53 ±2.22	13.70 ±1.25	13.47 ±1.31

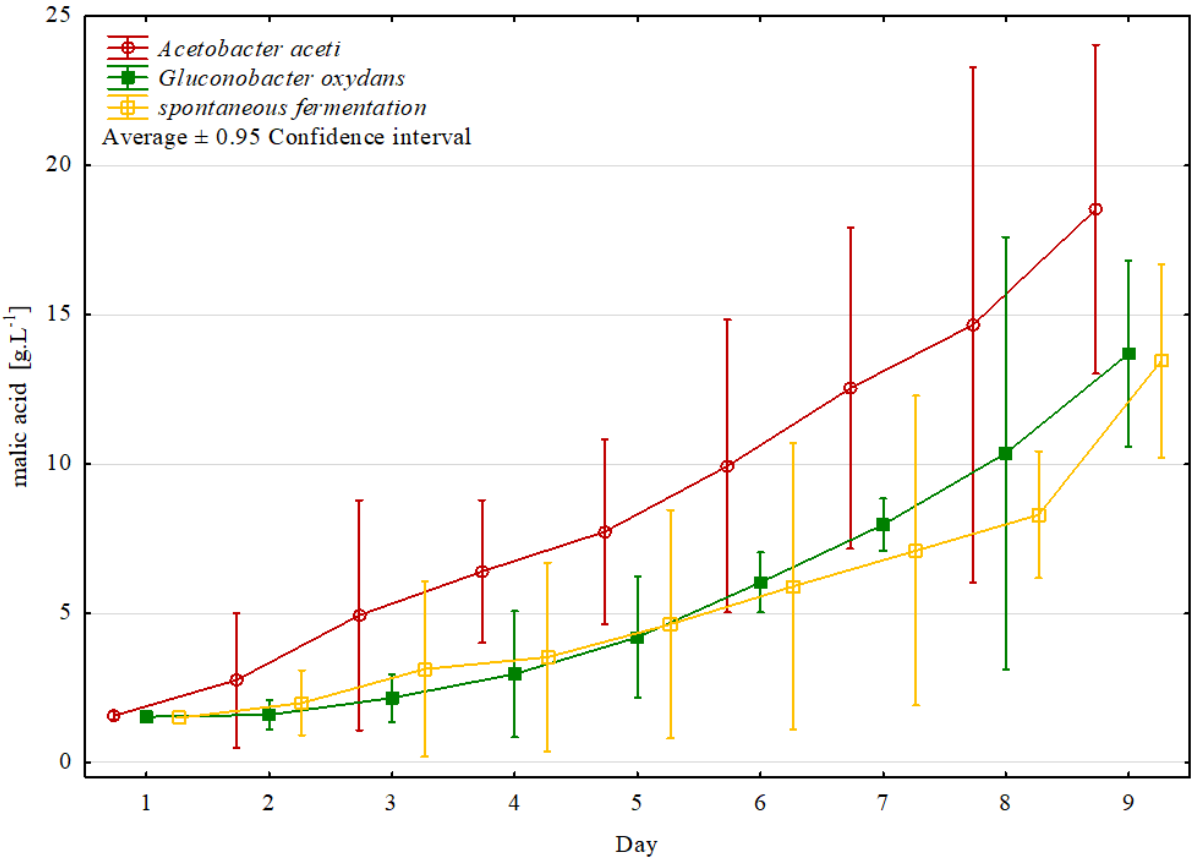

Figure 2 Statistical expression of changing malic acid values (expressed in $kg.m^{-3}$) during acetic fermentation.

Monitoring Tartaric Acid Content

Table 3 summarises the levels of tartaric acid that the acetic acid fermentation plants can also produce. However, compared with the previous organic acids' production, this acid's production is considerably less. Acetic acid bacteria only produces this organic acid to a small extent. In our experiment, after nine days of fermentation, the greatest increase in tartaric acid was observed in the variant fermented by *Acetobacter aceti*. This was an increase from 1.20 $g.L^{-1}$ to only 2.80 $g.L^{-1}$. In Figure 3, the spontaneously fermented variant and the variant fermented by *Gluconobacter oxydans* also showed an increase in tartaric acid. Still, again, the production of this organic acid was lower than in the variant fermented by *Acetobacter aceti*, which showed the greatest increase in measured tartaric acid values.

Table 3 Changing values of tartaric acid (expressed in g.L^{-1}) during acetic fermentation.

Tartaric acid	*Acetobacter aceti*	*Gluconobacter oxydans*	Spontaneous fermentation
Day No. 1	1.20 ±0.00	1.27 ±0.12	1.20 ±0.00
Day No. 2	1.40 ±0.10	1.33 ±0.06	1.37 ±0.12
Day No. 3	1.60 ±0.10	1.37 ±0.06	1.50 ±0.10
Day No. 4	1.70 ±0.17	1.43 ±0.06	1.57 ±0.06
Day No. 5	1.67 ±0.21	1.47 ±0.12	1.80 ±0.10
Day No. 6	2.00 ±0.26	1.73 ±0.12	1.73 ±0.15
Day No. 7	2.20 ±0.20	1.87 ±0.06	1.77 ±0.21
Day No. 8	2.27 ±0.15	1.80 ±0.17	1.90 ±0.17
Day No. 9	2.80 ±0.10	2.00 ±0.10	2.13 ±0.06

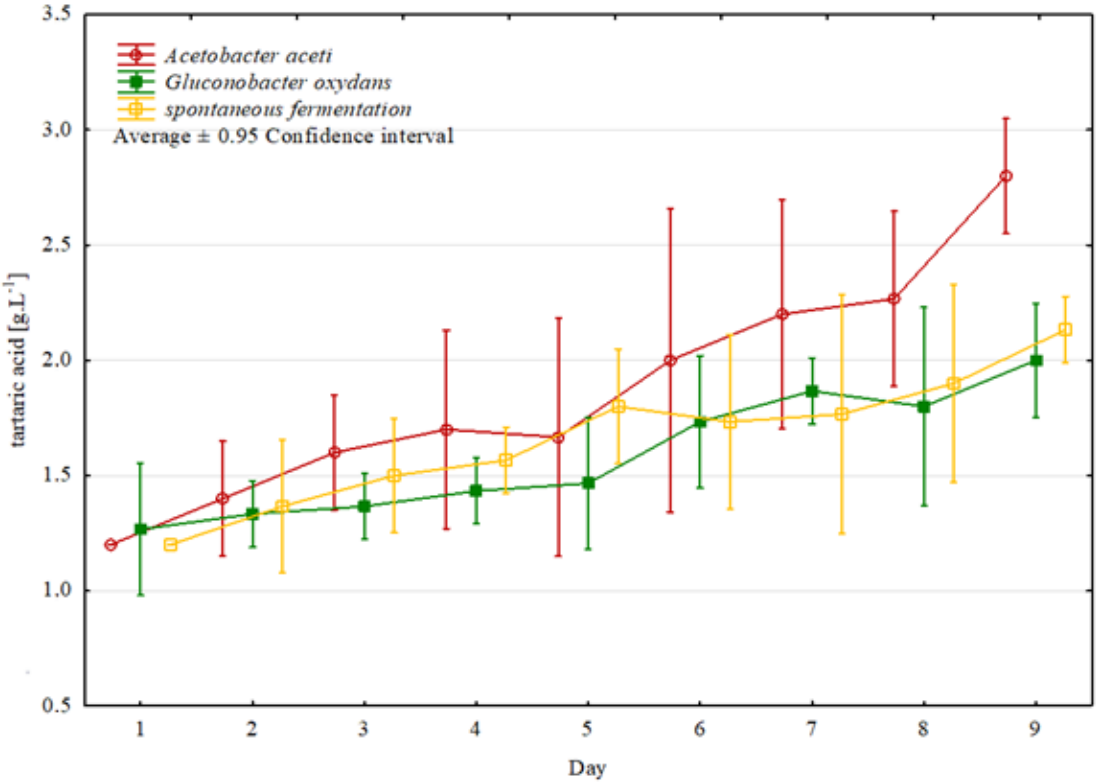

Figure 3 Statistical expression of changing tartaric acid values (expressed in g.L^{-1}) during acetic fermentation.

Monitoring Alcohol Content

Table 4 shows the decreasing values of alcohol content during the vinegar fermentation over the nine days. The biggest difference in the measured values between the first and last day can be seen in the variant fermented with *Acetobacter aceti.* In this variant, the alcohol content changed from 7.82% vol. to 2.14% vol. Figure 4 clearly shows that *Acetobacter aceti* can metabolize alcohol much more rapidly than *Gluconobacter oxydans*. The measured data show that the variant fermented spontaneously did not consume alcohol to the same extent as the variants inoculated with the different species of acetic acid bacteria.

Table 4 Changing values of alcohol (expressed in % vol.) during acetic fermentation.

Ethyl alcohol	*Acetobacter aceti*	*Gluconobacter oxydans*	Spontaneous fermentation
Day No. 1	7.82 ±0.18	7.71 ±0.18	7.70 ±0.14
Day No. 2	6.72 ±0.24	7.02 ±0.48	7.43 ±0.28
Day No. 3	4.90 ±0.75	6.42 ±0.49	6.66 ±0.74
Day No. 4	4.29 ±0.35	6.08 ±0.19	6.31 ±0.73
Day No. 5	3.98 ±0.07	5.80 ±0.32	6.56 ±0.78
Day No. 6	3.57 ±0.15	5.13 ±0.31	6.17 ±0.86
Day No. 7	3.15 ±0.08	3.80 ±0.93	5.82 ±0.95
Day No. 8	2.31 ±0.22	3.30 ±0.12	4.63 ±1.20
Day No. 9	2.14 ±0.19	3.19 ±0.21	4.42 ±1.12

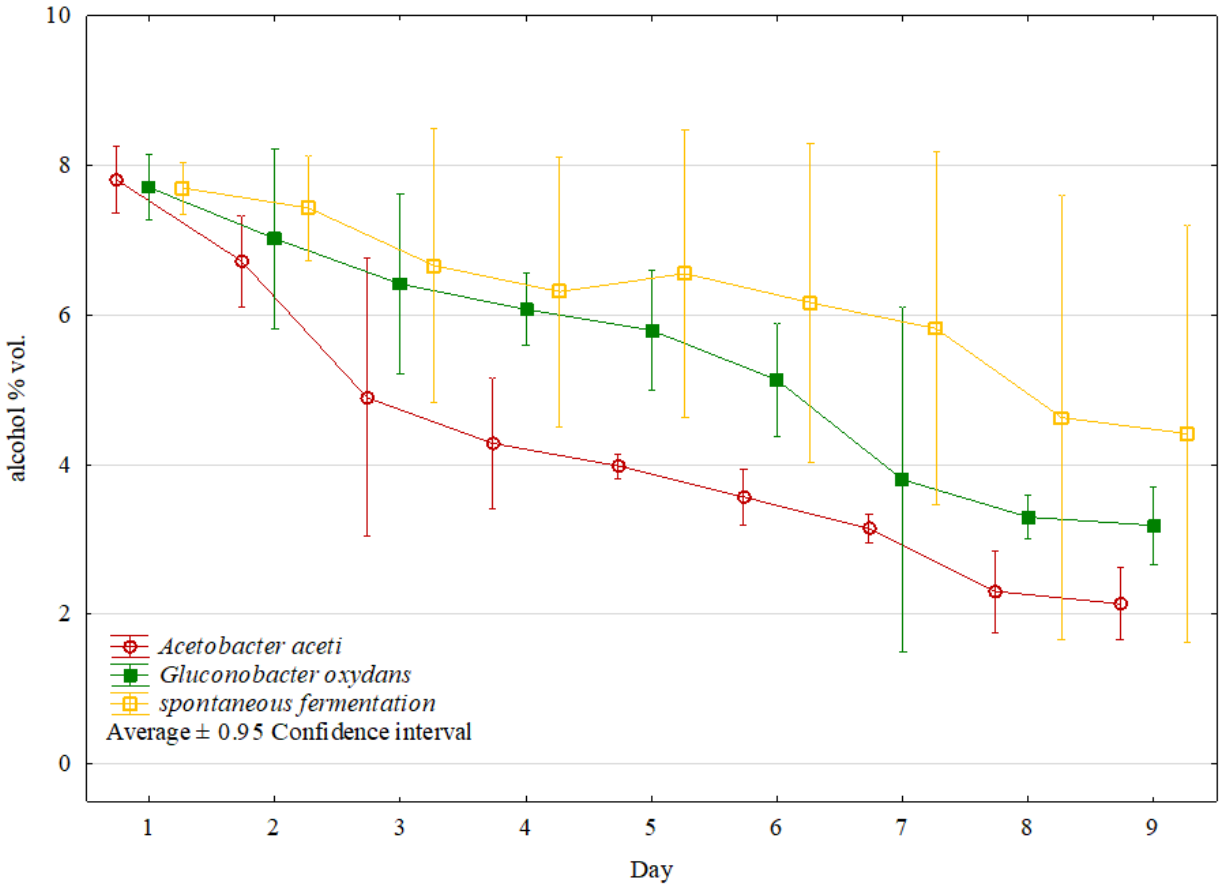

Figure 4 Statistical expression of the changing values of alcohol (expressed in % vol.) during acetic fermentation.

Acetic acid fermentation is a biochemical process in which acetic acid bacteria oxidize ethanol to acetic acid under strict aerobic conditions. Our research concluded that although both genera of *Acetobacter* and *Gluconobacter* are suitable for acetic acid fermentation, the former is much more suitable. This is also due to the fact that bacteria of the genus *Acetobacter* oxidize ethanol more strongly than glucose, while *Gluconobacter* oxidizes glucose more strongly than ethanol. Our original substrate was a white wine fermented completely dry with a low sugar content. The *Acetobacter aceti* strain was able to produce more organic acids and consumed ethanol faster than the other variants **[14]**. Our research focuses on the ability of acetic acid bacteria to produce various organic acids. These bacteria are capable of producing many organic biomolecules. Since there is a recent need to try to limit chemical synthesis, acetic acid bacteria are an ideal way to produce many organic substances. These properties can be used in the food, chemical, pharmaceutical, and medical industries. This work mainly focused on monitoring the increasing acid content during acetic fermentation. The ability of these bacteria to produce acetic, lactic and tartaric acid was also confirmed by this research, and the examined representatives produced different amounts of the aforementioned organic acids. These microorganisms can produce other organic acids such as malic, formic, citric, succinic, gluconic, and glucuronic acids. The production of organic acids also depends on the fermentation temperature since, at lower temperatures, these bacteria produce only limited amounts of the above compounds. This was confirmed in the optimization of this research. Proper acetic fermentation was achieved only when the appropriate fermentation medium temperature was maintained **[15]**, **[16]**, **[17]**. The importance of knowing the bacteria involved in vinegar fermentation is described in a 2006 study. Pure cultures of acetic acid bacteria are rarely used for vinegar fermentation. And when this does happen, it is very likely that other unwanted acetic acid bacteria subsequently contaminate precisely selected strains during fermentation, and these undesirable microorganisms can produce vinegar of inferior quality. In the industry, a mixed culture of *Acetobacter* is mainly used to produce acetic acid, but no attention is paid to its proper maintenance **[18**, **19]**. Following the experiment presented, our assertion that Gluconobacter bacteria are not suitable for total vinegar fermentation is well supported, as research from 2010 also shows that *Acetobacter* bacteria are predominant in traditional vinegar fermentation. In this research, the population dynamics of acetic fermentation bacteria were determined in two independent *Acetobacter* plants at both the species and strain levels. The effect of four different wood species of fermentation barrels on the diversity of acetic fermentation bacteria was also investigated. Vinegar fermentation bacteria were isolated on solid media. RFLP-PCR of 16S rRNA genes

then identified individual species, and confirmation was performed by 16S rRNA gene sequencing, while the strains were typed by ERIC-PCR and (GTG)5-rep-PCR. *Acetobacter pasteurianus* was the most frequently isolated species, accounting for almost 100% of all isolates detected during the whole vinegar fermentation. Representatives of the genus *Gluconacetobacter* appeared only at the end of the process and only in oak barrels from one of the vinegar plants investigated. The different *A. pasteurianus* showed a precise sequence with increasing acetic acid concentration. In all the vinegar houses, the dominance of the other strains changed with increasing acetic acid concentration, and the diversity of strains tended to decrease at the end of the process **[20]**. Surprisingly, a 2010 study showed that a representative from *Acetobacter* (*A. pasteurianus*) was not as successful as expected for total vinegar fermentation. On the contrary, higher oxygen and acetic acid concentrations seem to have promoted the development of *Gluconacetobacter* species (*G. europaeus* and *G. Intermedius*). This study indicated that mixed inoculum of *A. pasteurianus* and selected *Gluconacetobacter* species are the most likely candidates for use as initial cultures for suitable vinegar fermentation of wine. *Gluconacetobacter* species have the advantage of better tolerance to high concentrations of acetic acid **[21]**. The claim that *Gluconacetobacter* genera are partly involved in vinegar fermentation can also be found in a 2008 study. This study investigated the identification of the dominant genera of acetic fermentation bacteria in coconut wine, the so-called mnazi. First, the bacteria were isolated on GYP agar, and physiological and biochemical tests followed. Both *Acetobacter* and *Gluconobacter* strains detected were oxidase negative and catalase positive. *Acetobacter* strains could oxidize lactate and acetate, while *Gluconobacter* strains oxidized only lactate. The research shows that both of these genera are responsible for the spoilage of coconut wine by mnazi **[22]**. The suitability of strains of the genus *Acetobacter* for vinegar fermentation can also be found in a study from 1999 when the possibility of producing a new type of vinegar from onions that did not meet the quality standards required for marketing was investigated. Various kinds of onion were first tested as raw material for vinegar production, and vinegar was successfully produced from the juice of red onion, the Kurenai cultivar, using culture with yeast and *A. aceti* **[23]**. Another suitable strain for acetic fermentation is *Acetobacter europaeus*, isolated and described by molecular methods in 1992. This strain was isolated from an inoculum from a large vinegar factory in Hamburg **[24]**. In 1998, two new strains were described that carry out acetic fermentation. This time the strains were isolated directly from red wine in which acetic fermentation was already taking place. These were the acetic fermentation bacteria *Acetobacter oboediens sp*. and *Acetobacter pomorum sp*. Molecular techniques again carried out the identification of these strains. The closest relatives of these strains are *Acetobacter europaeus*, *Acetobacter xylinus,* and *Acetobacter pasteurianus* **[25]**. Another strain suitable for acetic fermentation is the strain described in 1985. This strain was named *Acetobacter polyoxogenes sp.* It is a strain capable of producing high amounts of acetic acid and was isolated from fermented vinegar broth with a high acidity **[26]**. In a 2013 study, blueberry wine was acetified using naturally occurring microorganisms in the first instance, and a second variant was fermented using an inoculated strain of *A. cerevisiae*. The acetification was carried out in three repetitions using the so-called Schützenbach method. It was found that the spontaneous fermentation processes lasted up to 66% longer than those of the variants involving inoculation with the *A. cerevisiae* strain. The isolation of the acetic fermentation bacteria and the subsequent analysis of these microorganisms by molecular methods made it possible to identify the main genotypes responsible for the acetification of blueberry wine. Although *A. cerevisiae* was the predominant strain isolated from samples from the inoculated processes, *A. pasteurianus* was isolated from samples for both processes and was the only species present for the spontaneous acetification variant. This study shows that the isolated strains of *A. pasteurianus* appear to be the most suitable representatives of acetic fermentation bacteria for the large-scale production of wine vinegar **[27]**. In a 2010 study, the volatile components of fruit vinegar fermented with two acetic acid bacteria were quantitatively analyzed. These were strains of *Acetobacter pasteurianus* AC2005 and *A. rancens* (AS1.41) by headspace solid-phase microextraction combined with gas chromatography and mass spectrometry. As a result, 45 species of volatile compounds were detected in the hawthorn vinegar produced by AC2005, including nine esters, 12 alcohols, three ketones, 12 acids, and nine other compounds. In vinegar fermented by AS1.41, 39 species were found, including 13 esters, nine alcohols, two ketones, 10 acids, and five other compounds. Of these, 26 species of volatile compounds detected in the two kinds of vinegar were the same. The characteristics of each *Acetobacter* strain were responsible for the variety of vinegars in taste **[9]**. In the 2016 study, *Acetobacter aceti* CCT 0190 and *Gluconobacter oxydans* CCMA 0350 were used simultaneously to produce jabuticaba vinegar. This combination of two species of acetic fermentation bacteria showed good results. In particular, these bacteria were able to produce high concentrations of citric acid (6.67 $g.L^{-1}$), malic acid (7.02 $g.L^{-1}$), and succinic acid (5.60 $g.L^{-1}$). Fruit wine produced from *Myrciaria jaboticaba* was used as starting material. The yeast species *Saccharomyces cerevisiae* CCMA 0200 was used to ferment the must for the production of jabuticaba wine **[28]**. In our research, spontaneous fermentation was used to produce the wine used for the subsequent acetic fermentation. Research from 1998 suggests that not every yeast always produces a suitable substrate for subsequent fermentation by acetic acid bacteria. It is also possible that *G. oxydans*

was not as suitable for wine vinegar production because of the unsuitable fermentate produced by *S. cerevisiae*, since, according to this research, the fermentate produced by *Candida stellata* positively affected the growth of acetic acid bacteria and the quality of the vinegar. In contrast, the wine produced by fermentation of *Kloeckera apiculata* was a good substrate for the growth of acetic acid bacteria and acetic acid production and could be used for 'ordinary' vinegar production **[29]**. A Slovenian industrial vinegar production plant was used for samples of unfiltered vinegar from three oxidation cycles of red wine and organic apple cider vinegar. *Gluconacetobacter oboediens* was the predominant species in all wine vinegar samples. At the beginning of fermentation, the acetic acid bacterial consortium was dominated by *Acetobacter*, with the genus *Gluconacetobacter* predominating over *Acetobacter* at the end of the oxidation cycle in all cider vinegar samples. Two dominant genera, *Lactobacillus* and *Oenococcus*, were identified among the lactic acid bacterial consortium, with *Oenococcus* predominating with increasing acetic acid concentration in the vinegar. Unexpectedly, the minor genus of the acetic acid bacterial consortium in organic apple cider vinegar was *Gluconobacter*, suggesting the possible evolution of a *Gluconobacter* population with tolerance to ethanol and acetic acid. The genus *Rhodococcus* was detected among the companion bacteria of wine vinegar but declined significantly towards the end of the oxidation cycles **[30]**. Our contention that the genus *Acetobacter* is a suitable genus for acetic fermentation is suggested by research from Garg, who produced vinegar from mango pulp by a dual fermentation and oxidation process using *Saccharomyces cerevisiae* and *Acetobacter aceti*. *S. cerevisiae* was recycled to increase the fermentation rate, while *A. aceti* was immobilized on wood shavings for semi-continuous vinegar production. The vinegar produced had 5.3% acidity as acetic acid. Conversion efficiency of 60% was achieved **[31]**. Acetic acid is an important basic chemical. It is mainly produced synthetically, and only 10% of the world's production is produced by bacterial fermentation for vinegar production. As reported in a 2018 study, several microorganisms can produce acetic acid, and some may incorporate CO_2 during production. To ensure proper acetic fermentation, choosing the right kind of acetic fermentation bacteria is important. Still, it is also essential to choose the right fermentation environment and original substrate **[32]**.

CONCLUSION

This work aimed mainly to determine a suitable acetic fermentation bacterium for the production of wine vinegar. For our experiment, we chose one bacterium of the genus *Acetobacter* (*Acetobacter aceti*) and one bacterium of the genus *Gluconobacter* (*Gluconobacter oxydans*). For the production of wine vinegar, white wine of the Veltliner Green variety was used. The vinegar fermentation was carried out in 5 L mini-acetators for nine days. Samples were taken at regular 24-hour intervals and analyzed. This experiment was further enriched by one variant, fermented by the so-called fermenter spontaneously. The alcohol, acetic, malic, and tartaric acid contents were monitored. The results revealed that all variants showed a strong acetic and malic acid increase. Tartaric acid was also produced in all three variants, but not to the same extent as the previous two organic acids. The results showed that *Acetobacter aceti* produced higher levels of all the organic acids studied than the other acetic acid bacteria mentioned. It was also found that *Acetobacter aceti* could metabolise ethanol more rapidly than *Gluconobacter oxydans*. Because of the above results, we can recommend *Acetobacter aceti* as a more suitable bacterium for wine vinegar production, as this bacterium produced up to 25 $g.L^{-1}$ acetic acid and up to 18 $g.L^{-1}$ lactic acid within nine days.

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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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Analysis of liability and protein content of soybean biscuits with Ambon banana as an alternative to emergency food for the elderly

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ABSTRACT

In disaster conditions, the elderly are vulnerable groups that require special attention. With increasing age, there is a decrease in biological function and psychological disorders. The elderly tend to have anxiety, especially during disaster conditions. This anxiety has an impact on the diet and health of the elderly. Soybean (*Glycine max* L.) flour biscuits and Ambon banana (*Musa paradisiaca var. sapientum* L.) flour is a food product that meets disaster emergency food requirements and food requirements for the elderly. It is appropriate to be used as an alternative disaster emergency food for the elderly. This study aims to determine the effect of the balance of the soybean flour biscuit formula Ambon banana flour on the organoleptic and protein properties. The research design used is an experimental study, with the research method used is a hedonic test for testing organoleptic properties and the Kjeldahl procedure for testing protein content. The formula of Ambon banana and soybean flour biscuits consisted of three balances, with the ratio of Ambon banana flour soybean flour (%) F1 (45:55), F2 (35:65), and F3 (25:75). From the study results, it can be concluded that there is a significant difference in the test results of organoleptic properties only for colour. Moreover, there are no significant differences in the test results of organoleptic properties, including aroma, taste, texture, and overall. In the test results of organoleptic properties, the balance (25:75) was declared superior overall. The 45:55 balance contains 8.01% protein, the 35:65 balance contains 7.23% protein, and the 25:75 balance includes 7.42%.

Keywords: biscuits; organoleptic; soybean flour; Ambon banana flour; elderly

INTRODUCTION

Law of the Republic of Indonesia Number 24 of 2007 concerning disaster management states that a disaster is an unexpected event because it negatively impacts victims and the environment, such as occurrence, loss of life, suffering, property loss, and environmental damage **[1]**. In 2018 in Indonesia, there were 4,089 natural disasters from 01-01-2018 to 31-12-2018, with 8,439 dead and missing, 5,125 injured, 1,365,956 affected, and displaced. In 2019, 9,392 natural disasters started from 01-01-2019 until 31-12-2019, with 912 deaths, 2,712 wounded, and 5,371,845 affected and replaced. In 2020, 1,285 natural disasters started on 01-01-2020 until 04-05-2020, with 53 dead and missing, 26 injured, and 529,818 affected and missing **[2]**. Assistance must be given immediately to deal with the adverse effects of post-disaster, such as evacuating the victim's property and fulfilling basic needs such as feeding to prevent refugees from feeling hungry and decreasing nutritional status. The United States Agency for International Development (USAID) states that emergency food must have sufficient nutritional value, be non-hazardous when consumed, be delicious, be given equitably to refugees, and be acceptable **[3]**. Most emergency food aid in disaster areas still requires a cooking process, such as rice and instant noodles. In an emergency, vulnerable groups, namely pregnant women, infants, toddlers, the elderly, and people with disabilities, require special attention. The elderly are residents who have an age of 60 years **[4]**. In disaster conditions, the elderly are vulnerable groups that require special attention, w. With creasing age, there is a decrease in biological function and psychological disorders. The elderly tend to have anxiety, especially during disaster conditions. This anxiety impacts the diet and health of the elderly **[5]**. Nina et al.'s 2015 research proved that bad psychological

conditions could cause a decrease in food intake, which then causes a decrease in nutritional status **[6]**. Nutritional problems often experienced by the elderly are overnutrition and undernutrition **[7]**. Soybeans and Ambon bananas are the choices because of the nutritional content and benefits that complement each other. Soybeans are a source of vegetable protein that is useful for increasing the body's immunity, replacing damaged body cells, and most importantly, helpful in producing energy. Soybeans have functional properties and can be further processed products **[8]**. Bananas contain several nutrients the body needs, such as potassium which can help cell growth, control blood pressure and smooth the functioning of the nervous system. Levels of antioxidants such as vitamins A and C can increase endurance. Vitamins B can help the process of protein metabolism and the content of tryptophan (an amino acid with a natural sedative effect), which can improve mood to reduce symptoms of depression and anxiety. Bananas contain 5.8 g of fibre in 100 grams of ingredients. In addition, when combined with banana soybeans, it can cover the unpleasant smell of soybeans **[9]**. Biscuits are snacks made from flour, margarine/butter, with or without adding other desired food ingredients **[10]**. Biscuits are preferred by the elderly because they are easy to chew, small in volume, and do not aggravate the work of the canal. It is suitable for the elderly disaster emergency victims because the biscuits meet the emergency food and nutritional requirements for handling disaster conditions in the elderly **[11]**. Faizah's 2015 research stated that the results of the preference test on soyaba (soya-banana) cookies which included aspects of colour, aroma, crispness, and overall obtained the highest average that the panellists liked the most was the balance of Anjasmoro soybean flour 80% banana mas 20% (A2B1). The selected soyaba cookies contain 3.46% water content, 2.18% ash content, 13.57% fat content, 38.14% protein content, and 42.66% carbohydrate content. The quality analysis results show that soybeans' colour, aroma, and taste perfectly conform to consumer expectations. In contrast, the aspects of the aroma and taste of mas bananas have a poor level of conformity, and the quality of crunchiness has a fairly good level of conformity **[12]**. The research of Abe et al. 2017 showed a comparison of the organoleptic assessment of Ambon banana flour and plantain flour with several aspects of assessment, namely the analysis of the colour assessment of Ambon banana flour at 3.48% and plantain flour at 3.25%. Analysis of the aroma assessment, Ambon banana flour 3.41% and 3.25% plantain flour. The assessment related to the taste of Ambon banana flour is 3.28%, and plantain flour is 3.38%. Assessment related to the texture of banana flour 3.30% and plantain flour 3.12%. Based on the proximate analysis, the protein content of Ambon banana flour was 0.55%, and plantain flour was 0.66%. Ambon banana flour is superior in colour, aroma, and texture **[13]**. Aris Pratomo's 2013 research stated that adding Ambon banana flour to dry sponge products positively affected the panellists' preference for colour, aroma, taste, and texture **[14]**. Ferawati 2009, research was related to alternative emergency food. The balance selected after the organoleptic test was 25:75 (ratio of soybean flour and Ambon banana (2:3) and cassava flour ratio (1:1). Shelf life with the aspect of moisture content at 28 °C has a shelf life of 2.16 months **[15]**, **[16]**, **[18]**.

Based on the description above, the authors are interested in making biscuits with a balance of soybean flour and Ambon banana flour and knowing the protein content following emergency food standards to be used as an alternative disaster emergency food for the elderly.

Scientific Hypothesis

Soybean biscuits and Ambon banana could serve as alternative emergency food to provide protein needs.

MATERIAL AND METHODOLOGY

Study Design

The research design used is an experimental study, with the research method used being hedonic test for testing organoleptic properties.

Samples

The independent variable consists of 3 balances of different Ambon banana and soybean flour that are balanced 1 (45:55), balance 2 (35:65), and balance 3 (25:75), while the dependent variables were organoleptic properties and protein content. Thirty panellists examined the hedonic test. Study this has to get agreement Commission Ethics Study Health Politeknik Kesehatan Kemenkes Bandung No. 31/KEPK/EC/XI/2020 dated November 20, 2020.

Preliminary research was conducted in January 2020 with the balance obtained from the preliminary test with three balances, namely 45%: 55%, 35%: 65%, 25%: 75%. Preliminary tests are carried out to determine the procedure for making the product and the amount of material used. The main research carried out in January-February 2021 includes making biscuits, collecting organoleptic test results and protein content, and then processing and analysing the data.

Chemicals

Pasundan University provided the chemicals. All chemicals were of analytical grade quality.

Animals and Biological Material

The animal and biological materials were not used.

Instruments

The distillation apparatus, Kjeldahl flasks.

Laboratory Methods

To analyse protein content, we used Micro Kjeldahl method **[29]**. Repeated analysis of Each Formula was examined three times by Micro Kjeldahl Method. Weighing samples that have been mashed as much as 1 g. Filling the sample into a Kjeldahl flask. Weighing 7 g K_2SO_4 and 0.8 g $CuSO_4$. Addition of 7 g K_2SO_4 and 0.8 g $CuSO_4$ to the Kjeldahl flask containing the sample. The addition of 12 mL of H_2SO_4 solution was carried out in a fume hood. The destruction process was carried out in an acid chamber by heating the sample in the Kjeldahl flask using an electric stove until it turned turquoise green. Cooling the Kjeldahl flask by allowing it to stand for 20 minutes. Add 25 mL of distilled water into the Kjeldahl flask containing the sample. Add 50 ml of 40% NaOH and a few grains of boiling rock into the Kjeldahl flask containing the sample. The addition of 30 mL of H_3BO_3 into the Erlenmeyer with 3 drops of BCG-MR indicator to capture the distillate from the distillation results. Distillation apparatus. The distillate obtained from the distillation was titrated using a standard solution of 0.1 N HCl until the colour of the solution changed to light pink.

Calculation: % Crude protein = % N x protein conversion factor **[28]**.

Description of the Experiment

Biscuit preparation: Preliminary research was conducted in January 2020 with the balance obtained from the preliminary test with three balances, namely 45%: 55%, 35%: 65%, 25%: 75%. Preliminary tests are carried out to determine the procedure for making the product and the amount of material used. The main research carried out in January-February 2021 includes making biscuits, collecting organoleptic test results and protein content, and then processing and analysing the data. In this study, three treatment groups were carried out. Each treatment for organoleptic testing was carried out once and 3 times for protein content testing.

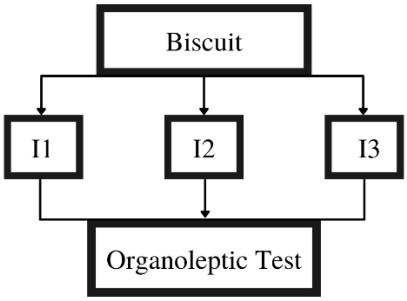

Figure 1 Biscuit organoleptic test scheme. Note: I1: Biscuit sample with a balance of 45%:55%, I2: Biscuit sample with a balance of 35%:65%, I3: Biscuit sample with 25%:75% balance.

Knowing substance biscuit protein nutrition on each balance with Kjeldahl method in the laboratory University Pasundan. In connection with the COVID-19 pandemic, the hedonic test was carried out from the home of each panellist. Then the panellists sent the results of the hedonic test through the prepared google form.

Randomization in this study was done by pressing the SHIFT Ran# 1000 button and then getting the numbers sequentially from the smallest number to the most significant number and ranking.

Number of samples analyzed: we analyzed 30 samples.

Number of repeated analyses: Protein analyses were conducted in triplicate analysis.

Number of experiment replication: 2 times.

Design of the experiment:

Table 1 Randomization of experimental units.

No	Random Number	Ranking	Treatment
1	377	1	I1
2	241	2	I2
3	202	3	I3

Then a plan for the experimental organoleptic unit was made and listed in Figure 2 below.

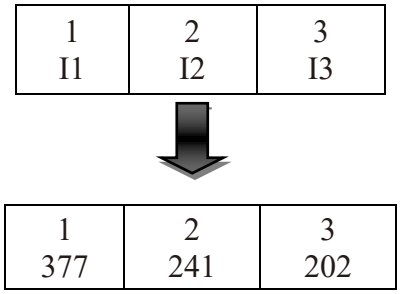

Figure 2 Organoleptic experiment unit plot.

Primary data were obtained from organoleptic test results by 30 moderately trained panellists for one test. Preliminary data on protein content was obtained from test results with three repetitions of observations for each treatment. He analysed the organoleptic data from the SPSS 15 program, namely the normality first test. The normality test used was Shapiro Wilk. If the Kruskal Wallis test is significant, the Mann-Whitney test continues.

Statistical Analysis

Significant differences (p <0.05) in the colour aspect of the biscuit were evaluated by the Kruskal Wallis test and continued by the Mann-Whitney test. The ANOVA test evaluated significant differences (p <0.05) in the protein content of the biscuit. This Statistical Analysis was calculated using SPSS 15 program.

RESULTS AND DISCUSSION

The biscuit balance in this study used a modified recipe based on the recommendation of emergency food nutritional value, where 10% was the provision of nutritional value for snacks or food consumed between main meals. In this study, the sample used was the balance between soybean flour and banana flour of 45%: 55%, 35%: 65%, and 25%: 75%. The ingredients that follow the percentage ratio are only soybean flour and Ambon banana flour. In contrast, other ingredients include egg yolks, liquid milk, baking powder, vanilla powder, salt, powdered sugar, cake ammonia, margarine, and food colouring using the same weight in each balance. The total weight of the ingredients of soybean flour and Ambon banana flour follows the weight of the ingredients in the original recipe is 100 grams; this aims to see a significant difference between the balance of soybean flour and banana flour on biscuits. The resulting Ambon banana and soybean flour biscuits are flat and round. Every 100 grams balance biscuit yields as much as 85%. Biscuits have a brownish colour. When biscuits added more banana flour, more panellists preferred the colour. The smell of biscuits gives rise to the distinctive aroma of biscuits. The texture of the resulting biscuit is soft, but its crunchiness and breaking power do not resemble the biscuits on the market. Various types of the resulting biscuits could see in Figure 3, below this:

Figure 3 Biscuits made from soybean flour with Ambon banana flour.

Influence Balance to Nature Organoleptic Biscuits Flour Peanut Soya Bean Flour Ambon Banana

Test organoleptic done for knowing level favourite to biscuits food emergency. Based on results testing from third balance by 30 panellists instead trained seen from whole indicators (colour, aroma, taste, texture, and overalls). The average result shows that the panellists own Mark's different likes from the three different sample balances. That formula are 45% : 55% (I), 35%: 65% (II), 25%: 75 % (III). The data can be seen in Table 2 below this:

Table 2 Distribution frequency likeness rating to colour, aroma, taste, texture, and overall.

Formula (%)	Liked Level	Colour		Aroma		Flavour		Texture		Overall	
		n	%	n	%	n	%	n	%	n	%
45:55	Very no like	0	0	0	0	1	3.3	0	0	1	3.3
	Not like	2	6.7	0	0	4	13.3	6	20.0	0	0.0
	Neutral	16	53.3	12	40.0	10.0	33.3	10	33.3	12	40.0
	Like	12	40.0	12	40.0	11	36.7	9	30.0	15	50.0
	Very like	0	0	6	20	4	13.3	5	16.7	2	6.3
35:65	Very no like	0	0	0	0	0	0.0	0	0	0	0.0
	Not like	0	0	1	3.3	2	6.7	6	20.0	2	6.7
	Neutral	13	43.4	9.0	30.0	9.0	30.0	11	36.7	11	36.7
	Like	14	46.7	15	50.0	16	53.3	12	40.0	15	50.0
	Very like	3	10.0	5	16.7	10	10.0	1	3.33	2	6.7
25:75	Very no like	0	0	0	0	0	0.0	0	0.0	0	0.0
	Not like	0	0	3	10.0	4	13.3	5	16.7	0	0.0
	Neutral	9	30.0	8.0	26.7	6.0	20.0	15.0	50.0	8	26.7
	Like	16	53.3	16	53.3	16	53.3	7	23.3	21	70.0
	Very like	5	16.7	3	10.0	4	13.3	3	10.0	1	3.3

Table 2 shows as many as 53.3% of panellists like 25:75 balance, 53.3% of panellists like 25:75 balance, 53.3% of panellists love the sense of balance between 35:65 and 25:75, 40% of panellists like 35:65 balance, 70% panellists like 25:75 balance. Results test hedonic has done, the average value of colour, aroma, taste, texture, and biscuit overalls flour soybean and banana Ambon as seen on Table 3 below this:

Table 3 Results analysis Kruskal Wallis difference balance flour peanuts soya bean flour Ambon banana.

Balance	n	Colour		Aroma		Flavour		Texture		Overall	
		median	*p*-value	median	*p*-value	median	*p*-value	median	*p*-value	median	*p*-value
45:55	30	3.33	0.016	3.80	0.777	3.43	0.539	3.43	0.752	3.56	0.422
35:65	30	3.66		3.80		3.66		3.26		3.56	
25:75	30	3.86		3.63		3.66		3.26		3.76	

Based on the results of statistical tests, there are significant differences in the colour aspect of the biscuits. At the same time, statistical tests for aroma, taste, texture, and overall aspects showed no significant differences.

Table 4 Results analysis of protein content between balance biscuits flour peanut soya bean flour Ambon banana.

Balance	Protein Content (%)
45:55	8.01
35:65	7.23
25:75	7.42

Based on Table 4 shows the balance of soybean flour biscuits. Ambon banana flour 45:55 has a protein content of 8.01%, the balance of soybean flour biscuits: Ambon banana flour 35:65 has a protein content of 7.23%, and the balance of Soybean flour biscuits: Ambon banana flour 25:75 has a protein content of 7.42%. The balance of soybean flour biscuits: Ambon banana flour 45:55 is a balance that has the highest protein content among other balances.

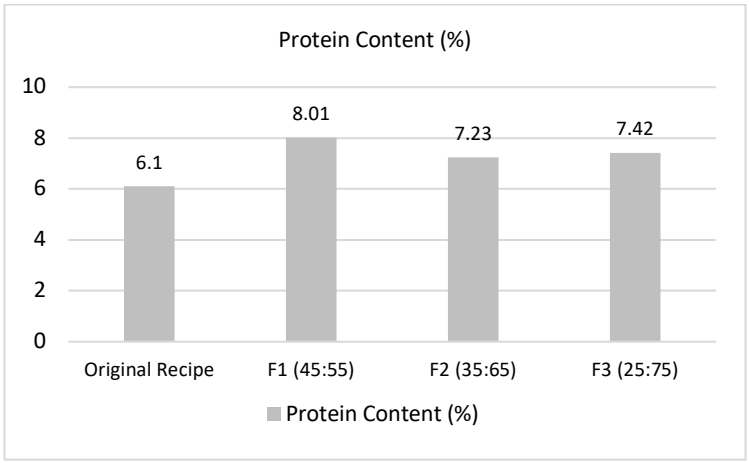

Figure 4 Compare protein content of original recipe with biscuits of soybean flour and ambon banana flour.

Based on chart 1 comparing the protein content of the original biscuit recipe with biscuits of soybean flour and Ambon banana flour for every 100 grams of the product, the original recipe contained 6.1%. In contrast, the ratio of 45:55 contained 8.01% protein, 35:65 comprised 7.23%, and 25:75 contained 7.42%. Therefore, the new formula has a higher protein content than the original recipe.

Table 5 Result Analysis ANOVA difference balance Addition of soybean flour and banana flour.

	Sum of Squares	df	Mean Square	F	Sig
Between Group	0.993	2	0.496	4963.000	0.000
Within Group	0.001	6	0.000		
Total	0.993	8			

Based on table 5, the ANOVA one-way ANOVA test results one-way ANOVA test results, there are significant differences in the addition of soybean flour and banana flour that affect the protein content of biscuits ($p = 0.000$). Biscuits food emergency is product biscuits made from flour peanut soya bean, and flour banana Ambon has substance nutrition good especially substance nutrition macro protein. The primary research was carried out in two stages, the first stage was testing the organoleptic properties with a hedonic test, and the second stage was testing the protein content. The first stage of testing for organoleptic properties was carried out on February 14, 2021, involving 30 moderately trained panellists who were students of Semester 6 and 8 of the Politeknik Kesehatan Kemenkes Bandung, Nutrition, and Dietetics Study Program. In connection with the Covid-19 Pandemic conditions, samples were delivered to the panellists' homes, and hedonic tests were carried out from panellists' homes. The hedonic test results were sent online via google-form. The second stage of protein content testing was carried out from February 15 to March 2, 2021, at the Food Lab at Pasundan University.

Test hedonic for knowing level favourite panellist to colour, aroma, taste, texture and overall biscuits. Food colour is the main attraction that will be judged by the panellists first by the sense of sight. Food is interesting not only because of its taste but also its appearance. If the display is not attractive, consumers will not be interested in tasting it **[22]**.

The desired colour of the biscuit is brown. The brown colour comes from the Maillard reaction between the amino acid lysine (found at the highest levels in soybean) and the reducing sugar syrup **[28]**. In addition, the baking process can affect the colour of the biscuits by considering the temperature time and affecting the colour of baked biscuits using an oven by paying attention to the right time and temperature. It can extend the shelf life of food products because it can remove anti-nutritional substances, stop the growth of microorganisms, and optimise the digestibility of nutrients **[19]**. Based on the results of the organoleptic test, the average panellists' ratings ranged between (neutral)-and (like). However, the balance of 25:75 has the highest average level of liking, with a like statement. The level of liking is estimated because, at the 25:75 balance, the addition of banana flour is more than the other balance. Banana flour can produce the colour of food products that panellists like. In line with Setyadi's research, adding Ambon banana flour with the right roasting time can produce a preferred product **[19]**. Furthermore, based on the research of Abe et al. 2017, the Ambon banana has the advantage of making products with good colour, aroma, and texture **[13]**.

The results of the Kruskal Wallis test showed that adding soybean flour to Ambon banana flour had a significant effect on the panellist's level of assessment of the colour of the biscuits produced. Then a follow-up test was

carried out with Man Whitney, and it was found that there was a significant difference (p=0.016) between the 45:55 balance and the 25:75 balance.

Aroma is the smell caused by stimuli captured by the sense of smell, by the olfactory nerves in the nasal cavity when food enters the mouth **[22]**. The smell received by the sense of smell can be in the form of a fragrant aroma, not a strong scent, aromatic, and charred **[23]**.

Based on the results of the organoleptic test, the panellists, on average, said they liked the smell of biscuits. The smell is thought to be due to soaking soybeans for 30 minutes, removing soybean husks roasting soybean flour, filtering soybean flour, adding vanilla and margarine to the dough, and successfully eliminating the unpleasant odour that the researchers did not like. Soybeans have a distinctive unpleasant odour caused by volatile compounds formed by fatty acids hydrolysed by the lipoxygenase enzyme **[24]**. However, the unpleasant smell can be reduced by heating and modifying pH **[17]**. In addition, the aroma of these emergency food biscuits can be influenced by vanilla and margarine. However, the organoleptic properties test results were not in line with Pratama and Ayustaningwarno **[27]** who stated that adding Ambon banana flour had a good effect on food products. The Ambon banana flour is thought to be because humans have different sensitivity levels, while the sense of smell is more easily affected by the surrounding environment than sight **[23]**. The sensitivity levels could be one of the causes of the organoleptic test results because the organoleptic test was carried out from the panellist's house without the researcher's supervision. Another cause is because Ambon banana flour is obtained from a third party, so efforts to maintain Ambon banana flour quality are beyond researchers' reach. The maturity level of Ambon banana influences the quality of Ambon banana flour as raw material and the process of making Ambon banana flour **[23]**.

The results of the Kruskal Wallis test showed that adding soybean flour to Ambon banana flour did not significantly affect the panellists' assessment of the aroma of the biscuits produced ($p = 0.777$).

Taste arises from food that is captured by the sense of taste. Taste is an essential factor in determining the decision for consumers to accept or reject a food or food product **[21]**. The taste captured by the sense of taste can be influenced by proton donors who give rise to a sour taste, inorganic salts, which give rise to a salty taste, and organic compounds, which give rise to a sweet taste **[19]**. The desired taste is sweetness. The sweet taste of biscuits is obtained from powdered sugar and banana flour.

Based on the results of the organoleptic test, the average rating ranged between (neutral)-and (like). The balance of 35:65 and the balance of 25:75 have the same intermediate level of preference. This thought is because banana flour gives a sweet taste to a balance of 35:65 and 25:75, more than the 45:55 balance. In addition, the sweet taste of biscuits is also influenced by the added powdered sugar. The results of the Kruskal Wallis test showed that adding soybean flour to Ambon banana flour did not significantly affect the level of panellists' assessment of the taste of the biscuits produced ($p = 0.539$).

The texture is the pressure that can be observed (eyes), bitten, chewed, and swallowed (mouth), and by touch with the ring finger **[22]**. Several factors affect the texture of food: water content, the addition of fat source raw materials, and the flexibility formed from the increase in gelation ability due to protein **[24]**. The desired texture is a texture that contributes to the level of the crispness of the biscuits, so margarine is added, and foods containing protein such as soybean flour, Ambon banana flour, wheat flour, and eggs are added. With the addition of these food ingredients, it is expected to produce a product texture favoured by panellists and increase the nutritional content of biscuits. Based on the results of the organoleptic test, the average rating given by the panellists was neutral. The results of the Kruskal Wallis test showed that adding soybean flour to Ambon banana flour did not significantly affect the level of panellists' assessment of the texture of the biscuits produced ($p = 0.752$). The results are thought to be because the biscuits are still not thin and crunchy. Some panellists stated that biscuits weighing 15 grams/piece were still too thick. Researchers are worried that if the biscuits are made too thin, the number of biscuits given to meet nutritional needs will increase.

Overall, assessing the organoleptic properties (taste, colour, aroma, and texture) shows the response of the sense of sight, taste, smell, and touch **[20]**. Based on the results of the organoleptic test, the panellists gave an average rating of liking for the overall biscuit. The initial process of making biscuits to distribution can affect the widespread biscuit. Overall, the balance of 25:75 is the balance that has the highest average value. Based on calculations from the Indonesian Food Composition Table TKPI per 100 g biscuits, the 45:55 balance contains 18.9 grams of protein. The 35:65 balance contains 15.6 grams of protein, and the 25:75 balance contains 12.3 grams. The overall aspect of the 45:55 balance has an average score of 3.56 for the panellists, the 35:65 has an average score of 3.56 for the panellists, and the 25:75 has an average score of 3.76. So, the 25:75 balance is an overall superior balance. In the Kruskal Wallis test, $p > 0.05$ (0.422), which means that there is no significant difference in organoleptic properties based on the overall organoleptic aspects between the three balances of soybean flour, Ambon banana flour.

The results of the protein content test using the Kjeldahl method showed that the ratio of 45:55 contained 8.01%

protein, 35:65 contained 7.23%, and 25:75 contained 7.42%. If observed that the 25:75 balance has a higher protein content than the 35:65 balance, while the 35:65 balance should have a higher protein content than the 25:75 balance because of more soybean flour. It can happen because the cooking process is too high and can cause damage to the protein content **[25]**.

However, temperatures above 100 °C and below 180 °C can remove anti-protein substances such as antitrypsin (antiprotease) 90% during the processing, interfering with protein function by binding and precipitating protein. In addition to heating, the 300-minute soaking process can remove anti-protein substances such as phytic acid and tannins because of their water-soluble properties **[26]**. Apart from the processing process, other causes could be unexpected during the protein content test because a third party carried out the protein content test, which was beyond the researcher's reach.

Fifteen grams of protein is a daily protein intake requirement that must be fulfilled through 3 snacks during a disaster emergency **[11]**. Compared with the calculation of protein content from TKPI, per 100 grams of biscuits, the 45:55 balance reaches 126% of the need, the 35:65 balance reaches 104% of the requirements, and the 25:75 balance reaches 83% of the condition. Meanwhile, compared with the results of the protein content test using the Kjeldahl method per 100 grams of biscuits, the 45:55 balance reached 53.4% of the requirement, and the 35:65 balance reached 48% of the need, and the 25:75 balance reached 49% of the condition.

The 45:55 balance is the balance that has the highest protein content due to the addition of more soybean flour than the other balances. The comparison between the calculation of protein content from TKPI and the protein content test using the Kjeldahl method is much smaller. This difference is because the foodstuff protein content will be reduced before processing due to heating **[25]**.

Based on comparing the protein content of the original recipe biscuit with soybean flour and Ambon banana flour, the new formula has a higher protein content than the original recipe. The protein content of the original recipe is 6.01%. At the same time, the biscuits of soybean flour and Ambon banana flour for every 100 grams of the product, the ratio of 45:55 contained 8.01% protein, 35:65 comprised 7.23%, and 25:75 contained 7.42%. Based on comparing the protein content of biscuits with soybean flour, Ambon banana flour, and SNI 2973-2011 regarding biscuit quality standards, biscuits must contain at least 5% protein for every 100 grams of the product. Therefore, the 45:65 balance, 35:65 balance, and 25:75 balance have met the biscuit quality requirements.

The Kruskal Wallis test results showed that adding soybean flour to Ambon banana flour significantly affects the protein content (p = 0.000). Therefore, soybean biscuits and Ambon banana could serve as alternative emergency food to provide protein needs supported by the fulfilment of protein adequacy for snacks and according to SNI 2973-2011 standards. The serving size of Ambon banana and soybean flour biscuits for a day with a frequency of eating three times, divided into morning, afternoon, and evening snacks, is 200 grams. Two hundred grams of biscuits is equivalent to 13 biscuits. Two hundred grams of biscuits can meet 30% of the daily adequacy's nutritional adequacy. 30% is the percentage of nutritional adequacy from 3x snacks.

To provide nutrition intake, the biscuit should be kept with the proper packaging. Based on Romani's research in 2014, the best packaging for the biscuit is multilayer polymeric materials without influencing the overall quality of the product during storage **[26]**. The storage time would be 2 months and 16 days; based on Ferawati's research, the shelf life of a biscuit is 2 months and 16 days, so the biscuit's best before date is 2 months and 16 days **[15]**.

This product will be recommended to the Indonesian Health Ministry as emergency food for the elderly. Then the Health Ministry can collaborate with the food industry to produce the biscuit. In advance, the product would have HACCP or ISO 22000 specifications. However, the biscuit should have an affordable price. The price for one biscuit serving is IDR 5400,00.

CONCLUSION

Based on the organoleptic properties test results, the panellists gave an average rating of liking for the overall biscuit. But the 25:75 balance is the most preferred overall. The protein content in 100 grams of F1, F2, and F3 biscuits is 8.01%, 7.23%, and 7.42%. There are significant differences in the biscuits' colour aspect on statistical tests (p = 0.000). At the same time, statistical tests for aroma, taste, texture, and overall aroma, taste, texture, showed no significant differences (p <0.05). Based on the result of statistical tests, there are significant differences in the addition of soybean flour and Ambon banana flour biscuits that affect the protein content for emergency food.

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Technological aspects of rice gluten-free bread production

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ABSTRACT

The article presents data on the study of the influence of hydrocolloids and protein additives on the technological aspects of gluten-free rice bread production. The method of full-factor experiment PFE 2 ^{3} determined the optimal conditions for bread production – the amount of yeast 1.5% by flour weight, dough moisture 60%, duration of fermentation, and proofing 70 minutes. The prescribed amount of yeast, salt, agar, and gelatin was dissolved in water at 35 ºC and mixed with the specified amount of rice flour. The dough was kneaded for 15 minutes. The dough was placed in the mould and left to ferment for 40 minutes and stand for 30 minutes at the temperature of 30 ºC. After fermentation, the dough was divided into pieces weighing 50 grams, placed in baking tins, and baked for 35 – 40 minutes at the temperature of 180 ºC. Since adding polysaccharides and protein improvers to the recipe of gluten-free dough to regulate its technological properties can significantly affect the intensity of fermentation and the activity of amylolytic enzymes of flour, studied the dynamics of carbon dioxide release gluten-free rice dough. It was found that additives of protein nature increase the amount of carbon dioxide accumulation in gluten-free dough by 33 – 44%. It is experimentally substantiated that the recommended duration of fermentation of rice flour dough with the addition of gelatin is 45 – 50 min, with the addition of agar 25 – 30 min, and the mixture of gelatin and agar 35 – 45 min. It is established that to achieve full readiness of bread based on rice flour, it is possible after 35 minutes of baking at 200 ºC. When extending the duration of heat treatment, the quality of bread does not change, so long-term heat treatment is not economically feasible.

Keywords: gluten-free rice bread, gelatin, agar, hydrocolloids, gas-forming ability, a specific bread volume, porosity, fermentation rate, baking, shrinkage.

INTRODUCTION

Dough-like masses are considered polydisperse systems consisting of solid, liquid, and gaseous phases. The solid phase is formed by starch, cellulose, and hemicellulose proteins. The liquid phase is a multi-component aqueous solution of organic and mineral substances of flour and recipe ingredients due to fermentation and capture of air bubbles **[1]**. During fermentation, basic biochemical and microbiological processes take place. Lactic acid bacteria produce lactic acid, which acidifies the environment, creating favourable conditions for developing yeast and suppressing other microorganisms whose products are toxic to yeast. Yeast enriches the environment with nitrogenous substances and vitamins necessary for the growth of bacteria. The main processes in the maturation of the dough are alcohol and lactic acid fermentation. As a result of these processes, the dough is loosened with carbon dioxin; saturation of the liquid phase with carbon dioxide with the formation of carbonic acid; increasing the acidity of the dough due to the formation of lactic, acetic, and other acids; lowering the pH of the dough; accumulation of flavouring and aromatic substances **[2]**, **[3]**, **[4]**, **[5]**, **[6]**, **[7]**. Baking yeast ferments sugars in the following sequence: glucose, fructose, sucrose, and maltose. Yeast fermentation enzymes directly ferment only glucose, others after hydrolysis into monosaccharides **[8]**.

In the process of life, yeast absorbs nitrogenous substances, mineral salts, and vitamins in the dough's liquid phase. Within 1 – 1.5 hours after kneading the dough, the yeast ferments its flour sugars feeds on other water-soluble dough substances. In the future, their activity depends on the accumulation of maltose, soluble nitrogen-containing compounds that are products of enzymatic hydrolysis of starch and dough proteins.

Thus, two processes coincide: the process of maltose formation and the process of fermentation of the dough microflora. The method of maltose formation should precede its fermentation **[3]**, **[4]**, **[5]**. The ripe dough must

contain at least 3% of fermentable sugars. This is the amount of sugars required for fermentation processes during the aging of the dough and for the reaction of melanoidin formation, which causes the colour of the crust **[7]**.

During the fermentation of the dough, pentosans are depolymerised under the action of flour enzymes, and pentoses are formed, which participate in the melanoidin formation reaction. Dough proteins continue to swell during fermentation. Swollen proteins are more accessible to proteolysis. The redox potential is shifted towards enhancing the reduction processes in the yeast dough. As a result, the proteinase of the dough is activated, the oxidised part of the proteolysis activators (glutathione, cysteine) is restored, and the disaggregation of the protein molecule in the dough is deepened. The content of high molecular weight fractions decreases, and the content of lower molecular weight gliadin, albumin, and globulins increases.

The products of protein hydrolysis pass into the dough's liquid phase, nourish the dough's microflora, and form aromatic and colour compounds during the baking process **[7]**, **[8]**, **[9]**.

As a result of proteolysis, the elasticity of the dough decreases, its elasticity improves, and specific structural and mechanical properties are formed. Biochemical processes in the dough intensify with increasing machining, increasing the dough's temperature. Their activity is influenced by the pH of the medium, the presence of activators and inhibitors of proteolysis, and the dough formulation.

In addition, during dough fermentation, the osmotic binding processes of water proteins, their swelling, and their increase in volume continue. Part of the proteins swells indefinitely, are peptised, and go into solution. This increases the content in the dough of the liquid phase and the dough thins.

Colloidal processes in the dough intensify with increasing structural and mechanical processing during kneading, acidity during fermentation, and fermentation temperature.

Thus, the analysis of the processes that occur during the dough's formation shows that the conversion of proteins and carbohydrates and their interaction with water fundamentally shape the quality of finished products. Therefore, when using proteins and hydrocolloids in gluten-free bread technology, it is necessary to consider changes in physicochemical, hydrocolloid, biochemical and microbiological processes, correct formulations, and justify technological modes of production.

The most common and widely used raw ingredients for gluten-free bread are rice flour and rice starch, corn flour and corn starch, potato, cassava, and wheat starch **[10]**, **[11]**, **[12]**, **[13]**. Gluten-free cereal flour (sorghum, millet, oatmeal) is offered as an alternative raw material **[14]**, **[15]**; gluten-free pseudo-grain flour (buckwheat, amaranth, quinoa); flour from roots and tubers (cassava, sweet potatoes); bean flour (soy, chickpeas, carob, beans, lentils, peas); other flour (flax, chestnut, banana, teffi, etc.) **[16]**, **[17]**, **[18]**. **[19]**, **[20]**, as well as flour mixtures.

The use of gluten-free flour raw materials to improve gluten-free bread's structural and mechanical properties **[21]** found that introducing flax, sorghum, sunflower flour, and quinoa flour reduces the irreversible relative deformation of dough by 36 – 68% and increase its elasticity. This reduces the relative plasticity - and increases the relative elasticity. In the presence of these additives, the amount of accumulated carbon dioxide in gluten-free dough increases by 10 – 30%.

Hydrocolloids are widely used as structuring agents to simulate the viscoelastic properties of gluten. These ingredients are usually used as a substitute for gluten due to their ability to thicken high water-binding and gelling properties. They can control the properties of the aqueous phase and stabilise the structure of emulsions, foams, suspensions, and multiphase systems **[22]**. Hydrocolloids increase the volume of the dough, stabilising its foam structure by increasing viscosity, flocculation, and coalescence. Hydrocolloids also prevent the effect of the aqueous phase on the foam structure, improving the stability of the liquid in the films surrounding the gas bubbles. Hydrocolloids can significantly affect the behaviour of the test, even if they are present in tiny quantities **[23]**, **[24]**.

Proteins are crucial in determining the structure of many foods, including gluten-free bread **[25]**. Due to their specific functional properties, proteins of animal origin are widely studied and proposed for use in food systems.

Scientists of Kharkiv National Technical University of Agriculture **[26]** found that using Na CMC at the concentration of 0.5% in the gluten-free unleavened dough is appropriate. The volume of bread increases compared to the control by 15%. The combined use of Na CMC and baking soda is inappropriate, as it leads to excessive loosening of the crumb structure and weakening its skeleton.

The positive effect of animal protein concentrates on bread's structural and mechanical properties is proved. For the use of concentrates of animal proteins (CoAP) as improvers of gluten-free unleavened dough, their concentration should be limited to 0.5 – 1.0% by weight of flour (higher concentrations lead to the slight deterioration of the structural and mechanical properties of the crumb, and there are inexpedient from the economic point of view). The proposed additives for improving the gluten-free yeast-free dough help improve the foam structure's porosity, forming the finely porous, uniform foam.

Other studies **[27]** show that introducing the solution of xanthan in the solution of gelatin structures increases the thermal stability of the flour whipped semi-finished product during heating. This is probably due to the

redistribution of associated and non-associated hydroxyl groups, forming many intermolecular hydrogen bonds. The catalytic effect of the enzyme transglutaminase in the gelatin-xanthan system on the interaction of lysine amino groups with the g-carboxamide group of peptide-linked glutamine residues is also proven. This effect provides a higher level of cross-linking of macromolecules of the protein framework and significantly slows down the dehydration of the semi-finished whipped flour product.

Thus, the combined use of hydrocolloids and protein supplements in gluten-free bread technology is justified and needs more in-depth research.

Scientific Hypothesis

The study aims to optimise the impact of protein and polysaccharide nature additives as structurants in their everyday use in the technology of gluten-free rice bread. Several interrelated tasks have been solved to solve the formulated goal:

- to determine rational technological parameters by conducting the full-factor experiment PFE 2^3 ;
- to study the influence of the amount and type of additive-structuring agent on the structural and mechanical properties of gluten-free bread;
- to study the peculiarities of microbiological processes in the dough with additives;
- to conduct a comprehensive assessment of the quality of finished bakery products.

MATERIAL AND METHODOLOGY

Samples

Sample 1	2.5% of yeasts; 60% of water; 90 min of fermentation and proofing.
Sample 2	1.5% of yeasts; 58% of water; 70 min of fermentation and proofing.
Sample 3	2.5% of yeasts; 60% of water; 70 min of fermentation and proofing.
Sample 4	2.5% of yeasts; 58% of water; 70 min of fermentation and proofing.
Sample 5	1.5% of yeasts; 58% of water; 90 min of fermentation and proofing.
Sample 6	1.5% of yeasts; 60% of water; 90 min of fermentation and proofing.
Sample 7	1.5% of yeasts; 60% of water; 70 min of fermentation and proofing.
Sample 8	2.5% of yeasts; 58% of water; 90 min of fermentation and proofing.

Chemicals

Agar	TM "Vprok", Ukraine.
Gelatin	TM "Deco", Ukraine.

Animals and Biological Material

Rice flour	TM "World's Rice".

Laboratory Methods

The volume of the finished products was measured with the volume meter. Baking was defined as the difference between the weight of the dough and hot bread and was expressed as the percentage by weight of the dough. Drying was defined as the difference between hot and uncooled bread as a percentage of the weight of hot bread.

Gas-forming ability and the rate of gas formation were determined in parallel with the degree of loosening of the dough **[28, 29]**. The calculation of dry matter consumption for fermentation was performed in terms of glucose by the amount of CO_2 released during fermentation, using the equation of glucose fermentation of Gay-Lussac **[30]**.

Loss of carbon dioxide during fermentation was calculated by formula 1:

$$B_{CO_2} = \frac{\int_a^b (F(x) - f(x))dx}{\int_a^b F(x)dx} \times 100 \quad (1)$$

Where:

B_{CO_2} – loss of carbon dioxide during fermentation (%), *a, b* – values of the integration limit; *F (x)* is the area of the curved trapezoid on the segment [a*, b*], limited by the equation of gas-forming capacity of flour and *OX* axis; *f (x)* is the area of the curvilinear trapezoid [a*, b*], limited by the gas holding capacity equation and *OX* axis.

The change in the volume of the dough during fermentation was determined using the measuring cylinder of 500 ml, which was placed 10 g of dough and kept at the temperature of 30 – 35 ºC. The change in dough volume was recorded every 60 s for 60 min.

Organoleptic assessment of bread quality was determined by the scale of quality assessment adopted by the Central Laboratory of the State Commission. The moisture content of bread was selected in the oven "Brabender" according to GOST 21094-75. The volume of the finished products was measured with the volume meter.

A comprehensive product quality assessment was performed using quality methods **[31]**, **[32]**.

Description of the Experiment

Sample preparation: The dough was kneaded as follows. The prescribed amount of yeast, salt, agar, and gelatin was dissolved in water at 35 ºC and mixed with the specified amount of rice flour. The dough was kneaded for 15 minutes. The dough was placed in the mould and left to ferment for 40 minutes and stand for 30 minutes at the temperature of 30 ºC. After fermentation, the dough was divided into pieces weighing 50 grams, placed in baking tins and baked for 35 – 40 minutes at the temperature of 180 ºC.

Number of samples analyzed: 8.

Number of repeated analyses: 3.

Number of experiment replication: 3.

Design of the experiment: In the course of experimental research and production tests, the following products were selected as objects, the obligatory general quality indicators of which corresponded to the indicators of the current normative documentation: rice flour, agar; gelatin; gluten-free bread; gluten-free dough.

The dough was kneaded as described according to conditions of FFE 2^3 (Table 1). The volume of the finished products was measured with the volume meter. Gas-forming ability and the rate of gas formation in the semi-finished product were determined in parallel with the degree of loosening of the dough.

The scale of quality assessment adopted by the Central Laboratory of the State Commission determined the organoleptic assessment of bread quality.

Statistical Analysis

For statistical analysis, absolute error was established by formula 2:

$$\Delta y_i = y_i - y_a \tag{2}$$

Where:

y_i – value of measured parameter; y_a – average value of measured parameter.

The next stage was calculation of dispersion – mean square of deviation of a random parameter from an average value of measured parameter by formula 3:

$$S_{(y_i)^2} = \frac{\sum_{i=1}^{n}(y_i - y_a)^2}{n-1} \tag{3}$$

Standard deviation was calculated by formula 4:

$$S_{(y_i)} = \sqrt{S_{(y_i)^2}} \tag{4}$$

And standard deviation of average result (formula 5):

$$S_{(y_a)} = \frac{S_{(y_i)}}{\sqrt{n}} \tag{5}$$

Checking the reliability of obtained results was made according to the Student's Criteria t_a for the number of conducted experiments f = n – 1 with the selected reliable probability α = 0.95 by formula 6

$$\varepsilon = t_a S_{(y_a)} \tag{6}$$

The establishment of reliable interval was conducted by formula 7:

$$y_a \pm \varepsilon \tag{7}$$

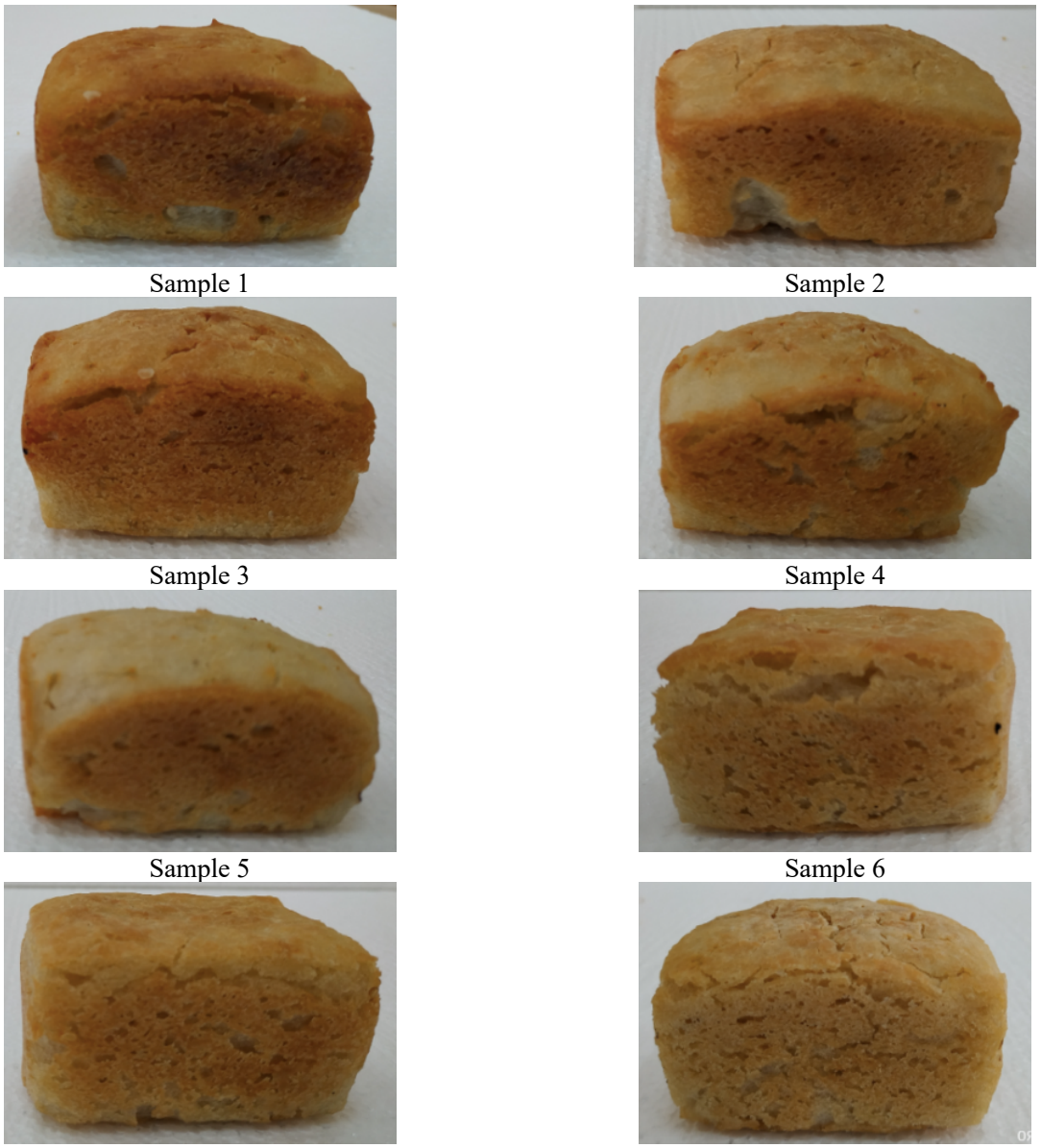
RESULTS AND DISCUSSION

To find the optimal modes of gluten-free bread production, we planned the full-factor FFE 2^3 **[28]**, **[29]**, **[30]**, **[31]**, **[32]** experiment. The factors of variation were selected, the amount of yeast,% (X_1), the amount of water, and % (X_2) the duration of fermentation and proofing, min. (X_3). The conditions of the experiment are presented in Table 1.

The specific volume was chosen as the criterion of optimality for implementing the full-factor experiment, which more fully characterises bread quality. In our opinion, determining the specific volume of bread is the most informative way to establish the influence of optimisation factors on making bread **[1, 2, 5]**. The maximum value of the particular volume of bread is chosen as the maximum developed porosity of products is reached at such value. The extreme criterion of optimality is achieved because after the maximum value of the specific volume of bread, with further movement in the direction of the optimization vector, the dough loses the ability to form a coherent structure. The results of trial laboratory baking are shown in Figure 1.

Table 1 Conditions for the full-factor experiment FFE 2^3.

Sample	Factor of variation		
	X_1	X_2	X_3
1	2.5	60	90
2	1.5	58	70
3	2.5	60	70
4	2.5	58	70
5	1.5	58	90
6	1.5	60	90
7	1.5	60	70
8	2.5	58	90



Sample 1 Sample 2 Sample 3 Sample 4 Sample 5 Sample 6 Sample 7 Sample 8

Figure 1 The results of trial laboratory baking of gluten-free rice bread.

Additionally, baking and shrinkage were determined. The results are presented in Table 2.

Table 2 Physico-chemical parameters of gluten-free rice bread.

Sample	Specific volume, $cm^3/100g$	Baked, %	Shrinkage, %
1	90	32.16	9.89
2	70	29.56	7.84
3	80	32.15	15.94
4	85	31.41	10.32
5	90	27.31	13.08
6	80	30.16	10.31
7	100	29.07	8.92
8	80	30.41	9.30

Note: (n = 3, $p \leq 0.05$).

The results show that adding yeast in the amount of 1.5% by weight of flour is advisable, as variants of samples with such a concentration have well-developed porosity, higher specific volume, and lower shrinkage. The optimum humidity of the dough is 60%, because when the humidity is reduced to 58% (samples 2, 4, 5, 8), have a dense crumb structure and a lower specific volume (average 8%). Prolonged spawning time leads to the formation of the dense crumb structure in samples 1, 5, 6, and 8, so it is not advisable.

Thus, gluten-free bread of a certain level of quality can be obtained by adding yeast in the amount of 1.5%, dough moisture of 60%, and the duration of fermentation and proofing of 70 minutes. However, even under these technological regimes, the prototypes do not have such quality indicators that are fully able to satisfy consumers **[6]**, **[7]**, **[9]**, **[9]**, **[10]**. Therefore, the next step was to determine the potential of gelatin and agar as gluten-free bread improvers. We recommend using polysaccharides and protein structurants **[11]**, **[12]**. To substantiate the type of additive and its concentration, the next stage of the study determined the effect of gelatin, agar, and their joint introduction on the specific volume and height of bread samples (Figure 2 and Figure 3).

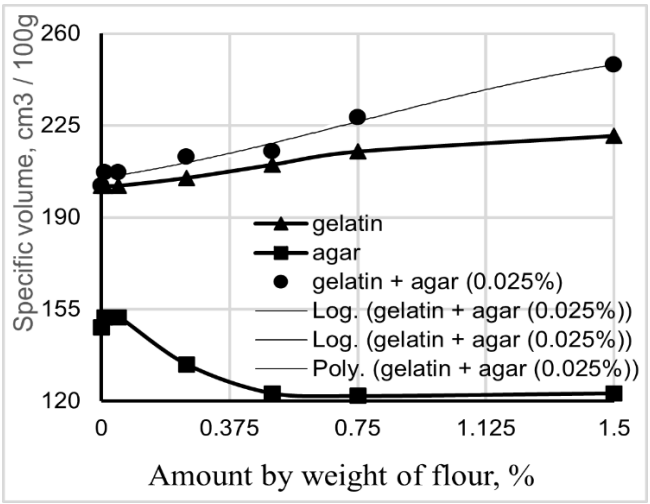


Figure 2 Dependence of specific volume of bread from the concentration and type of structure forming additives.

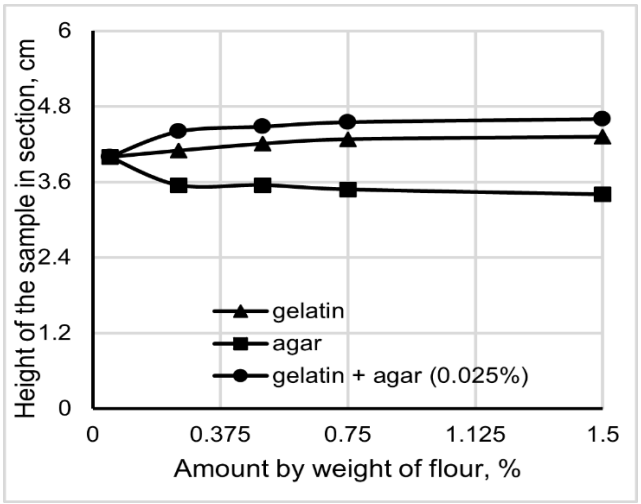


Figure 3 The dependence of the bread sample height from the concentration and type of structure forming additives.

The following samples were used for the study: samples with the addition of gelatin in the concentration of 0 to 1.5% (because the introduction of gelatin to more than 1.5% causes the unpleasant odor and darkening of bread crumbs **[13]**, **[14]**, **[15]**; sample with the addition of agar in the concentration of 0 to 0.050%; sample with the combined use of agar and gelatin (where the amount of agar 0.025% was taken as the constant, as previous studies have shown that increasing the amount leads to the decrease in the specific volume of bread) **[16]**, **[17]**, **[18]**, **[19]**, **[20]**. Two circumstances can explain this: firstly, in minimal amounts, agar can increase the volume of bread, and secondly, the ability of mixed jelly to improve significantly at lower concentrations of components in comparison with single-component jelly.

The research results show that the use of agar alone harms both the specific volume of bread and the height of the sample. Thus, when the agar content increases to 0.08%, the specific volume and height of the sample decrease

by 16.9% and 15.0%, respectively. Thus, increasing the agar content by more than 0.025% by weight of flour is not considered appropriate. This result may be due to the agar's high-water absorption and water holding capacity, resulting in competition for water between biopolymers of flour and additives. The addition of gelatin slightly increases the indicators by 9.4% and 8%, respectively, to increase the specific volume and height of gluten-free bread when adding gelatin and agar. At the same time, with increasing the number of additives to the mass of flour, the results improve.

The addition of polysaccharide and protein improvers to the formulation of the gluten-free dough to regulate its technological properties can significantly affect the intensity of fermentation and the activity of amylolytic enzymes in flour **[21]**, **[22]**, **[23]**, **[24]**, **[25]**, **[26]**.

It should be noted that the technological stage of fermentation for the gluten-free dough is significantly reduced than for wheat, so studies of the dynamics of CO_2 release were performed for 70 min, corresponding to the established duration of fermentation during PFE. The results show (Figure 4) that adding polysaccharides and protein additives lead to the accumulation of carbon dioxide in gluten-free dough by 33 – 44%.

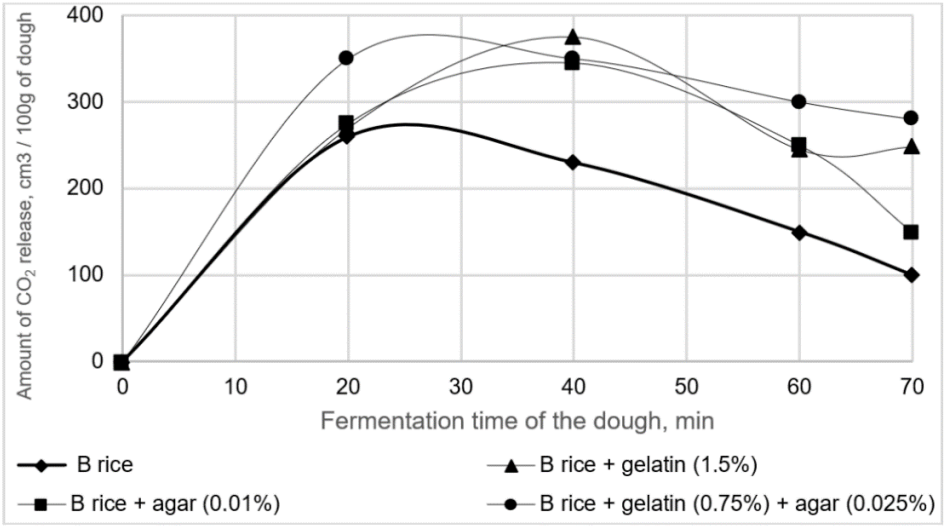


Figure 4 Amount of carbon dioxide released by gluten-free rice dough.

It is possible to assume that in the presence of supplements, the nutrition of yeast cells is improved (possibly by facilitating the transport of nutrients).

Also, research results show that adding additives leads to a slight slowdown in fermentation in the dough. In most cases, the peak of carbon dioxide accumulation is shifted by 15 – 20 minutes. In the case of using structurants, extending the fermentation to 40 minutes and settle to 30 minutes.

In order to establish the recommended modes of dough fermentation, a study of the change in dough volume was conducted. It was found that adding additives slightly shifts the peak of the fermentation process (Figure 5).

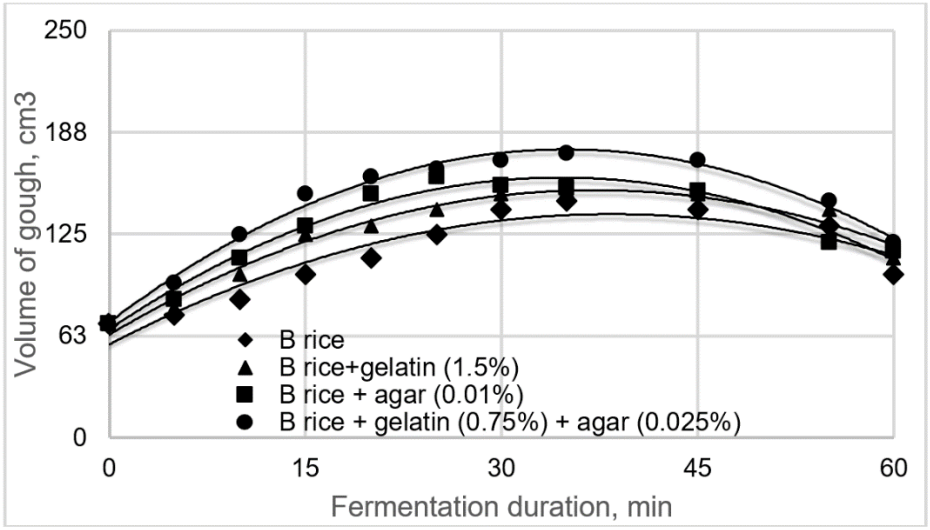


Figure 5 Changing the volume of gluten-free rice dough during fermentation.

Thus, it was determined that the recommended duration of fermentation of rice flour dough with the addition of gelatin is 30 – 35 min, with the addition of agar 25 – 30 min, with the addition of the gelatin mixture and agar 40 – 45 min. It should be noted that each sample was stand for 30 min after fermentation.

The increase in dough volume can be explained by the improvement of the rheological properties of the dough with additives and the higher ability of the dough to retain gas.

In addition, studies have shown that introducing additives changes the rate of accumulated CO_2 in the dough (Table 3).

Table 3 Rate of accumulated CO_2 in the dough, ml CO_2/min.

Composition of the sample	Fermentation duration, min								
	5	10	15	20	25	30	35	45	60
B rice	15.00	8.50	6.67	5.50	5.00	4.67	4.14	3.11	2.36
B rice + gelatin (1.5%)	16.00	10.00	8.33	6.50	5.60	5.00	4.34	3.33	2.55
B rice + agar (0.01%)	17.00	11.00	8.67	7.50	6.40	5.17	4.40	3.38	2.73
B rice + gelatin (0.75 %) + agar (0.025%)	19.00	12.50	10.00	8.00	6.60	5.67	5.00	3.78	2.64

Note: (n = 3, $p \leq 0.05$).

The study results show that the highest rate of accumulated CO_2 in the dough can be achieved by making gelatin in combination with agar. At the same time, after 5 minutes of fermentation, the amount increases by 26% compared to the sample without additives, and after 10 minutes by 47%. After 35 minutes of fermentation, the rate of CO_2 begins to decrease: which coincides with the peak of reaching the maximum volume of the dough. Therefore, the addition of additives in the mixture significantly improves the gas-forming and gas-holding capacity of the dough, which allows prolonging the fermentation operation accumulate more gas in the dough and get bread with a higher specific volume and better organoleptic properties **[27]**, **[28]**, **[29]**, **[30]**.

At the next stage of the study, the bread quality was assessed depending on the baking duration **[31]**, **[32]**. Based on the results of experimental research, quality stars were constructed, and the rational duration of baking was determined (Figure 6).

The assessment was performed according to the 5-point scale:

- crumb stickiness: 1 – sticky, viscous, 2 – sticky, 3 – sticky in the middle, baked from the edges, 4 – baked throughout, there is the slight stickiness, 5 – baked throughout, excessive no stickiness;
- crust stickiness: 1 – sticky, not separable from the form, 2 – sticky, partially separated from the form, 3 – completely separated from the form, but there is excessive stickiness, 4 – there is slight stickiness, 5 – no excessive stickiness;
- porosity: 1 – absent, 2 – slightly developed, 3 – developed from the edges of the crumb, absent in the middle, 4 – unevenly developed throughout the volume of the crumb, 5 – well and evenly developed;

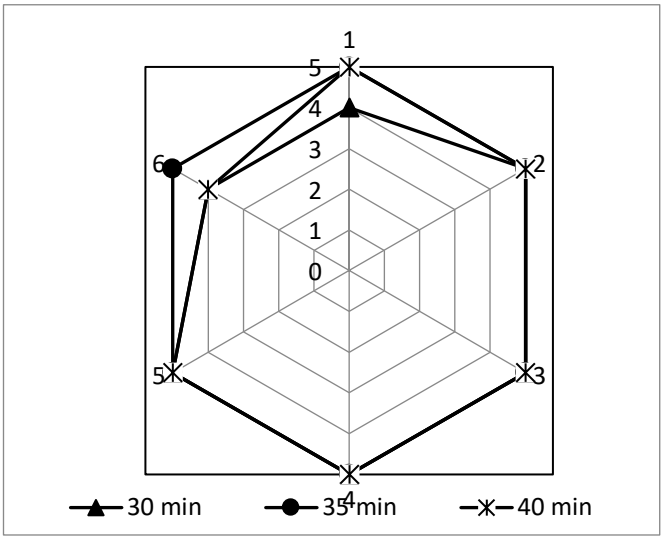


Figure 6 Stars of gluten-free bread quality based on rice flour (depending on the duration of heat treatment (1 – the stickiness of the crumb, 2 – the stickiness of the surface, 3 – porosity, 4 – the presence of crust, 5 – smell, 6 – the color of crust).

- Crust: 1 – completely absent, 2 – slightly developed in the upper part of the bread, 3 – developed in the upper part of the bread and absent in the lateral parts, 4 – unevenly developed over the entire surface of the bread, 5 – evenly developed over the entire surface of the bread.
- odor: 1 – side, unpleasant, 2 – not expressed, side, 3 – weakly expressed, characteristic, 4 – expressed, characteristic, 5 – intensely expressed, characteristic;
- crust color: 1 – pale or burnt, 2 – light beige or from yellow to brown, very uneven, 3 – yellow or intensely dark brown, not uniform enough, 4 – light golden or brown, fairly uniform, 5 – from golden to light brown, uniform.

The results show that the rice flour-based bread is fully baked after 35 minutes of baking at 180 ºC. When extending the duration of heat treatment, the quality of bread does not change, so long-term heat treatment is not economically feasible.

Thus, based on the studies carried out, it can be argued that the introduction of additives of the polysaccharide and protein nature does not lead to significant changes in the rate of evaporation of moisture from the dough. Therefore, the increase in the duration of heat treatment is inappropriate.

CONCLUSION

It is established that the optimal humidity of gluten-free rice bread is 60%. At such humidity, the porosity of the dough develops well, the specific volume increases, and the crumb's moisture remains sufficient. The study results show that adding polysaccharide and protein additives leads to an increase in the amount of carbon dioxide accumulation in gluten-free dough by 33 – 44%. It is experimentally substantiated that the recommended duration of fermentation of rice flour dough with the addition of gelatin is 45 – 50 min, with the addition of agar is 25 – 30 min, with the addition of the mixture of gelatin and agar is 40 – 45 min. It is established that to achieve full readiness of bread based on rice flour can be reached after 35 minutes of baking at the temperature of 180 ºC.

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The impact of the Russian embargo on the development and specialization of agri-food trade between Slovakia and Russia

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ABSTRACT
The paper examines and evaluates the impact of the Russian embargo on the development and specialization of agri-food foreign trade between Slovakia and Russia through the evaluation of the one-factor Lafay and Grubel-Lloyd indexes. As a result of the application of the Russian embargo on imports of agri-food products, based on the calculation of the indexes, we can state that the degree of specialization of Slovak agri-food foreign trade has changed. Although in 2013 Slovakia specialized in exporting a relatively wide range of agri-food products, in 2020, their number decreased. Also, in 2020 there was no overall increase in the volume of mutual trade. In general, Slovakia's exports to Russia decreased compared to 2013. The following factors have contributed to this situation: substantial attenuation, that is, the elimination of Slovak agri-food exports to Russia based on the impact of the Russian embargo with side effects and an increase in imports from Russia but not in absolute but relative terms in the context of its comparison with Slovak exports.

Keywords: agri-food foreign trade embargo, Grubel-Lloyd index, Lafay index, Slovakia, Russia

INTRODUCTION

The development of foreign trade in the Slovak Republic in the transformation process of the Slovak economy was characterized by gradual liberalization. The signing of several trade agreements with the independent Slovak Republic contributed to this development, the most important of which was the Association Agreement with the EU, the Central European Free Trade Agreement (CEFTA) and the customs union with the Czech Republic. These and other agreements predestined the development of Slovak agri-food foreign trade to be oriented mainly to the countries of the future EU.

The enlargement of the EU to include the countries of Central and Eastern Europe in 2004 meant a significant expansion of the European common agricultural market in which Slovakia has improved its position. However, it remained a net importer of agri-food commodities. Slovakia benefited from bilateral agreements even before it acceded to the EU. However, these advantages were limited and acted asymmetrically on the foreign trade of the Slovak Republic. Instead of increasing exports, they supported the increase in imports from EU countries. However, the volume of foreign trade in the Slovak Republic developed favourably. Nevertheless, from a territorial point of view, except for the Commonwealth of Independent States - CIS countries led by Russia, where there was a significant decrease in Slovak exports between 1996 and 2005 of up to 60% due to the reorientation of the Slovak Republic to EU countries and the negative economic situation in this group of countries. Agri-food trade played an important role before the accession of the Slovak Republic to the EU within the framework of total foreign trade of the Slovak Republic with EU countries. According to Gálik and Dome **[1]**, the accession countries had a significant position in the agri-food trade of the EU in 2004, which represented up to 85.8% of the total agri-food turnover. Several specific factors influence the current export of agricultural and food products from the Slovak Republic. One of them is the introduction of the RF embargo on imports of agri-food products from the EU, which is also a subject of this research **[2]**. After the European Union began to apply trade policy

sanctions in 2014, Russia's response to retaliatory sanctions was prompt. Russia has imposed an embargo on imports of certain agri-food products from these countries. The embargo entered into force on 7 August 2014 and is still valid today through its further extensions. Russia has banned imports of EU countries from EU countries to import beef, pork, poultry, meat, fish, shellfish, milk and dairy products, fruit, vegetables, nuts and other agricultural products **[3]**. On 1 November 2016, Russia's sanctions list was extended by an embargo on the import of edible salt and products containing sodium chloride **[4]**. This regime worked until the beginning of 2022, when Russia invaded Ukraine, resulting in the massive introduction of trade policy sanctions by western states against Russia. In general, the nature of the Russian embargo can be described as a measure targeted at the agricultural sector, where the assumption is that the reorientation of Russian importers to other suppliers will be easier. At the same time, it is also an effort to support domestic agricultural production **[5]**.

Agri-food sanctions and their impact on economies and foreign trade between the EU and Russia have been an object of research by many authors. Crozet and Hinz, for example, estimate the effects of sanctions and countersanctions on trade between the Russian Federation and 37 countries in 2014 and 2015. They concluded that most trade losses in European countries are not directly related to Russian countersanctions **[6]**. Skvarciany et al. (2020) estimate the total loss of exports from countries of the European Union to Russia at more than USD 226 billion from 2014 – 2018 **[7]**. Boulander et al. estimate that Russia bears the highest income loss (about €3.4 billion) while the EU recovers part of its lost trade by expanding exports to other markets **[8]**. Various one-factor evaluation indicators can be used to assess the impact of the Russian embargo on the development of mutual agri-food trade between Slovakia and Russia. The most important indicators of the international competitiveness of certain goods or industries include the Lafay Index of Revealed Comparative Advantages and the intra-industry trade index (IIT), also known as the Grubel-Lloyd index.

An increase or decrease in the values of indexes may reflect the dynamics of the development of competition between the studied countries **[9]**. Studies carried out on the similarity of food consumption patterns in selected EU countries, combined with the similarity of food production and imports, show that the EU models of food consumption patterns are similar to countries with the current increase in disparities in the structure of food production driven by its specialization, hence the need to meet part of the demand for food through imports **[10]**. The Lafay index (LFI) is used most frequently to assess the international specialization of foreign trade. It was first used by Lafay **[11]**, who also considered the impact of the gross domestic product on the disclosed comparative advantages. According to Zaghini **[12]**, to measure the degree of trade specialization, it is more advantageous to use the LFI over the Balassus Index of Uncovered Comparative Advantages (RCA) because the LFI allows us to control intra-industry trade and re-export, while at the same time shocks caused by cyclical factors can be avoided. Furthermore, the LFI does not only capture trade from an industry perspective but also considers various irregularities caused by macroeconomic effects through the use of the gross domestic product **[13]**. The Lafay index makes it possible to analyze each particular product's position within each country's foreign trade structure or group of analyzed countries.

Grubel and Lloyd **[14]** analyzed a possible anomaly that assumed that a country's high share of trade is made up of both internal and external trade. The IIT compares exports and imports of the same type of goods or the same industry between two countries or regions. Within the IIT calculation, a distinction is made between horizontal and vertical trade. Horizontal trade is mutual trade in the same products of the same quality, and vertical trade is a bilateral trade in vertically differentiated products that differ in quality and price **[15]**. This index is gaining importance in today's globalised world, as it follows the principle of exchanging similar products. The growing maturity of trade economies influences the growth of intra-industry trade in the world economy. Other authors developed the theory of intra-industry trade. Havrylyshyn and Kunzel **[16]** perceived it as a measure of diversity at the level of specialization or through the state of the country's industrial progress. Based on this assumption, they explain why this index was used to measure a country's ability to cope with competition in a changing environment. According to them, this idea is why IIT is recognized as a way to measure competitiveness.

The use of intra-industry trade was also addressed in the works of Hamilton and Kniest **[17]**, Brülhart **[18]**, Thom and McDowell **[19]**, Crespo and Fontoura and others **[20]**. The volume of intra-industry trade varies inversely with the level of trade restrictions and creates additional trade policy objectives that specifically influence the range of exported and imported commodities **[21]**. Baccini, Dür and Elsig **[22]** investigated the impact of intra-industry trade and global value chains on the political economy of trade. The impact of intra-industry trade on tariff reductions between countries is very diverse. Some studies show that the presence of intra-industry trade is accompanied by rapid and significant reductions in tariffs, while others indicate slow and insignificant reductions in tariffs. The findings of Milner **[23]** and Manger **[24]** reflect that intra-industry trade can directly reduce competition between products and thus reduce the number of domestic companies that perceive foreign imports as a threat. Another group of authors points out that the presence of intra-industry trade can strengthen narrow protectionist groups, which can result in lobbying for the protection of private goods **[25]**.

Scientific Hypothesis

This paper aims to examine and evaluate the impact of the Russian embargo on the development and specialization of foreign agri-food trade between Slovakia and Russia through the evaluation of the one-factor Lafay and Grubel-Lloyd index. To refine scientific research, in addition to research goals, we also set a scientific hypothesis (H), which is based on our assumptions based on a long-term study of the research issues:

H: The Russian embargo imposed on the import of agri-food goods from the EU resulted in a change in the specialization of Slovak agri-food foreign trade in the Russian Federation and reciprocal intra-industry trade in 2013 – 2020.

MATERIAL AND METHODOLOGY

Data from the EUROSTAT database and the Slovak Republic Statistical Office (SO SR) were used for this research. The structure of the foreign trade commodities of agri-food was classified according to the Harmonized System (HS) at the level of HS2. The limitation of the investigation was as follows: The analysis of the development of agri-food foreign trade between the Slovak Republic and Russia was determined from 2010 to 2020.

Research has been established for using LFI and GLI indexes since 2013, when the Russian embargo was implemented in 2014. The adverse effects of the embargo on Slovak agri-food exports are reflected in various indicators. We decided to point out these adverse effects through the Lafay index, with which we analyzed the specialization of Slovak agri-food exports concerning Russia. We chose 2013 as the reference year, as it was the last year before introducing the Russian agri-food embargo. We compare the LFI index values with those for 2020. EUROSTAT data was used as a relevant data source. We calculate the LFI index for individual commodity groups according to the HS2 classification. The evaluation of the specialization of mutual agri-food trade between the Slovak Republic and the Russian Federation is based on the calculation of the Lafay index **[11]**:

$$LFI_j^i = \left(\frac{x_j^i - m_j^i}{x_j^i + m_j^i} - \frac{\sum_{j=1}^{N}(x_j^i - m_j^i)}{\sum_{j=1}^{N}(x_j^i + m_j^i)}\right)\frac{x_j^i + m_j^i}{\sum_{j=1}^{N}(x_j^i + m_j^i)} * 100 \quad (1)$$

Where:

x^i_j – export of the commodity "j" of the country "i" to the rest of the world; m^i_j – the import of commodities from the country and the rest of the world; N - number of commodities for which LFI is calculated.

$$\sum_{j=1}^{N} LFI_j^i = 0 \quad (2)$$

The LFI provides information on the existence of comparative advantages at the bilateral level, where xij and mij represent exports and imports of the product of a given country or integration group i, to the world. If the LFI value is positive, there is a comparative advantage, and the higher the value of this index, the higher the degree of specialization of the country. On the contrary, the negative value of the LFI index points to a marked lack of specialization and comparative advantages. Therefore, by calculating the index, we can point out how the specialization of Slovak agri-food exports to Russia has changed due to the embargo. Negative values of the index indicate de-specialization, while positive values indicate the specialization of exports.

The index could not be calculated for some commodity groups due to the absence of trade in the given year. To deepen the analysis of mutual agri-food trade between the Slovak Republic and Russia, we supplemented the research with the results of the Grubel-Lloyd index of intra-industry trade, through which we find out whether there is an intra-industry trade between the Slovak Republic and Russia within the monitored group of agri-food products, i.e. what changes in this indicator occurred during the period under review. The Grubel-Lloyd index of intra-industry trade measures the value of trade between countries with products that are similar or slightly different **[14]**:

$$GLI = [(Xj + Mj) - |Xj - Mj|]/(Xj + Mj), \quad (3)$$

Where:

Xj represents the export of commodity j and Mj the import of commodity j, the index can reach values in the range 0 to 1.

If:

- GLI = 0, meaning that the country analyzed is a net exporter or importer of the commodity, and there is no intra-industry trade;
- GLI = 1, which means that there is intra-industry trade between countries; that is, the country exports the same amount of goods as it imports.

The closer the GLI value is to 0, the higher the degree of specialization in trade relations between the countries studied **[26]**.

Statistical analysis

Calculations have been performed by using Microsoft Excel 2010 software in combination with statistical software XLSTAT. The measured and calculated values were statistically evaluated. Statistical analyzes and calculations were performed using the Microsoft Excel 2010 application program. Procedures and methods of MS Excel application were used in the evaluation of the measurements. Subsequent statistical analysis of the data was conducted by Microsoft Excel - XLSTAT.

RESULTS AND DISCUSSION

Development of agri-food foreign trade between the Slovak Republic and Russia

The subject of this article is to examine the impact of the Russian embargo on agri-food trade between the Slovak Republic and Russia. We primarily focus on Slovakia's exports, as it has been negatively affected by the Russian agri-food embargo. Slovakia's foreign trade is focused primarily on EU countries, and non-member countries represent only alternative markets. Russia is one of the important trading partners of the Slovak Republic in this sector among third countries. A group of factors determines the demand for the current agri-food production of the Slovak Republic. The determining factor is the current embargo of the Russian Federation on imports of agri-food products from the EU, which automatically applies to the Slovak Republic as well as the development of prices of agri-food products.

Exports of agri-food products from the Slovak Republic in 2020 reached the value of 2,718.0 million (Figure 1). EUR and imports reached a value of 4,489.3 mil. EUR. The total turnover of foreign trade of the Slovak Republic with agri-food products in 2020 reached a value of 7,207.3 million EUR. In 2020, agri-food products, therefore, accounted for 4.86% of the total foreign trade of the Slovak Republic (exports 3.60%; imports 6.17%). The Slovak Republic has a long-term passive balance of foreign trade in agri-food products, which is caused by the export of products with lower added value than in the case of imports **[28]**. The highest volume of foreign trade was recorded in 2019 during the review period (EUR 7,757.6 million), caused by a significant increase in the imports of live animals and animal products, as well as products from the food industry and beverages.

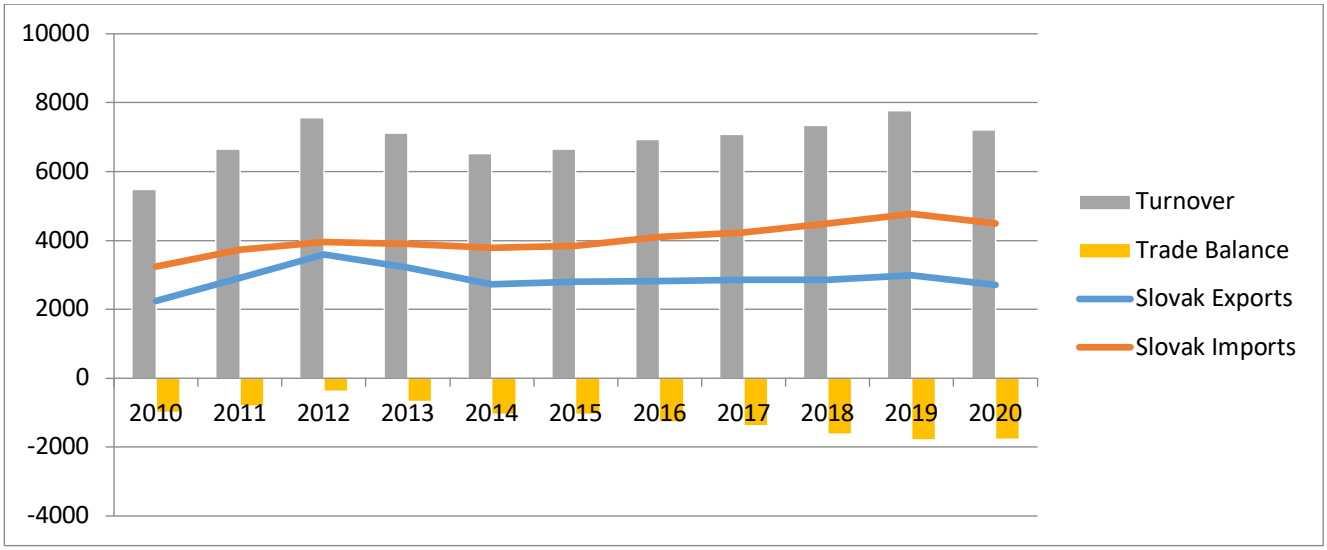


Figure 1 Foreign trade in agri-food of the Slovak Republic with third countries between 2010 – 2020. Note: Source **[27]**.

However, between 2012 and 2014, there was a significant decrease in foreign trade turnover by 13.7%. However, since 2014, there has been a gradual increase in interest rates. There was an increase in indicators of foreign trade

of the Slovak Republic with agri-food products, mainly due to increased imports. An overview of the territorial structure of Slovakia's agri-food foreign trade in 2020 is clearly shown in Figure 2.

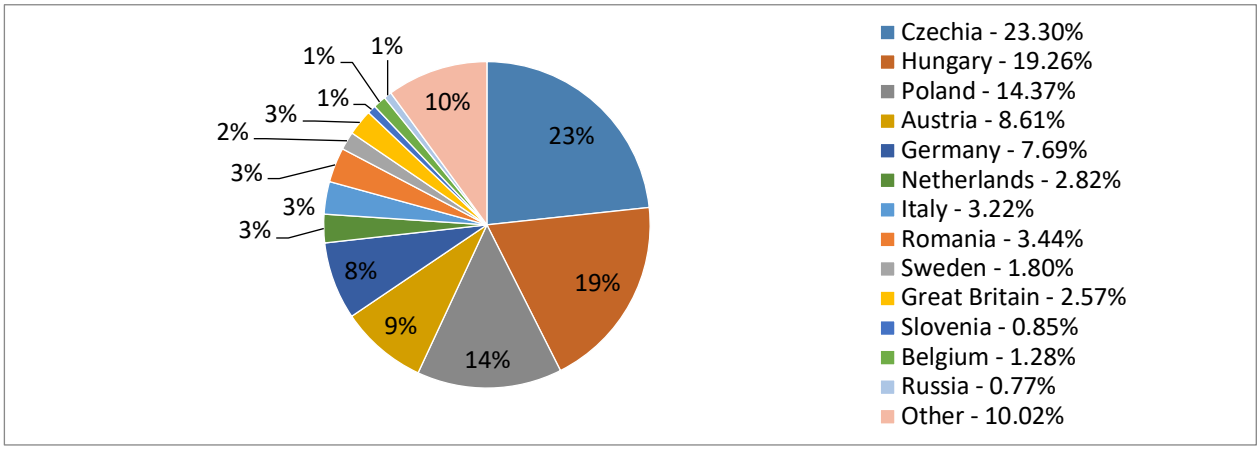
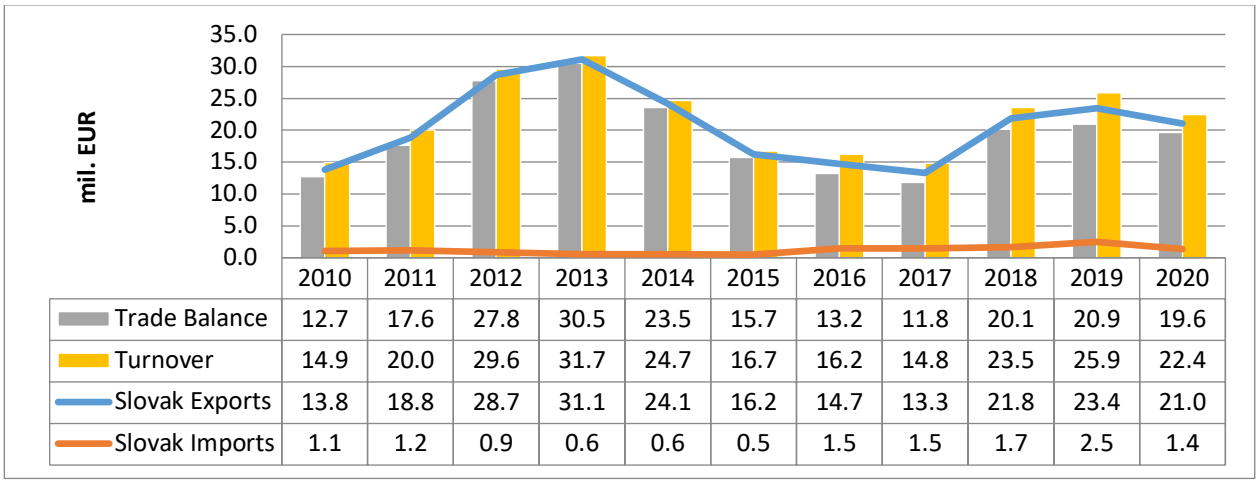


Figure 2 Territorial structure of Slovak agri-food export in 2020. Note: Source **[28]**.

Slovak exports of agri-food products are relatively less diversified from a territorial point of view, with more than 90% of exports going to EU countries. The largest share of 22.30% of exports in 2020 went to the Czech Republic (633.7 million EUR). This is followed by the countries neighbouring Slovakia, namely Hungary (19.26%), Poland (14.37%) and Austria (8.61%). Together, these four countries absorb 64.5% (EUR 1,751.6 million) of Slovak exports. In addition, there are EU countries such as Denmark, the Netherlands and Italy. Regarding agri-food exports to the Slovak Republic, Russia was Slovakia's 13th largest trading partner. The total export reached the value of 21 mil. EUR, which represented a 0.77% share in Slovakia's exports. However, it should be noted that Russia was the second most important export partner of the Slovak Republic among non-EU countries (after the United Kingdom). The total turnover of agri-food trade between the Slovak Republic and Russia reached 22.4 million EUR in 2020 (see Figure 3). There was a slight decrease in foreign trade turnover by 13.6% compared to 2019. The export of agri-food products from the Slovak Republic to the Russian Federation reached a value of 21 million EUR in 2020, and imports 1.4 million EUR. The foreign trade balance was active in the amount of 19.6 mil. EUR.

The amount of the balance itself points to a significant predominance of Slovakia's agri-food exports over its imports. From 2010 to 2013, mutual trade grew at an average rate of 36%, mainly due to export growth. Exports had a dominant impact on the overall development of trade turnover during the entire period under review. Significant fluctuations did not characterize the development of imports, its value oscillated for most of the period in the range of 0.6 – 2.5 mil. EUR. The year 2013 reached the highest value of mutual agri-food trade, and its future development seemed highly promising. However, in 2014, the Russian agri-food embargo started a period of a relatively significant decline in Slovak exports, which lasted until 2017. It again had an immediate effect on the decline in mutual trade turnover.



	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Trade Balance	12.7	17.6	27.8	30.5	23.5	15.7	13.2	11.8	20.1	20.9	19.6
Turnover	14.9	20.0	29.6	31.7	24.7	16.7	16.2	14.8	23.5	25.9	22.4
Slovak Exports	13.8	18.8	28.7	31.1	24.1	16.2	14.7	13.3	21.8	23.4	21.0
Slovak Imports	1.1	1.2	0.9	0.6	0.6	0.5	1.5	1.5	1.7	2.5	1.4

Figure 3 Development of agri-food trade between Slovakia and Russia during the years between 2010 – 2020 (EUR million). Note: Source **[27]**.

For comparison, Cheptea **[29]** calculated that the Russian food embargo cost monthly losses of 125 million EUR to the exports of the European Union. In 2018, a significant recovery in Slovak exports was recorded (an increase of 63.9%), while this trend was also reflected in 2019 (an increase of 7.3% compared to 2018). The Russian embargo on imports of selected agri-food products and commodities undoubtedly had a significant share in the decline in exports in 2014 – 2017 (either directly or indirectly). Its extent is the subject of analysis in the following graphs and tables.

Table 1 Development of exports of 25 groups of agri-food products from the Slovak Republic to Russia according to the HS2 classification (2013, 2017, 2020; EUR million).

HS2	Goups of agri-food products	2013	2017	2013/2017	2020	2013/2020
		thousands of EUR	% change		thousands of EUR	% change
	Agri-food export from SR to RF	31 319	13 415	-57.2	21 167	-32.5
01	Live animals	2 456.2	972.2	-60.5	1 331.5	-45.8
02	Meat and edible meat offal	16.0	0	-100.0	0	-100.0
03	Fish and crustaceans, molluscs, and others	0	0	-	0	-
04	Milk and milk products	5 225.2	3 435.3	-34.3	7 844.3	+50.9
05	Products of animal origin	0	46.9	-	1.2	-
06	Live trees and other plants, flowers	0	0	-	24.8	-
07	Vegetables, edible plants, roots and tubers	0	0	-	0	-
08	Edible fruits and nuts	14.8	0	-100.0	0	-100.0
09	Coffee, tea, spices	140.1	0	-100.0	3.7	-97.4
10	Cereals	79.4	208.8	+163.0	12.8	-83.9
11	Mill products, starches	2 820.4	1 426.9	-49.5	441.9	-84.3
12	Oil seeds and oleaginous fruits	0	1.8	-	43.8	-
13	Shellac, gums, resins and others	0	0	-	0	-
14	Products of vegetable origin, non - specific	0	0	-	0	-
15	Animal or vegetable fats and waxes	252.3	17.8	-92.9	65.4	-74.1
16	Preparations of meat, fish and crustaceans	6 698.2	250.8	-96.4	0	-100.0
17	Sugar and confectionery	310.4	900.8	+190.2	1 011.8	+226.0
18	Cocoa and cocoa preparations	1 082.7	45.4	-95.9	3 031.5	+180.0
19	Preparations of cereals, flour, starch and milk	502.4	1 449.8	+188.6	893.8	+77.9
20	Preparations of vegetables, fruits and nuts	263.5	1.2	-99.5	16.4	-93.8
21	Various edible preparations	2 939.6	656.3	-77.7	356.1	-87.9
22	Nonalcoholic and alcoholic beverages, vinegar	713.0	1 017.5	+42.7	1 791.0	+151.2
23	Residues and waste from the food industries	7 804.7	2 967.7	-62.0	4 297.2	-44.9
24	Tobacco and tobacco products	0	16.8	-	0	-
	Others	1	0	-100	2	+100.0

Note: Source **[27]**.

Table 1 analyses the development of the Slovak Republic's agri-food exports to Russia based on the classification into 25 commodity groups according to the chapters of the harmonised system. It compares the year before the embargo (2013), when the agri-food export of the Slovak Republic to Russia was the highest ever during the 2017 year when exports reached the lowest values and the current year 2020.

The commodity group HS 04 – Milk and dairy products had the largest share in Slovak agri-food export to Russia in 2020. The total export volume of this group was 7.8 mil. EUR, which represents a 37.2% share of total agri-food exports. Even though the embargo also applies to items in this group, compared to 2013, exports increased by 50.9%, which is because within this commodity group there are a number of exceptions to the embargo, i.e. the embargo focuses only on selected subheadings of the Combined Nomenclature. The second most important was the commodity group HS 23 – Residues and food industry waste, with a share of 20.3% (EUR 4.3 million) in the exports of the Slovak Republic. This was followed by HS 18 – Cocoa and cocoa preparations (14.3%); HS 22 – Nonalcoholic and alcoholic beverages (8.5%), and HS 01 – Live animals (6.3%).

Compared to 2013, when Slovak exports were not yet affected by the embargo, the commodity structure in 2020 was different in quantitative and qualitative terms. Although exports of certain commodity groups, such as HS 04 – Milk and milk products (+ 50.9%), HS 17 – Sugar and confectionery (+ 226.0%), HS 18 – Cocoa and cocoa preparations (+180, 0%) or HS 22 – Nonalcoholic and alcoholic beverages (+ 151.2%) increased quite significantly, exports of other commodity groups have stopped completely or partially. The effect of the Russian embargo completely eliminated the export of meat, fruit and nuts, fish and crustaceans, while there was a significant reduction in the exports of coffee, tea, spices, cereals, mill products, vegetable and fruit preparations. As a result of the direct and indirect effects of the embargo, Slovakia's total agri-food exports to Russia decreased by 32.5%. Thus, on the one hand, the embargo caused the effect of disappearance of trade and on the other hand, the effect of trade diversion. Smutka, Ľ., Rovný, P., & Hambalková, M. **[2]** and Zábojník, S., & Hamara, A **[5]** came to similar conclusions in their research, claiming that the introduction of the Russia embargo on the import of agri-food products from the EU was one of the factors that affected the export of agri-food in the Slovak Republic after 2014.

For comparison, we also present in Table 1 the values of agri-food exports from the Slovak Republic to Russia in 2017, when it reached its lowest ever value. In 2017, it reached the value of 13.4 mil. EUR, which is 57.2% less than in 2013. The uncertainty caused by the imposition of the embargo, the sanctions war and the political tensions between the EU and Russia has caused a decrease in exports not only of those commodity groups that directly fall under the sanctions lists but also the export of other commodities groups was negatively affected. Here we would allow ourselves to disagree with the authors' claim **[6]** that sanctions do not have a direct impact on the development of exports in EU countries, because our research on the example of Slovakia confirmed this, in contrast. However, it should be taken into account that their research was limited to the years 2014 – 2015. Since 2017, exports have gradually grown, which may be due to the stabilization of trade relations and a reduction in the degree of uncertainty in mutual trade relations. Skvarciany et al. **[7]** and Boulanger, P., Dudu, H., Ferrari, E., & Philippidis, G. **[8]** also came to similar conclusions, although their research was focused on the analysis of the EU markets as a whole, in contrast to our research, which focuses solely on the Slovak market.

Figure 4 compares the development of the total exports of all goods from the Slovak Republic to Russia with total agri-food exports from the Slovak Republic to Russia and with the export of groups of goods whose exports were affected by the Russian embargo. Figure 4 also shows the development of individual indicators in parallel in their development. The value of exports of commodity groups whose exports were affected by the embargo reached 6.4 million EUR in 2013, representing 20.4% of the total agri-food exports from the Slovak Republic to Russia. In 2017, this share decreased to 4.5% and in 2020 reached only 1.4% of the total agri-food exports of the Slovak Republic to Russia. As mentioned in the previous part of the analysis, the agri-food export from the Slovak Republic to Russia has decreased by 32.5% since 2013 (from EUR 31.3 million in the year to 21.2 million EUR in 2020). Exports of the commodity groups covered by the embargo fell between 2013 and 2020 by 95.4% or 6.1 million EUR. Until 2017, the development of commodities exports affected by the embargo and the development of total agri-food exports was symmetrical, as the values of both indicators decreased. Since 2018, we have seen an asymmetry in the fact that, while exports of affected agri-commodities have stagnated, total agri-food exports have begun to grow. Gabrisch, H., & Segnana, M. L. **[15]** and Kunzel, P., & Havrylyshyn, O. **[16]** encountered a similar problem in their research which show the influence between the growing maturity of business economies and the growth of intra-industry trade, and at the same time, they also perceived it as a measure of diversity at the level of specialization and industrial progress of the country.

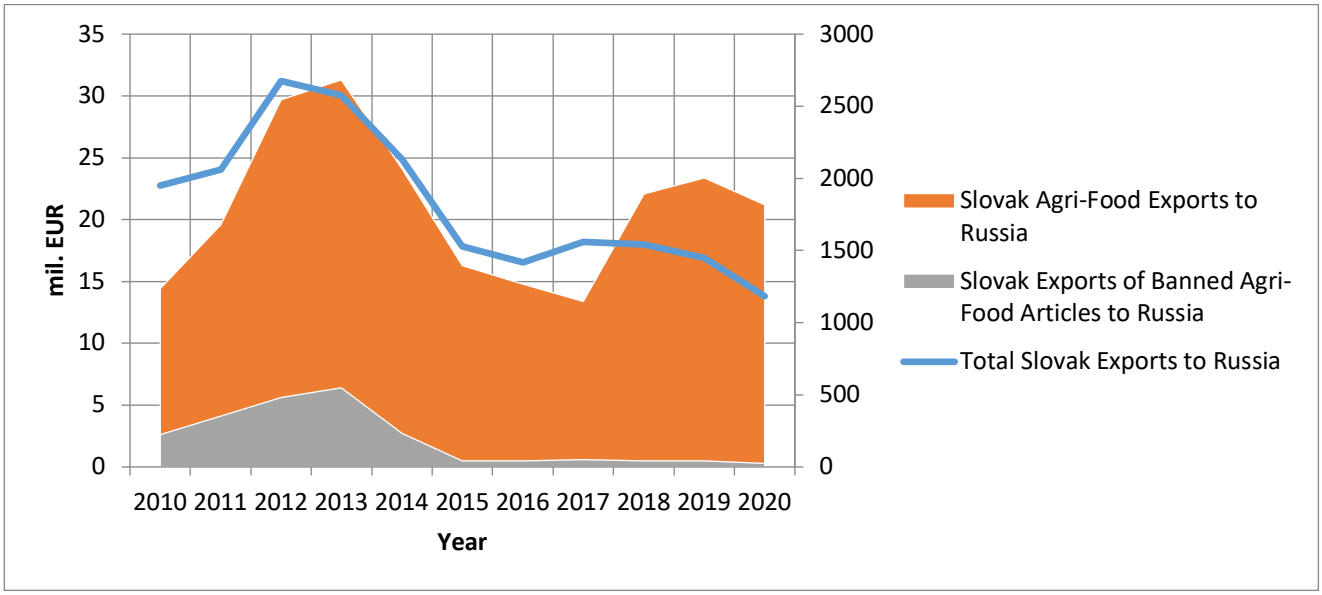


Figure 4 Comparison of total Slovak exports to Russia with total Slovak agri-food exports to Russia and with Slovak agri-food exports of articles banned by the Russian embargo (EUR million). Note: Source **[27]**.

Figure 4 also compares the agri-food exports of the Slovak Republic to Russia with the development of the total exports of the Slovak Republic to Russia. As in the case of the previous two indicators, there was a significant decrease in the case of total exports. In the following years, there was a relatively significant decline, which was characterized by a slight recovery in 2017. However, in 2020, exports reached the lowest value during the review period (EUR 1,183.5 million). Ultimately, exports in 2020 represent only 44.2% of the value of exports in 2012. However, it should be noted that the decline in the value of exports is affected not only by the Russian embargo but also by the decline in the purchasing power of Russian consumers due to the devaluation of the RUB against the strong Euro, which harmed not only Russian consumers but also Slovak exporters **[30]**. The decline in the exports has also been caused by the transformation of Russian external demand for agri-food products into internal demand, the Russian government's efforts to diversify foreign suppliers of agri-food products and the decline in Russia's GDP growth, which in 2014 was 2.5% instead of the expected 5.9%.

Specialization of agri-food trade in the Slovak Republic with the Russian Federation affected by the Russian embargo

Through the calculation of the LFI, we can point out the specialization of the agri-food trade of the Slovak Republic with the Russian Federation affected by the Russian embargo. The values in Table 2 shows how the specialization of individual groups of Slovak agriexport to Russia has changed according to HS 02 before the introduction of the Russian embargo (2013) and in 2020. We supplemented the LFI with the calculation of the Grubel-Lloyd index, which also indicates the degree of specialization of trade between the studied countries. The zero value of the index indicates that one of the countries is a net exporter or importer of goods and a value equal to one indicates that the country exports the same amount of goods as it imports, and thus there is intra-industry trade between the countries.

The values of the specialization index for 2013 indicate that the degree of specialization itself, that is, the trade specialization of individual commodity groups, was relatively low, as the values oscillate around 0. The highest degree of specialization was achieved in the case of commodity groups HS 23 – residues and waste from the food industry (0.75); HS 04 – Milk and milk products (0.75) and HS 16 – Meat, fish and crustacean preparations (0.73). On the contrary, the highest degree of despecialization was achieved in the group of HS 21 – Miscellaneous edible preparations (-1.36); HS 03 – Fish and crustaceans (-0.98) and HS 15 – Animal and vegetable fats (-0.48). However, from an overall point of view, it must be stated that in the case of HS 14 commodity groups, Slovak exports reached a certain level of specialization, and in the case of HS 07 commodity groups, exports were despecialized. Similarly to the results of the study by the Zaghini, A **[12]**, Alessandrini, M., Fattouh, B., & Scaramozzino, P. **[13]**, Grubel, H. G., & Lloyd, P. J. **[14]**, it is more advantageous to use the LFI and GLI indexes to measure the degree of specialization compared to the usual single-factor indicators of revealed comparative advantages.

Table 2 Analysis of agri-food trade between Slovakia and Russia using the Lafay and GL index.

HS2	Groups of agri-food products	LFI		GLI	
		2013	2020	2013	2020
01	Live animals	0.319	0.085	0.004	0
02	Meat and edible meat offal	0.002	n/a	0	n/a
03	Fish and crustaceans, molluscs and others	-0.981	-1.908	0	0
04	Milk and milk products	0.747	11.908	0	0
05	Products of animal origin	-0.010	-0.029	0	0.677
06	Live trees and other plants, flowers	n/a	n/a	n/a	n/a
07	Vegetables, edible plants, roots and tubers	-0.0022	-0.667	0	0
08	Edible fruits and nuts	0.002	-0.131	0	0
09	Coffee, tea, spices	0.019	-0.001	0.002	0
10	Cereals	0.011	0.297	0	0
11	Mill products, starches	0.401	2.057	0.001	0.0001
12	Oil seeds and oleaginous fruits	-0.058	-2.606	0	0.003
13	Shellac, gums, resins and others	n/a	-0.066	n/a	0
14	Products of vegetable origin, not specified	n/a	n/a	n/a	n/a
15	Animal or vegetable fats and waxes	-0.480	-0.588	0.485	0.754
16	Preparations of meat, fish and crustaceans	0.728	-0.084	0.011	0.335
17	Sugar and confectionery	0.0448	0.602	0	0.029
18	Cocoa and cocoa preparations	0.155	1.348	0	0.001
19	Preparations of cereals, flour, starch and milk	0.072	2.115	0	0.005
20	Preparations of vegetables, fruits and nuts	0.038	-0.008	0	0.893
21	Various edible preparations	-1.360	-2.667	0.173	0.647
22	Nonalcoholic and alcoholic beverages, vinegar	-0.397	-11.693	0.197	0.673
23	Residues and waste from the food industries	0.751	1.984	0.015	0.058
24	Tobacco and tobacco products	n/a	0.051	n/a	0.001
	Others	0.319	0.084	n/a	n/a

Note: Source **[27]**.

In 2020, they reached the highest level of specialization of the commodity group HS 04 – Milk and milk products (11.91); HS 19 – Preparations of cereals, flour, starch and milk (2.11) and HS 11 – Mill products (2.06). In terms of despecialization, they were among the most important HS 22 – Nonalcoholic and alcoholic beverages (-11.69); HS 21 – Miscellaneous edible preparations (-2.67) and HS 12 – Oil seeds and oleaginous fruits (-2.61).

Compared to 2013, there were some changes in 2020. First of all, it is clear that the circle of specialized commodity groups has narrowed to 10 commodity groups, while the circle of non - specialized commodity groups has expanded to 12. In addition, the degree of specialization and despecialization has increased for several commodity groups. Therefore, we can agree with the results of the studies **[9]**, **[10]**, **[11]** that the increase or decrease in the values of the GLI or LFI index can reflect the dynamics of the development of competition between the countries studied.

This can be attributed to the application of the Russian embargo on imports of selected agri-food commodities from the EU. Fewer commodity groups with a specialization index at the expense of commodity groups with a specialization index reflect the fact that Slovakia's exports decreased as a result of the embargo, while imports were not affected in any way by the embargo, which also reflects the development of Slovakia's foreign trade with Russia, which is shown in Table 2. The deterrence of index values from low to higher levels can be interpreted as the effect of some trade substitution in commodities whose exports were not affected by the embargo. Although exports of agri-food commodities covered by the embargo have disappeared, exports of other commodities have increased. The effect of the embargo is also clear in the case of de-specialized commodity groups, which are also higher than in 2013. As exports were restricted, imports increased relatively. It is obvious that the agri-food trade between the Slovak Republic and Russia is also affected by other factors, such as the changing price of agri-food products or market factors. However, their specific impact is not the subject of analysis in this article.

During the monitored periods, changes in the values of the Gruber-Lloyd index are also noticeable. As in the case of the Lafay index, the index values could not be calculated for all commodity groups due to the absence of trade. In 2013, zero values of the GL index were recorded for 12 commodity groups, which means that Slovakia was a net exporter or importer of commodities from the given group. In 2020, zero was recorded for only eight commodity groups. A closer analysis of export and import data shows what caused this development. In 2013, Slovakia was a net exporter of commodity groups. HS 02 – Meat and edible meat offal, HS 04 – Milk and milk products, HS 08 – Edible fruit and nuts, HS 10 – Cereals, HS 17 – Sugars and sugar confectionery, HS 18 – Cocoa and cocoa preparations, HS 19 – Preparations of cereals, flour, starch or milk, HS 20 – Preparations of vegetables, fruits, nuts while the net importer for the four commodity groups: HS 03 – Fish and crustaceans, molluscs and others, HS 05 – Products of animal origin, HS 07 – Vegetables, edible plants, roots and tubers and HS 12 – Oil seeds and oleaginous fruits. This is confirmed by research **[17]**, **[18]**, **[19]**, **[20]**, **[21]** in which intra-industry trade changes inversely proportionally under the influence of the sanction and creates other trade policy objectives that specifically affect the range of exported and imported commodities.

In 2020, Slovakia was a net exporter only in the case of two groups: HS 5 – Products of animal origin and HS 11 – Mill products, starches and a net importer in the case of five groups: HS 04 – Milk and milk products, HS 08 – Edible fruits and nuts, HS 09 – Coffee, tea, spices, HS 10 – Cereals and HS 14 – Products of vegetable origin, not specified. Between 2013 and 2020, there was an inversion of the ratio of the number of commodity groups for which Slovakia is a net exporter to the number of commodity groups for which Slovakia is a net importer. The introduction of the Russian embargo on the import of selected agri-food products undoubtedly contributed to this change, as Slovakia has stopped exporting them since its introduction. Looking at the opposite end of the achievable GL index values range, there have also been changes over the years. Looking at Table 2, it is clear that for several commodity groups, the value of intra-industry trade increased significantly, especially for HS commodity groups as follows: HS 05 – Products of animal origin, HS 15 – Animal or vegetable fats and waxes, HS 16 – Preparations of meat, fish and crustaceans, HS 20 – Preparations of vegetables, fruits, nuts, HS 21– Various edible preparations, HS 22 – Nonalcoholic and alcoholic beverages, vinegar.

In 2013, a certain level of intra-industry trade was recorded for eight commodity groups and in 2020 for 13 commodity groups. Paradoxically, however, in 2020 there was no overall increase in the volume of mutual trade. In general, Slovakia's exports to Russia decreased compared to the year 2013. Although its imports from Russia increased, this increase did not offset the decline in exports. This confirms the findings of the Baccini, L., Dür, A., & Elsig, M. **[22]** that the impact of intra-industry trade on the reduction of tariffs between countries is very diverse. A decrease in Slovak exports thus caused an increase in intra-industry trade within the monitored commodity groups and, at the same time, an increase in its imports. In contrast to the findings of Milner **[23]** and Manger **[24]**, who, on the contrary, pointed out that intra-industry trade can reduce the number of domestic companies, and thus cause a decrease in foreign imports, or, according to Kono, D. Y. **[25]**, strengthen narrow protectionist groups in the import of specified goods.

Currently, it is not clear to what extent the Russian-Ukrainian conflict will reach its peak and what consequences it will bring. In his research, Lam **[31]** pointed out the effectiveness of only hard restrictions. However, we can state that the EU's restrictive measures against the Russian Federation are among the most complex trade and political sanctions imposed since the Second World War and have not yet deterred the military activities taking place in Ukraine. Our research also supports the fact that sanctions measures are not always able to change the behavior of a given country, as they are insufficient and do not include a whole range of factors that influence political events, as stated in his study by Hufbauer **[32]**.

Kašťáková, Baumgartner & Žatko **[33]** in their earlier research) also addressed the impact of the Russian embargo on agri-food products from the EU to Russia in the period 2010 – 2016 after the first Russian-Ukrainian conflict in 2014. The authors came to the conclusion that if the sanctions persist, the EU will not be able to achieve the same volumes of mutual agri-food trade as they were in the years 2010 – 2013, even though the mitigation of

the influence of the sectional policy helped the use of indirect reexports of EU production through Belarus to Russia. Currently, such possibilities are not possible under the influence of the second Russian-Ukrainian military conflict from 2022.

Therefore, the hypothesis follows. The Russian embargo imposed on the import of agri-food goods from the EU resulted in a change in the specialization of the Slovak agri-food to RF and mutual intra-industry trade during the years between 2013 – 2020, based on the results of the LFI and GLI calculation, can be accepted.

CONCLUSION

We have come to the following conclusions based on research on the impact of the Russian embargo on the development and specialization of agri-food trade between the Slovak Republic and Russia. Even though Slovakia's agri-food foreign trade in the period under review was focused primarily on EU countries, in 2020 Russia ranked second among non-EU countries after the United Kingdom. The determining factor that influences the development of the prices of agri-food products and the change in the specialization of foreign trade in Slovak agri-foods is the ongoing embargo of the Russian Federation on the import of agri-food products from the EU, which automatically applies to the Slovak Republic. Between 2013 and 2020, due to the direct and indirect effects of the embargo, total agri-food exports from the Slovak Republic to Russia decreased by 32.5%. Political tensions between the EU and Russia and the established sanction policy have caused a decline in Slovak exports of not only those commodity groups which directly fall under the sanction lists but has also negatively affected other commodity groups of Slovak exports. As a result of the application of the Russian embargo on imports of agri-food products based on the calculation of the Lafay and Grubel-Lloyd indices, we can state that the degree of specialization of Slovak agri-food foreign trade has changed. Although in 2013 Slovakia specialized in the export of a relatively wide range of agri-food commodities, in 2020 this number of products decreased. Two factors contributed to this development. The first factor is substantial attenuation, i.e. the elimination of Slovak agri-food exports to Russia. The main reason can be identified as the Russian embargo and its side effects in the form of a decline in exports of other agri-food commodities. The second factor is the increase in imports from Russia but not in absolute but relative terms - we can talk about a substantial increase in imports in the context of its comparison with Slovak exports.

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

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The influence of beet pectin concentrate and whole-ground corn flour on the quality and safety of hardtacks

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ABSTRACT

Currently, the main task of food manufacturers is to continuously improve quality while complying with legal regulations primarily related to ensuring product safety for consumers. In this regard, using pectin substances as natural detoxifiers and wholemeal flour in the production of hardtacks will solve the problem of meeting the population's needs for safe food products with high nutritional and biological value. The article substantiates the sequence and parameters of technological operations for producing pectin concentrate from 'Ardan' sugar beet. The effectiveness of the use of beet pectin concentrate and whole-ground corn flour in the production of hardtacks has been substantiated experimentally based on a study of their qualitative characteristics, chemical composition and safety. The optimal dosage of pectin concentrate was determined at 10% and whole-ground corn flour at 15% in the production of hardtacks from first-grade wheat flour, where the properties of the gluten and the quality of finished products were similar to the control samples. The use of 'Ardan' sugar beet pectin concentrate made it possible to alter the dough's properties to increase its firmness and elasticity. It was found that the food and biological value of the developed hardtacks was higher than that of the control samples. The products obtained complied with the safety requirements of TR CU 021/2011 Technical Regulations of the Customs Union 'On Food Safety'.

Keywords: beet pectin concentrate, wholemeal corn flour, hardtacks, quality, safety

INTRODUCTION

One of the pressing problems of the modern development of states, considering the prospects for the coming years, is ensuring food security. The food industry, which encompasses industries producing goods for consumption by the population, is central to ensuring food security. In this regard, high-quality, balanced and safe food, considering standards, is of paramount importance. The quality of food products and their safety for the country should become a national priority, a national idea that must be enshrined in legislation.

In conditions of the rapid development of industry, transport, intensive development of minerals and the active chemicalisation of agriculture, the ecological conditions of human living are sharply deteriorating: air, water, and soil. Foodstuffs contain an excessive amount of environmentally harmful substances, among which radionuclides, pesticides, heavy metals salts and others are particularly important **[1]**, **[2]**.

In this regard, the problem of detoxification of the human body with the help of special substances, for example, pectin, is very urgent. Detoxification of the body is the basis of a healthy life. This process can be briefly described as cleansing and resting the body and nourishing it from the inside. Eliminating and removing toxins and nourishing the body helps protect against disease and restore natural maintenance of optimal health. The main physiological property of pectin, which predetermines its use in the production of dietary food, is the ability of pectin to bind and remove heavy metals and radionuclides from the body. The mechanism of action of pectin concerning the removal of metals is as follows: Entering the gastrointestinal canal, pectin forms gels. When swollen, the mass of pectin dehydrates the alimentary canal and, moving along the intestine, captures toxic substances. Many experts call pectin the orderly of the human body for its unique ability to remove harmful substances such as radioactive elements, toxic metal ions and pesticides from the body without disturbing the bacteriological balance of the body **[3]**, **[4]**.

The group of dietary fibers includes polysaccharides, mainly of plant origin. The group of dietary fibers includes cellulose, hemicellulose, lignin, phytin, chitin, pectin, gums (gum), mucus, protopectins, and alginates. Dietary fibers perform a number of important biological functions, not only in relation to the digestive system but also in terms of systemic metabolism. In unstructured ballast substances (pectin, etc.), water binding occurs by turning into gels. Dietary fiber increases the binding and excretion of bile acids, and neutral steroids, including cholesterol, from the body and reduces the absorption of cholesterol and fats in the small intestine. Due to the absorption capacity, dietary fiber adsorbs or dissolves toxins, thereby reducing the risk of contact of toxins with the intestinal mucosa, the severity of intoxication syndrome and inflammatory-dystrophic changes in the mucous membrane. Due to their ion-exchange properties, dietary fiber removes heavy metal ions (lead, strontium) and affects electrolyte metabolism in the body. Some opportunistic bacteria absorb nutrients through the biochemical processes of decay and fermentation. Pectins suppress the vital activity of these microorganisms, which contributes to the normalization of the composition of the intestinal microflora. Dietary fibers stimulate the growth of lactobacilli, and streptococci and reduce coliform growth, affecting the metabolic activity of normal microflora. Finally, dietary fiber increases the synthesis of vitamins B_1, B_2, B_6, PP, folic acid by intestinal bacteria. The physiological need for dietary fiber for an adult is 20 g/day, for children over 3 years old 10 – 20 g/day **[5]**, **[6]**.

Recent studies have shown that pectin has a beneficial effect, not only under acute exposure to metals but also during their long-term intake into the body, which is characteristic of an environmental load of residents of an industrial region or modern metropolis. It was found that modified citrus pectin significantly increased urinary lead excretion in adults **[7]** and is especially recommended for children as a safe and harmless chelator **[8]**. There is information in the literature that when exposed to pectins, the antioxidant activity of the blood and liver tissues increases **[9]**.

The degree of esterification of pectin determines its ability to influence intestinal biocenosis. In the first stage, the growth of conditionally pathogenic enterobacteria is inhibited; in the second, normal intestinal microflora is restored. The degree of pectin esterification determines the immunopotentiating effect of pectin **[10]**. Clinical studies have found that there were no side effects when taking pectin. Pectin, intended for treating acute intestinal diseases, has a distinct and persistent positive effect on intestinal dysbiosis.

Thus, the analysis of literary sources shows that pectin substances can bind and remove stable and radioactive metals from the human body. At the same time, the best complexing properties are possessed by low-esterified pectin substances, which include beet pectin.

According to modern trends in nutritional science **[11]**, **[12]**, the range of food products should be expanded with improved quality, increased nutritional value and preventive and dietary prescription products. Biologically active additives are effective in creating such products, increasing the body's resistance to adverse environmental influences.

In recent years, natural biological additives, including those of plant origin, have been increasingly used for these purposes **[13]**, **[14]**. Among plant crops, cereals take the leading place in terms of production volume and growth rates. Oats, barley, rice, sorghum, corn, millet and buckwheat are such cereals. Among the variety of non-traditional raw materials, corn flour is of interest.

Corn, in comparison with other cereals, contains little protein (7 – 8) but more fat (4 – 5 %), and the amount of carbohydrates is the same as that of wheat, oats and other crops (70 – 75 %). Corn contains vitamins A (510 IU), B1 (thiamine) - 0.2%, C - 5.1 %, folic acid - 260 mg, nicotinic acid - 1.3 mg, phosphorus, magnesium, potassium, zinc, calcium, manganese, iron, aluminium, copper, arsenic, cobalt, bromine and gold **[15]**, **[16]**. In the endosperm, the most valuable amino acids are formed – tryptophan and lysine – found in scant amounts in wheat and cannot be synthesised in the human body.

According to the analysis of data published in the scientific and technical literature, corn deservedly takes one of the prime places among cereals. It can rightfully be called a miniature chemical plant. It selectively processes and accumulates a quarter of the elements of Mendeleev's periodic system and belongs to the group of medicinal plants **[17]**, **[18]**.

Some literature **[19]** scientifically substantiates the connection between diseases and eating habits that have changed the lives of millions of people. Arguments in favour of a diet based on whole plant foods and new research results into the effects of animal and plant foods on the human body are presented. In the production of whole grain food, all parts of the grain are used – the germ, grain shells and endosperm. This food category is high in protein, complex carbohydrates, fibre, vitamins and minerals. The use of wholemeal flour and wholegrain cereals containing all the protein, fibres, vitamins and mineral substances necessary for the human body will solve the problem of meeting the population's needs for high-quality food products with high nutritional and biological value.

All of the above determined the direction of this study and its relevance. Using beet pectin concentrate and whole-ground corn flour in hardtack production opens up broad prospects for creating new safe food products with pronounced functional properties.

Scientific Hypothesis

Improving the quality and safety of hardtacks will depend on the properties of the sugar beet pectin concentrate.

MATERIAL AND METHODOLOGY

Samples

The objects of the study were: beet pectin concentrate from sugar beet, variety 'Ardan', whole-ground corn flour from corn, variety Budan 237 harvested in 2019 and hardtacks, wheat flour of the first grade. All analyzes were carried out in an accredited laboratory of the Almaty Technological University.

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Animals and Biological Material

Animal and biological materials were not used in this study.

Instruments

We used automatic fat extractor SER 148/3 (Velp Scientifica, Italy), Kjeldahl VELP UDK 129 (Velp Scientifica, Italy), atomic absorption spectrometer KVANT-Z-ETA-T (OJSC Kortek, Russia), Capillary electrophoresis systems "KAPEL®-105M" (Lumex, Russia), convection oven UNOX XB693 (UNOX, Italy).

Laboratory Methods

The pectin content was determined following GOST 29059–91 **[20]**. The method is based on alkali titration of previously isolated and prepared pectin substances before and after hydrolysis. The titration results are proportional to the number of free and esterified carboxyl groups and, when multiplied by the corresponding equivalents, give the content of polyuronides in the pectin substances of the product. All the necessary organoleptic and physicochemical indicators of flour quality were determined according to the methods given in the relevant regulatory and technical documents. The Color, smell, taste and crunch of flour were determined according to GOST 27558–87 **[21]**. The color of the flour or bran is determined by comparing the test sample with an established sample or with the color specification specified in the relevant product standards. At the same time, attention is paid to the presence of individual particles of shells and impurities that violate the uniformity of the color of the flour. To determine the smell, a sample of flour or bran weighing about 20 g is taken from the sample intended for analysis, poured onto clean paper, warmed with breath, and the smell is established. To enhance the sensation of smell, a portion of flour or bran is transferred to glass and doused with hot water at a temperature of 60 °C. The water is drained, and the smell of the product is determined. The taste and the presence of a crunch are determined by chewing 1 – 2 portions of flour weighing about 1 g each. The odor, taste, and crunch were determined following the characteristics specified in the standards for flour and bran. To improve human health, all ethical principles were observed: research was conducted in compliance with ethical standards and opened up prospects for raising the standards of everyone's health. When conducting a study of organoleptic indicators were:

- current laws, regulatory documents in the field of quality, environmental protection measures, standardization, metrology, certification, and consumer protection are observed;
- the safety and integrity of the selected samples (samples) are ensured when they are sent for testing;
- demonstrated objectivity and independence during the examination;
- reasoned evidence of the correctness of the assessments made and the reliability of the results obtained are provided;
- ethical standards are observed, and the confidentiality of the information obtained as a result of the verification is ensured.

Flour moisture by an accelerated method according to GOST 9404–88 **[22]**. The essence of the method lies in the dehydration of flour and bran in an air-heating cabinet at fixed temperature and drying time parameters. The ash content of flour, according to GOST 27494–87 **[23]**, the essence of the methods is the combustion of flour and bran, followed by the determination of the mass of non-combustible residue. Metal-magnetic impurities, according to GOST 20239–74 **[24]**, the essence of the method consists in separating a metal-magnetic impurity (particles of metals, ores, etc., with magnetic properties) by a magnet in a mechanized way or manually, followed by weighing and measuring its particles. Pest infestation of grain stocks following GOST 27559–87 **[25]**, the essence of the method for determining infestation is to isolate insects and mites by sieving on sieves and visually detecting living individuals, and contamination - dead individuals. Infected pests are flour and bran with the

presence of live insects and mites in all stages of their development. The content of wet gluten following GOST 27839–88 **[26]**. This standard applies to wheat flour and establishes methods for determining the amount of gluten by washing it out of the dough using mechanical means or manually and the quality of gluten by measuring its elastic properties. Gluten is a complex of protein substances capable of forming a coherent elastic mass when swollen in water. The acidity of flour in accordance with GOST 27493–87 **[27]**, the essence of the method lies in the titration with sodium hydroxide of all acid-reactive substances of flour and bran. Organoleptic (colour, surface) and physicochemical (moisture, acidity, wetness) indicators of hardtacks were determined according to the methods described in the literature **[28]**. The chemical composition and safety of pectin concentrate, flour, and hardtacks were determined according to the methods described in the literature **[29]**.

Description of the Experiment

The degree of esterification is the ratio of the number of esterified carboxyl groups to the total content of carboxyl groups in pectin (esterified and unesterified). Determination of the degree of esterification was carried out by a titrimetric method **[30]**, **[31]**.

The complexing ability is determined by the coefficient of selectivity of cation exchange, which characterises the affinity of pectin molecules for divalent cations **[32]**. The complexing ability of pectin extracts was determined by the amount of bound lead when processing a solution of pectin extract with a solution of lead acetate.

Number of samples analyzed: We analyzed 2 samples.

Number of repeated analyses: All tests were performed in triplicate.

Number of experiment replication: 2 times.

Design of the experiment: The development of technology for producing beet pectin with desired properties as an effective complexing and structuring agent is of particular relevance in modern conditions. In addition, one should consider the cheapness and availability of beet pulp **[33]**, **[34]**. Enzymatic treatment is the most effective method of producing pectin concentrate. It leads to an increase in the purity of the pectin with a simultaneous increase in its jelly-forming ability.

The technological scheme for obtaining pectin concentrate from sugar beet of the 'Ardan' variety, used in future work as an additive in the production of hardtacks, is shown in Figure 1.

The preparation of raw materials (beet pulp) is to remove sugar, aromatic, dyes, salts, etc. The pulp is dried at a temperature of 50 – 60 °C for 12 h, and the resulting dry pulp is ground in a mill. The following process is dilution with distilled water at 20 – 25 °C, followed by filtration to remove carbohydrates. Swelling is carried out at a temperature of 50 °C for 12 h.

To carry out enzymatic extraction, an enzyme preparation of pectinase from *Aspergillus niger* is added to the resulting mixture at a level of 2%, and the extraction is carried out at a temperature of 38 – 40 °C for 4 h. Then, every 30 min, the samples are mixed for 5 min. The extract is filtered off, and the pulp is added to water at a temperature of 65 – 70 °C and kept for 40 min, after which the solution is filtered and combined with the first extract. The extract is a transparent liquid of light grey colour containing 0.5 – 0.8% of pectin substances with a density of 1.01 – 1.02 and pH of 0.6 – 0.7. The resulting pectin-containing extract is centrifuged at 8000 g/min for 15 min. The enzyme is inactivated at a temperature of 75 °C for 15 min, and the total pectin content in the obtained samples is determined by the classical method (precipitation of pectin in the form of Ca pectate). Concentration is carried out by vacuum evaporation using an RV 05 basic 2B brand at 75 °C and a reduced pressure of 0.7 atm. The maximum total pectin content in the 'Ardan' beet pulp concentrate was 5.86 ±0.004%. Sterilisation of the obtained pectin-containing concentrates from the beet pulp is carried out at a temperature of 75 – 78 °C for 30 min.

Statistical Analysis

The data obtained during the experiments were processed using the mathematical method of variation statistics using the Statistika 10.0 developer: StatSoft, USA. Also, the data were analyzed using MS Excel for Windows version 10 Pro, 2010. The data collected during the study were subjected to independent testing, and questionnaires were conducted to assess the organoleptic characteristics of control and test samples. The analysis process used absolute and relative statistical indicators and tabular and graphical methods for presenting the results. Values were estimated using mean and standard deviations.

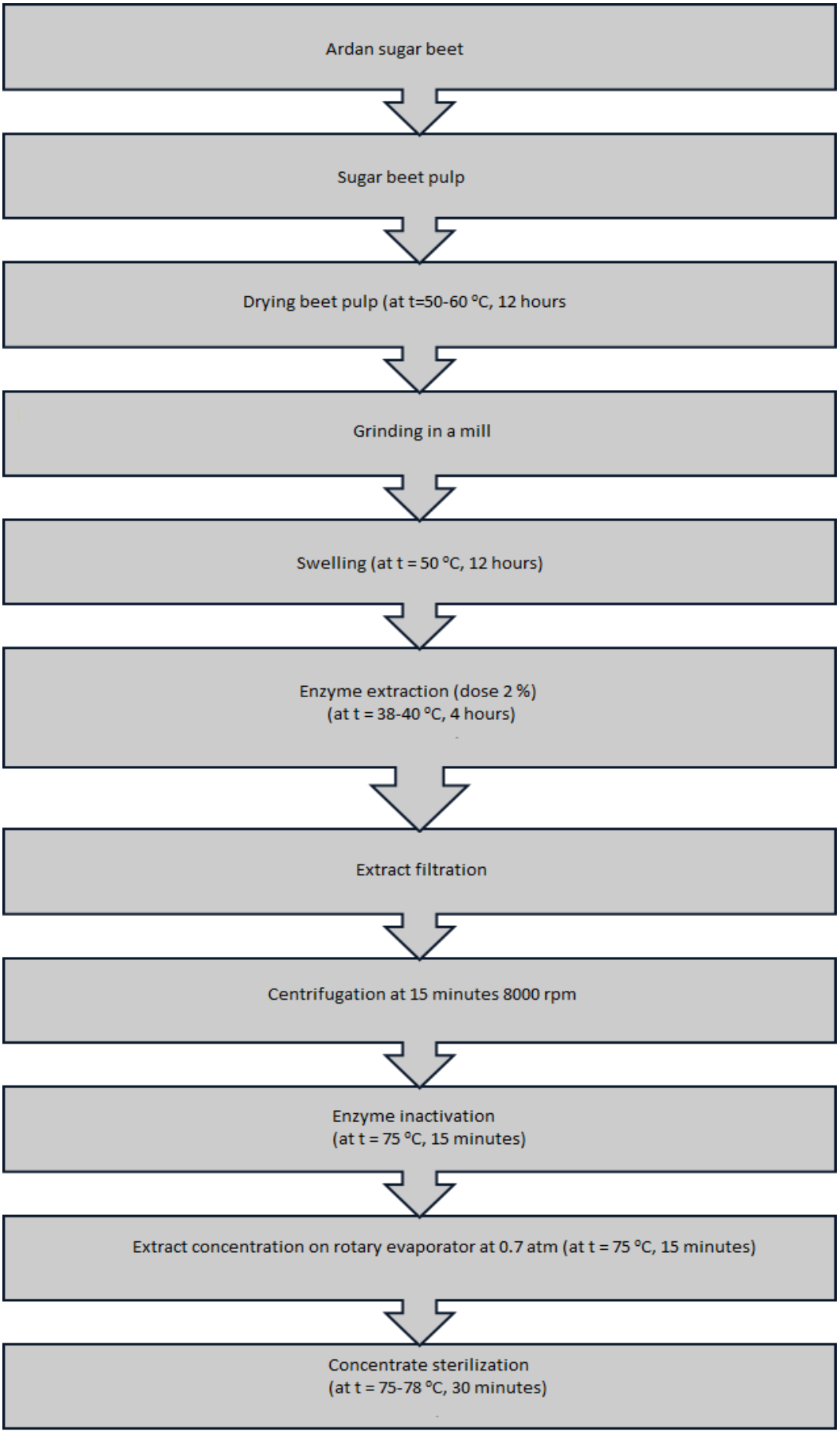

Figure 1 Technological scheme of obtaining pectin concentrate from sugar beet 'Ardan'.

RESULTS AND DISCUSSION

Hardtacks, like most foods, are a possible source of a wide range of contaminants, which may cause potential health risks. The selection of raw materials represents the most critical step of the production process. The successive processing steps must also be monitored because if they cannot improve the initial safety condition, they could worsen it. The most effective mitigation strategies involve product reformulation and alternative baking technologies to minimize the thermal load **[35]**.

One of the most important biologically active properties of pectin-containing products is their complexing ability based on the interaction of pectin with heavy and radioactive metal ions. It is the basis for the design of food products based on pectin-containing raw materials.

To assess the quality indicators of the pectin concentrate from 'Ardan' sugar beet, its complexing ability and the degree of esterification were determined (Table 1). The table shows that the degree of esterification was 31.4%, and the complexing ability of the pectin-containing concentrate was 270.0 mg Pb^{2+}/g. Therefore, the resulting concentrate can be described as a low-esterified pectin substance.

Table 1 Qualitative indicators of pectin concentrate from sugar beet variety 'Ardan', % in terms of absolute dry weight.

No.	Indicator name	Pectin concentrate from sugar beet variety 'Ardan'
1	Esterification degree, %	31.4 ±0.07
2	Complexing ability, mg Pb^{2+}/g	270 ±0.11
3	Total pectin content, %	5.86 ±0.004

Note: ± – standard deviation.

For the manufacture of hardtacks and the experiments, first-grade wheat flour and whole-ground corn flour, obtained by grinding whole grains of corn of the Budan 237 variety, harvested in 2019, were used.

In the finished flour, organoleptic (colour, smell, taste and crunch) and physicochemical (moisture, quantity and quality of gluten, ash content, acidity, metal impurity content and pest infestation of grain stocks) indicators were determined.

The quality characteristics of first-grade wheat bakery flour and wholemeal corn flour are shown in Table 2.

Table 2 Indicators of flour quality.

Indicators	First-grade wheat flour	Whole-ground corn flour
Organoleptic:		
Colour	white	yellow
Taste and smell	peculiar	
Mineral impurity content	not detected	
Physicochemical:		
Humidity, %	12.4 ±0.02	12.0 ±0.09
Crude gluten content, %	31.36 ±0.04	-
Gluten quality according to the Gluten deformation meter-1, units of the device	74 ±0.08	-
Ash content, %	0.72 ±0.01	1.12
Acidity, degree	2.8 ±0.3	4.2 ±0.2
Content of metal impurities, mg/kg flour	not detected	
Pest infestation of grain stocks	not detected	

Note: ± standard deviation.

As can be seen from Table 2, the moisture content of the first-grade wheat flour and wholemeal corn flour was within normal limits. The gluten content in the first-grade wheat flour was 31.36%, and the gluten quality according to the gluten deformation meter-1 was 74 units. The ash content for first-grade wheat flour was 0.72%, and for wholemeal corn flour 1.12%. Metallomagnetic impurity and pest infestation of grain stocks in the first-grade wheat flour and wholemeal corn flour were not detected **[36]**, **[37]**, **[38]**.

Based on the analyses of organoleptic and physicochemical indicators, it can be argued that wheat flour of the first grade and whole-ground corn flour meet the requirements of the Normative and Technical Documents.

Analysis of the chemical composition of whole-ground corn flour would allow evaluation of the effectiveness of its use in hardtack production and consideration of the possibility of its use as a food additive for enriching hardtacks with valuable nutrients **[39]**, **[40]**, **[41]**. In connection with the above, to substantiate the practicality of

using products of processing of grain crops as food additives, studies were carried out to study the chemical composition of whole-ground corn flour and a comparative analysis with wheat bakery flour.

The results of the analysis of the chemical composition of first-grade wheat flour and wholemeal corn flour are shown in Table 3.

Table 3 Chemical composition of flour.

Indicators	Content per 100 g of product	
	First-grade wheat flour	Wholemeal corn flour
Protein, g	12.3 ±0.09	10.2 ±0.07
Amino acids, mg		
Essential:		
Isoleucine	550 ±1.41	406 ±1.32
Valine	550 ±1.11	460
Leucine	880 ±0.89	1300 ±1.33
Lysine	260 ±1.35	316 ±0.29
Methionine	141 ±1.54	216 ±1.28
Threonine	353 ±1.22	314 ±0.47
Tryptophan	125 ±0.74	83 ±1.15
Phenylalanine	630 ±1.08	554 ±1.52
Nonessential:		
Alanine	380 ±1.74	702 ±1.86
Arginine	560 ±1.21	500 ±0.74
Aspartic acid	450 ±1.38	654 ±0.99
Histidine	230 ±1.49	271 ±1.83
Glycine	430 ±1.51	352 ±1.49
Glutamic acid	3382 ±2.09	1860 ±2.44
Proline	1130 ±1.88	942 ±1.72
Serine	515 ±1.31	500 ±1.11
Tyrosine	340 ±1.98	361 ±1.75
Cystine	240 ±1.20	181 ±1.36
Fat, g	1.22 ±0.04	3.43 ±0.25
Carbohydrates, g	69.4 ±1.08	65.3 ±1.09
Ash, g	0.72 ±0.04	1.02 ±0.07
Mineral substances, mg		
Ca	20.8 ±1.22	71.5 ±1.01
Mg	47.8 ±1.73	103.0 ±1.25
Fe	1.86 ±1.09	3.76 ±1.42
K	167.0 ±1.28	252.4 ±1.66
Vitamins, mg		
β-carotene	-	0.315
E	2.912 ±1.17	0.62 ±1.48
C	-	4.08
PP	1.2 ±0.49	1.85 ±0.55

Note: ± standard deviation.

As seen from the data in Table 3, a significant part of dry matter (approximately 70%) of the studied samples was represented by a carbohydrate complex, consistent with the scientific and technical literature data. A characteristic feature of cereals is their low protein content compared to legumes. Thus, the protein content in first-grade wheat flour was 11.3% and in corn flour 10.2%.

It is known that the amino acid composition of plant raw materials and processed products largely determines their biological value and affects their organoleptic properties **[42]**, **[43]**. Since amino acids are reactive compounds, they easily undergo various transformations during the processing of raw materials, participate in the processes of melanoid formation and the browning of products and undergo destruction **[44]**, **[45]**, **[46]**, the composition of irreplaceable and nonessential amino acids was determined (Table 3).

When comparing the balance of essential amino acids of proteins of wheat and corn flour, significant differences are noted: in corn flour, lysine is 1.22 times, methionine 1.53 times and leucine 1.48 times higher than in wheat

flour of the first grade. Isoleucine in corn flour is 1.35 times, valine 1.2 times, threonine 1.12 times and tryptophan 1.5 times lower than in wheat flour. According to the data presented in Table 2, corn flour is not inferior to wheat flour of the first grade in terms of the content of nonessential amino acids, except for glycine, glutamic acid, cystine and proline.

Analysing the vitamin composition of whole-ground corn flour in comparison with first-grade wheat flour, it can be concluded that corn flour is rich in vitamins **[47]**, **[48]**, **[49]**. In particular, the content of niacin (vitamin PP) in corn flour is 1.54 times higher than in wheat flour.

It is known that mineral substances play the role of the most important catalysts in a number of biochemical processes and function together with enzymes and vitamins, influencing the course and direction of metabolic processes. Information about the complex of mineral substances makes it possible to evaluate the raw materials under study in biological respect. Considering the above, the mineral composition of first-grade corn and wheat flour was studied.

As can be seen from Table 3, the amount of calcium in corn flour is 3.44 times higher than in wheat flour of the first grade. Magnesium is 2.15 times, iron 2.1 times and potassium 1.51 times higher than wheat flour.

Sugar beet pectin concentrate, corn and wheat flour are the main ingredients in hardtack making. In this regard, their safety was investigated. Table 4 shows the results of a study of some safety indicators. The following safety indicators have been determined in the pectin concentrate from sugar beet variety 'Ardan', corn and wheat flour: microbiological indicators (QMAFanM, coliform bacteria), the content of toxic elements (lead, cadmium, arsenic, mercury), pesticides and mycotoxins. The research results showed the safety of pectin concentrate from sugar beet, corn and wheat flour and compliance with TR CU 021/2011.

Table 4 Safety indicators of pectin concentrate from sugar beet, corn and wheat flour.

Name of indicators, units of measurement	Actual results		
	Pectin concentrate	Wheat flour Grade 1	Corn flour
Microbiological indicators:			
QMAFAnM, CFU / g, no more	not detected	1×10^1	2×10^2
Coliform bacteria (coliforms) in 1.0 g of product	not detected	not detected	not detected
Heavy metals, mg/kg:			
Lead	0.0737 ±0.008	0.102 ±0.001	0.115 ±0.008
Cadmium	0.0017 ±0.005	0.023 ±0.011	0.019 ±0.004
Mercury	not detected	not detected	not detected
Arsenic	not detected	0.018	not detected
Pesticides, mg/kg:			
Hexachlorocyclohexane (HCH) (α-, β-, γ-isomers)	not detected	not detected	not detected
Heptachlor	not detected	not detected	not detected
Dichlorodiphenyltrichloromethylmethane (DDT) and its metabolites	not detected	not detected	not detected
Mycotoxins, mg/kg:			
Aflatoxin B1	not detected	not detected	not detected
Deoxynivalenol	not detected	not detected	not detected
Zearalenone	not detected	not detected	not detected
T-2 toxin	not detected	not detected	not detected

Note: ± standard deviation.

To prepare hardtacks from first-grade wheat flour using Ardan sugar beet pectin concentrate and whole-ground corn flour, it is important to determine how the properties of the dough and the quality of the finished product would change. In this regard, the effect studied of pectin concentrate in dosages of 5.0, 10.0 and 15.0% on the quality of gluten flour from mixtures of first-grade wheat and whole-ground corn flour in the ratios of 95.0:5.0; 92.5:7.5; 90.0:10; 87.5:12.5; 85.0:15; 82.5:17.5; 80.0:20; 77.5:22.5 and 75.0:25. As a control, samples were taken without the addition of pectin concentrate and wholemeal corn flour. The results are shown in Figure 2.

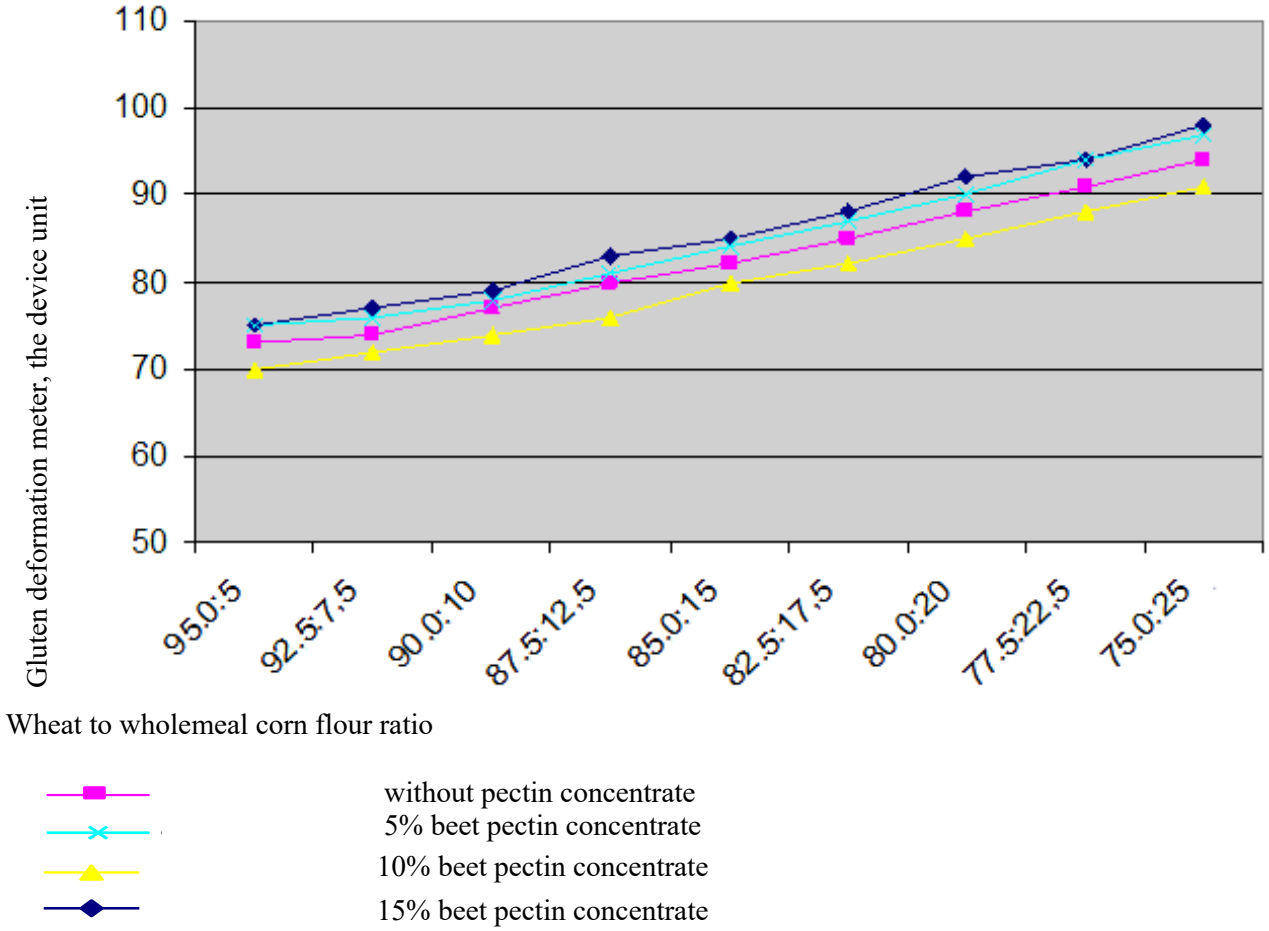

Figure 2 Influence of pectin concentrate on the quality of gluten flour from a mixture of first-grade wheat and wholemeal corn flour.

The data in the figure indicate that with an increase in the dosage of whole-ground corn flour without the use of pectin concentrate, the properties of gluten deteriorate, which is reflected in a decrease in its quality. This is due to the virtual absence of gluten proteins in corn flour **[50]**, **[51]**, **[52]**. However, when using pectin concentrate, gluten quality changes in the direction of increasing firmness and elasticity. The best results are obtained with the addition of 10% pectin concentrate. In this case, the optimal dosage of corn flour is 15%.

When studying the effect of pectin concentrate on the quality of hardtacks from a mixture of wheat flour and wholemeal corn flour, the dough was prepared by the sponge. As a control, samples of hardtacks from first-grade wheat flour without pectin concentrate and wholemeal corn flour were selected (Table 5).

Table 5 Influence of beet pectin concentrates on the quality of hardtacks from a mixture of wheat and wholemeal corn flour.

The ratio of wheat and wholemeal corn flour, %	Colour	Surface	Humidity, %	Acidity, degree	Wetness, %
Control	straw yellow	smooth with punctures, without foreign inclusions and stains	10.01 ±0.54	2.0 ±0.08	205.0 ±1.74
without pectin concentrate					
92.5:7.5	light yellow	smooth with punctures, without foreign inclusions and stains	10.03 ±0.08	2.0 ±0.01	210.0 ±0.88
90:10	light yellow	smooth with punctures, without foreign inclusions and stains	10.05 ±0.77	2.0 ±0.08	180.0 ±1.72
87.5:12.5	light brown	a little scabrous	10.04 ±0.09	2.5 ±0.02	160.0 ±1.29
85:15	light brown	a little scabrous	10.02 ±0.17	2.5 ±0.10	165.0 ±1.37
82.5:17.5	dark brown	scabrous	10.01 ±0.61	3.0 ±0.05	159.0 ±1.75
80:20	dark brown	scabrous	10.04 ±0.28	3.5 ±0.06	150.0 ±0.99
10% beet pectin concentrate					
92.5:7.5	light yellow	smooth with punctures, without foreign inclusions and stains	10.01 ±0.14	2.0 ±0.07	210.0 ±1.05
90:10	light yellow	smooth with punctures, without foreign inclusions and stains	10.01 ±0.25	2.0 ±0.11	200.0 ±1.14
87.5:12.5	light brown	smooth with punctures, without foreign inclusions and stains	10.04 ±0.05	2.5 ±0.03	188.0 ±1.17
85:15	light brown	smooth with punctures, without foreign inclusions and stains	10.05 ±0.09	2.5 ±0.07	180.0 ±0.88
82.5:17.5	dark brown	a little scabrous	10.01 ±0.14	3.0 ±0.07	165.0 ±1.49
80:20	dark brown	scabrous	10.03 ±0.07	3.5 ±0.08	156.0 ±1.33

Note: ± standard deviation.

The results show that the use of pectin concentrate from sugar beet variety 'Ardan' when kneading dough made from a mixture of wheat and grain flour improves organoleptic and physicochemical indicators of hardtack in comparison to samples without pectin concentrate. The best-quality hardtacks were achieved using 10% pectin concentrate and adding whole-ground corn flour at 15% of the mass of first-grade wheat flour (Table 6).

The study of hardtacks' food and biological value. Hardtacks' food and biological value determines the feasibility and validity of using new types of raw materials in the production of hardtacks **[53]**, **[54]**, **[55]**.

To study the nutritional and biological value and safety, hardtacks were prepared from a mixture of wheat flour and whole-ground corn flour in a ratio of 85:15 using pectin concentrate at 10 % when kneading the dough. Samples of hardtacks prepared from first-grade wheat flour without pectin concentrate and wholemeal corn flour were taken as controls.

Table 6 Recipe for the control and experimental sample of the hardtacks.

No.	Name of raw materials	Consumption of raw materials per 10 kg of finished products, in kind

		Control sample	Experimental sample
1	Wheat flour of the first grade	101.6	86.36
2	Whole-ground cornmeal	0	15.14
3	Sugar	2.04	1.836
4	pectin concentrate	0	0.204
5	Salt	1.5	1.5
6	Sodium bicarbonate	0.4	0.4
7	Yeast	2.03	2.03
8	Lactic acid (40%)	0.19	0.19
	Total	107.6	112.09
	Product yield	100	100

The results of the study of the chemical composition of the developed hardtacks are shown in Table 7.

Table 7 Chemical composition of hardtacks using whole-ground corn flour and beet pectin concentrate.

Nutrients	Content in 100 g of product	
	First-grade wheat flour hardtacks (control)	Hardtacks from first-grade wheat flour with the use of whole-ground corn flour (15 %) and beet pectin concentrate (10 %)
Protein, g	9.8 ±0.01	9.68 ±0.05
Amino acids, mg:		
Essential:		
Isoleucine	493 ±0.80	482 ±0.39
Valine	501 ±0.54	494 ±0.88
Leucine	804 ±0.78	839 ±0.41
Lysine	229 ±0.02	234 ±0.29
Methionine	131 ±0.68	136 ±0.74
Threonine	317 ±0.25	315 ±0.22
Tryptophan	114 ±0.47	109 ±0.63
Phenylalanine	580 ±0.98	572 ±1.08
Nonessential:		
Alanine	340 ±0.43	362 ±0.72
Arginine	510 ±0.88	504 ±0.57
Aspartic acid	411 ±0.64	403 ±0.98
Histidine	215 ±0.38	219 ±0.28
Glycine	390 ±0.69	383 ±0.23
Glutamic acid	3112 ±1.54	3090 ±1.29
Proline	1045 ±2.14	1032 ±2.43
Serine	472 ±0.75	471 ±0.34
Tyrosine	312 ±0.94	315 ±0.25
Cystine	224 ±0.38	220 ±0.82
Fat, g:	1.17 ±0.04	1.44 ±0.06
Carbohydrates, g:	67.8 ±0.29	67.0 ±0.42
Ash, g:	0.78 ±0.04	0.92 ±0.08

Table 7 Cont.

Nutrients	Content in 100 g of product	
	First-grade wheat flour hardtacks (control)	Hardtacks from first-grade wheat flour with the use of whole-ground

		corn flour (15 %) and beet pectin concentrate (10 %)
Mineral substances, mg:		
Ca	20.1 ±0.84	25.8 ±0.25
Mg	45.0 ±0.09	49.1 ±0.06
Fe	1.65 ±0.07	2.12 ±0.01
K	161.0 ±0.8	167.7 ±0.2
Vitamins, mg:		
β-carotene	-	0.019 ±0.002
E	2.50 ±0.07	2.39 ±0.09
C	-	0.25 ±0.05
PP	1.02 ±0.03	1.26 ±0.02

Note: ± – standard deviation.

The data analysis shows that in hardtacks prepared with the addition of whole-ground corn flour and beet pectin concentrate, the content of vitamins and minerals increased compared with the control. The amino acid composition of hardtacks is influenced by the chemical composition, the type and grade of flour from which it was prepared, the composition of other recipe components and losses associated with the technology of preparing hardtacks **[56]**, **[57]**. According to the data presented in Table 7, hardtacks with whole-ground corn flour and beet pectin concentrate are not inferior to the control sample in terms of the content of essential and nonessential amino acids.

Whole-ground corn flour and beet pectin concentrate are new raw materials for hardtacks; therefore, the safety of the developed products prepared using whole-ground corn flour and beet pectin concentrate was investigated.

Table 8 shows the study results of safety indicators, which were determined according to the methods described in Section 2.

Table 8 Safety indicators for hardtacks.

Name of indicators, units of measurement	Actual results	
	First-grade wheat flour hardtacks (control)	Hardtacks from first-grade wheat flour with the use of whole-ground corn flour (15 %) and beet pectin concentrate (10 %)
Microbiological indicators:		
QMAFAnM, CFU/g, no more:	not detected	1×10^1
Coliform bacteria (coliforms) in 1.0 g of product	not detected	not detected
Heavy metals, mg/kg:		
Lead	0.100 ±0.008	0.093 ±0.001
Cadmium	0.020 ±0.006	0.015 ±0.004
Mercury	not detected	not detected
Arsenic	0.020 ±0.008	0.015 ±0.009
Pesticides, mg/kg:		
Hexachlorocyclohexane (HCH) (α-, β-, γ-isomers)	not detected	not detected
Heptachlor	not detected	not detected
Dichlorodiphenyltrichloromethylmethane (DDT) and its metabolites	not detected	not detected

Table 8 Cont.

Name of indicators, units of measurement	Actual results	
	First-grade wheat flour hardtacks (control)	Hardtacks from first-grade wheat flour with the use of whole-ground corn flour (15%) and beet pectin concentrate (10%)
Mycotoxins, mg/kg:		
Aflatoxin B1	not detected	not detected

Deoxynivalenol	not detected	not detected
Zearalenone	not detected	not detected
T-2 toxin	not detected	not detected

Note: ± standard deviation.

Photos of first-grade wheat flour hardtacks (control) and hardtacks from first-grade wheat flour with the use of whole-ground corn flour (15%) and beet pectin concentrate (10%) are shown in Figures 3 and 4.

Analysis of the research results of hardtacks made from whole-ground corn flour and beet pectin concentrate showed their safety and compliance with TR CU 021/2011.

Pectin concentrate does not dissolve in the human digestive system, helping to cleanse the body of toxins. Passing through the body, pectin does not enter into chemical reactions with sorbed substances. Therefore, it does not change blood biochemistry **[58]**, **[59], [60]**. Thus, using a pectin product helps gently stabilize the metabolism.

Enterosorbent lowers cholesterol, improves blood circulation and stimulate the intestines. Pectins are natural enterosorbents, which are practically not absorbed by the body's digestive system. The detoxifying properties of pectin are because when it enters the intestines, the substance swells, enveloping the mucous membrane of the stomach and intestines, thereby leading to a decrease in inflammation, preventing the formation of ulcers and damage, slowing down the destructive effects of some toxic substances that enter with food. Therefore, using hardtacks with petite concentrate in 500 g per day completely replenishes the recommended amount of polysaccharides and is safe for the human body.

Figure 3 First-grade wheat flour hardtacks (control).

Figure 4 Hardtacks from first-grade wheat flour with whole-ground corn flour (15%) and beet pectin concentrate (10%).

Modern nutritional science considers food a source of energy, plastic substances, and a complex natural pharmacological complex. This is especially important in connection with the impact on an individual of the polluted nature of his/her habitat. The main physiological property of pectin, which predetermines its use in the production of dietary food, is its ability to bind and remove heavy metals and radionuclides from the body. At the same time, low-esterified pectin substances, which include beet pectin, have the best complexing properties. The use of wholemeal flour and wholegrain cereals containing all the protein, fibres, vitamins and mineral substances necessary for the human body will solve the problem of meeting the population's needs with food products with high nutritional and biological value.

The research carried out makes it possible to substantiate the sequence and parameters of technological operations for the production of pectin concentrate from sugar beet variety 'Ardan', consisting of the following main stages: preparation of pectin-containing raw materials (obtaining beet pulp, drying, grinding); enzymatic extraction; filtering the extract; centrifugation; enzyme inactivation; concentrating the extract; and sterilisation of the obtained pectin-containing concentrates. For the enzymatic extraction, the enzyme pectinase from Aspergillus niger was used at 2%. The total pectin content in the concentrate was 5.86 ±0.004%.

It has been established that beet pectin concentrate is characterised by a low degree of esterification of 31.4% and high complexing ability of 270 mg Pb^{2}+/g, which makes it possible to recommend the use of the developed pectin product as a natural detoxifier capable of forming strong chelate bonds with heavy metals.

Based on the analysis of the chemical composition of whole-ground corn flour and comparison with the composition of bakery wheat flour of the first grade, it was found that whole-ground corn flour is rich in essential amino acids, vitamins and micro- and macroelements. Thus, the calcium content in corn flour is 3.44 times, magnesium 2.15 times, iron 2.1 times, potassium 1.51 times, and niacin (vitamin PP) 1.54 times higher than wheat flour of the first grade.

Pectin is an indigestible dietary fiber capable of forming a gel-forming mass that naturally collects toxic substances from the intestinal walls and removes them from the body. The use of the resulting hardtack from sugar beet pectin concentrate normalizes metabolism by normalizing intestinal motility, and maintains the bacteriological balance of the human body.

CONCLUSION

1. The food safety of pectin concentrate from 'Ardan' sugar beet and whole-ground corn flour has been determined.

2. An optimal dosage of pectin concentrate of 10% and whole-ground corn flour of 15% in the production of hardtacks from first-grade wheat flour, in which the gluten properties and the quality of finished products were similar to control samples, have been substantiated and determined. The use of 'Ardan' sugar beet pectin concentrate made it possible to change the properties of the dough towards an increase in firmness and elasticity.

It was found that the food and biological value of the developed hardtacks was higher than that of the control samples. The products obtained complied with the safety requirements of TR CU 021/2011 Technical Regulations of the Customs Union 'On Food Safety'. Numerous clinical studies have shown that pectin reduces diarrhoea, improves the absorption function of the intestine, and promotes the proliferation of the mucous membrane. Pectin plays a significant protective role in preventing oxidative damage induced by hydroxyl radicals in the mucosa of the jejunum. In general, pectin promotes intestinal adaptation, reduced diarrhoea and improved absorption.

The products obtained are recommended especially for patients with diarrhea, children, and general for functional purposes.

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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 a scientific concept of industrial storage systems for environmentally safe apples

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ABSTRACT
The research project has developed and justified the storage modes of apples in a modified gas environment by creating an isolated "closed loop" of high-pressure polyethylene; the expediency of creating highly efficient technologies for storing fresh fruits in a controlled atmosphere, in bioactive bactericidal packages and by creating microfilm on the surface of fruits has been confirmed. The prospects of using a progressive method of storing fruits in a modified gas atmosphere by creating an isolated "closed circuit" in a separate refrigerating chamber without using expensive equipment (in normal and subnormal gas environments) are proved. New technologies have been developed for storing apple fruits susceptible to infectious and physiological diseases based on improved storage methods with minimal losses. The consumption rates of *Phytosporin-M* for the surface treatment of fruits were determined and optimized to control the intensity of biochemical and microbiological processes during storage. The modes and technologies of post-harvest fruit processing with the *Phytosporin-M* biopreparation have been substantiated.

Keywords: apple, storage, gas environment, closed loop, bioactive coatings, bactericidal packaging

INTRODUCTION
A significant part of the grown fruit products is lost during storage. For some types, these losses often reach 30% and sometimes 50% **[1]**, **[2]**. Therefore, developing highly efficient storage technologies is an urgent task today **[3]**, **[4]**, **[5]**, **[6]**. All methods of storing fruits in a modified gas atmosphere can be classified according to the type of medium used, the method of management, methods of creating the medium, etc. **[7]**, **[8]**. The history of developing methods for storing fruit and vegetable products shows that atmospheric air and its components are used in most cases. Storage in artificially created gases (for example, ozone) has not found wide application in practice due to their high cost or low efficiency (for fruits) **[9]**, **[10]**, **[11]**.
Based on the gas composition of atmospheric air and depending on the type of environment created on its basis, we conducted research on the storage of fruits and vegetables:
- in a controlled atmosphere (CA);
- in bioactive bactericidal packaging;
- with a surface coating of fruits with biologically active preparations.

The advantages of storing fruit products in a controlled atmosphere (CA) are well known. However, such storage does not replace storage in the refrigerator (its temperature is usually 3 – 5 °C higher than in the refrigerator). The storage of fruit products in the CA slows down the processes of maturation and overripe, as a result of which its shelf life is lengthened, the quality and yield of marketable products are increased, immunity is preserved, microbial spoilage and physiological damage are reduced **[12]**, **[13]**, **[14]**. Biological oxidation (respiratory gas exchange or respiratory activity) is at the center of physiological and biochemical processes during fruit ripening. All physiological and biochemical changes in fruit products are made due to the energy released during breathing **[15]**, **[16]**, **[17]**.

In plant cells, cytochrome oxidase, polyphenol oxidase, ascorbate oxidase and flavin enzymes can perform the functions of O_2 activators at the final stages of respiration. With a change in the concentration of O_2 and CO_2, the proportion of participation of individual oxidases in the respiration process changes. At very low concentrations of O_2, cytochrome oxidase and peroxidase activity increases sharply, and the activity of flavoprotein enzymes decreases. The middle position is occupied by polyphenol oxidase and ascorbate oxidase. High concentrations of CO_2 (7 – 10%) block respiration due to a sharp decrease in carboxylase, cytochrome oxidase, pyruvate dehydrogenase, etc., and also disrupt many other processes of fruit metabolism **[18]**, **[19]**, **[20]**. The composition of the gaseous medium (CO_2 and O_2) and temperature are important factors regulating the rate of biological oxidation in plant cells. Therefore, data on the effect of individual components of the gaseous medium on the rate of fetal respiration are of particular interest **[21]**, **[22]**. The transition of one phase of development (maturation) to another (overripe) is characterized by a change in the predominance of certain enzymatic systems that catalyze this process during respiration **[23]**, **[24]**, **[25]**, **[26]**.

Therefore, developing a method for storing fruits in a modified gas atmosphere is promising and relevant by creating an isolated "closed loop" and a system for reducing fruit losses based on complex post-harvest treatments with biologically active preparations.

Scientific Hypothesis

Creating an isolated "closed loop" and carrying out complex post-harvest treatments of apples with biologically active preparations can ensure a reduction in fruit losses during storage.

MATERIAL AND METHODOLOGY

Samples

The samples were the fruits of promising varieties of domestic and foreign breeding, grown in the soil-climatic conditions of the Krasnodar Territory (Russia): Jonathan, Reinette Simirenko, Idared and Korey.

Chemicals

Ethanol, Sodium carbonate, Sodium phosphoric acid, Sodium sulphate, Potassium ferruginous, Zinc acetic acid, Zinc sulphate, Lead acetic acid, Nitric acid, Sulfuric acid, Hydrochloric acid, Oxalic acid, Sodium hydroxide, Potassium permanganate, Phenolphthalein. All chemicals above were purchased by LenReactive LLC (Sants Petersburg, Russia) and were of analytical grade quality).

Apple samples were treated with Phytosporin-M preparation (BashInkom Inc., Russia).

Animals and Biological Material

The apple variety *Malus domestica* (var. Idared, Korey, and Jonathan).

Instruments

GHP-75 gas analyzer (Alta LLC, Stavropol, Russia), FT - 372 penetrometer with plunger (Alitus LLC, Moscow, Russia), Abbe NAR-4T Refractometer (ATAGO Inc., Tokyo, Japan), UV-1800 spectrophotometer (Shimazy Inc., Tokyo, Japan), Climate camera BINDER KBW 240 (BINDER GmbH, Tuttlingen, Germany).

Laboratory Methods

Analytical and experimental studies were based on the macroscopic theory of gases, modern ideas about the physiological state of storage objects, mass transfer theory, and various fruits' storage technologies.

Fruit quality assessment was conducted according to the "Program and methodology of variety study of fruit, berry and nut crops" **[27]**. The fruits for analysis were selected at removable maturity. During the technical analysis, the mass, the size of the fetus, and the shape index, i.e., the ratio of height and diameter, were measured. Biochemical studies were carried out in 3-5-fold repetition in the biochemical laboratory of fruit storage and processing. The hardness of the apple pulp was determined by an FT - 372 penetrometer with a plunger with a diameter of 11 mm; tasting evaluation – according to GOST 8756.1-79 **[28]**; the chemical composition of apples was determined by methods generally accepted in fruit biochemistry: soluble dry substances – by the refractometric method (GOST 28562-90) **[29]**; sugars – by the Bertrand spectrophotometric method (GOST 8756.13-87) **[30]**; titrated acidity – by titration with 0.1N NaOH solution (GOST 25555.0-82) **[31]**; vitamin C – with potassium iodate (GOST 24556-89) **[32]**; pectin substances – by titrometric method (GOST 29059-91) **[33]**; alcohols and aldehydes – by spectrophotometric carbazole method **[34]**, polyphenolic substances – by vanillin method **[34]**. Natural decline, spoilage, total losses, and output of marketable products were determined according to recommendations of GOST 34314-2017 **[35]**.

Description of the Experiment

Sample preparation: To prepare the apples for the experiment, the samples were packed in a polyethylene film with a thickness of 40 – 120 microns or lowered into a solution of the drug Phytosporin-M to form an active nanofilm.

Number of samples analyzed: 100 in each experimental group.

Number of repeated analyses: 3.
Number of experiment replication: 2.
Design of the experiment:

Analytical and experimental studies were based on the macroscopic theory of gases, modern ideas about the physiological state of storage objects, mass transfer theory, and various fruits' storage technologies. To prepare the apples for the experiment, the samples were packed in a polyethylene film with a thickness of 40 – 120 microns, or lowered into a solution of the drug Phytosporin-M to form an active nanofilm (Figure 1).

Figure 1 Experimental samples of apple.

Fruit quality assessment was conducted according to the "Program and methodology of variety study of fruit, berry and nut crops" **[27]**. The fruits for analysis were selected at removable maturity. During the technical analysis, the mass, the size of the fetus, and the shape index, i.e., the ratio of height and diameter, were measured. Biochemical studies were carried out in 3-5-fold repetition in the biochemical laboratory of fruit storage and processing. The hardness of the apple pulp was determined by an FT-372 penetrometer with a plunger with a diameter of 11 mm; tasting evaluation – according to GOST 8756.1-79 **[28]**; the chemical composition of apples was determined by methods generally accepted in fruit biochemistry: soluble dry substances – by the refractometric method (GOST 28562-90) **[29]**; sugars – by the Bertrand spectrophotometric method (GOST 8756.13-87) **[30]**; titrated acidity – by titration with 0.1N NaOH solution (GOST 25555.0-82) **[31]**; vitamin C – with potassium iodate (GOST 24556-89) **[32]**; pectin substances – by titrometric method (GOST 29059-91) **[33]**; alcohols and aldehydes – by spectrophotometric carbazole method **[34]**, polyphenolic substances – by vanillin method **[34]**. Natural decline, spoilage, total losses, and output of marketable products were determined according to recommendations of GOST 34314-2017 **[35]**.

Statistical Analysis

Statistical processing of experimental data was carried out using the NSR.BASE Excel software and analysis of variance using Student's criterion with confidence probability ($\alpha = 0.95$) **[36]**. Statistically significant values were assumed at $p < 0.05$. Values with $p > 0.05$ were not taken into account in the experiment.

RESULTS AND DISCUSSION

Our research has shown that under optimal storage conditions in CA, the respiration of apples of various varieties proceeds evenly and slowly. So, after 5 – 7 months, the respiration rate of Jonathan and Reinette Simirenko apples was 55 – 77% compared to fruits stored under normal conditions (control). A similar trend was previously established by other researchers in the study of different varieties and fruits **[37]**, **[38]**, **[39]**.

In the most long-stored variety of Korey apples, the initial state is characterized by the lowest respiratory intensity (2.3 mg/kg/h), which is slightly higher in Idared apples (2.7 mg/kg/h), but different periods of climacteric rise. In the control storage variant, the climacteric rise of respiration in the Korey variety began after 5.5 months, in Idared after 4 months. When stored in a "closed loop" with increased CO_2 content, the climacteric rise in respiration was weakly pronounced and stretched over time, which corresponds to the results of other researchers **[40]**, **[41]**.

According to Calegario et al. (2001), the timing of the climacteric rise of fetal respiration and the steepness of the fall correlates with the rate of entering the gas regime in a "closed loop" and with the rate of biochemical processes **[42]**.

To clarify the respiratory gas exchange during the storage of apples in a "closed loop", the gas composition was determined using the GHP-75 gas analyzer. The results obtained are presented in Table 1.

Table 1 The content of O_2 and CO_2 in the "closed loop" (p <0.05).

Apple variety	The composition of the air in a "closed loop" during storage, %							
	1 month		2 months		4 months		7 months	
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
Idared:								
p/e film 120 μm	14.5	6.5	13.0	8.0	12.0	9.0	nitrogen purging or perforation	
p/e film 40-60 μm	18.0	3.0	14.2	6.8	12.8	8.2	12.8	8.2
Korey								
p/e film 120 μm	15.2	5.8	14.0	7.0	12.5	8.5	nitrogen purging or perforation	
p/e film 40-60 μm	19.0	1.0	15.3	5.7	13.5	7.5	13.0	8.0

From the data in Table 1, it can be seen that when storing fruits in a "closed loop", the gas composition changes in the direction of increasing the concentration of carbon dioxide. The degree of change in the gas environment depends on the varietal characteristics of the fruits **[43], [44]**. According to our data, significant changes in the gas composition in the package occur during the first month of storage: a decrease of O_2 from 1.5 to 3.7% and an increase of CO_2 from 1.5 to 4.7%. For further storing, there is a gradual decrease in oxygen and an increase in carbon dioxide. In the contour of a 40 – 60 μm film, the steady-state mode (due to fruit respiration) is stable until the end of storage (7 months). In a "closed loop" of a 120 μm film, the gas medium was also created as a result of fruit respiration, but after 4 months of storage, it was maintained by blowing or opening the valve.

Biochemical quality indicators are presented in Table 2. After 7 months of storage in a "closed loop" (120 μm), Fruits had good preservation of dry substances and sugars and a sourer taste than fruits stored with free access to air. At the same time, the higher acidity of the fruit is mainly due to the content of malic acid, which is explained by the inhibitory effect of carbon dioxide on the intensity of respiration **[45]**, **[46]**.

Changes in the mass fraction of ascorbic acid in apples under the influence of a gaseous medium differed little from control samples. This confirms the assumption of Sapei and Hwa (2014) that the dynamics of vitamin C is less dependent on the composition of the medium and a greater correlation with the temperature regime of storage **[47]**. The increased content of carbon dioxide and the reduced oxygen content in the medium contributed to the ripening of the fruits to a lesser extent, as evidenced by the data on the content of alcohol and acetaldehydes after 7 months of storage. The mass fraction of them, when stored in a "closed loop" was minimal compared to the control (Table 2).

It should be noted that apples stored in a modified gas atmosphere in p/e films of 40 and 60 μm also had good storage results in terms of commodity and chemical quality indicators (in comparison with the control) but were inferior to apples in a "closed loop" (120 μm) in all indicators.

Processing of apples of the Idared variety before laying for storage with the biological preparation *Phytosporin-M* (40, 60 μm) also positively affected the quality of storage, reducing losses from microbial spoilage and reducing the gap in the yield of marketable products between fruits stored in a "closed loop" (120 μm).

Table 2 Biochemical indicators of apple fruit quality after 7 months of storage in a modified gas environment (p <0.05).

The variety of apples with the method of storage	Soluble dry substances, %	Total sugars, %	Total acidity, %	Alcohol, %	Aldehydes, %, mg/dm^3	Pectin substances, %		Polyphenolic substances, mg %	Vitamin C, mg %
						instant pectin	Proto-pectin		
Korey (when laying for storage)	13.0	12.0	0.33	–	–	0.29	0.62	240.0	7.0
Korey control (without treatment) storage in containers	12.2	10.9	0.22	0.150	11.65	0.26	0.40	190.0	4.7
Korey in p/e film 40 µm	12.4	11.2	0.23	0.091	11.42	0.25	0.44	190.0	5.0
Korey in p/e film 60 µm	12.5	11.4	0.24	0.069	9.64	0.23	0.45	196.0	5.5
Korey in p/e film 120 µm ("closed loop")	12.7	11.7	0.26	0.058	6.90	0.22	0.50	220.0	5.9
Idared (when laying for storage)	12.0	10.8	0.58	–	–	0.34	0.50	150.0	8.8
Idared control (without treatment) storage in containers	10.3	9.8	0.36	0.173	17.35	0.13	0.27	123.3	6.9
Idared in p/e film 40 µm	10.4	9.8	0.38	0.104	14.62	0.13	0.30	124.5	7.4
Idared in p/e film 60 µm	10.8	10.0	0.38	0.092	9.00	0.12	0.32	126.3	7.4
Idared in p/e film 120 µm ("closed loop")	11.3	10.4	0.44	0.068	7.80	0.13	0.42	131.5	7.7

From the results obtained, summarized in Table 3, it can be seen that the method of storing apples in an "isolated circuit" gives a pronounced positive effect since losses are significantly reduced (from 4.5% to 0.4%) from weight loss and, very importantly, from microbiological spoilage from 4.6% to 0.8% and physiological diseases, which guarantees the safety of nutrients. At the same time, the yield of commercial varieties increased by 7 – 8.5%, depending on the variety.

When storing apples in a modified gas atmosphere, the following requirements must be observed: to store fruits with high-quality indicators at optimal maturity; to load chambers with products homogeneous in keeping quality and immediately after harvesting; varieties susceptible to sunburn must be treated with antioxidants or biological preparations **[48]**, **[49]**. Failure to comply with these conditions reduces the shelf life, losses, and quality of fruits.

Thus, studies have shown that our proposed method of storing apples in an isolated "closed loop" using high-pressure polyethylene with a film thickness of 120 µm, without the use of expensive equipment and materials for sealing chambers, allows us to achieve storage results close to storing apples in a controlled atmosphere **[50]**, **[51]**, **[52]**. Also, we used existing fruit storage capacities and parts of them while maintaining constant access to the chambers, which is very important for farms that grow small volumes of apples and cannot build separate refrigeration facilities **[53]**, **[54]**.

Infectious and physiological diseases of fruits annually cause huge economic damage to the industry **[55**, **56]**. This problem is solved by treatment with harmless and effective drugs that reduce the microbiological load in food technologies.

Apples of winter varieties were used: Idared, Korey, Jonathan. A prolonged-acting microbiological fungicide with anti-stress properties, *Phytosporin-M*, was used for surface treatment. The apple surface treatment was carried out according to the method proposed by Gvozdenko et al., 2022 **[57]**. The processed apples were dried and sent to refrigerated storage for 7 days. To determine the optimal consumption rates of the *Phytosporin-M* fungicide for post-harvest processing of raw materials, a solution of 1 – 1.5 liters per ton was used.

Table 3 Commercial quality of apples when stored in a modified gas environment (p <0.05).

The variety of apples with the method of storage	For 4 months of storage				For 7 months of storage			
	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %
Idared control, storage in containers	2.4	1.4	3.8	96.2	4.9	3.7	8.6	91.4
Idared in p/e film 40 μm (treated with a bactericidal preparation)	0.0	0.0	0.0	100.0	0.7	3.1	3.8	96.2
Idared in p/e film 60 μm (treated with a bactericidal preparation)	0.0	0.0	0.0	100.0	0.6	2.4	3.0	97.0
Idared in p/e film 120 μm	0.0	0.0	0.0	100.0	0.5	1.1	1.6	98.4
Korey control, storage in containers	21	2.7	4.8	95.2	4.2	5.1	9.3	90.7
Korey in p/e film 40 μm	0.0	0.0	0.0	100.0	0.6	3.9	4.4	95.6
Korey in p/e film 60 μm	0.0	0.0	0.0	100.0	0.5	2.0	2.5	97.5
Korey in p/e film 120 μm	0.0	0.0	0.0	100.0	0.4	0.7	1.1	98.9

The results of the optimal solution concentration were judged by the commodity assessment of the quality of raw materials during storage: the presence of rot, diseased, and sprouted specimens. The experimental batch of plant raw materials received the best commodity rating and was studied according to organoleptic, biochemical, and microbiological quality indicators.

Comparative data on the quality of fruits treated with a solution of Phytosporin-M and control (without treatment) are presented in Table 4, Table 5, and Figure 2 and Figure 3.

Table 4 Commercial quality of apples treated with *Phytosporin-M* during storage at 0 +2° C (p <0.05).

The variety of apples with the method of storage	For 4 months of storage				For 7 months of storage			
	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %	Natural decline, %	Spoilage, %	Total losses, %	Output of marketable products, %
Korey control, storage in containers	2.1	2.7	4.8	95.2	4.2	5.1	9.3	90.7
Korey, treated with a bactericidal preparation, storage in containers	1.4	1.0	2.4	97.6	3.0	2.3	5.3	94.7
Idared control, storage in containers	2.4	1.4	3.8	96.2	4.7	2.9	7.6	92.4
Idared, treated with a bactericidal preparation, storage in containers	1.8	1.1	2.9	97.1	3.7	2.0	5.7	94.3
Jonathan control, storage in containers	3.5	2.0	5.5	94.5	7.8	4.6	12.4	87.6
Jonathan, treated with a bactericidal preparation, storage in containers	2.5	1.2	3.7	96.3	5.6	2.6	8.2	91.8

According to research results, pretreatment with *Phytosporin-M* provides the best yield of marketable products, minimal microbial spoilage (Figure 2 and Figure 3), and loss of biologically active substances (Figure 4).

To preserve the natural stability of fruits and prolong their shelf life, preventing the intensive accumulation of alcohol is of great importance **[58]**. At the next stage of the experiment, apple samples were packed in a polyethylene film with a thickness of 40 – 120 microns or lowered into a solution of the drug Phytosporin-M to form an active nanofilm **[57]**, **[59]**, **[60]**, **[61]**. According to our data (Table 5), the alcohol content in processed fruits by the end of storage (7 months) is 1.5 times lower than in the control.

Table 5 Biochemical quality indicators of apple fruits treated with *Phytosporin-M* after 7 months of storage (p <0.05).

The variety of apples with the method of storage	Soluble dry substances, %	Total sugars, %	Total acidity, %	Alcohol, %	Pectin substances, %		Polyphenolic substances, mg %	Vitamin C, mg %
					instant pectin	Proto-pectin		
Korey (when laying for storage)	13.0	12.0	0.33	–	0.29	0.62	240.0	7.0
Korey control (without treatment) storage in containers	12.2	10.9	0.22	0.150	0.26	0.43	190.0	4.7
Korey processed Phytosporin-M (in containers)	12.5	11.5	0.29	0.093	0.24	0.45	215.0	5.5
Idared (when laying for storage)	12.0	10.8	0.58	–	0.34	0.50	150.0	8.8
Idared control (without treatment) storage in containers	10.3	9.9	0.38	0.173	0.13	0.27	123.3	6.9
Idared processed Phytosporin-M (in containers)	11.0	10.2	0.42	0.114	0.12	0.30	133.8	7.5
Jonathan (when laying for storage)	12.5	11.5	0.60	–	0.50	0.38	170.0	8.0
Jonathan control (without treatment) storage in containers	10.2	10.0	0.39	0.185	0.16	0.22	130.0	5.9
Jonathan processed Phytosporin-M (in containers)	11.2	10.9	0.44	0.125	0.13	0.25	145.0	6.4

The microbiological studies of apple fruits during storage fully confirm the advantages of storing fruits with their pretreatment with *Phytosporin-M* fungicide (Figure 4). This technology makes it possible to reduce losses from microbial spoilage by 2 times for the Korey and Jonathan varieties and by 1.6 times for the Idared variety (Figure 2 and Figure 3).

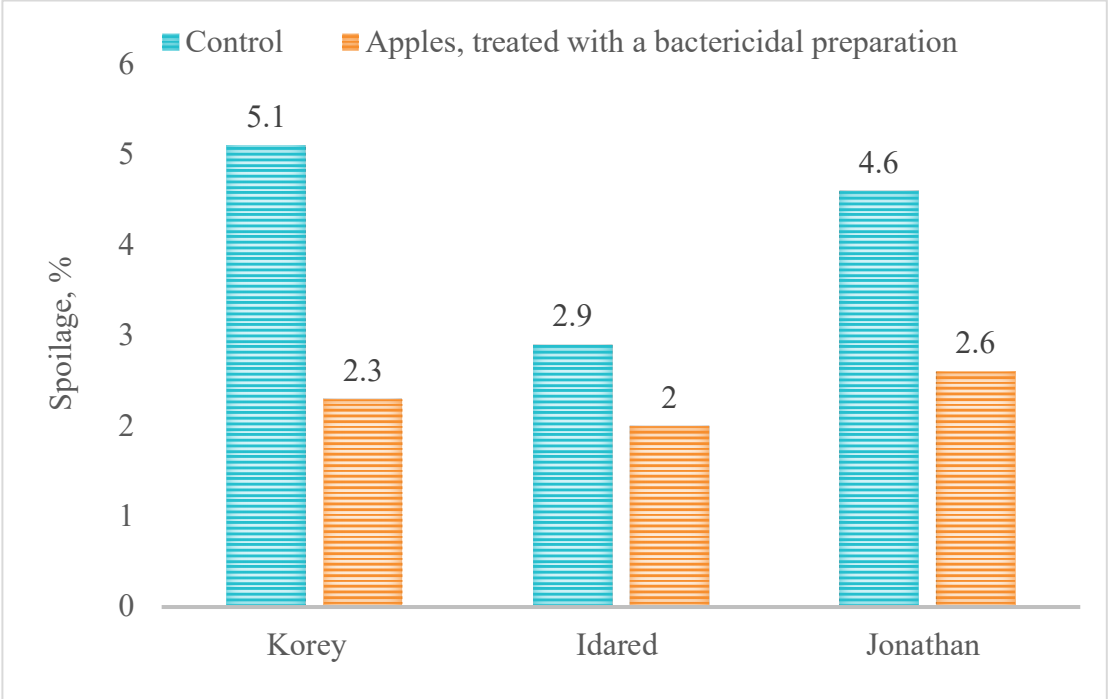

Figure 2 The effect of processing apples with a biological preparation on the amount of microbiological spoilage during 7 months of storage (p <0.05).

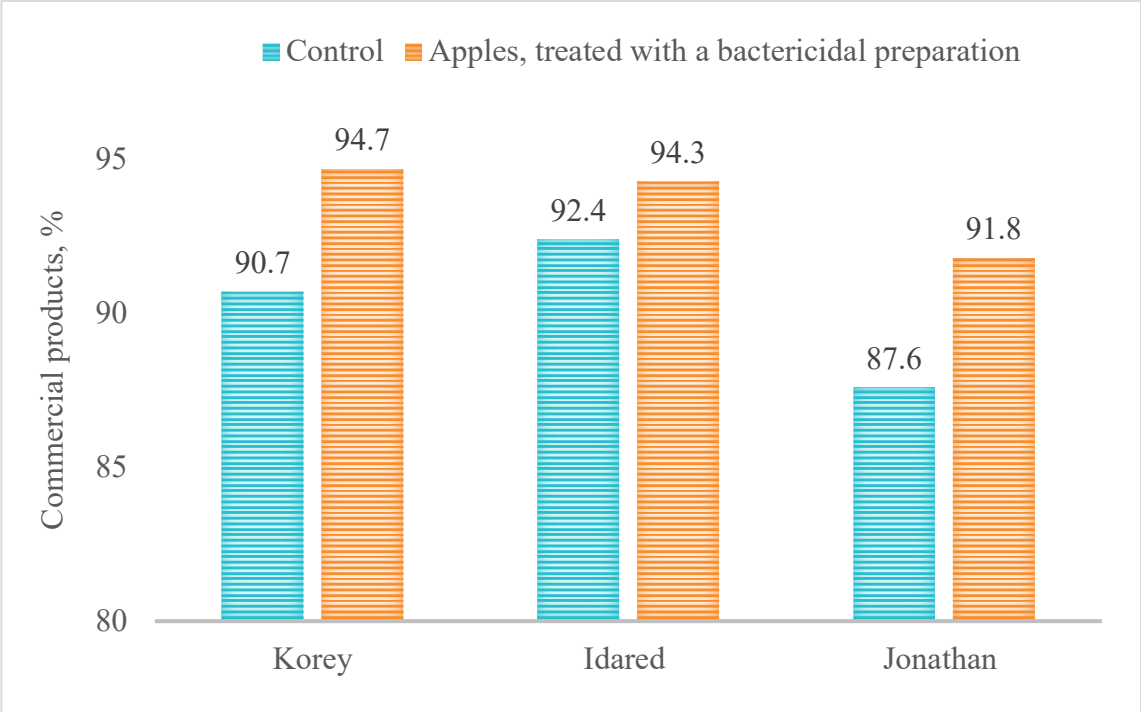

Figure 3 The effect of processing apples with a biological product on the yield of marketable products during 7 months of storage (p <0.05).

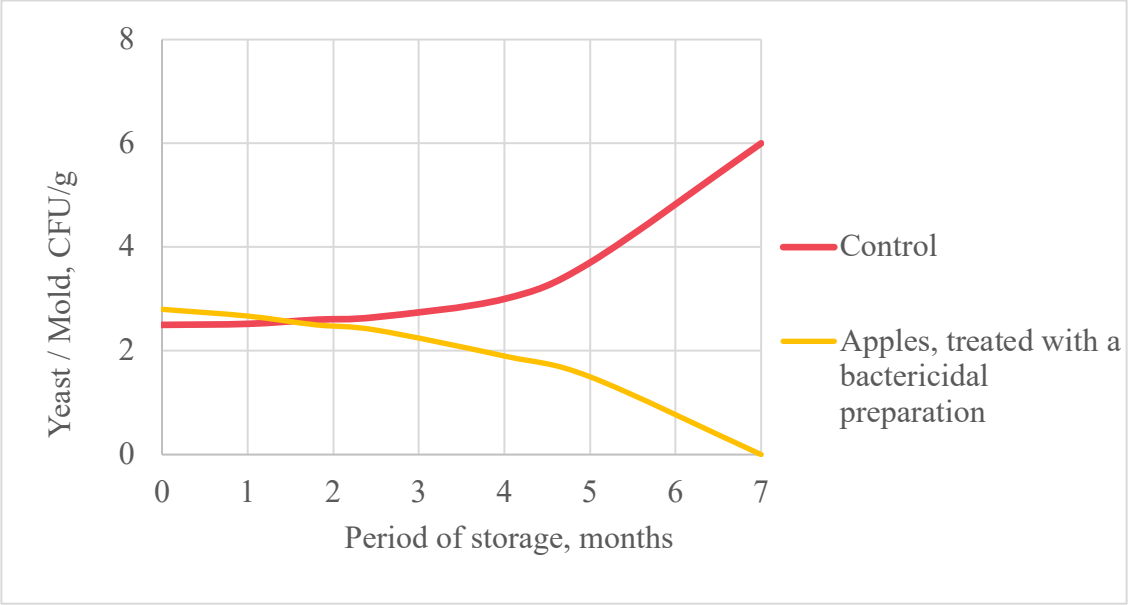

Figure 4 The effect of the apple storage method on microbiological indicators.

The tasting evaluation of apple fruits after 7 months of storage shows that the experimental samples exceeded the control ones in taste, presentation, and dense consistency (turgor of tissues) and no signs of wilting.

Thus, the results show that during long-term storage of apple fruits, there was a pronounced positive effect for fruits treated with a solution of the *Phytosporin-M* biological preparation, which allowed to increase the yield of high-quality commercial products by 8 – 9%, which positively affected the economic indicators of production.

CONCLUSION

A low-cost technology of non-generator storage of apple fruits in an isolated "closed loop" has been developed. It allows you to implement the controlled atmosphere (CA) technology in a typical environment (7 – 8% CO_2) and (13 – 14% O_2) and a subnormal environment (3% O_2 and 5% CO_2) without using a gas generator. The technology of apple fruit storage with pretreatment of raw materials, containers, and internal storage surfaces with a solution of the biological preparation Phytosporin-M are scientifically substantiated. The developed technology made it possible to reduce the moisture loss of the studied raw materials significantly, losses from microbial spoilage, and increase the yield of high-quality commercial products by 8 – 13%, which positively affected the economic indicators of production. Thus, our research aimed at extending the shelf life and reducing the loss of raw materials will provide an opportunity to increase the output of valuable canned fruit, improve the assortment, reduce the cost and generally increase the efficiency of their production.

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The potential of Curcuma extract to alleviate muscle damage in amateur soccer players

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ABSTRACT

Compounds with high bioactive are commonly used as a nutritional approach for accelerating muscle damage recovery after strenuous exercise. There are still inconsistent results of post-exercise antioxidant supplementation on the circulating muscle damage biomarker. This study aimed to examine the effect of post-exercise Curcuma extract supplementation in ice cream on muscle damage and inflammatory markers in amateur soccer players. Male amateur soccer athletes (aged 14 – 18 years) participated in a randomized double-blind placebo-controlled study under two conditions: control group (n = 10) and treatment group (n = 10). The treatment group was treated with Curcuma extract ice cream (250 mg/100 g) for 21 days. Blood samples were drawn before training, considered baseline, and 3 h after training on day 21. The level of creatine kinase, IL-6, haemoglobin (Hb), and lactic acid were quantified. There was a significant decrease in creatine kinase change in the treatment group compared to the control group (p <0.05). No change in IL-6 and Hb levels in the treatment group. Lactic acid decreased by 16.3% from baseline in the treatment group (p <0.05). Curcuma extract ice cream potentiates to ameliorate exercise-induced muscle damage.

Keywords: Curcuma extract, inflammation, muscle damage, soccer, exercise

INTRODUCTION

Muscle damage mediated by inflammation is a key feature in response to exercise **[1]**. Creatine kinase is an enzyme found at a high level under high energy demands (i.e., after the high intensity of exercise) and is considered an indirect indicator of muscle damage **[2]**. Exercise-induced muscle damage stimulates the bone marrow to release immune cells, which can express cytokines such as interleukin-6 (IL-6). A marked increase in IL-6 level was observed immediately after a soccer game in elite competitive-level players **[3]**. Inflammation in response to exercise-induced muscle damage can be featured by pain feelings **[4]** that may subsequently decrease athlete performances. A nutritional approach has been conducted to alleviate inflammation-associated muscle damage after exercises, such as ginger (*Zingiber officinale*), ginseng (*Panax quinquefolium*), and Curcuma **[5]**. The inflammation phase and degree of oxidative stress in response to exercise-induced muscle damage are interrelated. Increasing oxidative stress levels indicated by excessive free radical production have been widely investigated and considered a signal to activate the immune system **[6]**. The production of free radicals after exercise is required to induce muscle rejuvenation.

On the other hand, the magnitude of oxidative stress reflects the degree of exhaustion state **[7],** and athletes need to accelerate muscle recovery. The overwhelming free radical could be ameliorated by exogenous antioxidants that help the body to produce endogenous antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase **[8]**. Exercising muscle in high intensity produces lactate in muscle and consequently releases it into circulation. Blood lactate has been reported to elevate in the first 15 min during a soccer match and remained high throughout the game **[9]**. Antioxidant supplementation was previously reported to lower blood lactate after performing maximal aerobic exercise in amateur athletes **[10]**. Since the soccer game requires a combination of aerobic and anaerobic energy systems, evaluating the antioxidant supplementation effect in soccer athletes is required. Curcumin is an antioxidant substance in Curcuma and has been reported for

its antioxidant capacity. The antioxidant capacity in curcumin is better than other components, such as *demetoksicurcumin* and *bisdemetoksicurcumin.*

Additionally, curcumin has an efficient reaction with superoxide radicals and lipid antioxidants, and the response leads to superoxide catalytic degradation, which curcumin represents as a SOD **[11]**. Supplementation in food products may increase acceptability. Ice cream is one of the products with a broad market segment, and every single person, regardless of age, is fond of it. The intervention of curcumin supplementation on the exercise-induced oxidative stress marker in recreationally participants has been previously reported **[12]**. The study revealed that significantly lower serum derivatives of reactive oxygen metabolites after exercise were observed in the curcumin group.

Furthermore, a systematic review and meta-analysis have recently reviewed the effect of curcumin supplementation on inflammatory markers and muscle damage in sedentary participants **[13]**. Nevertheless, the effect of curcumin supplementation post-training on the circulating muscle damage, inflammatory marker, lactate, and haemoglobin in soccer athletes has not been investigated. Therefore, this study aimed to examine the effect of Curcuma extract in the ice cream form supplementation on creatine kinase, IL-6, lactate, and haemoglobin.

Scientific Hypothesis

We hypothesized that post-training Curcumin intervention alleviated muscle damage-related to training, indicated by a significant difference in circulating creatine kinase and inflammatory markers.

MATERIAL AND METHODOLOGY

Samples

Blood samples were taken in the prior study before subjects conducted the training. The Curcuma extract ice cream was given daily for 21 days after subjects ran 1 km consisting of a repeated sprint, six maximal 15-m runs with a 30-s recovery, an intermittent endurance test (IET) consisting of forty 15-s bouts of high-intensity running interspersed by 10-s bouts of low-intensity running. At the end of the study, at least 3 h after training, the blood samples were taken to assess IL-6 and creatine kinase.

Chemicals

Human IL-6 Elisa Kit (Elabscience, USA) and human creatine kinase (Elabscience, USA) were used in this study.

Animals and Biological Material

The raw Curcuma was purchased from the local market Purworejo, Semarang Province, Indonesia, where well-recognized for having a high-quality Curcuma.

Laboratory Methods

Based on the previous study, the ice cream composition had 11% fat and 15% sugar **[14]**. The flowchart for making ice cream showed in Figure 1. In brief, the same ripe of Curcuma rhizome was selected. The selected Curcuma rhizome was sliced ±5 – 7 mm thickness after washing. The Curcuma rhizome was dried in the oven at 50 °C until the water content was 10% and was filtered using a 40-mesh filter to obtain the Curcuma powder. The ingredients (milk, Curcuma powder, sugar, Carboxymethyl cellulose) were mixed, then lemon juice (3 mL/100 g ice cream) and cinnamon (10 mL/100 g ice cream) were added after it was heated at 80 °C for 20 min. The final compound (Figure 2) was kept at the freezer temperature.

Description of the Experiment

The inclusion criteria of this study were male, 14 – 18 years, no acute and chronic diseases identified, Hb level ≥13 g/L, nutrition status normal (BMI ranged from 18.5 – 25.0 kg/m^2), no coffee, no alcohol, and no drugs consumption, no smoking, no vitamin, and other supplements consumption at least two weeks before this study was conducted, no additional exercise except the programmed exercise. Written informed consent was obtained from all participants. All of soccer athletes in Pusat Pendidikan dan Pelatihan Pelajar (PPLP) Semarang (Indonesia) became the subject in this study (n = 20). Participants were divided into two groups: the control group (n = 10), treated with ice cream without any Curcuma extract supplementation, and the treatment group (n = 10), treated with Curcuma ice cream. The confounding variable in this study (the antioxidant-sourced food) was controlled by recording the athletes' dietary intake. The ice cream selected in the present study was the ice cream contained 250 mg of Curcuma since we considered the sensory evaluation result of this study (unpublished data). The procedure of this study was according to the guidelines laid down in the Declaration of Helsinki and has been reviewed and approved Bioethical Commission of Research of Faculty of Medicine Universitas Sultan Agung, Semarang, Indonesia with No. 208/IV/2018/Komisi Bioetik and registered at ClinicalTrials.gov ID: NCT04439981. Daily subject intakes during the study were recorded in the recall form (data not shown).

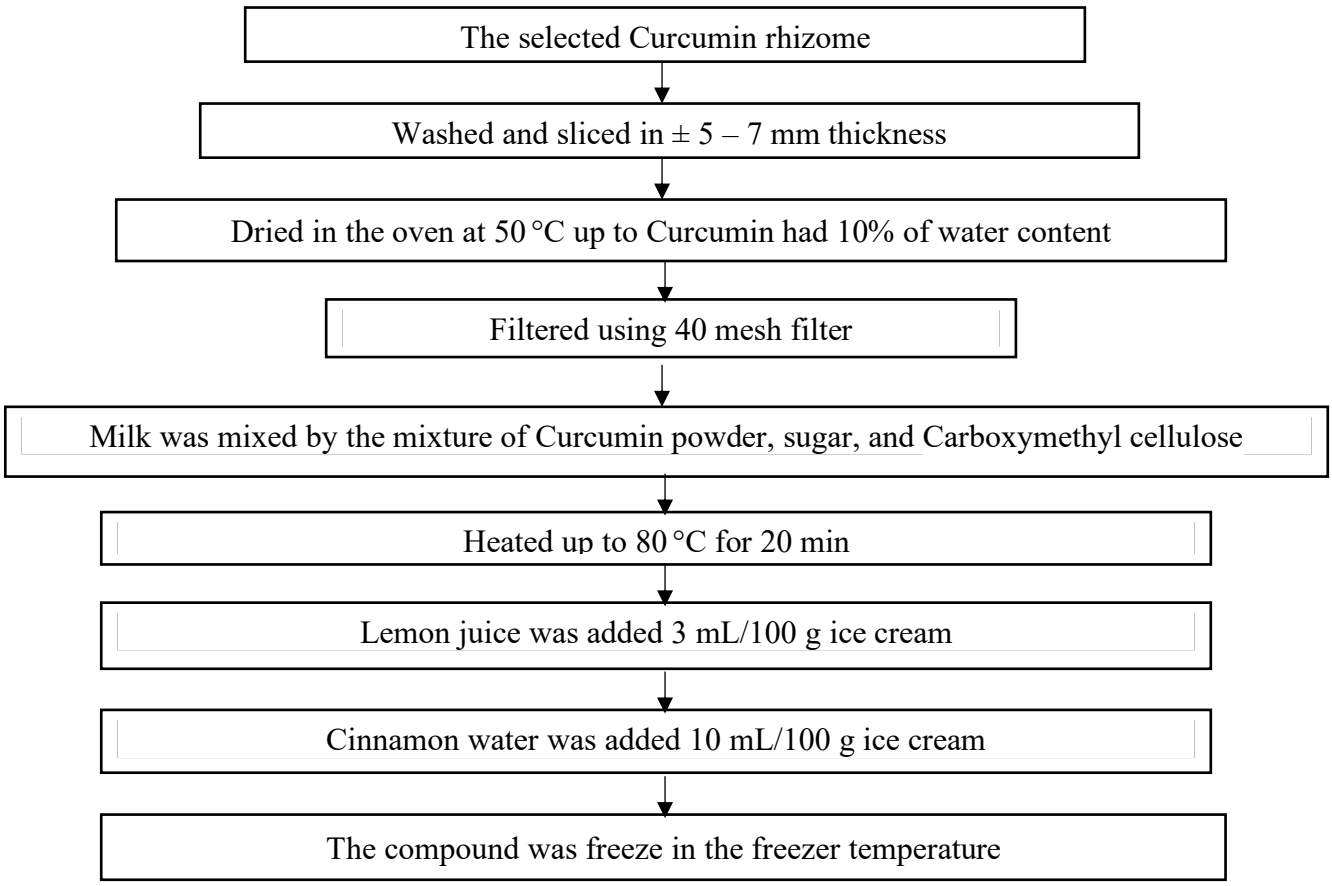

Figure 1 The workflow of the Curcuma ice cream-making process.

Figure 2 The representative ice cream consumed by the control group (left) and intervention group (right). The physical appearance of ice cream in the control and intervention groups was identical.

Statistical Analysis

Statistical analyses were performed using SPSS software for IBM 27.0 version. Since the data are normally distributed (indicated by $p > 0.05$ on the Shapiro-Wilk test), parametric tests were performed. The comparison between groups was analysed by ANOVA followed by an LSD post-hoc test to assess the Curcuma ice cream. For the supplementation effect on the haematological biomarkers, the comparison before and after the intervention was analysed using paired t-test. At the same time, the comparison between the group was analyzed by an independent t-test. The data were expressed as mean ±SE and were considered significant at 95% CI ($p < 0.05$).

RESULTS AND DISCUSSION

To our knowledge, this study was a novel study giving curcumin supplementation after training amateur soccer athletes. Curcumin has been comprehensively reported to significantly enhance total antioxidant capacity with a significant effect in humans **[15]**; therefore, the present study aimed to answer whether the antioxidant supplementation post-exercise in the fatigue indicated by circulating creatine kinase. The present study has three main findings: 1) 250 mg of Curcuma/100 g ice cream decreased lactic acid level significantly by 16.3%. 2) The Curcumin content in this study may not be sufficient to reduce fatigue biomarkers indicated by circulating creatine kinase.

The effect of Curcuma extract ice cream on muscle damage

The baseline characteristics of the participants are summarized in Table 1. Decreasing creatine kinase after 21 days of intervention of curcumin supplementation was observed by 10%.

Table 1 Baseline characteristics of participants.

Variables	Control group (n = 10)	Treatment group (n = 10)	*p*-value
Age (y)	16.2 ±0.2	16.8 ±0.9	0.75
BMI (kg/m^2)	22.1 ±2.5	21.1 ±1.9	0.61
Haemoglobin (g/L)	13.5 ±1.6	14.1 ±2.5	0.89

Note: Data are expressed as mean ± SD; BMI – Body Mass Index; data are analysed by independent t-test.

Curcuma extracts ice cream, as seen in Table 2, reduced creatine kinase levels at the end of the study by -32.50 ±73.56 U/L. The creatine level of the treatment group differed significantly from the creatine level of the control group (p <0.01). Creatine kinase has widely increased significantly after muscle contraction, indicating exercise-related muscle damage **[2]**, **[16]**. Increasing creatine kinase related to strenuous exercise **[2]**, **[17]** is mediated by the inflammation phase **[18]**, **[19]** indicated by neutrophil infiltration as a first immune response to muscle damage **[20]**, **[21]**. The sport of soccer requires a combination of energy from aerobic and anaerobic energy systems **[22]**, and antioxidant supplementation has been highlighted as a method to accelerate recovery after training **[23]**. The previous studies showed that antioxidant supplementation, either in bioactive or vitamins, lowered creatine kinase, which may be due to endogenous antioxidant increases **[24]**, **[25]**, **[26]**. Even though the intervention of post-training antioxidant supplementation slightly decreased creatine kinase, curcumin potentiates to alleviate circulating creatine kinase when it is provided in a higher concentration.

The effect of Curcuma extract ice cream on inflammation

Even though there was no significant difference between the two groups at the end of the study, curcumin extract ice cream decreased IL-6 level in the treatment group by 0.007 ±0.03 pg/mL (Table 2). IL-6 is a standard inflammatory marker released by muscle due to muscle contraction and low muscle glycogen **[27]**, **[28]**. The mobilization of circulating IL-6 is seemingly time-dependent after exercise, which is still inconsistent compared to the previous study **[28]**. A previous study showed the circulating IL-6 returned to baseline the 1.5 h after a single bout of exercise **[29]**, indicating the rapid kinetic response of IL-6 to muscle damage. The concentration of circulating IL-6 depends on the intensity of training, which might lead to numerous folds from 5 to 100 folds, and it reaches a peak in 30 minutes after strenuous exercise **[30]**. We speculate the circulating IL-6 in the present study has returned to baseline since the blood draw was taken at least 3 h after training.

The effect of Curcuma extract ice cream on Hb

Table 2 shows an increasing level of Hb in the treatment group. In contrast, decreasing levels of Hb were observed in the control group. The difference in the haemoglobin level had a significant level between those two groups (p = 0.017). Hb on the sports field may be a crucial haematological biomarker to carry oxygen from the lungs to the tissue **[31]**, **[32]**, which is proportional to athletes' decreased performance **[33]**. Increasing demand for oxygen in the contracting muscle led to bulk blood flow into exercised muscle tissue to maintain a sufficient oxygen level **[34]**. Therefore, decreasing haemoglobin was observed after exercise **[35]**. This study resulted in the improvement of haemoglobin levels in the treatment group compared to the control group. Curcuma in the herb form with 30 mg/100 g has proven to maintain Hb to normal levels in animal experiments **[36]**. The present study used the lowest antioxidant content since we consider the preference of the participants based on the sensory acceptability of the Curcuma ice cream. Further examination is required asking whether Curcumin supplementation affects the muscle damage and inflammatory markers in skeletal muscle of soccer athletes since Curcuma supplementation potentiates to alleviate exercise-induced muscle damage. Sequential time analysis is

required in a future study to delineate the magnitude change of the markers in response to Curcumin supplementation.

Table 2 Creatine kinase, IL-6, haemoglobin, and lactic acid level at the beginning and end of the study.

	Control Group	Treatment Group	*p*-value
Creatine kinase level (U/L)			
Before	157.60 ±29.98	317.20 ±243.36	0.009[b*]
After	253.40 ±133.79	284.70 ±196.63	0.971[b]
Difference	95.80 ±116.95	-32.50 ±73.56	0.010[b]*
p-value	0.037[a*]	0.203[a]	
IL-6 level (pg/mL)			
Before	0.508 ±0.76	0.159 ±0.05	0.739[b]
After	0.538 ±0.78	0.152 ±0.04	0.165[b]
Difference	0.030 ±0.06	-0.007 ±0.03	0.190[b]
p-value	0.139[a]	0.574[a]	
Haemoglobin level (g/dL)			
Before	15.35 ±0.80	15.75 ±1.21	0.393[b]
After	14.98 ±0.67	15.98 ±0.99	0.017[b]*
Difference	-0.37 ±0.37	0.23 ±0.62	0.017[b]*
p-value	0.012[a*]	0.268[a]	
Lactic acid level (mmol/L)			
Before	3.73 ±0.47	3.25 ±0.90	0.152[b]
After	4.76 ±2.33	2.72 ±0.68	0.015[b]*
Difference	1.03 ±2.15	-0.53 ±0.79	0.050[b]*
p-value	0.185[a]	0.06 [a]	

Notes: * – indicates data significantly different ($p < 0.05$); [a] – data were analysed by paired t-test; [b] – data were analysed by independent t-test; data are presented as mean ±SD. IL-6 – interleukin-6.

The effect of Curcuma extract ice cream on lactic acid

As shown in Table 2, the level of lactic acid was significantly decreased in the treatment group (-0.53 ±0.79; $p = 0.06$), but there was an increasing level of lactic acid in the control group. There was also a significant difference between the two groups at the end of the study ($p = 0.015$). Blood lactate level can be used to predict exercise intensity **[37]**, evidenced by the proportional increases of blood lactate concentration as the increase in work rate **[38]**. This study highlighted the increase of lactic acid levels in the control group by 27.6% from baseline. Lactate can be considered as an intracellular shuttle that provides the raw material for ATP resynthesis in mitochondria during the high physical effort of exercise **[39]**. The reduction of lactic acid in the intervention group may be explained by increasing lactate clearance through lactate dehydrogenase (LDH). Therefore, lactate can be converted into pyruvate **[40]**. The potential ability of curcumin to maintain the redox state via ameliorating reactive oxidative species (ROS) in skeletal muscle damage cannot be excluded. Strenuous muscle contraction generates free radicals **[41]** as a signal to recruit neutrophils from bone marrow **[42]**. Curcumin can be considered an antioxidant as β-diketone groups can enhance the activity of catalase, SOD, and GPx, which act as endogenous antioxidants **[43]**. In this study, the antioxidant activity has been observed and may potentiate to stimulate endogenous antioxidant release.

CONCLUSION

Curcumin extract ice cream (250 mg/100 g) has the potency to reduce muscle damage in soccer athletes after conducting 21 day-strenuous training. Curcuma extract ice cream intervention might suppress the inflammation phase following exercise. We postulate that the decrease in lactic acid in the intervention group relates to the amelioration of free radicals from exercise. The present study brings the novel for the long-term effect on muscle recovery after training. Further research is required to see the effectiveness of the acute impact to see an inflammatory response after training.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

The study protocol has been reviewed and approved by Bioethical Commission of Research of Faculty of Medicine Universitas Sultan Agung, Semarang, Indonesia with No. 208/IV/2018/Komisi Bioetik. The present study was also registered at ClinicalTrials.gov identifier: NCT04439981.

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The extrusion process of poly-cereal mixtures: study and calculation of the main parameters

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ABSTRACT
Theoretical prerequisites for the extrusion of bulk components for the production of high-readiness products have been developed, which formed the basis for calculating and optimizing the main technological parameters of the extrusion process. It has been experimentally confirmed: firstly, the design parameters of the extruder and the initial humidity of the poly-cereal mixture have the greatest influence on the melt pressure of the product; secondly, the geometric characteristics of the working body, the frequency (speed) of the screw rotation and the pressure of the product maximally affect the temperature in the pre-matrix zone of the extruder. It was found that an increase in the rotation speed of the working organ (screw) from 80 to 250 min^{-1} leads to the highest value of the optimization criterion – the energy value of a poly-cereal food product of a high degree of readiness, respectively, for the poly-cereal mixture Fitness – 332.34 kcal and the poly-cereal mixture Health – 334.09 kcal.

Keywords: calculation, extrusion, extrudate, poly-cereal, technological parameters

INTRODUCTION

Currently, the role of fast food products (ready-to-eat) and dietary, therapeutic, and preventive, health-improving products based on grain crops is sharply increasing in the population's diets. The demand for fast food products with high protein and dietary plant fibers has increased. The same is true with the main grains: corn and wheat is processed mainly for flour and cereals, and oats have recently been used to produce oat flakes. Other grains are not used at all, except for the production of ordinary cereals **[2]**, **[4]**, **[6]**, **[8]**, **[10]**, **[12]**. In this regard, the development of technology for producing high-readiness products based on poly-cereal mixtures balanced in the content of individual nutrients (fiber), vitamins, macro- and microelements with certain functional properties is an urgent task. Fast food has changed eating habits and has become one of the traditional forms of nutrition worldwide. They are very widely used by the population of many countries as ready-made breakfasts and healthy food products. For example, the USA's market for ready-to-eat foods and light snacks is growing by 3% annually. In our country, this demand and the demand for protein food products are met by imports. Extrusion technology is most often used for the preparation of fast food **[2]**, **[3]**, **[10]**, **[12]**. During extrusion processing of starch and protein-containing raw materials, several advantages can be obtained compared to traditional technologies. These advantages consist in the fact that with single short-term processing of dry grain, it is possible to obtain ready-to-use food products or components with high water and fat retention capacity, as well as ready-to-eat products;

- to increase the digestibility of raw materials and the degree of their use;
- to reduce the microbial contamination of raw materials and neutralize the thermolabile anti-nutritional components of legumes.

Scientific Hypothesis

An increase in the rotation frequency of the working organ (screw) will increase the energy value of a poly-cereal food product with a high degree of readiness. Moreover, the influence of the moisture content of the processed poly-cereal flour mixture on the energy value of the finished product is insignificant.

MATERIAL AND METHODOLOGY

Samples

The following grain crops were identified as objects of study, which were conditionally divided into three groups:

- grain raw materials as scientific products of domestic breeders;
- bulk raw materials, flour from a whole ground grain of cereals and poly-cereal mixture based on it, which is a valuable source of nutrients and minerals;
- products of a high degree of readiness that strengthen the functional status, i.e., products of directed action with maximum preservation of biologically active substances concentrated in the peripheral parts of grain raw materials.

The samples of grain raw materials selected for the study comply with the requirements set out in the ST RK ISO 7970-2013 and GOST ISO 24333-2017 standards. At the same time, control and certification tests of prototypes were studied in the conditions of a testing laboratory or a certification body in the field of food production of Nutritest Academy of Nutrition LLP.

Chemicals

The chemical composition of the poly-cereal mixture (mass fraction of protein, starch and fiber content, ash content) was determined using an infrared analyzer. Chemicals were not used in this research.

Animals and Biological Material

this study, raw materials of plant origin were used (poly-cereals: based on grains of wheat, barley, rye, oats, buckwheat, corn and their composite mixtures).

Instruments

The research laboratory of the KazNARU Center for the extrusion of poly-cereal mixtures includes the following equipment: 1 – universal crusher DU-500 (Russian Federation); 2 – mixer of the poly-cereal flour mixture (China); 3 – twin-screw extruder LT 65L with control panel (China); 4 – filling filler (China) and 5 – micronizer UTZ-4 (Russian Federation).

Laboratory Methods

Extrusion is a special method of processing raw materials in which the flour mixture is amenable to mechanical action (grinding) on the screw part of the extruder. This process occurs under the influence of high temperatures (approximately 150 °C) and pressure. Furthermore, the crushed heated mass under high pressure falls under the influence of low pressure. As a result of a sharp drop, a so-called «explosion» occurs: the finished product increases in volume and acquires a porous structure.

When studying the process of mixing various poly-cereal mixtures, after the expiration of every 10 seconds, an experimental sample of a flour poly-cereal mixture was taken, where the chemical composition (protein, fiber, fat content) was determined by near-infrared spectroscopy, after which the caloric content of all organic compounds in the test sample was calculated according to the mathematical formula.

$$E_v = 4.0 \cdot x_p + 9.0 \cdot x_f + 3.75 \cdot x_c$$

Where:

E_v – energy value (calorie content); x_p, x_f and x_c – protein, fat and carbohydrate content (fiber + starch).

INFRAMATIC 8611/8620 infrared spectrometer was used to determine the chemical composition of the selected mixture sample to analyze ground grain and grinding products.

For rational construction of the technological production process of high-readiness products based on a poly-cereal mixture, the physicomechanical and biochemical properties of poly-cereal raw materials were studied, which assess the technological advantages of grain raw materials.

Description of the experiment: Experimental studies on the process of extrusion of a poly-cereal flour mixture were carried out under the conditions of the International Research Center «Technology of Food and Processing Industries» of the Kazakh National Agrarian Research University and the research laboratory of the Astana branch of the Kazakh Research Institute of Processing and Food Industry LLP.

Instrumental methods for studying the rheological properties of food products were used to determine the quality indicators, rheological properties (elasticity, extensibility, elasticity, energy intensity) of dough based on whole grain flour, as well as the safety of grain raw materials of Kazakhstan selection and flour from poly-cereal raw materials.

Studies were carried out in experimental installations to evaluate the efficiency of technological processes of grinding grain raw materials, mixing, and extrusion of a poly-cereal mixture.

The efficiency of the raw process of grinding grain raw materials in experimental facilities was evaluated by the following indicators: the degree of grinding (granulometric composition) of the objects of study, the productivity of the grinding device (*Q*, kg/hour), and the specific energy costs of conducting the grinding process (*N*, kWh/t).

The efficiency of the technological process of grinding grain raw materials depends on many factors. The humidity of the crushed material significantly affects (–the calculation of the degree of influence of the moisture content of the crushed material is reflected in the matrix of the multifactorial experiment according to Table 1) the efficiency of the technological process of processing grain raw materials. Also, the rotation frequency of the working body and the number of cycles of repeated processing has a significant impact (–the calculation of the degree of influence of the rotational speed of the working body and the number of reprocessing cycles are reflected in the matrix of the multifactorial experiment according to Table.1) on the efficiency of the grain processing process. Therefore, experimental studies were carried out at different values of the moisture content of the crushed grain and different rotational speeds of the working organ **[16]**, **[17]**, **[18]**.

A preprepared experimental sample of the object of study (wheat, barley, oats, corn, buckwheat, and millet) weighing 10 kg is alternately loaded into the receiving device of the grinding plant and subjected to grinding at variable values of the rotational speed of the working bodies, the multiplicity of grinding, and the humidity of the crushed material.

In addition, the mixing process was investigated, which is a complex technological process, on the effectiveness of which the homogeneous distribution of all nutrients in any volume of the poly-cereal mixture depends.

As an indicator of the efficiency of the mixing process, variable values of the caloric content of the selected samples of the poly-cereal mixture were used, with fixed values of the mixing time and the rotation frequency of the working organ.

The efficiency of the mixing process was evaluated by controlling the following parameters: the values of the caloric content of selected samples of flour poly-cereal mixture (E_v, kcal) and the mixing time (*t*, sec) of the bulk components of the mixture, the rotation speed of the working organ (*n*, min^{-1}) at fixed values of the energy intensity of the mixing process.

The pre-prepared bulk components of the poly-cereal mixture are loaded alternately into the mixing tank of the device, then the installation starts. Experimental studies were carried out at various fixed values of the rotation speed of the mixer's working body. The rotation frequency *n* of the working body was changed by replacing the pulleys' diameter on the electric motor's driveshaft.

The results obtained from the experimental studies were entered into the tables of the Microsoft Excel word processor, based on which a graph was constructed of the dependence of the caloric content of the selected samples of flour poly-cereal mixture (E_v, kcal) and the mixing time (*t*, sec) of the bulk components of the mixture at different rotational speeds of the working organ (*n*, min^{-1}).

Next, the extrusion process was investigated, an important technological process for giving unique food and taste advantages to the products produced.

The methodology of conducting experimental studies on the study of the extrusion process is as follows. The pre-prepared poly-cereal mixture, by the developed recipe, is loaded into the receiving device of the experimental extruder. After that, the installation starts at fixed values of the rotation speed of the working body – the pressing screw. At the same time, variable values of pressure and temperature of the extrudate at the outlet of the matrix of the pressing screw are fixed.

Number of samples analyzed: we analyzed 27 samples.

Number of repeated analyses: repeated analyses = 3.

Number of experiment replication: triple.

Statistical Analysis

Mathematical statistics, methodological approach and methods of comparative analysis were used to conduct experimental studies. The obtained experimental data were processed by the methods of mathematical statistics in the STATISTICA editor and Microsoft Excel. The accuracy of the obtained experimental data was determined using the Student's t-test with a confidence probability of ≤0.05 with a set of parallel determinations of at least 5 (confidence probability $p = 0.95$). Linear programming tasks were solved using the *MS Excel* spreadsheet processor setting "Search for a solution" with the Excel Solver setting.

RESULTS AND DISCUSSION

In practice, the Ostwald-de Ville power equation is more often used to describe the flow of bulk material during extrusion **[1]**, **[5]**:

$$\tau = \mu' \cdot \dot{\gamma}^n \quad (1)$$

Where:

τ – is the shear stress in the material; μ'– the consistency coefficient of the material; γ is the shear rate; n is the flow index.

The power law has become widely used to express the flow of various non-Newtonian materials **[1]**, **[5]**, **[11],** which is due to its simple mathematical form, the minimum number of rheological parameters (two), and a fairly good approximation of the results in practical use. It makes it easy to describe the rheological behaviour of the material.

This allows us to conclude that determining the rheological properties of the bulk mass in the extrusion process, which directly affects the quality of the finished product, is of considerable interest and is an urgent task at present.

While studying the flow of viscoplastic materials in channels of various shapes, the possibility of their movement with slippage on contact surfaces was found. At the same time, the physical meaning of the slippage phenomenon is not considered.

Hypothetically, the possibility of the pressed material slipping along the bottom of the screw channel was considered by Bostanjian and Stolin **[5]**. This hypothesis was confirmed by an experimental study of some modes of extrusion of grain components **[9]**, **[12]**, **[15]**.

It was previously shown **[4]**, **[12]**, that the "piston" motion of the material pressed in a cylindrical channel can be represented as a layered flow when the viscosity of the boundary layer of the material is less than the viscosity of the core of the flow. We apply this approach to determine the rate of material slipping along the bottom of the screw channel.

Ignoring the influence of the blades, let's imagine the screw channel with two parallel planes correlated with the Cartesian coordinate system, as shown in Figure 1.

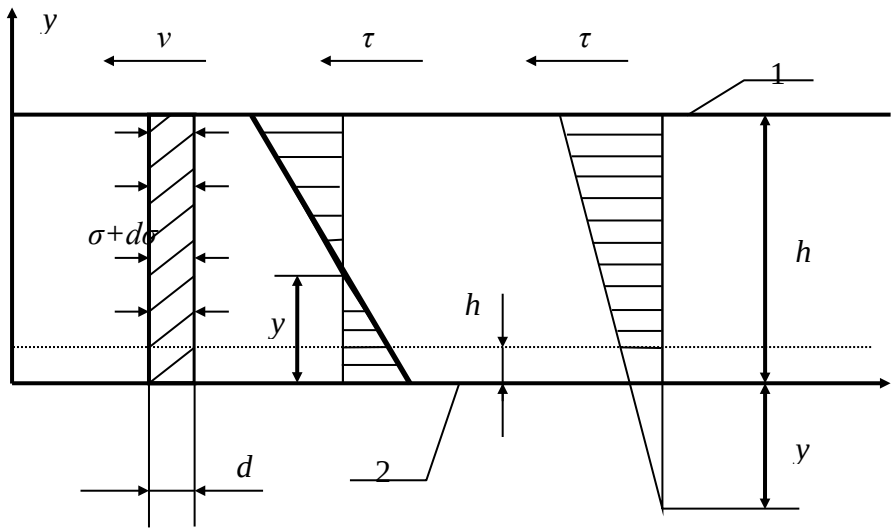

Figure 1 Diagram of the screw channel model. Note: 1 – plane replacing the bottom of the screw channel; 2 – plane replacing the screw cylinder.

The upper plate moves at a velocity v_c relative to the lower one. There is no material slippage on the upper plate, and tangential stress acts τ_c. Compression stresses modulo increase in the direction of velocity v_c.

The equilibrium equation for this case has the form (2) **[1]**, **[5]**:

$$\tau_{xy} = \frac{d\sigma}{dx}(y - y_0) \quad (2)$$

Where:

τ_{xy} – is the shear stress in the pressed material; $\frac{d\sigma}{dx}$ – the gradient of normal stresses in the pressed material; y_0- the coordinate of the plane on which the tangential stresses $\tau_{xy} = 0$.

Select a boundary layer with a thickness of h_n adjacent to the lower plate. A dotted line indicates the border of this layer (see Figure 1).

We assume the dependence of the shear stress τ_{xy}on the shear rate $\dot{\gamma}_x$ (velocity gradient $\frac{dv_x}{dy}$) in the boundary layer is satisfactorily described by the Oswald-de Ville equation **[1]**, **[11]**. Then equation (1) is transformed into the form (3):

$$\tau_{xy} = \mu'_n \dot{\gamma}_x^{n_n} = \mu'_n \left(\frac{dv_x}{dy}\right)^{n_n} \quad (3)$$

Where:
μ'_n – is the consistency coefficient of the pressed material in the boundary layer; n_n is the index of the flow of the pressed material in the boundary layer.

The Ostwald-de Ville equations (1) and (3) are valid outside the boundary layer. At the same time, its parameters do not have a subscript **[1]**, **[5]**.

Denote the velocity of the material in the region $y \geq y_0$ by v_{x1}, and in the region. $y \geq y_0$ by v_{x2}.

Consider the motion of the material in the boundary layer when the velocity derivative changes its sign in the flow region between the plates outside the slip layer, that is, when the condition $h_n < y_0 < h_w$ is met. For this case, equation (2), taking into account the dependence (3) in the region $0 < y_0 < h_n$, has the form (4) **[1]**, **[5]**, **11]**:

$$\frac{dx_{x1}}{dy} = a_{wn}(y_0 - y)^{m_n} \quad (4)$$

Where:

$$a_{wm} = \left(\frac{1}{\mu'_n}\right)^{m_n} \left|\frac{d\sigma}{dx}\right|^{m_n}, \quad m_n = \frac{1}{n_n}$$

We assume the initial condition $v_{x1} = 0$ at $y = 0$ and, integrating equation (4) within the boundary layer, we obtain equation (5) **[1]**, **[5]**:

$$v_{xn} = \frac{a_{wn}}{m_n+1}\left[y_0^{m_n+1} - (y_0 - h_n)^{m_n}\right] \quad (5)$$

For the case $y_0 < 0$, taking into account the direction of the tangential stress $\tau < 0$, equation (2) is transformed into equation (6) **[1]**, **[5]**:

$$\frac{dv_{x2}}{dy} = a_{wn}(y - y_0)^{m_n} \quad (6)$$

Integrating it under the same conditions as equation (4), we obtain (7) **[1]**, **[5]**:

$$v_{xn} = \frac{a_{wn}}{m_n+1}[(-y_0)^{m_n+1} - (h_n - y_0)^{m_n+1}] \quad (7)$$

Equations (5) and (6) allow us to determine the velocity of wall sliding in the boundary layer at a known thickness and rheological parameters of the pressed material.

It is possible to distribute tangential stresses in the pressed material at which $0 < y_0 < h_n$. For this case, the velocity of wall sliding is determined by solving the differential equation (3) under initial conditions $v_{x2} = 0$ at $y = 0$, and the differential equation (6) under initial conditions $v_{x2} = v_{xn}$ at $y = h_n$. Taking **[1]**, **[5]**:

$$v_{x1} = v_{x2} \text{ at } y = y_0 \quad (7)$$

We will get:

$$v_{xn} = \frac{a_{wn}}{m_n+1}\left[y_0^{m_n+1}(h_n - y_0)^{m_n+1}\right] \quad (8)$$

To illustrate the nature of the movement of the pressed material in the screw channel, velocity plots are constructed according to the previously obtained solution of equations (3) and (5) dependences **[1]**, **[5]**:

$$v_{x1} = v_{xn} + \frac{a_w}{m+1}[(y_0 - y)^{m+1} - y_0^{m+1}] \quad (9)$$

$$v_{x2} = v_c + \frac{a_w}{m+1}[(h_w - y_0)^{m+1} - (y - y_0)^{m+1}] \quad (10)$$

If $h_n < y_0 < h_w$, using the boundary condition $v_{x1} = v_{x2}$ at $y = y_0$ it is possible to determine from equations (9) and (10) the value y_0, given the velocity v_c of the upper plate (see Figure 1), or to determine the value necessary for this velocity distribution v_c, given the value y_0. If $y < h_n$, similar solutions can be obtained from equations (9) and (7) or (9) and (8) using the boundary $v_n = v_{x2}$ condition at $y = h_n$.

As a result of experimental studies conducted by **[13]**, **[14],** it was found that the assumption about the origin of the slip layer due to local heating of the material is not confirmed, since in this case there is no noticeable slippage along the bottom of the screw channel.

As a result, the authors of the experiment explain the occurrence of a boundary layer with rheological parameters different from the parameters of the main material in the screw channel, which consists of the distribution of the power of the layered flow in the material.

Thus, the equation of the specific power of the layered flow of the magnitude will take the following form **[7]**, **[9]**.

$$N_U = \tau_{xy}(v_{xi} - v_c), i = 1.2 \quad (11)$$

Where:

τ_{xy}– shear stress in compressed material.

Formula (11) considers that the pressed material's speed was considered higher in the reversed movement of the screw pressing mechanism. The velocity is determined by equations (9) and (10). Taking into account equation (2), formula (11) is transformed to the form (12): **[7]**, **[9]**:

$$N_U = \frac{d\sigma}{dx}(y - y_0)(v_{xi} - v_c) \quad (12)$$

Where:

$\frac{d\sigma}{dx}$ – the gradient of normal stresses in the compressed material; y_0 – the coordinate of the plane on which shear stresses take place.

$$\tau_{xy} = 0$$

The study of the casts of the pressed material extracted from the screw channel suggests that the thickness of the boundary layer can be neglected compared to the height of the screw channel h_n. Therefore, when determining the flow of material in the channel $h_ш$, the flow in the boundary layer can be neglected **[1]**, **[19]**.

The above theoretical prerequisites formed the basis for calculating and optimizing the main technological indicators of the process of extrusion of bulk components for the production of products with a high degree of readiness **[16]**, **[17]**, **[18]**.

Following the planning of the multifactorial experiment, Table 1 was compiled. A list of variable factors was determined, and their variation intervals and levels were established. The degree of influence of the values of the selected factors on the criteria for optimizing the process of extrusion of a loose poly-cereal mixture is calculated. The criterion for optimizing the extrusion process is the energy value of «Fitness» and «Health» of high-readiness products **[16]**, **[17]**, **[22]**, **[23]**.

Table 1 Experimental values of variable factors and optimization criteria according to the planning matrix.

Natural values		**Experimental values of technological parameters and criteria for optimizing the extrusion process**					
						E_v, **kcal**	
***W*, %**	***n*, rpm**	***P*, MPa**	***t*, °C**	***Q*, kg/h**	***N*, *kW***	**Poly-cereal product «Fitness»**	**Poly-cereal product «Health»**
1	2	3	4	5	6	7	8
3.5	120	18.25	152.7	210	44	314.52	316.68
3.5	210	21.05	212.6	370	76.5	322.38	324.54
16.5	120	14.45	151.9	205	41.0	314.40	316.70
16.5	210	20.95	211.6	365	73.0	321.20	323.50
12.0	170	17.88	183.3	295	64.5	316.01	318.30
18.0	170	17.75	181.9	280	56.0	315.45	320.60
15	80	12.0	130.0	144	28.8	313.85	315.60
15	250	25.0	250.0	450	90.0	332.34	334.09
15	170	18.48	189.4	306	61.2	317.50	319.25

High-readiness poly-cereal products «Fitness» and «Health» are shown in Figure 2. They differ only in the composition of the poly-cereal mixture from which they are made **[16]**, **[17]**.

a *b*

Figure 2 High-readiness poly-cereal products *a)* «Fitness» and *b)* «Health».

The results of experimental studies were entered into a table of a *Microsoft Excel* word processor, and then, based on the data obtained, three-dimensional graphs of the dependence of the pressure created in the pre-matrix zone (*P*, MPa), the temperature of the finished product at the exit from the working zone (*t*, °C), the productivity of the extruder (*Q*, kg/h), the power consumption of the electric drive during the extrusion process (*N*, kW h), the energy value of high-readiness products «Fitness» and «Health» (E_v, kcal) on variable values of the rotation speed of the extruder screw *n*, (min^{-1}) and humidity of the extruded poly-cereal mixture, *W* (%) **[17]**, **[20]**.

Analysis of the experimental data presented in Table 1 showed that an increase in the rotation speed of the working organ n from 80 to 250 min^{-1} leads to an increase in the pressure values in the pre-matrix zone. At the same time, the humidity of the processed flour poly-cereal mixture reduces during the extrusion process.

In addition, an increase in the rotation speed of the working body (screw) *n* from 80 to 250 min^{-1} leads to an increase in the temperature of the extrudate at the outlet of the working area of the device (*t*, °C). And the humidity of the extruded flour multicomponent mixture changes the temperature values during the extrusion process.

It was also observed that an increase in humidity up to 15% led to an increase in t values up to 130 °C. A further increase in humidity to 18% reduced the temperature of the extrudate at the exit from the working area of the device.

It has been experimentally established that an increase in the rotation speed of the working body (screw) *n* from 80 to 250 min^{-1} increases the productivity of a twin-screw extruder (*Q*, kg /hour). At the same time, the humidity of the processed flour poly-cereal mixture changes the *Q* values during the extrusion process.

And also, an increase in the rotation speed of the working body (screw) n from 80 to 250 min^{-1} leads to an increase in the power consumption of the electric drive of the extruder (*N*, kW/hour). At the same time, the humidity of the processed flour poly-cereal mixture reduces the *N* values during the extrusion process.

Experimental data indicate that an increase in the rotation frequency of the working organ (screw) *n* from 80 to 250 min^{-1} leads to the most significant increase in the energy value of a poly-cereal food product of a high degree of readiness (E_v, kcal), respectively, for «Fitness» 332.34 kcal and for «Health» - 334.09 kcal. At the same time, the humidity of the processed flour poly-cereal mixture slightly changed the E_v values during the extrusion process **[16]**, **[17]**.

Thus, the analysis of the experimental data is presented in Table. 1 showed that an increase in the speed of rotation of the working body n from 80 to 250 min^{-1} leads to an increase in the pressure values in the pre-matrix zone. This also increases the temperature of the extrudate at the exit from the working area of the device; the productivity of a twin screw extruder, and the power consumption of the extruder electric drive. An increase in the moisture content W of the polycereal flour mixture from 15% to 18% reduces the pressure values in the pre-matrix zone; extrudate temperature during extrusion; productivity, and power of the electric drive of a twin-screw extruder.

An analysis of the experimental data also indicates that an increase in the speed of rotation of the working body (screw) n from 80 to 250 min^{-1} leads to the largest increase in the energy value of a highly prepared poly-cereal food product, respectively, for "Fitness" 332.34 kcal and for "Health" - 334.09 kcal. At the same time, the influence of moisture content W of the processed poly-cereal flour mixture on the energy value of the finished product is insignificant.

An analysis of the obtained three-dimensional surfaces of extruded products showed that the performance characteristics of the extruder at all values of the screw speed are the same, i.e. the extruder first increases and then decreases from a certain value of *Q*. It is obvious that in the completely closed exit mode at $Q = 0$, the pressure in pre-matrix zone continuously increases, and in the open output mode $Q = Q_{max}$ – continuously decreases. In a real extrusion process, with increasing productivity, the pressure of the product reaches a certain value, the maximum possible for the given operating conditions of the extruder, and then steadily decreases **[16]**, **[21]**, **[24]**.

As a result of the analysis, the influence of the factors taken into account on the temperature and pressure of the food medium has been experimentally confirmed: the design parameters of the extruder (the diameter of the through a section of the matrix), as well as the initial humidity of the mixture, have the greatest influence on the melt pressure of the product; the geometric characteristics of the working body, the frequency (speed) of the screw rotation and the pressure of the product maximally affect the temperature in the pre-matrix zone of the extruder. They allow us to find out the dominant value of each studied factor (*W, n*) on kinetic parameters and describe with a sufficient approximation the kinetics of the extrusion process of flour poly-cereal mixture in the production of high-readiness products «Fitness» and «Health».

CONCLUSION

The developed theoretical premise made it possible to calculate and optimize the main technological parameters of the extrusion process of bulk components for the production of high-readiness products «Fitness» and «Health». Experimental data indicate that an increase in the rotation frequency of the working organ (screw) *n* from 80 to 250 min^{-1} leads to the greatest increase in the energy value of a poly-cereal food product of a high degree of readiness (E_v, kcal), respectively, for Fitness product 332.34 kcal and for Health product - 334.09 kcal. The influence of the factors taken into account on the temperature and pressure of the food medium has been experimentally confirmed: the design parameters of the extruder (the diameter of the cross-section of the matrix), as well as the initial humidity of the mixture, have the greatest influence on the melt pressure of the product; the geometric characteristics of the working body, the frequency (speed) of the screw rotation and the pressure of the product maximally affect the temperature in the pre-matrix zone of the extruder. They allow us to find out the dominant value of each studied factor (*W, n*) on kinetic parameters and describe with a sufficient approximation the kinetics of the extrusion process of flour poly-cereal mixture in the production of high-readiness products Fitness and Health.

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A fish market survey using a novel PCR-sequencing-based protocols for the identification of commercial significant fish species

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ABSTRACT

This study developed a simple, specific, and affordable PCR-sequencing-*COI* gene-based protocol for the simultaneous identification of some important commercial fish species: *Merluccius merluccius*, *Lates niloticus*, *Gadus morhua*, *Ruvettus pretiosus*, *Pangasianodon hypophthalmus*, *Epinephelus* spp. For this study, a local market survey on fish was carried out to evaluate the application of labelling laws and to detect fraudulent actions using the developed PCR protocols. Ten specimens of each fish species of interest were obtained from wholesale fishery plants and were utilized for the protocol development. DNA was extracted from the individual samples and quantified. DNA isolates were subjected to end-point PCR and the PCR products were sequenced. For the identification of fish species, novel species-specific primers were developed by the program "Primer Express 3.0" and by the software "Primer-BLAST" to amplify fragments of 200 bp, 250 bp, 300 and 562 bp, 350 bp, 400 bp and 522 bp within the *COI* gene for *M. merluccius*, *L. niloticus*, *G. morhua*, *R. pretiosus*, *P. hypophthalmus*, *Epinephelus* spp., respectively. Single PCR was performed using DNA isolates and developed primers for each fish species of interest. After sequencing, the isolates were compared with the selected sequences of the COI gene and showed a similarity ranging from 99 to 100%. Among 43 samples obtained for the survey, 19 (44.2%) were mislabelled, with 18 (41.9%) mislabelled samples from local fisheries and fish marketplaces and 1 (2.32%) from hypermarket stores. Among fish samples purchased at local fisheries and fish marketplaces, fraudulent actions were observed more frequently in fish slices (100%) than fish fillets (65%). Regarding fish fillets, out of four samples labelled as grouper, three were *L. niloticus* and one *P. hypophthalmus*. Two fillets marketed as cod were substituted with *L. niloticus*. Five samples labelled as "fillet" and two samples labelled as "perch" were identified as *P. hypophthalmus*. Regarding fish slices, all samples marketed as grouper (*E. marginatus*) were slices of *R. pretiosus*. The single case of mislabelling detected from fishery products purchased at hypermarket stores was a sample of "Spinycheek grouper" (*Epinephelus diacanthus*) that was indicated on label as "Grouper" (*Epinephelus marginatus*). In summary, our work highlights the need for continuous surveillance of the commercialization of fishery products, to reduce the number of fraud cases that happen in the market. Furthermore, our protocols based on PCR techniques could be useful for quality control of fresh finfish and to strengthen controls on the most frequent fraudulent actions of marketed fishery products.

Keywords: fishery products, fish frauds, Multiplex PCR, *COI* gene

INTRODUCTION

The seafood consumption has increased several folds during the last 50 years, including wild and aquaculture products **[1]**. The species substitution in which low value fish is replaced with high value ones is a prominent phenomenon in the international seafood trade and is a leading cause of fraud in the fishery sector, leading to economic and health concerns. Fishery products present a valid alternative to other types of animal-origin food (terrestrial animal meat, eggs, dairy products, etc.) especially for their high digestibility due to the lower presence of connective tissues and lipid components **[2]**, **[3]**, **[4]**. Despite its increasing popularity, seafood is one of the prominent products associated with food frauds. Authentication studies and market monitoring of commercial

products show that fish products are more vulnerable to mislabelling than other consumer goods **[5]**, **[6]**. This phenomenon regards both the acquisition of fishery products traditionally and new products, for example fillets, slices, fish burger, "ready to cook" breaded products, or „ready to eat products". In this situation, fish are not easily identifiable from a phenotypic perspective with the increase in commercial and sanitary frauds. Victims of such frauds can be both consumers and the fishery industry. In fact, cases of seafood fraud are reported in almost all countries although the rate can vary. Mislabelling was detected in 50% fish products in Germany **[6-8]**, 22% seafood products in India **[9]**, 24% in South Brazil **[10]**, **[11]** and almost 80% commercial fish fillets in Italy **[11]**, **[12]**, **[13]**, **[14]** and several other countries. The EU enforced the Regulation (EU) 2013/1379 that lay down to the fish economic operators to report on the label of the fish products some information such as the commercial and scientific designation name, the production method (catch or breeding), its origin (the FAO fishing area for sea products and the name of the Country for breeding products) and the fishing gear **[15]**. Similar regulations are in force in many countries; however, despite these regulations, widespread seafood mislabelling has been identified in the United States and Canada **[16]**, **[17]**, in Europe **[14]**, in Asia **[18]** and south Africa **[19]** indicating the need for stringent control measures to generate efficient species identification **[20]**. The identification of species represents a key aspect both for food control and food safety and it is an important tool to ascertain frauds. DNA-based identification methods present several advantages over protein analysis, including increased specificity, sensitivity, and reliable performance with processed samples. In fact, DNA molecules are more resistant and thermo-stable than proteins. For the simultaneous amplification of many targets of interest, Multiplex-PCR is often performed using more than one pair of primers in one reaction **[21]**. Multiplex-PCR can produce considerable savings of time and effort within the laboratory **[22]**, **[23]**.

This study developed a set of original primers for the molecular identification of valuable fish species, using the PCR. To test the suitability of the developed protocols, a local market survey was done. The final objective of the study was to provide multiplex-PCR-based protocols suitable for the quality and safety assessment of some valuable fishery products.

Scientific Hypothesis

Designing set of primers for the molecular identification of valuable fish species and its validation by PCR and authenticate various fish species sold in the market.

MATERIAL AND METHODOLOGY

Samples

Ten specimens of each fish species of interest (*Merluccius merluccius*, *Lates niloticus*, *Gadus morhua, Ruvettus pretiosus*, *Pangasianodon hypophthalmus*, *Epinephelus* spp.) were obtained from wholesale fishery plants and were utilized for the protocol development. Forty-three fishery products were purchased in some cities located in Apulia Region (Southern Italy). Among these, 18 samples (42%) were obtained at four hypermarket stores and 25 samples (58%) at five fisheries and at six local markets. All samples were stored at -20 °C until analysed.

Chemicals

All chemicals were purchased by IZSPB were of analytical grade.

Animals and Biological Material

Samples purchased at hypermarket stores consisted of 6 fish skewers containing Nile perch (labelled as *Lates niloticus*), 2 breaded hake fillets (labelled as *Merluccius merluccius*), 2 fish burgers (labelled as *Gadus morhua*), 1 cod fillet (labelled as *G. morhua*), 1 breaded Nile perch fillet (labelled as *L. niloticus*), 3 Nile perch fillets (labelled *L. niloticus*), 2 salted cod fishes (labelled as *G. morhua*) and 1 grouper fillet (labelled as *Epinephelus marginatus*).

Instruments

The PCR machine, electrophoresis apparatus, weighing balance, microcentrifuge, laminar air flow, were used in this research.

Laboratory Methods

DNA extraction, PCR, gel electrophoresis and sequencing were used.

Description of the Experiment

Sample preparation: The fish samples were collected from different stores. 50-100 g samples were cut and stored at -20 °C until analysed. 20-25 mg tissue were taken from stored sample for DNA extraction and PCR analysis.

Number of samples analyzed: 43

Number of repeated analyses: two repetitions.

Number of experiment replication: two repetitions.

Design of the experiment: To develop novel protocols based on the PCR for the genetic identification of some significant commercial fish species, we created specific primers for the identification of the following: *Merluccius merluccius, Lates niloticus, Gadus morhua, Ruvettus pretiosus, Pangasianodon hypophthalmus* and *Epinephelus* spp. Mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene was selected to identify fish species. This genetic fragment presents very low intraspecific variability, thus permitting the unequivocal identification of fish species. Then, we applied the developed protocols to a local survey and ascertained fishery products' correct labelling at local retail outlets.

Primer design: *Merluccius merluccius, Lates niloticus, Gadus morhua, Ruvettus pretiosus, Pangasianodon hypophthalmus* and *Epinephelus* spp. were the six fish species of interest subjected to the study. Mitochondrial cytochrome oxidase subunit 1 (*COI*) gene was used to identify the above fish species. For each fish species, *COI* sequences were obtained from the GenBank database and aligned and compared by the program BioEdit. The primers were developed via two methods. Firstly, species-specific primers to amplify fragments of 200 bp, 250 bp, 300 bp, 350 bp and 400 bp within the COI gene for *M. merluccius, L. niloticus, G. morhua, R. pretiosus, P. hypophthalmus*, respectively, were designed by the program "Primer Express 3.0". The program "Primer Express 3.0" was set according to the parameters reported in Table 1. Secondly, *COI*, FASTA sequences for *Epinephelus* spp. and *G. morhua* were inserted in the software "Primer – BLAST" to develop primers to amplify fragments of 522 bp and 562 bp, respectively. The software "Primer – BLAST" was set to create primers according to the parameters reported in Table 2.

Table 1 Parameters inserted in the software "Primer Express 3.0" in order to obtain a pair of primers for the identification of *M. merluccius, L. niloticus, G. morhua, R. pretiosus, P. hypophthalmus*.

Parameter	Value
Primer Tm	
Min Primer Tm	58
Max Primer Tm	60
Max difference in Tm of two primers	2
Primer GC Content	
Min Primer % GC Content	30
Max Primer % GC Content	80
Max Primer 3' GC's	2
Primer 3' End Length	5
Primer 3' GC Clamp Residues	0
Primer Length	
Min Primer Length	9
Max Primer Length	40
Optimal Primer Length	20
Primer Composition	
Max Primer G Repeats	3
Max Num Ambig Residues in Primer	0
Primer Secondary Structure	
Max Primer Consec Base Pair	4
Max Primer Total Base Pair	8
Primer Site Uniqueness	
Max % Match in Primer	75
Max Consec Match in Primer	9
Max 3' Consec Match in Primer	7
Amplicon	
Min Amplified Region Tm	0
Max Amplified Region Tm	85
Min Amplified Region Length	200 (variable)
Max Amplified Region Length	400 (variable)
Penalty	close to zero

Table 2 Parameters inserted in the software "Primer – BLAST" to obtain a pair of primers for the identification of *Epinephelus* spp. and *Gadus morhua*.

Parameter	Value
PCR product lenght	
Min product lenght	500
Max product lenght	600
Primer melting temperatures (T_m)	
Min Primer Tm	57
Optimum Primer Tm	58
Max Primer Tm	59
Max Tm difference	1

Sample collection and DNA Extraction: Forty-three fishery products were purchased in some cities located in Apulia Region (Southern Italy). Among these, 18 samples (42%) were obtained at four hypermarket stores and 25 samples (58%) at five fisheries and at six local market. Samples purchased at hypermarket stores consisted of 6 fish skewers containing Nile perch (labelled as *L. niloticus*), 2 breaded hake fillets (labelled as *M. merluccius*), 2 fish burgers (labelled as *G. morhua*), 1 cod fillet (labelled as *G. morhua*), 1 breaded Nile perch fillet (labelled as *L. niloticus*), 3 Nile perch fillets (labelled *L. niloticus*), 2 salted cod fishes (labelled as *G. morhua*) and 1 grouper fillet (labelled as *Epinephelus marginatus*). Samples purchased at fisheries and fish marketplaces consisted of 20 fish fillets and 5 fish slices. Regarding fish fillets, four were labelled as grouper, two as cod, three as Nile perch, four as striped catfish, five reported as "fillet" from local fisheries and fish marketplaces and two as "perch" (both without the indication of fish species). All fish slices were labelled as grouper (Table 7). After collection samples were subjected to DNA extraction with NucleoSpin Tissue Kit (Macherey-Nagel). All DNA samples were quantified (about 20 ng/μL) by Nanodrop (Thermo Scientific) and subjected to PCR with original species-specific primers developed for the identification of fish species. Primers were commercially synthesized by Sigma Aldrich (Milan, Italy). Primers were diluted to a final concentration of 100 nM. PCR primers for each fish species of interest were created. Both methods developed two pairs of primers for G. morhua (Table 3).

Table 3 Original species-specific primers developed for the identification of fish species.

Method	Fish species	Primers sequences	Length (bp)	Product size (bp)
Primer Express 3.0	*Merluccius merluccius*	FWD 5'- ATAATTGGAGGCTTCGGAAACTG -3' RVS 5'- CCAGCGTGGGCAAGATTACT -3'	23 20	200
Primer Express 3.0	*Lates niloticus*	FWD 5'- GGAGCTGGAACCGGTTGAA -3' RVS 5'- CAGCTAAGACTGGGAGGGAAAG -3'	19 22	250
Primer Express 3.0	*Gadus morhua*	FWD 5'- GGTGCACTTCTTGGTGATGATC -3' RVS 5'- ATCAACAGATGCCCCAGCAT -3'	22 20	300
Primer Express 3.0	*Ruvettus pretiosus*	FWD 5'- CGGCACATGCCTTCGTAATAA -3' RVS 5'- GGCTGCGGGTTTCATATTAATAA -3'	21 23	350
Primer Express 3.0	*Pangasiodon hypophthalmus*	FWD 5'- CCTTCTAGGCGACGACCAAA -3' RVS 5'- ATATTGTGAAATTGCTGGTGGTTTT -3'	20 25	400
Primer – BLAST	*Epinephelus* spp.	FWD 5'- TCTTGTATTTGGTGCCTGGG -3' RVS 5'- ACTGCTGTAATTAGGACGGC -3'	20 20	522
Primer – BLAST	*Gadus morhua*	FWD 5'- TCTCGTATTTGGTGCCTGAG -3' RVS 5'- GATACCAGCTGCTAAGACGG -3'	20 20	562

Polymerase chain reaction (PCR): All samples were subjected to end-point PCR in a Thermal Cycler Eppendorf. The PCR mixture (total volume 25 μL) contained 1X PCR buffer containing 1.5 mM $MgCl_2$ (20 nm Tris-HCl pH 8.4, 50 mm KCl), 0.2 mM dNTPs, 0.5 μM of each primer, 2 U of Hot Start II DNA Polymerase (Thermo Scientific) and approximately 5 ng of DNA (Table 4). PCR conditions were 98 °C for 30 s, 34 cycles of 98 °C for 5 s, 58 °C for 30 s, and 72 °C for 15 s, with a final extension at 72 °C for 1 min (Table 5).

Table 4 PCR Master Mix for the identification of fish species of interest.

Reaction Component	Final Concentration	Amount for each Reaction
Water	-----	16.85 μL
PCR Buffer	1X	5 μL
dNTP's	0.2 mM	0.5 μL
Primer Forward	0.5 μM	0.5 μL
Primer Reverse	0.5 μM	0.5 μL
Taq DNA Polymerase	2 U	0.15 μL
DNA	-----	1.5 μL
		Final Volume: 25 μL

Table 5 PCR Amplification Program performed in a Thermal Cycler Eppendorf.

Step	Temperature (°C)	Time	Number of Cycle
Initial Denaturation	98	30 sec	1
Denaturation	98	5 sec	29
Annealing	58	30 sec	
Extension	72	15 sec	
Final Extension	72	1 min	1

The PCR amplicons were analysed by agarose gel electrophoresis by using a horizontal 2% (wt/vol) agarose gel in lX TBE buffer (pH 8.3; 0.09 M Tris, 0.09 M boric acid, 2.0 mM EDTA) and with 0.003% (wt/vol) ethidium bromide for DNA staining. PCR products were mixed with a sample buffer of 1X TBE and then applied to each well. Gel ran in 1X TBE buffer at 200 V for 30 min. The DNA marker used was Amplisize molecular ruler, 50% GC content, 50-2000 bp, 10 bands (Bio Rad, Hercules, Spain). The PCR products were visualized and photographed by a Gel Doc XR+ System transilluminator (Bio Rad, Milan, Italy).

Sequencing: PCR products were purified using Montage PCR filter units (Millipore, Milan, Italy) and sequenced by BigDye 3.1 Ready reaction mix (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions (Tables 4 and 5). Sequences were imported and assembled with the BioNumerics 7.5 software (Applied Maths, Saint-Martens-Latem, Belgium) and searched for homologous sequences by BLAST search analysis (http://www.ncbi.nlm.nih.gov).

Multiplex-PCRs: Primers were developed to obtain amplicons with different lengths (at least 50 base pairs). Duplex and Triplex PCR protocols were developed to simultaneously analyse more fish species using the designed primers with several combinations (Table 6).

Table 6 Combinations of Duplex and Triplex PCR protocols by using the developed primers.

Multiplex-PCR	Fish Species	Amplicon Length
Duplex-PCR	*Lates niloticus*	250 bp
	Epinephelus spp.	522 bp
Duplex-PCR	*Lates niloticus*	250 bp
	Gadus morhua	300 bp
Duplex-PCR	*Merluccius merluccius*	200 bp
	Gadus morhua	562 bp
Duplex-PCR	*Ruvettus pretiosus*	350 bp
	Gadus morhua	562 bp
Duplex-PCR	*Ruvettus pretiosus*	350 bp
	Epinephelus spp.	522 bp
Duplex-PCR	*Pangasianodon hypophthalmus*	400 bp
	Epinephelus spp.	522 bp
Duplex-PCR	*Pangasianodon hypophthalmus*	400 bp
	Gadus morhua	562 bp
Triplex-PCR	*Merluccius Merluccius*	200 bp
	Lates niloticus	250 bp
	Pangasianodon hypophthalmus	400 bp

Table 6 Cont.

Multiplex-PCR	Fish Species	Amplicon Length
	Merluccius merluccius	200 bp
Triplex-PCR	*Ruvettus pretiosus*	350 bp
	Epinephelus spp.	522 bp
	Lates niloticus	250 bp
Triplex-PCR	*Pangasianodon hypophthalmus*	400 bp
	Gadus morhua	562 bp
	Lates niloticus	250 bp
Triplex-PCR	*Ruvettus pretiosus*	350 bp
	Epinephelus spp.	522 bp

Specificity tests: Single PCRs were performed using the designated primers for each fish species of interest with the DNA extracted from the non-target fish species (negative controls).

Statistical Analysis

Statistical Analysis is not required for this study.

RESULTS AND DISCUSSION

Specificity of the developed protocols

PCR assay allowed the detection of DNA extracted from all specimens of each fish species of interest, giving fragments of the expected length. At the end of the running, the electrophoresis agarose gel showed a clear separation of amplicons due to their different sizes (Figures 1, 2, 3, 4 and 5). Single PCRs performed for the specificity tests gave the expected results. After sequencing, the isolates were compared with the selected sequences of *COI* gene and showed a similarity ranging from 99 to 100%. Grouper samples subjected to Epinephelus spp. authentication, showed 97.5% homology to Epinephelus costae GenBank entry (KM077928.1) and 100% homology to Epinephelus marginatus GenBank entry (KC500692.1). The results of the experiment are shown in Table 1 and Figure 1.

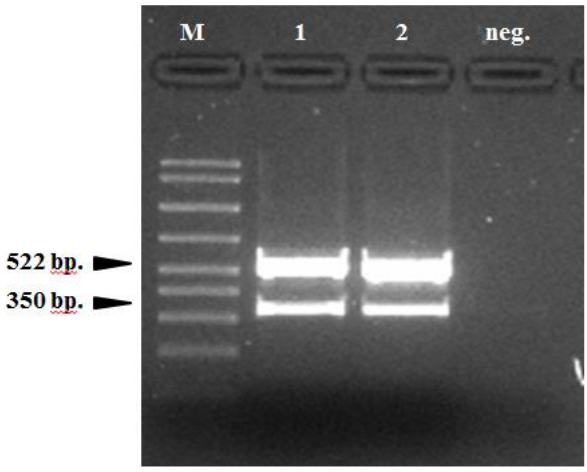

Figure 1 Gel electrophoresis of PCR products obtained from Duplex PCR assays (lanes 1 and 2) for identification of *Ruvettus pretiosus* (350 bp) and *Epinephelus* spp. (522 bp.). Lane M: AmpliSize™ Molecular Ruler (50–2000-bp ladder; Bio-Rad). Lane (neg.): negative control.

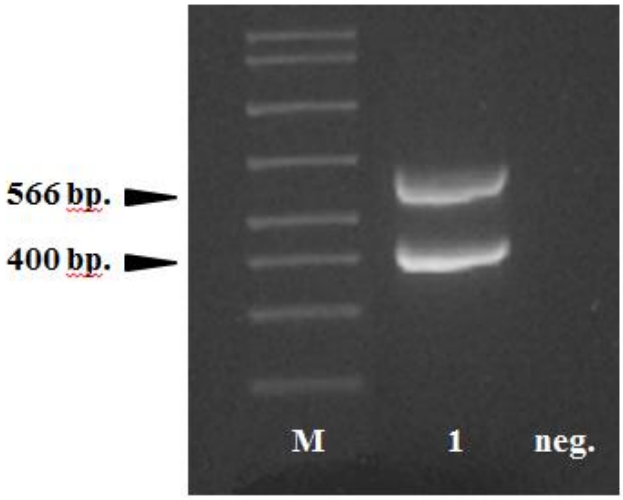

Figure 2 Gel electrophoresis of PCR products obtained from Duplex PCR assays (lane 1) for identification of *Pangasianodon hypophthalmus* (400 bp.) and *Gadus morhua* (566 bp.). Lane M: AmpliSize™ Molecular Ruler (50–2000-bp ladder; Bio-Rad). Lane neg.: negative control.

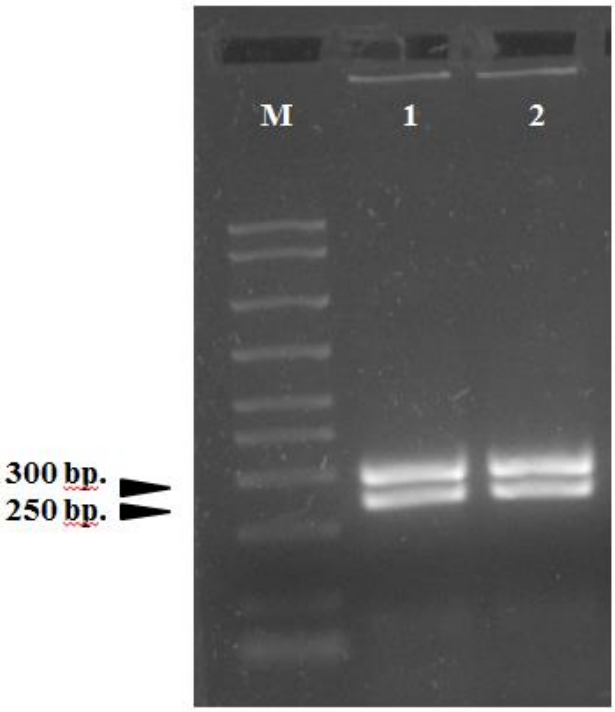

Figure 3 Gel electrophoresis of PCR products obtained from Duplex PCR assays (lanes 1 and 2) for identification of *Lates niloticus* (250 bp.) and *Gadus morhua* (300 bp.). Lane M: AmpliSize™ Molecular Ruler (50–2000-bp ladder; Bio-Rad).

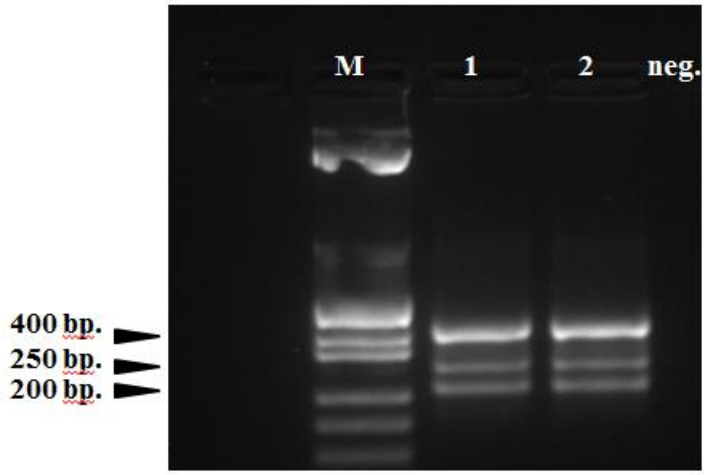

Figure 4 Gel electrophoresis of PCR products obtained from Triplex PCR assays (lanes 1 and 2)for identification of *Merluccius merluccius* (200 bp.), *Lates niloticus* (250 bp.) and *Pangasianodon hypophthalmus* (400 bp.). Lane M: AmpliSize™ Molecular Ruler (50–2000-bp ladder; Bio-Rad). Lane neg.: negative control.

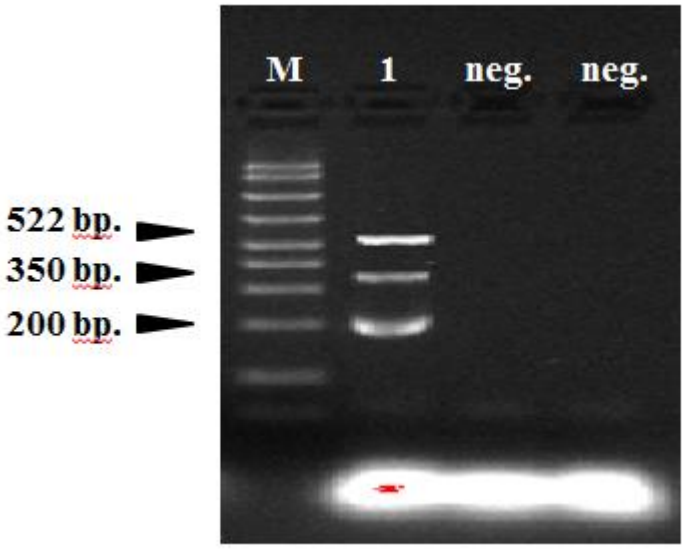

Figure 5 Gel electrophoresis of PCR products obtained from Triplex PCR assays (lane 1) for identification of *Merluccius merluccius* (200 bp.), *Ruvettus pretiosus* (350 bp.) and *Epinephelus* spp. (522 bp.). Lane M: AmpliSize™ Molecular Ruler (50–2000-bp ladder; Bio-Rad). Lane neg.: negative controls.

Identification of the samples used for the survey

Overall, out of 43 fish samples analysed, 19 (44.2%) resulted mislabelled, with 18 (41.9%) mislabelled samples from local fisheries and marketplaces and 1 (2.32%) from hypermarket stores (Table 7). As Regarding fish samples purchased at hypermarket stores, all cod samples tested positive for *G. morhua* showing an amplicon of 562 bp; all Nile perch samples tested positive for *L. niloticus* showing an amplicon of 250 bp; all hake samples tested positive for *M. merluccius* showing an amplicon of 200 bp; the grouper sample tested positive for *Epinephelus* spp. showing an amplicon of 522 bp. To ascertain the existence of false positives, identifications were confirmed by sequencing. After sequencing, *Epinephelus* spp. isolates showed 100% homology to *Epinephelus diacanthus* GenBank entry (EF609520.1). Out of 25 fish samples purchased at fisheries and fish marketplaces, 18 (72%) were mislabelled. Cases of mislabelling regarded more fish slices (100%) than fillets (65%). Regarding fish fillets, three Nile perch fillets (15%) and four striped catfish fillets (20%) were correctly labelled. The DNA analysis on the remaining fillets showed that thirteen samples were mislabelled (65%). All

samples marketed as grouper fillets showed fraudulent actions. In fact, out of four samples labelled as grouper, three (75%) tested positive for L. niloticus showing an amplicon of 250 bp., and one (25%) positive for *P. hypophthalmus* showing an amplicon of 400 bp. Both cod fillets (100%) resulted to be *L. niloticus*, showing an amplicon of 250 bp. The 5 samples from local fisheries and fish marketplaces labelled as "fillet" and the 2 samples labelled as "perch" were identified as *P. hypophthalmus* showing an amplicon of 400 bp (Table7). As regards grouper slices, all samples (100%) showed fraudulent species substitutions; in fact, *R. pretiosus* was marketed as grouper (amplicon of 350 bp.).

Table 7 Results of the survey on the application of the labelling laws and for the detection of fraudulent actions.

Retail outlet	Fishery products	N.	Species labelled	Species identified by PCR	Result
Hypermarket stores	Fish skewer	6	Nile perch (*Lates niloticus*)	*Lates niloticus*	Correctly labelled
Hypermarket stores	Fillet	2	Hake (*Merluccius merluccius*)	*Merluccius merluccius*	Correctly labelled
Hypermarket stores	Fish burger	2	Cod (*Gadus morhua*)	*Gadus morhua*	Correctly labelled
Hypermarket stores	Fillet	1	Cod *(Gadus morhua)*	*Gadus morhua*	Correctly labelled
Hypermarket stores	Fillet	1	Nile perch (*Lates niloticus*)	*Lates niloticus*	Correctly labelled
Hypermarket stores	Fillet	3	Nile perch (*Lates niloticus*)	*Lates niloticus*	Correctly labelled
Hypermarket stores	Salted fish	2	Cod (*Gadus morhua*)	*Gadus morhua*	Correctly labelled
Hypermarket stores	Fillet	1	Grouper (*Epinephelus marginatus*)	*Epinephelus diacanthus*	**Mislabelled**
Local fisheries and fish marketplaces	Fillet	4	Grouper	*Lates niloticus (75%)* *Pangasius hypophthalmus (25%)*	**Mislabelled**
Local fisheries and fish marketplaces	Fillet	2	Cod	*Lates niloticus*	**Mislabelled**
Local fisheries and fish marketplaces	Fillet	3	Nile perch	*Lates niloticus*	Correctly labelled
Local fisheries and fish marketplaces	Fillet	4	Striped catfish	*Pangasius hypophthalmus*	Correctly labelled
Local fisheries and fish marketplaces	Fillet	5	Reported as "fillet"	*Pangasius hypophthalmus*	**Mislabelled**
Local fisheries and fish marketplaces	Fillet	2	Perch	*Pangasius hypophthalmus*	**Mislabelled**
Local fisheries and fish marketplaces	Fish slices	5	Grouper	*Ruvettus pretiosus*	**Mislabelled**

Recently, several studies have demonstrated the vulnerability of the fish supply chain to fish fraud, particularly species substitution and mislabelling **[24]**. An investigation was done by INTERPOL_EUROPOL which demonstrated fish fraud as 3rd highest risk category of food vulnerable to fraud **[25]**, **[26]**. In the fish sector, the identification of fish species throughout the production chain is of main importance, even if fishery products have already been processed. In fact, there are different ways to purchase fish and fishery products: whole, fillets, slices, skewers or mixed with other species for gastronomic dishes (seafood salad, risotto mix, fish fingers, etc.). Furthermore, the presence of similar fish species, but very different from a nutritional and organoleptic perspective, is more frequent. Currently, commercial fishery products in Europe come from all parts of the world, meaning that accurate species identification is not always easy. In this situation, both sanitary and quality control and product traceability could be obstructed because fish are not easily identifiable, with the increase in commercial (*aliud pro alio*) and sanitary frauds (commercialisation of toxic organisms). Further, food poisoning due to the consumption of toxic fishery products belonging to *Tetradontidae*, *Molidae*, *Diodontidae* and *Canthigasteridae* families may occur **[27]**, although their marketing is forbidden by European Regulations (EC Reg. 853/2004). For example, oilfish (*Ruvettus pretiosus*) is seldom marketed in conformity with the current EU Regulation (EC Reg. 1021/08) and it is often commercialized in place of the most popular, expensive, and precious species, such as grouper (*Epinephelus* spp.). The problem of fraudulent actions in the commercialization of foods is strongly felt at the European Union level; in fact, recently a recommendation was enacted in the need to establish a "coordinated plan of supervision designed to determine the prevalence of fraudulent practices in the marketing of certain foodstuffs", including fishery products (EU Recommendation n. 1558 – 12 March 2015).

EU enforced the Regulation (EU) 1379/2013 that lay down to the fish economic operators to report on the label of the fish products some information such as the commercial and scientific designation name, the production method (catch or breeding), its origin (the FAO fishing area for sea products and the name of the Country for breeding products) and the fishing gear **[28]**. The objective of this regularity policy was to generate safe supply for consumers and the food processing industry as well as to give consumers a more detailed information about food products to protect fraud, prevent illegal fishing and promote sustainable aquaculture. Similar regulations are in force in many countries; however, despite these regulations, widespread seafood mislabelling has been identified in the United States and Canada **[16]**, **[17]**, In Europe **[14]**, Asia **[9]** and south Africa **[19]** indicating the need for stringent control measures to generate efficient species identification **[20]**. In fact, in 2016, Oceana published a major report by reviewing more than 200 published studies across 55 countries and found 20% mislabelling in catering and related sectors **[20]**. In 2021, Oceana Canada observed 46% mislabelling in seafood products, which is just 1% less compared to a study conducted during 2017-2019 **[29]**. In 2021, a Guardian Seascape analysis of 44 recent surveys of more than 9,000 seafood samples from restaurants, fishmongers, and supermarkets in more than 30 countries conducted and found that 36% samples were mislabelled, exposing a large amount of seafood fraud at global scale **[30]**. All studies conducted indicate that species substitution and mislabelling are serious problems in international fish trade. Other studies conducted in countries like Italy **[31]**, **[32]** Germany **[6]**, **[7]**, India **[9]**, South Brazil **[10]**, **[11]** show the concerns related to fish fraud **[33]**, **[34]**, **[35]**.

The development of PCR protocols has allowed a rapid and specific response for identifying fish species. In fact, the time required from the arrival of the fish sample to the end of the analysis was about 6-8 h. Thanks to the development of Duplex and Triplex PCR protocols, additional information may be gained from a single test run with considerable saving of time, reagents, and efforts within the laboratory. Furthermore, the applicability of the assay to commercial fishery products has been demonstrated. In fact, in our survey, of the 43 investigated samples, we detected 19 (44.2%) mislabelled samples. Most of the mislabelled samples derived from local fisheries and marketplaces (41.9%) and one sample (2.32%) from hypermarket stores. Our findings are similar to the results obtained from a national seafood fraud investigation carried on in the United States from 2010–2012. In this survey, out of 1200 seafood samples from 674 retail outlets in 21 States, DNA testing found that one-third (33 per cent) were mislabelled **[36]**. Forty-four per cent of the retail outlets visited sold mislabelled fish. Also, a recent Italian investigation revealed numerous commercial frauds; for example, Cutarelli et al., found that a sample marketed as "frozen grouper fillet" was made from halibut (*Hippoglossus hippoglossus*) instead of grouper (*E. marginatus*) **[37]**. Given consumers' high demand for grouper, the prices at the subsequent wholesale and retail market levels are also high relative to other finfish species. Additionally, the importation of large quantities of grouper from many foreign sources must meet the ever-growing demand for grouper. The strong demand for grouper and its high market value, which continues to be evident in the market, is also a motivation for economic frauds. The most prevalent economic fraud associated with grouper is the selling of a cheaper finfish as grouper. In fact, the most common types of mislabelling among the grouper samples collected in the US were substitutions with farmed Asian striped catfish (*Pangasianodon hypophthalmus*), freshwater perch (*Macquaria novemaculeata*), weakfish (*Cynoscion regalis*), bream (*Abramis brama*), and king mackerel (*Scomberomorus cavalla*). It is important to underline that grouper is a precious fish species often an item of fraud; in fact, when grouper is sold as fillet, its main features completely disappear, and its identity cannot be established on the basis of morphological features **[38]**.

A survey carried out by the Eurofishmarket (www.ilfattoalimentare.it) showed that around 15% of fresh/frozen grouper fillets sold on the market belonged to other species. These facts are strongly confirmed in our survey, in fact, we found that all samples marketed as grouper slices (*E. marginatus*) were slices of *R. pretiosus*. Such fraud could be considered both a commercial and a sanitary fraud because *R. pretiosus* is a fish known for its potential dangerousness for consumer. In fact, *R. pretiosus*, also known as "oilfish," is a deep-sea fish that stores a large amount of wax esters in its body for buoyancy control. In humans the accumulation of the indigestible wax esters in the rectum through the consumption of these fish produces discharges or leakage per rectum as orange or brownish green oil, but without noticeable loss of water; this response is called keriorrhea **[39]**. Outbreaks of keriorrhea have been repeatedly reported across continents. In the EU, the marketing of *R. pretiosus* is regulated by the EC Reg. 1021/08 (EC Reg. 1021/08). According to this regulation, food business operators must sell oilfish products in packaged form and provide information on label to the consumer about their gastrointestinal adverse effects.

In conclusion, our method based on PCR constitutes an effective molecular tool for detecting fraudulent substitution of fish species of interest applicable to raw finfish. These protocols could be applied to both quality control and official sanitary control of fishery products and to help the anti-fraud actions control fishery products' traceability and labelling.

CONCLUSION

The seafood industry is one of the imported traded products globally. Due to several health benefits, availability, less religious concerns, and possibility options, its demand and consumption increased exponentially. Due to increasing trade value, it is continuously vulnerable to frauds where costly fish can be replaced with cheap fishes, particularly in products where morphological identification is lost. Fish frauds may have health and environmental concerns. It must be authenticated before serving customers. Due to the limitations of protein-based methods, DNA-based methods like multiplex PCR provide a better alternative for species identification and tracking food fraud. It can help food control authorities to ensure food safety and the rights of consumers.

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How to target millennials as beer consumers through social responsibility? The case of Plzenský Prazdroj

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ABSTRACT

The paper evaluates the consumer attitude of millennials as beer consumers through social responsibility. Various CSR activities are applied by beer producer companies that target different age groups, gender, etc., through different communication channels. The main subject of the paper is the beer producer company, Plzenský Prazdroj (PP), which has an ambitious strategy related to the environment, waste management, underage alcohol drinking, and other aspects. Even though the company has a promising vision relating to CSR, the effect on consumer awareness can be different than expected. Therefore, a general hypothesis was set on whether there is or is not a difference between millennials in their attitudes towards CSR activities of PP. The characteristics of the research sample are displayed on the set of general factors, such as gender, age, monthly income, and more; beer factors like beer preferences, place of drinking, disposal of plastics, etc.; and attitudes of the monitored millennials towards the selected CSR activities of PP, such as recyclable packaging, Promile app, support of communities and more. The results of the paper assist in understanding the consumer attitude of this age group, and their perception of the CSR activities of PP, and can contribute to a successful marketing strategy creation of Plzenský Prazdroj oriented toward targeted cohort. Concerning the results, we created suggestions and recommendations for PP such as diversification of product portfolio and/or even business activities, diversification of non-alcoholic beer products, strengthening the CSR activities relating to the environment and waste management, and creating CSR activities that enable the engagement of millennials via their smartphones. The outcomes can also benefit other brewing companies in terms of CSR activities and marketing strategy creation.

Keywords: consumer, attitude, beer, Corporate Social Responsibility, Plzenský Prazdroj – PP

INTRODUCTION

Consumer behavior is influenced by various factors, including but not limited to pricing, product qualities or traits, and CSR engagement **[1]**. The prevailing paradigm behind corporate social responsibility, or CSR, is currently centred on "shared value." According to this concept, the role of a business is to generate value for its shareholders while simultaneously generating value for society, resulting in a solution where everyone benefits **[2]**. Several studies are researching the concept of CSR in the food and beverage industry **[3]**, **[4]**, **[5]**. The executive branch of the EU (European Union) is also dealing with CSR, as it states that companies can voluntarily decide if they want to contribute to a better society and sustainable environment. At the same time, companies develop CSR strategies and form their identity, which signifies the responsibility toward all the stakeholders affected by the companyhas become a rising trend in all industries. In contrast, companies develop CSR strategies and form their identity, which signifies responsibility to all the affected stakeholders **[6]**. Another goal of CSR is to protect the company's reputation and identity by engaging with stakeholders and responding to various institutional pressures **[7]**, **[8]**. By eliminating information asymmetry and boosting stakeholder decision-making, CSR contributes to long-term profitability **[9]**. Based on Porter and Kramer **[10]** there are four key reasons to engage in CSR activities: moral obligation, sustainability, license to operate, and reputation. Third-party endorsements, such as collaborations with non-profit organizations or NGOs, and certificates granted by reliable third parties, should be actively shared by corporations and organizations **[11]**. According to studies **[12]**, **[13]**,

CSR fulfilment has a favorable impact on consumer evaluations of corporate operations, positively impacting consumers' purchasing behavior and future purchase intention. This also indicates that consumers who are more aware of CSR are more inclined to buy a company's products, which is the primary reason corporations must engage in CSR-related activities **[14]**.

Marketing communication serves various purposes for customers or target markets, including informing and demonstrating how and why a product is utilized, the target market, and where and when the product is available **[15]**. As an emerging topic within corporate marketing communication, CSR management and marketing communication is fully recognized and considered a long-term investment **[16]**. With the rise of social media in today's digital age, studies have long demonstrated that traditional channels and communication techniques are losing their effectiveness **[17]**. As a result, businesses are forced to interact with customers via social media sites in any circumstances **[18]**. Based on a study from the UK, during the pandemic caused by COVID-19, alcohol corporations quickly modified their marketing to address health and social concerns related to the pandemic. This included the support of social distancing, hosting of online live-streamed events, and CSR initiatives such as philanthropic donations and linking products with the efforts of key workers, e.g., donations to health care and the hospitality sector **[19]**. Young millennials place a higher value on philanthropic initiatives because they perceive themselves as a socially committed population to humanitarian efforts and exhibit social awareness in their daily routines, which translates into a favorable attitude toward brands that promote social causes **[20]**. They devote more attention to the content these companies provide because they are more likely to trust them and buy their products or services **[21]**. In addition, millennials differ from the previous generations in several ways, including how they purchase and make decisions. This is hardly surprising because each demographic generation has distinct characteristics **[22]**. Millennials are a digitally naive and tech-savvy age group who utilize messaging platforms and the internet to access various media. They are often innovators or early adopters who use a variety of food sales channels and want firms to have a relevant online presence to be accessible. For them, food purchase priorities have shifted to a healthy profile and freshness **[23]**. According to several findings from the 20th century **[24], [25]**, the age of business students was associated with ethical beliefs and behavior, with older students displaying stronger ethics than their younger counterparts. To understand the consumer behavior of millennials, it is necessary to examine the influence of varied factors, such as the impact of advertising **[26]**,**[27]**, **[28]**, **[29]**, type of packaging **[30]**, **[31]**, **[32]** and others. According to Bakewell and Mitchell **[33]**, millennials' buying habits differ from preceding generations. It is critical to recognize their differences and recognize that employing the same marketing methods will be ineffective. As a result of an intense sense of personal identity, millennials participate in socially responsible activities, and to themselves and others, millennials employ socially responsible behavior to demonstrate their compassion **[34]**.

Although the alcohol business recognizes that its products can provide significant personal pleasure and societal value, they can also inflict major personal and social harm if drunk irresponsibly **[35]**. Over the years, industry members have contributed to innovative initiatives to prevent drunk driving and underage drinking. It explains why Oh et al. **[36]** call controversial industries such as tobacco, alcohol, gaming etc. “sinful firms”. Even though such organizations are stigmatized, several types of research show that despite their nature of operating CSR activities can support firm value and decrease risk **[37]**, **[38]**. On the other hand, the contradiction between their industry and CSR needs to be considered **[39]**, **[40]**. Evidence suggests that tobacco businesses exploited CSR operations to boost profits by improving their image, deflecting criticism, gaining access to policymakers, and mitigating legal risks **[40]**, **[41]**. When it comes to alcohol producers’ social responsibility, it is a challenge that must be faced and creatively overcome. For such companies, the promotion of responsible drinking is a fundamental initiative **[42]**. According to Mialon and McCambridge´s **[43]** research, there are five main types of CSR initiatives by alcohol industry actors: alcohol information and education provision, drunk driving prevention; research involvement; policy involvement, and the creation of social aspects organizations. Besides activities preventing harmful drinking, philanthropy is considered with non-alcohol issues, such as arts, culture, and emergency humanitarian aid. According to Jones et al. **[44]**, the leading spirits and beer companies are working to integrate CSR into their core business. While they emphasize their commitment to promoting responsible drinking, they also address various impacts in the marketplace, communities where they operate, the environment, and the workplace. Alcohol industry participants (producers, distributors, and so on) think integrating CSR and social marketing into their business operations can improve their economic, social, and environmental performance **[45]**. As the number of breweries and product developments has grown, new products benefit customers, society, and the environment. Diversification toward low-alcohol and non-alcoholic beer has created opportunities for breweries of all sizes, increasing sales. The availability of ecological beer is also steadily rising **[46]**.

Scientific Hypothesis

The study aimed at the specific problem of targeting communication of the selected brewing producer on millennial consumers. Our analysis is based on the general premise that millennials are responsive to ethical and responsible issues **[47]**, **[48]**, **[49]**, **[50],** and therefore we analyzed the differences in their attitudes towards the socially responsible scope of the monitored company.

For this purpose, we created a set of general factors (GF1-GF8) and factors of beer consumption (BF1-BF10) that affect the attitudes of millennials towards socially responsible activities of the monitored company (PP1-PP17).

Research is based on the general hypothesis H0 and connected alternative hypothesis H1:

- H0: There is no difference between millennials in their attitudes towards CSR activities of the monitored company.
- H1: There is a difference between millennials in their attitudes towards CSR activities of the monitored company.

The study included the set of characteristics (which describe millennials and their relation to beer drinking) and a set of factors (which describe their attitudes towards CSR activities of company PP). Regarding this, the alternative hypothesis was extended to the specific hypotheses derived from Ha (Table 1).

Table 1 Alternative hypothesis derived from Ha.

Ha		Factors		Attitudes	
Ha1	There is a statistically significant difference between millennials of ...	GF1 Gender	...in their attitudes towards CSR activity...	PP1 Most Important CSR Activity	...of monitored company Plzenský Prazdroj (PP).
Ha2		GF2 Age		PP2 Extent Consideration of Environment	
Ha3		GF3 Social Status		PP3 Extent Consideration CSR Activities	
Ha4		GF4 Monthly Income		PP4 CSR Attitude – Reduction of Water	
Ha5		GF5 Place of Living		PP5 CSR Attitude – Local Suppliers	
Ha6		GF6 Relationship Status		PP6 CSR Attitude – Recyclable Packaging	
Ha7		GF7 District		PP7 CSR Attitude – Reduction of Plastics	
Ha8		GF8 Origin		PP8 CSR Attitude – Use of Renewable Resources	
Ha9		BF1 Beer Likeness		PP9 CSR Attitude – Economical Technologies	
Ha10		BF2 Beer Frequency		PP10 CSR Attitude – Support of Communities	
Ha11		BF3 Place of Drinking		PP11 CSR Attitude – Work Safety	
Ha12		BF4 Beer Preferences		PP12 CSR Attitude – Garden Program	
Ha13		BF5 Dispose of Cans		PP13 CSR Attitude – Beer Alley	
Ha14		BF6 Dispose of Glass Cottles		PP14 Promile APP	
Ha15		BF7 Dispose of Plastic Bottles		PP15 Promile APP - CSR	
Ha16		BF8 Amount Single Occasion		PP16 Respect 18	
Ha17		BF9 End Up Single Occasion		PP17 Respect 18 – CSR	
Ha18		BF10 Increased Beer Expenses – COVID-19			

Note: Source: Own processing.

Specific alternative hypotheses (Ha1-Ha18) pointed to the differences between factors (general factors GF1-GF8 and beer factors BF1-BF10) and attitudes of the monitored millennials towards socially responsible activities of the company Plzenský Prazdroj (PP1-PP17). A computed *p*-value lower than the significance level alpha = 0.05 indicates rejection of the null hypothesis H0 and acceptance of the alternative hypothesis Ha and *vice versa*.

MATERIAL AND METHODOLOGY

This study aims to determine the company's CSR activities, Plzenský Prazdroj which can be effectively used in marketing communication towards millennial consumers. Plzenský Prazdroj´s sustainable development strategy is closely coordinated with Asahi Europe and International Group, including different CSR activities such as supporting the development of regions, reducing the average water consumption for production, purchasing resources from sustainable farms, switching to only circular packaging, reducing waste, preventing underage alcohol consumption and more. Therefore, analysis is based on statistically significant differences in millennials' attitudes towards CSR activities of (variables) regarding their general characteristics and beer-drinking habits (factors).

Picture 1 Logo of Plzenský Prazdroj. Note: Source: www.prazdroj.cz.

The study was conducted as an online questionnaire on a sample of 726 Czech and Slovak millennials. Because most of the product range of monitored company Plzenský Prazdroj contains alcohol, only persons 18+ years were included. The online questionnaire form (Google Forms) was made public through social media (Facebook primarily). Questionnaires consist of three parts: general (classification) questions – later used as general factors (GF1-GF8), questions regarding consumption habits of beer – later used as beer factors (BF1-BF10) and questions towards attitudes of respondents towards specific CSR activities of PP company – later used as variables (PP1-PP17).

Characteristics of the research sample can be displayed on the set of general factors:

- GF1 Gender (1 – Man (288), 2 – Woman (438)),
- GF2 Age (1 – 18-24 (506), 2 – More than 24 (220)),
- GF3 Social Status (1 – Student (355), 2 – Student with job (163), 3 – Job (208)),
- GF4 Monthly Income (1 – Under 300 EUR (336), 2 – More than 300 EUR (390)),
- GF5 Place of Living (1 – Dormitory/Rent (167), 2 – Mama Hotel (387), 3 – Own household (172)),
- GF6 Relationship Status (1 – Single (320), 2 – In Relationship (406)),
- GF7 District (1 – BA – Bratislava (100); 2 – TR – Trnava (134); 3 – TT – Trenčín (63); 4 – NR – Nitra (64); 5 – ZA – Žilina (110); 6 – BB – Banská Bystrica (53); 7 – PE – Prešov (112); 8 – KE – Košice (90)),
- GF8 Place of Living (1 – City (353), 2 – Village 373)).

Drinking habits of monitored millennials were described through a set of ten beer factors:

- BF1 Beer Likeness (1 – Very weak (27), 2 – Weak (55), 3 – Average (47), 4 – Strong (255), 5 – Very strong (342)),
- BF2 Beer Frequency (1 – Couple times a year (155), 2 – Once a month (69), 3 – Couple times a month (248), 4 – Couple times a week (236), 5 – Every day (18)),
- BF3 Place of Drinking (1 – Pub (353), 2 – Home (173), 3 – Outdoor activities (42), 4 – Restaurant (with meal) (70), 5 – At friend's place (88)),
- BF4 Beer Preferences (1 – Tapped (609), 2 – Can (63), 3 – Glass Bottle (48), 4 – Plastic Bottle (6)),
- BF5 Dispose of Cans (1 – Mixed waste (187), 2 – Separated waste (539)),
- BF6 Dispose of Glass Bottles (1 – Mixed waste (27), 2 – Separated waste (296), 3 – Refund (403)),
- BF7 Dispose of Plastic Bottles (1 – Mixed waste (91), 2 – Separated waste (635),
- BF8 Amount Single Occasion (1 – Less than 0.3 L (46), 2 – 0.3-0.5 L (157), 3 – 0.5-1.5 L (350), 4 – 1.5-3.5 L (153), 5 – 3.5 L and more (20)),
- BF9 End Up Single Occasion (1 – Single glass (164), 2 – Tipsy (475), 3 – Move to harder alcohol (71), 4 – K.O. (16)),
- BF10 Increased Beer Expenses – COVID-19 (1 – Yes (30), 2 – No (696)).

Attitudes of monitored millennials towards selected CSR activities of PP were identified through the following questions:

Multiple choice type

PP1 Most Important CSR Activity of PP (1 – Packaging circularity, 2 – Raw materials from natural sources, 3 – Carbon neutrality, 4 – Reduction of waste production, 5 – Reduction of water consumption, 6 – Higher production of non-alcoholic beers, 7 – Increase in the number of women in leadership positions).

Likert scale type

The Likert scale was used in the two groups of questions depending on the answers:

A. Answers: 1 – Very weak, 2 – Weak, 3 – Averagely, 4 – Strong, 5 – Very strong (PP2 Extent Consideration of Environment – PP; PP3 Extent Consideration CSR Activities – PP).
B. Answers: 1 – Very irresponsible, 2 – Rather irresponsible, 3 – I can't judge, 4 – Rather responsible, 5 – Very responsible (PP4 CSR Attitude – Reduction of Water; PP5 CSR Attitude – Local Suppliers; PP6 CSR Attitude – Recyclable Packaging; PP7 CSR Attitude – Reduction of Plastics; PP8 CSR Attitude – Use of Renewable Resources; PP9 CSR Attitude – Economical Technologies; PP10 CSR Attitude – Support of Communities; PP11 CSR Attitude – Work Safety; PP12 CSR Attitude – Garden Program; PP13 CSR Attitude – Beer Alley; PP15 Promila APP – CSR; PP17 Respect 18 – CSR.

Dichotomy type

Answers: 1 – Yes, 2 –No (PP14 Promila APP; PP16 Respect 18).

Statistical Analysis

The data obtained in the questionnaire survey were later examined by statistical analysis conducted on the sample of 726 Slovak millennials – beer consumers. The first step includes computing the coefficient of reliability Cronbach's alpha, **[51]** which measures the model's internal consistency. The analysis confirmed a total outcome higher than 0.7 (Cronbach's Alpha = 0.763; Cronbach's Alpha Based on Standardized Items = 0.778), which we considered acceptable for further statistical analysis. Also, the partial outcomes for selected variables – general factors (GF1-GF8) and factors of beer consumption (BF1-BF10) indicate acceptable outcomes for keeping the model without changes since Cronbach's Alpha if Item Deleted for all included variables are above 0.7 limits.

The next step includes confirmation of data distribution using the Shapiro Wilk normality test **[52]**. This test confirmed non-normal distribution for all used variables (general factors (GF1-GF8) and factors of beer consumption (BF1-BF10), since their sig. values were below 0.05 so data significantly deviates from a normal distribution. This result also indicates the use of non-parametric tests in the later examination.

The Durbin–Watson test on autocorrelation **[53]** was used to indicate autocorrelation between included variables. Procedure computed on variables GF1-GF8 and BF1-BF10 shows outcomes between 1.5 and 2.5; therefore, we can conclude that the data are not auto-correlated.

Regarding indicated non-normal data distribution, the Kruskal-Wallis nonparametric statistical test was used for further analysis. This assesses the differences among samples on a single non-normally distributed variable **[54]**, in this case with an assumption of statistically significant differences between millennials in their attitudes towards CSR activities of the monitored company. In case of two optional sorting questions (GF1, GF2, GF4, GF6, GF8), the Mann–Whitney U test **[55]** was used as an alternative to Kruskal Wallis. There were two sets of tests: tests using grouping variables GF1-GF8 (Table 2) and tests using BF1-BF10 (Table 3). Tables are displayed in two sets according to the type of variables, allowing us to highlight significant differences in every cohort. Regarding better readability, the summary outcomes of all conducted Kruskal – Wallis and Mann–Whitney U tests were summarized in Table 4. This displays significant differences only and according to their number; it indicates the significance of the selected factor. We used the simple premise that the more significant testing individual factors according to the individual variable that can be found, the more significant factor is. According to this, the outcome of summary significances (Figure 4 and Figure 5) was included in the conclusion of this study. Statistical analysis was conducted using IBM SPSS Statistics Subscription 1.0.0.1447 software.

RESULTS AND DISCUSSION

Nowadays, corporate social responsibility is a widely used managerial tool whose implementation can potentially positively affect customers' attitudes towards the company **[56]**. It has long been known that corporations are expected to meet societal expectations of contributing to social good to legitimize their existence **[57]**. When considering social responsibility, the food industry is a specific sector in which current trends of healthy and responsible lifestyles play a significant role in consumer preferences **[58]**, **[59]**, **[60]**. As an example, we can use an upward tendency in the consumer demand for plant-based analogues **[61]**, the fact that a healthy way of life and environmental knowledge jointly influenced young consumer ecological behaviour **[62]** or that

health is an important motivation for buying organic food products **[63]**. In the case of the alcohol industry, CSR activities can support the firm value and decrease risks, even if it is a controversial industry **[37]**,**[38]**, **[39]**, **[40]**. CSR is an effective tool for building corporate goodwill **[64]** and connecting the brand with positive emotions. Nagyová, et al. **[65]** confirmed that emotions are a significant factor 'influencing consumers' decisions and even changing them. This fact creates space for the growth of selected companies and the needed development of Slovak agricultural foreign trade **[66]**. This study aims to determine the CSR activities of the company Plzenský Prazdroj which can be effectively used in marketing communication toward millennial consumers.

Many types of research study the importance of CSR in the food industry **[3]**, **[4]**, **[5]**. PP has created various CSR activities and as it is illustrated in Figure 1, 436 of the asked millennials consider raw materials attained from sustainable natural sources as the essential CSR activity. This is followed by the importance of packaging circularity (158), carbon neutrality (75), and reduction of waste production (57).

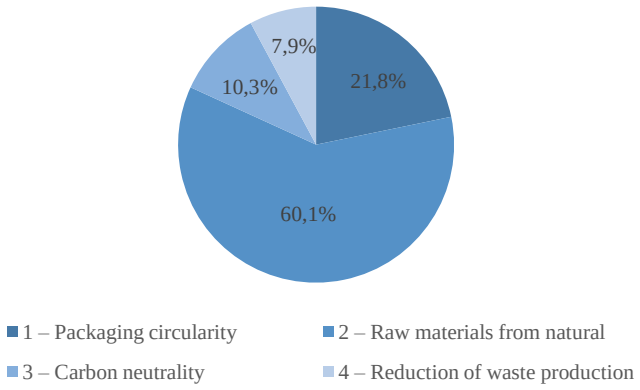

Figure 1 Frequencies PP1 Most important CSR activity of PP in percentages – Multiple choice style question. Note: Source: own processing.

Figure 2 represents the results of the consideration of millennials about how responsible PP is based on distinct aspects. Various research papers deal with targeting millennials and consider this age group as a specific segment **[22]**, **[34]**. Based on our results, in most of the cases, more than half of the respondents could not judge if PSS is responsible or not, which means that if this company wants to improve its CSR activities, it needs to improve its communication strategy through different communication channels. These aspects were work safety, support of communities, economic technologies, local suppliers, and water reduction. In addition, more millennials answered "I can´t judge" than the other possibilities in the case of beer alley, Garden program, use of renewable resources, reduction of plastics, and recyclable packaging.

On the other hand, most respondents consider PP´s warning of society about underage alcohol consumption as very responsible, and the Promile APP is considered positively since there were 236 answers "very responsible" and 225 "rather responsible". Most of the aspects were answered positively, except for one CSR activity – the reduction of plastics. The results are: 182 very responsible, 0 rather responsible, 353 I can´t judge, 183 rather irresponsible, and 8 very irresponsible. Donoghue et al. **[67]** also point out that 6.8% of the used packaging materials by the European breweries were PET bottles in 2010, only 0.7% of them were returnable, and 6.1% were non-returnable, and bottles and cans are packed in plastic foil when delivering and exporting.

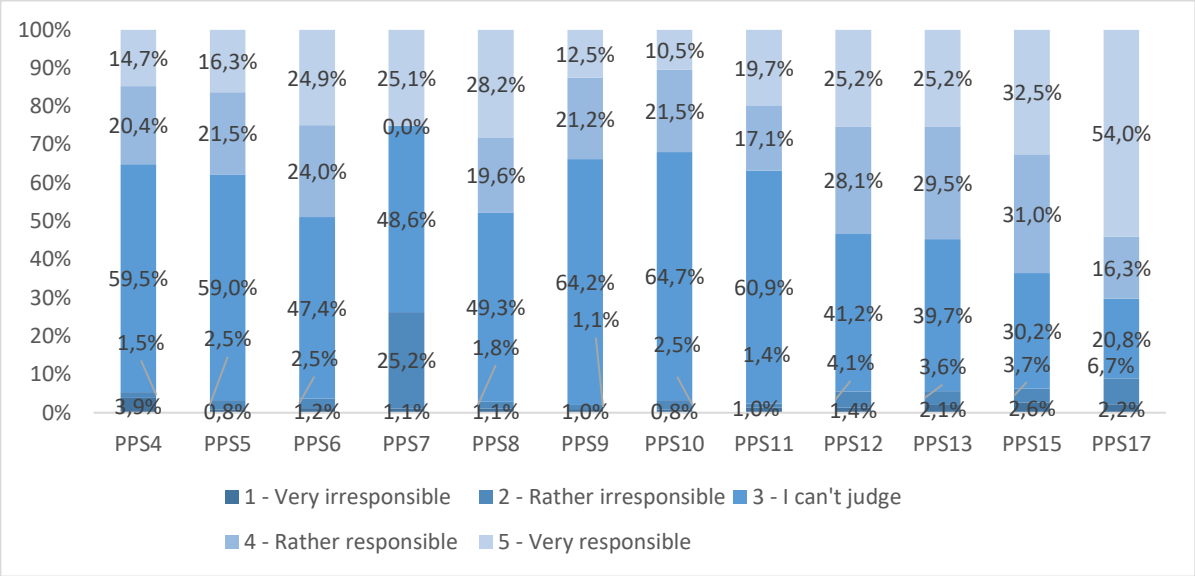

Figure 2 Frequencies PP4-PP13, PP15, PP17. Note: Source: own processing.

Next, in Figure 3 the attitudes of monitored millennials towards the selected CSR activities of PP are illustrated. PP introduced two main campaigns – "Respect 18" and an app "Promile INFO" – as CSR activities related to millennials, their effectiveness must be considered. In the case of the campaign "Respect 18!" only 29.89% of the respondents were familiar with the campaign and 70.11% of the respondents were not. The app "Promile INFO" has also had a negative result, since 25.34% of the monitored millennials were familiar with the app and 74.66% were not. Other beer producers and multinationals also applied such ideas associated with technology and innovation. AB InBev organized one-day social events in Argentina, Brazil, Bolivia, and Portugal to celebrate alcohol responsibility through various CSR activities. Besides the TV and radio campaigns for responsible drinking, stickers with legal age enforcement were distributed in Argentina, and breath analyzers were donated for educational purposes. In Brazil, non-governmental organizations, a company that engaged employees and partners, ran educational activities in economically disadvantaged urban areas **[68]**.

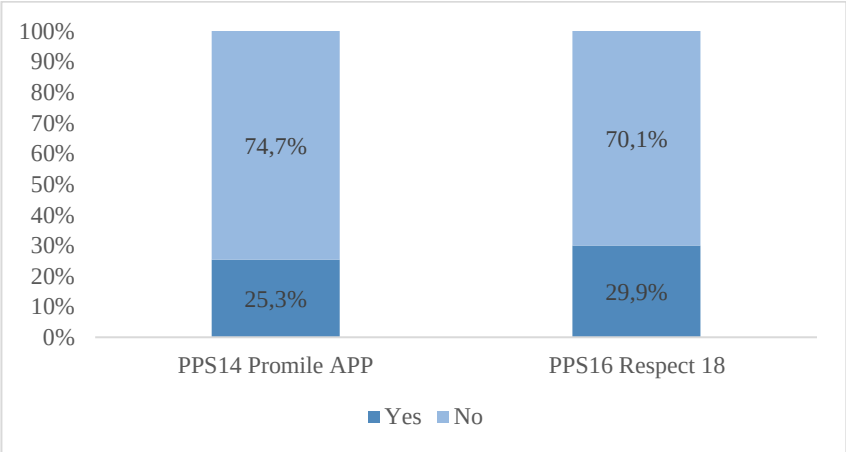

Figure 3 Frequencies PP14 and PP16 – Dichotomy style questions. Note: Source: own processing.

Table 2 shows the results of the verification of statistically significant differences among grouping variables GF1 – GF8, which find more significant differences between the selected general factors and selected CSR activities of PP. In the case of GF1 – Gender, we accept Ha1 for PP2, PP3, PP4, PP5, PP6, PP8, PP9, PP10, PP11, PP12, PP13, PP14, and PP17 and reject the null hypothesis H0. Therefore, we accept H0 for PP1, PP7, PP15, and PP16 and reject the alternative hypothesis. There is a statistically significant difference between gender and the following CSR aspects: the extent of consideration of the environment, the extent of consideration of CSR activities, reduction of water, local suppliers, recyclable packaging, use of renewable resources, economic technologies, support of communities, work safety, Garden program, beer alley, Promile app, Respect 18. For GF2 – Age we accept Ha1 for PP2, PP11, and PP17, reject H0, and for the other CSR activities, reject Ha1 and

accept H0. This means a statistically significant difference between age and extent in consideration of the environment, attitude – work safety and respect 18. For GF3 – Social Status, we accept Ha1 for PP14 – Promile app, reject H0 and for all the other CSR activities, reject Ha1 and accept H0. Therefore, there is a statistically significant difference between social status and Promile app. In the case of GF4 – Monthly Income, we accept Ha1 for PP4 and PP14, reject H0 and for the other CSR activities, accept H0 and reject Ha1. There is a statistically significant difference between monthly income and reduction of water and Promile app. For GF5 – Place of Living, we accept Ha1 for PP2 and PP3, reject H0, and for the other CSR activities, accept H0 and reject Ha1. There is a statistically significant difference between the place of living, the extent of consideration of the environment, and the extent of consideration of CSR activities. In the case of GF6 – Relationship Status, we accept Ha1 for PP3, PP15, and PP17, reject H0, and for the other CSR activities, accept HO and reject Ha1. There is a statistically significant difference between relationship status and extent of consideration of CSR activities, Promile app, and respect 18. For GF7 – District, we accept H0 for all the observed CSR activities and reject Ha. There is no statistically significant difference between district and CSR activities among Slovak millennials. In the case of the last general factor, GF8 – Origin, we accept Ha1 for PP5, PP13, and PP16, reject H0, and for the other CSR activities, accept H0 and reject Ha1. There is a statistically significant difference between origin and CSR attitudes - local suppliers, CSR attitude – beer alley, and Respect 18.

Table 2 Verification of statistically significant differences among grouping variables GF1-GF8.

		PP1	PP2	PP3	PP4	PP5	PP6	PP7	PP8	PP9	PP10	PP11	PP12	PP13	PP14	PP15	PP16	PP17
GF1	KW H	0.876	12.549	7.480	8.997	17.420	12.719	1.507	3.914	9.177	15.690	17.071	7.993	7.315	13.406	0.055	**0.000**	13.240
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.349	**0.000**	**0.006**	**0.003**	**0.000**	**0.000**	0.220	**0.048**	**0.002**	**0.000**	**0.000**	**0.005**	**0.007**	**0.000**	0.815	0.989	**0.000**
GF2	KW H	0.300	4.130	2.301	1.955	2.272	0.268	0.484	0.997	0.643	0.379	**0.003**	0.264	0.437	0.486	2.137	1.419	**0.027**
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.584	**0.042**	0.129	0.162	0.132	0.605	0.486	0.318	0.423	0.538	0.953	0.608	0.508	0.486	0.144	0.234	0.870
GF3	KW H	0.254	1.592	0.105	0.566	0.216	2.190	0.501	1.401	1.793	0.730	1.099	0.404	0.211	**0.031**	3.445	3.989	0.905
	df	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Asymp. Sig.	0.881	0.451	0.949	0.754	0.898	0.334	0.778	0.496	0.408	0.694	0.577	0.817	0.900	0.984	0.179	0.136	0.636
GF4	KW H	0.055	0.120	0.070	**0.015**	1.424	2.674	2.011	0.371	1.892	1.256	3.249	0.697	0.557	**0.021**	0.052	3.449	0.287
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.814	0.729	0.791	0.902	0.233	0.102	0.156	0.543	0.169	0.262	0.071	0.404	0.456	0.885	0.820	0.063	0.592
GF5	KW H	1.751	9.642	6.926	1.898	1.792	1.404	2.313	0.314	0.235	1.850	0.181	1.684	4.833	1.258	5.212	2.030	0.446
	df	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Asymp. Sig.	0.417	**0.008**	**0.031**	0.387	0.408	0.496	0.315	0.855	0.889	0.396	0.914	0.431	0.089	0.533	0.074	0.362	0.800
GF6	KW H	0.478	0.068	**0.039**	0.172	0.850	0.327	0.405	0.317	0.527	0.567	0.088	0.471	0.596	0.496	**0.047**	0.575	4.108
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.489	0.794	0.843	0.678	0.356	0.567	0.525	0.573	0.468	0.452	0.767	0.493	0.440	0.481	0.828	0.448	**0.043**
GF7	KW H	3.908	6.988	4.760	0.588	9.336	9.147	12.164	9.835	8.541	8.400	11.553	4.566	8.794	6.472	5.547	10.321	4.409
	df	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	Asymp. Sig.	0.689	0.322	0.575	0.997	0.156	0.166	0.058	0.132		0.210	0.073	0.601	0.186	0.372	0.476	0.112	0.622
GF8	KW H	0.473	0.079	0.788	3.516	3.956	3.381	2.924	3.570	3.040	1.301	1.786	0.694	**0.001**	0.364	2.665	4.781	0.180
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.492	0.779	0.375	0.061	**0.047**	0.066	0.087	0.059	0.081	0.254	0.181	0.405	0.970	0.547	0.103	**0.029**	0.671

Note: bolded numbers represent the statistically significant difference. Source: own calculations.

Table 3 Verification of statistically significant differences among grouping variables BF1-BF10.

		PP1	PP2	PP3	PP4	PP5	PP6	PP7	PP8	PP9	PP10	PP11	PP12	PP13	PP14	PP15	PP16	PP17
BF1	KW H	10.041	3.301	1.913	0.991	5.914	4.163	4.915	7.200	5.742	3.224	3.851	4.081	14.422	7.186	5.414	0.741	4.867
	df	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Asymp. Sig.	**0.040**	0.509	0.752	0.911	0.206	0.384	0.296	0.126	0.219	0.521	0.427	0.395	**0.006**	0.126	0.247	0.946	0.301
BF2	KW H	10.819	7.620	14.347	1.746	3.839	4.752	1.296	2.016	0.626	3.037	1.993	12.449	10.051	9.253	2.292	6.578	7.862
	df	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Asymp. Sig.	**0.029**	0.107	**0.006**	0.782	0.428	0.314	0.862	0.733	0.960	0.552	0.737	**0.014**	**0.040**	0.055	0.682	0.160	0.097
BF3	KW H	6.144	1.412	5.014	5.561	9.244	4.456	2.070	3.082	4.710	5.773	6.165	7.287	3.688	6.634	7.492	4.642	6.063
	df	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Asymp. Sig.	0.189	0.842	0.286	0.234	0.055	0.348	0.723	0.544	0.318	0.217	0.187	0.121	0.450	0.157	0.112	0.326	0.195
BF4	KW H	9.033	0.775	9.207	2.699	4.173	2.277	7.029	3.142	3.667	4.039	4.700	1.872	0.367	4.784	1.164	0.791	4.212
	df	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Asymp. Sig.	**0.029**	0.856	**0.027**	0.440	0.243	0.517	0.071	0.370	0.300	0.257	0.195	0.599	0.947	0.188	0.762	0.852	0.239
BF5	KW H	0.913	2.420	3.594	0.486	10.109	9.911	5.159	**0.042**	2.395	2.874	6.830	0.384	3.253	0.074	3.036	4.856	2.155
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.339	0.120	0.058	0.486	**0.001**	**0.002**	**0.023**	0.838	0.122	0.090	**0.009**	0.535	0.071	0.786	0.081	**0.028**	0.142
BF6	KW H	4.640	3.218	4.415	5.318	7.367	4.051	6.832	11.559	12.663	4.690	7.164	4.262	7.395	1.500	1.090	4.913	5.977
	df	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Asymp. Sig.	0.098	0.200	0.110	0.070	**0.025**	0.132	**0.033**	**0.003**	**0.002**	0.096	**0.028**	0.119	**0.025**	0.472	0.580	0.086	0.050
BF7	KW H	1.766	5.385	4.139	0.302	4.646	9.805	5.615	2.742	1.221	2.611	3.291	1.031	7.253	0.058	0.114	8.911	9.242
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.184	**0.020**	**0.042**	0.583	**0.031**	**0.002**	**0.018**	0.098	0.269	0.106	0.070	0.310	**0.007**	0.809	0.735	**0.003**	**0.002**
BF8	KW H	4.165	4.428	12.048	8.167	2.379	2.428	8.244	3.045	4.080	1.742	2.835	10.820	16.090	29.470	0.394	7.455	7.143
	df	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	Asymp. Sig.	0.384	0.351	**0.017**	0.086	0.666	0.658	0.083	0.550	0.395	0.783	0.586	**0.029**	**0.003**	**0.000**	0.983	0.114	0.129
BF9	KW H	3.752	3.058	10.601	0.390	2.461	0.458	15.918	2.664	3.953	6.800	2.872	5.105	2.022	9.272	7.791	3.201	17.568
	df	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Asymp. Sig.	0.290	0.383	**0.014**	0.942	0.482	0.928	**0.001**	0.446	0.267	0.079	0.412	0.164	0.568	**0.026**	0.051	0.362	**0.001**
BF10	KW H	0.067	1.171	0.424	0.556	0.661	2.055	12.776	0.107	0.288	0.557	0.148	1.192	0.951	0.067	0.190	0.685	0.121
	df	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Asymp. Sig.	0.796	0.279	0.515	0.456	0.416	0.152	**0.000**	0.743	0.592	0.456	0.701	0.275	0.330	0.796	0.663	0.408	0.728

Note: bolded numbers represent the statistically significant difference. Source: own calculations.

In Table 3, the verification results of statistically significant differences among grouping variables BF1 – BF10 are presented with more significant differences between the selected beer factors and CSR activities of PP. For BF1 – Beer Likeness, we accept Ha1 for PP1 and PP13 and reject H0, and for the other CSR activities, we accept H0 and reject Ha1. There is a statistically significant difference between beer likeness and the most important CSR activity and CSR attitude – beer alley. For BF2 – Beer Frequency, we accept Ha1 for PP1, PP3, PP12, and PP13, reject H0, and for the other CSR activities, accept H0 and reject Ha1. There is a statistically significant difference between beer frequency and most important, CSR activity, the extent of consideration of CSR activities, CSR attitude – Garden program, and CSR attitude – beer alley. In the case of BF3 – Place of Drinking, we accept H0 and reject Ha1 for all the observed CSR activities of PP by millennials in Slovakia. Therefore, there is no statistically significant difference between beer factors and CSR activities. For BF4 – Beer Preferences we accept Ha1 for PP1 and PP3, reject H0, and for the other CSR activities, accept H0 and reject Ha1. That means a statistically significant difference between beer preferences and, most importantly, CSR activity and the extent of consideration of CSR activities. For BF5 – Dispose of Cans, we accept Ha1 for PP5, PP6, PP7, PP8, PP11, and PP16, reject H0, and for all the other observed factors, accept H0 and reject Ha1. Therefore, there is a statistically

significant difference between disposing of cans and the following CSR activities: CSR attitude – toward local suppliers, CSR attitude – toward recyclable packaging, CSR attitude – toward reduction of plastics, CSR attitude – toward the use of renewable resources, CSR attitude – work safety and Respect 18. In the case of BF6 – Dispose of Glass Bottles we accept Ha1 for PP5, PP7, PP8, PP9, PP11, and PP13, reject H0, and for the other CSR activities, accept H0 and reject Ha1. There is a statistically significant difference between disposing of glass bottles and the CSR attitude – toward local suppliers, CSR attitude – reduction of plastics, CSR attitude – use of renewable resources, CSR attitude - toward economic technologies, CSR attitude – toward work safety, and CSR attitude – beer alley. For BF7 – Dispose of Plastic Bottles, we accept Ha1 for PP2, PP3, PP5, PP6, PP7, PP13, PP16, and PP17, reject H0, and for the other factors, accept H0 and reject Ha1. That means that there is a statistically significant difference between the disposal of plastic bottles and the following: the extent of consideration of the environment, the extent of consideration of CSR activities, CSR attitude – toward local suppliers, CSR attitude – toward recyclable packaging, CSR attitude – reduction of plastics, CSR attitude – beer alley, respect 18 and respect 18 – CSR. Next, for BF8 – Amount Single Occasion, we accept Ha1 for PP3, PP12, PP13, and PP14, reject H0, and for the other CSR activities, accept H0 and reject Ha1. There is a statistically significant difference between amount single occasions and the following CSR activities: extent consideration of CSR activities, CSR attitude – Garden program, CSR attitude – beer alley, and Promile app. For BF9 – End Up Single Occasion, we accept Ha1 for PP3, PP7, PP14, and PP17 reject H0, and for the other factors, accept H0 and reject Ha1. There is a statistically significant difference between ending up on a single occasion and the extent consideration of CSR activities, CSR attitude – reduction of plastics, Promile app and Respect 18 – CSR. For the last beer factor, BF10 – Increased Beer Expenses – COVID-19, we accept Ha1 for PP7, reject H0, and for the other CSR activities, accept H0 and reject Ha1. There is a statistically significant difference between Increased Beer Expenses – COVID-19 and CSR attitude – reduction of plastics.

Table 4 Kruskal – Wallis test: summary outcomes.

	GF1	GF2	GF3	GF4	GF5	GF6	GF7	GF8	BF1	BF2	BF3	BF4	BF5	BF6	BF7	BF8	BF9	BF10	TOTAL
PP1									X	X		X							3
PP2	X	X			X										X				4
PP3	X				X					X		X			X	X	X		7
PP4	X																		1
PP5	X							X					X	X	X				5
PP6	X												X		X				3
PP7													X	X	X		X	X	5
PP8	X													X					2
PP9	X													X					2
PP10	X																		1
PP11	X												X	X					3
PP12	X									X						X			3
PP13	X								X	X				X	X	X			6
PP14	X															X	X		3
PP15																			0
PP16								X					X		X				3
PP17	X					X									X		X		4
TOTAL	13	1	0	0	2	1	0	2	2	4	0	2	5	6	8	4	4	1	55

Note: Source: own calculations.

Table 4 presents the results of the Kruskal – Wallis summary outcomes. Statistically significant differences between attitudes of monitored millennials towards selected CSR activities of PP (PP1 – PP17) and characteristics of the research sample1 – GF8), drinking habits of monitored millennials through the set of beer factors (BF1 – BF10) were analyzed. There are statistically significant differences between the CSR activities and observed variables in all cases, except for PP15 Promile APP – CSR. The highest number of statistically significant differences are confirmed in the case of PP3 (7), PP13 (6), PP5 (5), and PP7 (5). From the other point of view,

there is no statistically significant difference in the case of GF3, GF4, GF7, and BF3. The highest number of statistically significant differences are in the case of GF1 (13), BF7(8), and BF6 (6).

The study was conducted as an online questionnaire distributed by social media. This form was used not just regarding the relative easiness of obtaining data but also because of limitations connected with the COVID-19 pandemic **[11].** Still, the form of data obtained can affect the characteristics of analyzed samples. We believe that study aimed at millennials is minimally affected by this phenomenon because this category is characterized by an elevated level of computer literacy **[69]**, high involvement in online studies **[70]**, and overall high use of social media **[71]**, **[72]**.

Based on the study results, it is easier to understand what the preferences of this age group are, and the results should be considered when deciding on a further marketing communication strategy. Since PP has created more types of CSR activities, their effectiveness needs to be considered and analyzed from various aspects. Based on our research, the majority of the millennial respondents selected raw materials attained from sustainable natural sources (436) as the most important ones, followed by the importance of packaging circularity (158), carbon neutrality (75), and reduction of waste production (57). The next part of the study dealt with the degree of responsibility of PP from several types of CSR activities. In most of the cases, more than half of the respondents could not tell if PSS is responsible or not, which means that the communication strategy of PP is not sufficient enough, and communication towards millennial consumers should be made accurate. These activities were work safety, support of communities, economic technologies, local suppliers, and water reduction. In addition, more millennials answered "I can´t judge" than the other possibilities for beer alley, Garden program, use of renewable resources, reduction of plastics, and recyclable packaging, which also supports the idea that communication should be reconsidered.

On the other hand, most respondents considered the warning about underage alcohol consumption very responsible, and the Promile APP was ranked positively. Most of the factors were answered positively, except for one CSR activity – reduction of plastics. The following part of the study presented the attitudes of monitored millennials towards the selected CSR activities of PP. Next, the effectiveness of two campaigns related to CSR - "Respect 18" and the app "Promile INFO" – were analyzed. In the case of the campaign "Respect 18!" the majority of the respondents were not familiar with the campaign, and it was the same with the app "Promile INFO", which also had a negative result. In addition, statistically significant differences between attitudes of monitored millennials towards PP (PP1 – PP17) selected CSR activities and characteristics of the research sample based on the Kruskal – Wallis test. As illustrated in Figure 4, the highest degree of statistically significant differences is confirmed in the case of extent consideration of CSR activities (7), CSR attitude – beer alley (6), CSR attitude – local suppliers (5), and CSR attitude – reduction of plastics (5). Therefore, these CSR activities should be improved based on the factors with statically significant differences provided in this study.

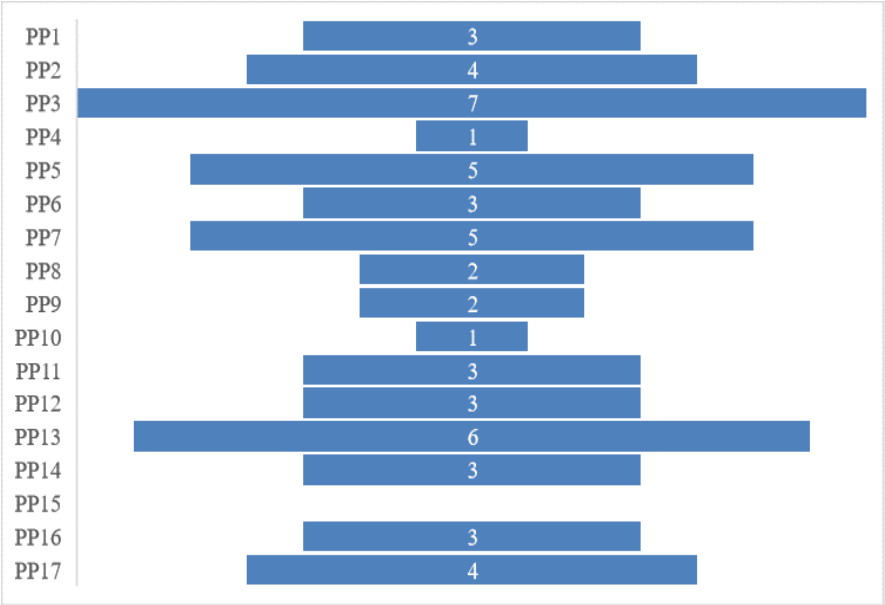

Figure 4 Statistical significances – variables PP1-PP17. Note: Source: own processing.

General and beer factors also need to be analyzed when considering the statistically significant differences. As provided in Figure 5, there is no statistically significant difference in the case of social status, monthly income,

district, and place of drinking. On the other hand, the highest number of statistically significant differences are in the case of gender (13), disposal of plastic bottles (8) and disposal of glass bottles (6).

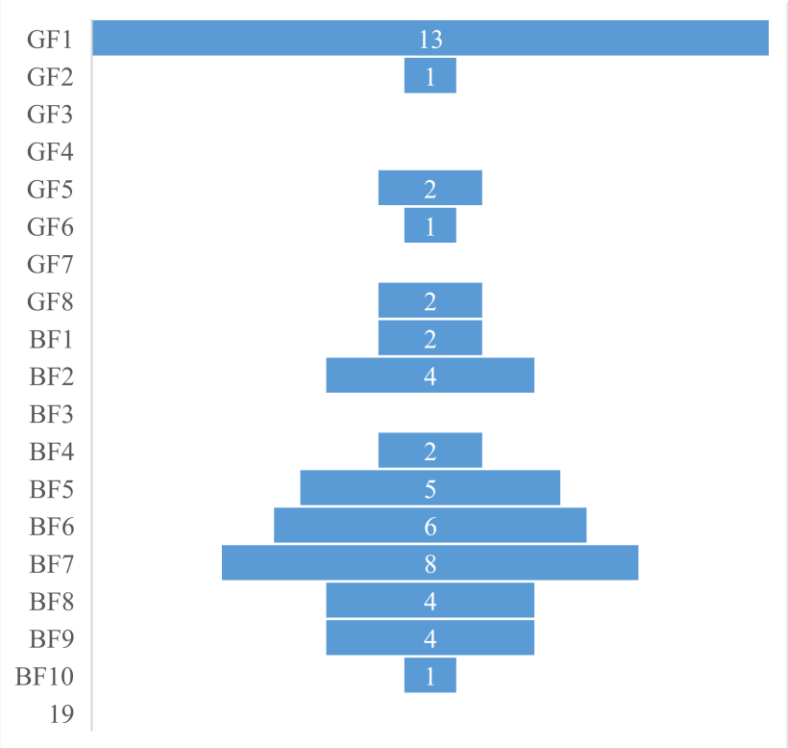

Figure 5 Statistical significances – factors GF1-8; BF1-10. Note: Source: own processing.

Based on the results, there are a few recommendations that should be considered by PP and can be beneficial for other brewing companies when creating marketing communication and CSR strategy, as well. Therefore, we recommend considering and applying the following steps to marketing strategy: diversification in the form of enlarging of product portfolio and/or even business activities of brewers would be beneficial and could stabilize their incomes during a difficult period such as an ongoing pandemic or war conflict nearby; diversification of non-alcoholic beer products can improve the image of PP since various research papers studied the rising popularity of such non-alcoholic beverages **[46]**, **[73]**. Beer producers need to promote responsible drinking and discourage underage alcohol consumption, a hot topic in different papers **[35]**, **[43]**. As a very perspective activity, we can see beer tourism **[74]** and effectively connect with the company's social responsibility. Younger generations, including millennials, are reacting positively to technological innovations accessible through their smart devices and, therefore, can be applied as a communication channel that strengthens the perception of CSR activities. According to several research papers, interactive technologies such as smartphones are an effective way of CSR communication **[75]**, **[76]**.

On the other hand, our results show a statistically significant difference between the CSR attitude and the "older" and "younger" respondents among millennials. Therefore, we recommend dividing the age group of millennials into two categories: "younger millennials" and "older millennials". Younger millennials have a close relation to digital technologies just like generation Z, therefore, we suppose that their consumer behavior is similar, too. Secondly, older millennials are close to generation X and can have similar consumer behavior. Further research would evaluate different results based on these two categories. Also, targeting would be more specific and understandable in the case of PP and other beer businesses. We also recommend including "younger millennials" when researching generation Z and "older millennials" when researching generation X. This could create more realistic research and results. Our further general contributions to theory and practice recommend active engagement on social media sites such as Facebook, Instagram, LinkedIn and more; engagement in humanitarian activities; Besides the online appearance, it is still very important to be present at festivals and other events since the generation of millennials being physically active and like to socialize. The PP Promile app should be upgraded and communicated through communication channels that are the most accessible to younger generations, such as social media sites and other digital marketing tools. Our results suggest circularity and waste management should be considered when improving the existing marketing communication strategy relating to CSR activities. Millennials are sensitive to the negative impacts of climate change and massive waste production; therefore, strict but realistic strategic goals should be set. The CSR activities are all about trust and responsibility, therefore, we

highly recommend PP and other beer producers be serious when publishing their CSR strategy. When millennials see that the CSR goals were met, the particular company earns the consumers' trust, strengthens its brand identity, and increases its sales.

For further research, we recommend enlarging the sample size and conducting similar research on beer consumers of various age groups to find significant differences between various age groups. Also, we can see a possibility of finding differences between various groups based on their gender, income level, occupation, or other characteristics. Furthermore, there is a possibility to continue and build broader research based on this one methodically. Variables, as well as factors, can be enlarged into a broader data matrix. Finally, the post hoc test can further evaluate significant differences and selected differences can be identified and described.

The conducted study opens space for diverse benefits. First, this study sets the methodical and data basis for further research. Second, there is a possibility of using obtained results in the business practice of various entities since our outcomes suggest beneficial results for any responsible-oriented marketing activity aimed at the millennial cohort. Third, there is a value for policymakers responsible for the official incorporation of corporate social responsibility into business practice across EU member states. Fourth, results will be used in the academic sphere and teaching at SUA – Department of Economics and Management, which is involved in the project VEGA-1/0525/2.

CONCLUSION

Consumer attitude of millennials as beer consumers through social responsibility was the paper's main focus. The research aimed at the brewing company, Plzenský Prazdroj (PP), which created an ambitious strategy related to the environment, waste management, underage alcohol drinking, and other CSR aspects. The study was conducted as an online questionnaire on a sample of 726 Czech and Slovak millennials, only 18+ individuals. Questionnaires consisted of three parts: classification questions, questions regarding consumption habits of beer and questions about the attitudes of respondents towards specific CSR activities of PP. Based on the statistical analysis results, we came up with a few suggestions, such as product portfolio enlargement, diversification of non-alcoholic beer products, strengthening environment-related CSR activities, promotion of responsible drinking and app creation. To target more effectively, we proposed dividing the age group of millennials into two categories: “younger millennials” and “older millennials”, since the consumer behaviour of these two groups could be slightly different. Considering the outcomes of the paper could be beneficial for marketing strategy and CSR strategy creation not only in the case of PP but also in the case of other beer producers.

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Antimicrobial susceptibility of mastitis pathogens of dairy cows in Ukraine

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ABSTRACT

Mastitis is one of the most common diseases on dairy farms. It causes significant economic damage associated with the cost of treating sick cows, reduced milk yield and quality indicators of dairy products, and the risk of premature culling of animals. Treatment of cows with mastitis on dairy farms is carried out mainly with antimicrobial drugs, which are usually used without a preliminary test to identify the causative agent of the disease and determine its sensitivity to antimicrobial substances, which is an important part of the effectiveness of therapy. Increasing the resistance of bacteria to antimicrobial substances poses a threat not only to the animal but also to humans, as a consumer of dairy products. The availability of data on the sensitivity of mastitis pathogens to antimicrobial drugs makes it possible for veterinary doctors to choose the most effective antibiotic for treating animals with the shortest duration of treatment. The presented results of studies of breast secret samples taken from cows indicate that in 57.5% of cases, contagious pathogens of mastitis were identified. In particular, *Streptococcus agalactiae* made 24.1%, *Staphylococcus aureus* – 18.4%, *Corynebacterium* spp. – 7.2%, *Streptococcus dysgalactiae*– 5.6%, *Streptococcus uberis* – 2.2%. Environmental pathogens accounted for 42.5% of the total number of isolated isolates, among which *Streptococci* represented gram-positive microflora at 11.5 *Streptococcuscus* spp. (6.2% *Streptococcuscus parauberis* (4.4% *Streptococcuscus Bovis* (0.9%) and *Staphylococcus* spp. – 10.3%. Gram-negative microflora is 20.6%, among which the largest percentage belongs to *E. coli* – 8.4% and *Klebsiella pneumonia* – 1.9%. Mastitis caused by yeast accounted for 1.4% of all diagnosed pathogens. Antimicrobial sensitivity was evaluated using the disk diffusion method (Kirby-Bauer). According to the results of determining the sensitivity of mastitis pathogens to antimicrobial substances, it was found that the highest sensitivity of the isolated isolates was to Ceftiofur, Amoxicillin/clavulanic acid, Rifampicin, Amoxicillin, Gentamicin, Ampicillin, Bacitracin, Cephalexin, Cloxacillin, Enrofloxacin, Trimethoprim/sulfamethoxazole, Oxytetracycline, Lincomycin. The least sensitive – to Spiramycin, Tylosin, streptomycin, neomycin, Marbofloxacin, Tilmicosin, and Danofloxacin.

Keywords: mastitis, antimicrobial substances, contagious, environmental, the causative agent of mastitis

INTRODUCTION

Farm owners and producers of dairy products suffer significant economic losses due to various infectious and non-infectious diseases, among which one of the main one is inflammation of the mammary gland. Mastitis, by its nature, is a complex, reasonably common, and expensive disease of cows on dairy farms **[1]**. Economic losses are associated with treatment costs, reduced milk production, and the quality of milk obtained, as well as the risks of premature culling of highly productive animals **[3]**, **[5]**, **[6]**. According to data **[3]**, the total cost of expenses caused by bovine mastitis is estimated at an average of USD 147 per cow per year. Bovine mastitis therapy is the most common reason for using antimicrobials on dairy farms **[7]**, **[11]**. In addition, it is known that broad-spectrum antimicrobials affect the development of resistance to a greater extent than narrow-spectrum antimicrobials **[20]**, **[47]**. Antimicrobial drugs for the treatment of animals with mastitis have been used for about sixty years and are often prescribed without a preliminary test to identify the pathogen and determine its sensitivity, which is a fairly important part of therapy **[2]**. Pathogens of mastitis are divided into two groups, the so-called contagious and environmental. Contagious pathogens are transmitted mainly from one cow to another, especially through milking equipment. In contrast, environmental pathogens enter the mammary gland from the external environment (through bedding, flies, or even cow skin) **[8]**, **[13]**. Contagious pathogens

include such types as *Staphylococcus aureus* and *Streptococcus agalactiae* and less common ones – such as *Mycoplasma bovis* and *Corynebacterium,* which are localized on the udder and skin. Environmental pathogens such as *Escherichia coli* or *Streptococcus uberis* penetrate and reproduce in the udder of cows, induce an immune response, and are rapidly eliminated **[9]**. Monitoring the resistance of mastitis pathogens to antimicrobials over time becomes extremely important to ensure the long-term effectiveness of antibacterial drugs. Access to antimicrobial sensitivity data helps veterinary doctors choose the most effective drug for treating animals with mastitis, especially given that therapy for this pathology usually begins before testing the sensitivity of the pathogen **[10]**, **[12]**. Increasing the resistance of bacteria to antimicrobial substances poses a threat to both animals and humans, as consumers of dairy products. Therefore, the World Organization for Animal Health (WOAH) recommends monitoring the resistance of pathogens and commensal bacteria if necessary. Such monitoring provides significant information for therapeutic measures and, at the same time, shows trends in the development of bacterial resistance, which can be taken into account when using individual antimicrobial drugs in practice **[11]**, **[14]**, **[15]**.

This study aimed to identify pathogens of excretion from samples of cow mammary glands secretions and determine the sensitivity of the main pathogens of mastitis to commonly used antimicrobial substances.

Scientific Hypothesis

We expect that isolated isolates of pathogens from the secretion of cows with mastitis will show different sensitivity to a wide range of antimicrobial substances, which will make it possible to isolate those with the highest antibacterial activity and recommend them for animal therapy. Testing the secretion of cows suffering from mastitis for antimicrobial substances is an effective tool in increasing the indicators of obtaining high-quality and safe dairy products.

MATERIAL AND METHODOLOGY

Samples

Samples of cow mammary glands secretions were submitted for research to the laboratory of bacteriology and path anatomy of LLC "Center for veterinary diagnostics" from different regions of Ukraine in sterile test tubes.

Chemicals

Blood agar (Oxoid, UK), MacConkey Agar (Oxoid, UK), Muller-Hinton Agar (Oxoid, UK), Condalab antimicrobial discs (Spain), Erba lachema indole test (Czech Republic), oxidase test HiMedia Laboratories (India), catalase test of Technopharm LLC (Ukraine), Química Clínica Aplicada S. A. Gram dye. (Spain).

Animals and Biological Material

The animals were of different breeds (Holstein, Ukrainian black, and piebald), age, and had different lactation duration and productivity. There was no information about the size of livestock, diet, maintenance, watering, milking system, or milk supply. The secret of the udder was taken from cows with mastitis.

Instruments

Petri dishes, microbiological loop.

Laboratory Methods

Udder secretion samples were examined microbiologically using standard laboratory methods **[16]**. Mammary gland secretions (approximately 0.1 mL) were applied in a loop to the surface of blood agar (agar-based medium enriched with 5% sterile sheep's blood) (Biocorp, Poland). Bacterial dishes were incubated at 37 °C for 24 – 48 hours under aerobic conditions. After that, the morphology of the colony was evaluated and described. Samples that produced more than three types of microorganisms were identified as contaminated. Individual bacterial colonies were subcultivation to produce pure isolates by repeated bacteriologic culture technique. Pure isolates were identified using phenotyping tests, including Química Clínica Aplicada S. A. (Spain) gram staining, HiMedia Laboratories oxidase test (India), indole tester Lachema (Czech Republic), and Technopharm LLC (Ukraine) catalase. Bacterial species were identified based on biochemical profiles using the API 20E BioMerieux system (France) and Streptotest 16 erga Lachema (Czech Republic). Gram-negative bacteria were identified based on growth on MacConkey Agar (Oxoid, UK), indole, and oxidase tests. Blood agar (Oxoid, UK) was used to cultivate yeast and mold. Determination of the sensitivity of isolated isolates to antimicrobial substances was performed using the Kirby Bauer Disk Diffusion method **[17]**, **[18]**, **[19]**, **[21]** in vitro on Muller-Hinton Agar (Oxoid, UK), using commercial Condalab disks (Spain).

Description of the Experiment

Sample preparation: According to the bacteriological study of 346 samples of udder secretions selected from cows with clinical and subclinical forms of mastitis, 264 samples were found to be positive. 21 samples with a negative result- no growth of microorganisms. Contamination was found in 61 samples of udder secretions (Figure 1).

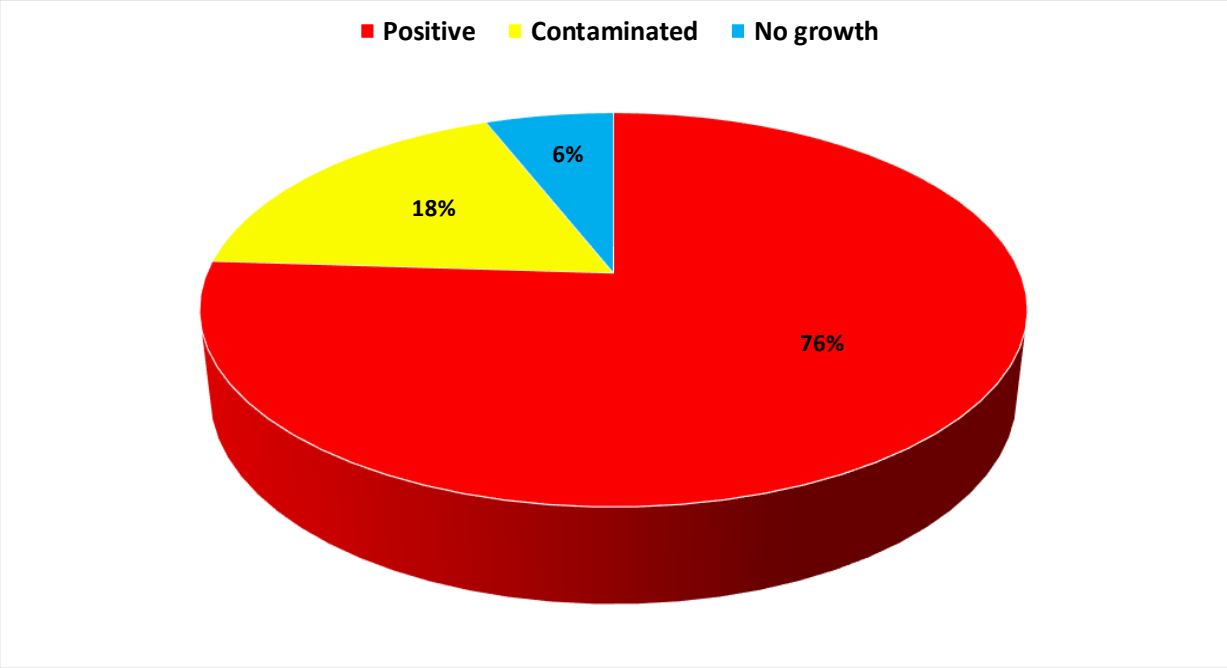

Figure 1 Results of the study of individual samples of udder secretions.

Number of samples analyzed: 320 samples were analyzed.

Number of repeated analyses: Each study was carried out five times, with the number of samples being four, which amounted to twenty repeated analyses.

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

Design of the experiment: The study was conducted on 3 dairy farms, in separate research units of the National University of Life and Environmental Sciences of Ukraine, "Velikosnityn educational and research farm named after O. V. Muzychenka", "Agronomic Research Station", "Educational and Research Farm "Vorzel" of Kyiv Region, Ukraine.

All research in research farms was conducted by a group of researchers consisting of 5 people in the period from July 2021 to October 2022. Management practices, housing conditions and milking procedures were assessed and documented in a standardized data collection form. Milking patterns were recorded by observing regular milking during one milking period. Observations during the visit were recorded during the keeping of cows with mastitis.

After conducting a clinical examination of the udder of cows and a laboratory study of its secretion, using the California mastitis test, samples of secretion from animals with mastitis were collected in sterile test tubes.

Then the samples were cooled to a temperature of +2 to +4 °C and immediately transported to the laboratory. Selected samples of udder secretions were subjected to bacteriological examination, followed by testing of selected isolates for antimicrobial substances.

Statistical Analysis

Simple descriptive statistics were used. The results of bacteriological cultures were expressed as a percentage of individual microbial species isolated. Sensitivity results were expressed as a percentage – as the percentage of sensitive isolates to each type of antimicrobial substance.

RESULTS AND DISCUSSION

Studies conducted in Slovakia showed that 21 yeast strains and 500 bacterial strains of 25 types were isolated from 633 samples of mammary gland secretions. The most common pathogens were coagulase-negative staphylococci, which made up 35.9% of positive results; the second most common was *E. coli* – 14.8%, followed by *S. aureus* (12.5%), *Str. uberis* (10.9%) and *Streptococcus agalactiae* (5.8%). We found that contagious pathogens of mastitis in cows accounted for 184 (57.5%) of isolated isolates: *Streptococcus agalactiae* – in 77 (24.1%), *Staphylococcus aureus* – in 59 (18.4%), *Corynebacterium* spp. – in 23 (7.2%), *Streptococcus dysgalactiae* – in 18 (5.6%), *Streptococcus uberis* – in 7 (2.2%) isolates, and environmental (non-infectious) mastitis pathogens – in 136 (42.5%) isolates. Most of the bacteria belonged to Gram-positive microflora, in particular to staphylococci in 33 samples (*Staphylococcus* spp. – 10.3%) and streptococci in 37

(11.5%) samples (*Streptococcus spp.* – 20 (6.2%), *Streptoccocus parauberis* – 14 (4.4%) samples, *Streptoccocus bovis* – 3 (0.9%) isolates. Gram-negative bacteria accounted for 66 (20.6%) isolates, among which the largest percentage was accounted for by *E. coli* 27 (8.4%) samples and *Klebsiella pneumonia* 6 (1.9%) samples.

The results of the bacteriological study of individual samples of udder secretions (from the affected udder lobes) showed (Table 1, Figure 2), which was most often isolated from the studied samples *Streptococcus agalactiae* (Figure 3 and 4), *Staphylococcus aureus* (Figure 5 and 6), *Staphylococcus spp.* (Figure 7 and 8) and *E. coli* (Figure 9 and 11).

Table 1 Isolated microflora from samples of cow udder secretions for mastitis.

It. no.	Microflora	RESULT	
		Total	Total
1	*Streptococcus agalactiae*	77	24.1
2	*Staphylococcus aureus*	59	18.4
3	*Staphylococcus* spp.	33	10.3
4	*E. coli*	27	8.4
5	*Corynebacterium* spp.	23	7.2
6	*Streptococcus* spp.	20	6.2
7	*Streptococcus dysgalactiae*	18	5.6
8	*Streptococcus parauberis*	14	4.4
9	*Trueperella pyogenes*	10	3.1
10	*Bacillus* spp.	10	3.1
11	*Streptococcus uberis*	7	2.2
12	*Klebsiella pneumoniae*	6	1.9
13	*Yeast*	5	1.6
14	*Enterobacteriaceae*	4	1.3
15	*Klebsiella terrigenous*	4	1.3
16	*Streptococcus Bovis*	3	0.9
	Total	**320**	**100**

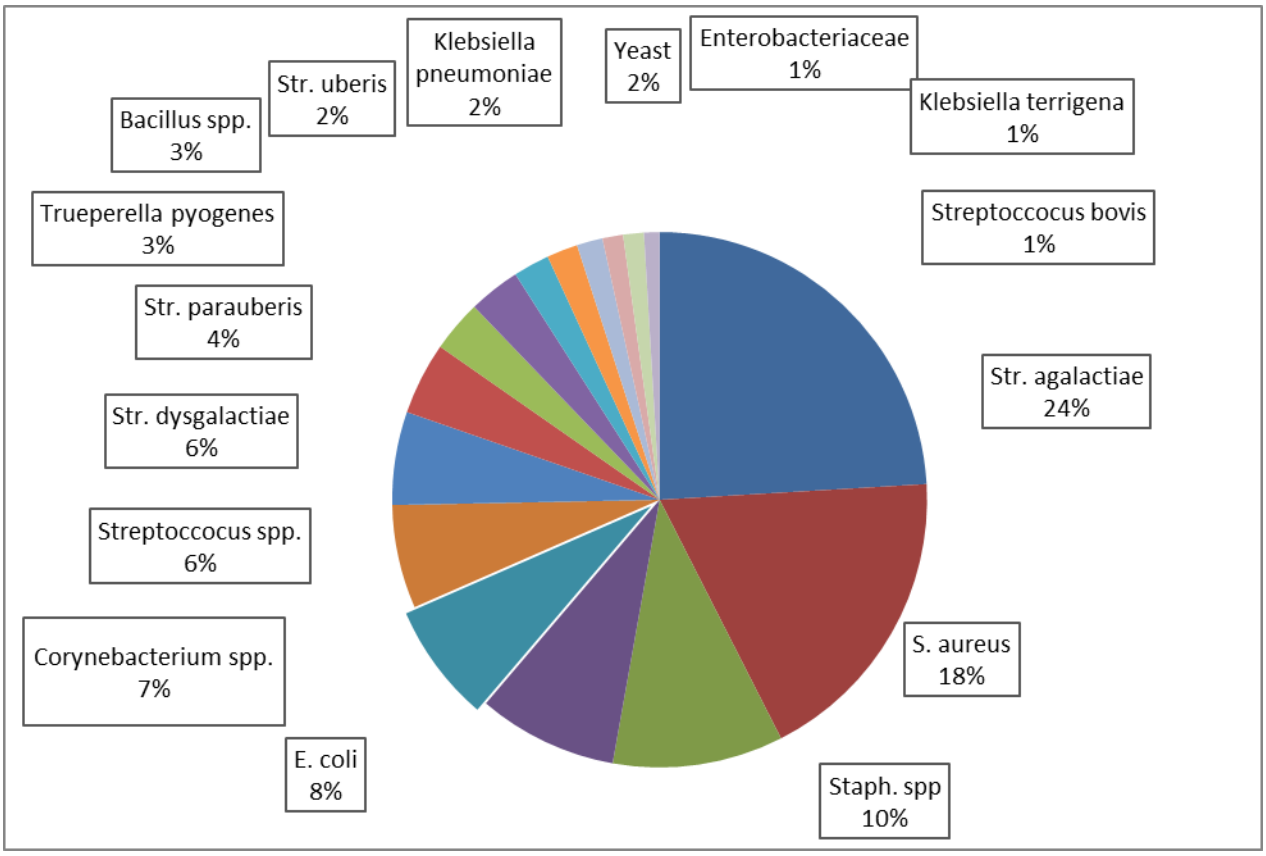

Figure 2 Total number of isolated isolates from milk samples from cows with mastitis.

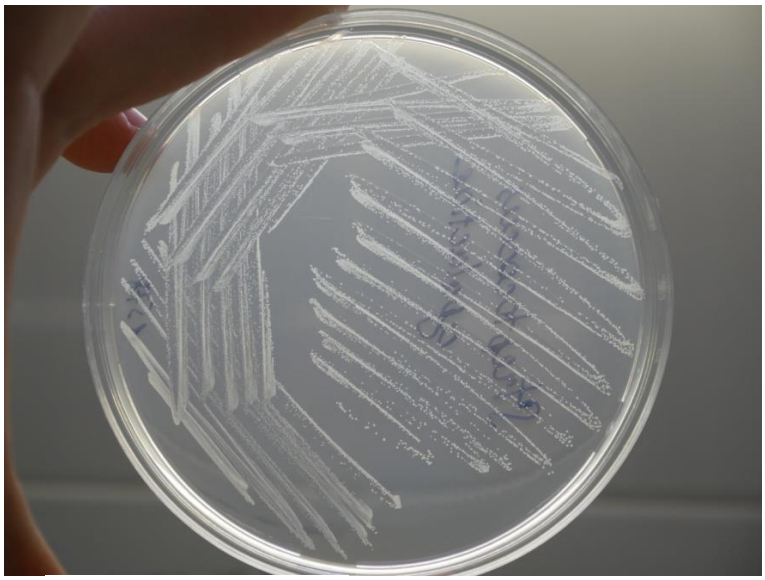

Figure 3 Bacterial colonies *Streptococcus agalactiae* on Muller-Hinton agar.

Figure 4 Bacterial colonies *Streptococcus agalactiae* on blood agar.

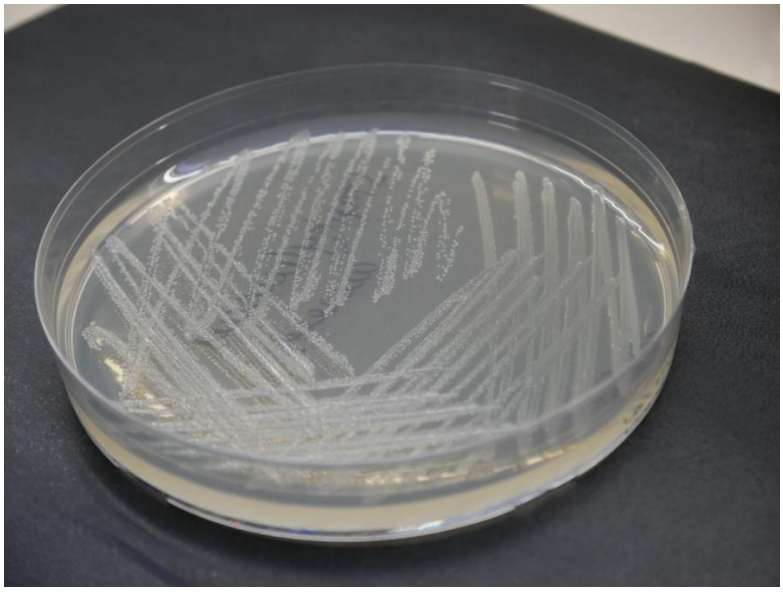

Figure 5 Bacterial colonies *Staphylococcus aureus* Muller-Hinton agar.

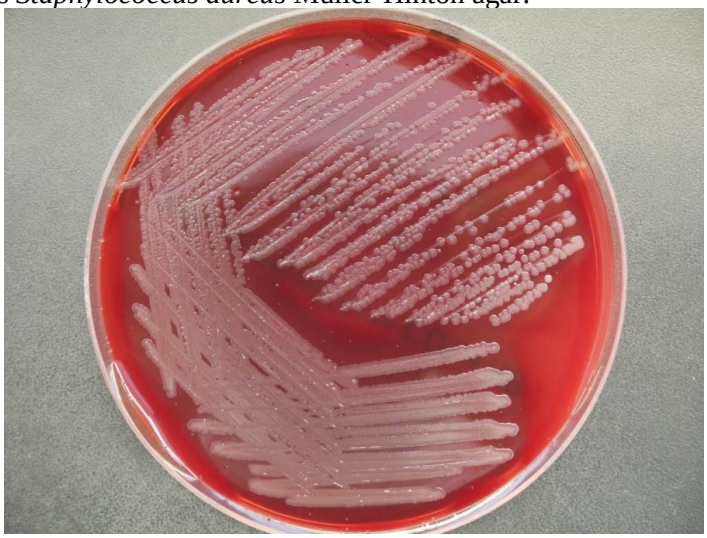

Figure 6 Bacterial colonies *Staphylococcus aureus on* Blood agar.

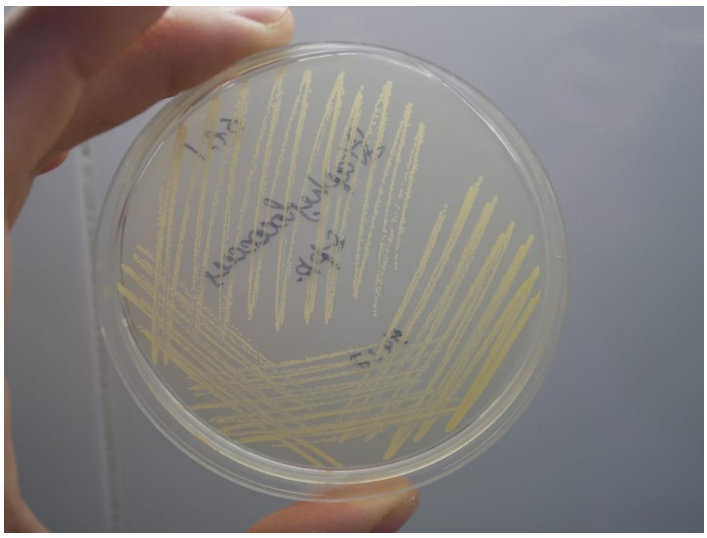

Figure 7 Bacterial colonies *Staphylococcus spp.* on Muller-Hinton agar.

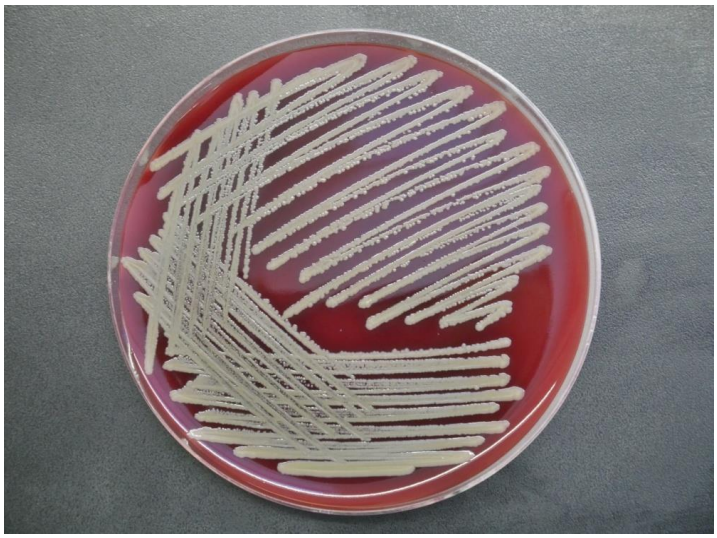

Figure 8 Bacterial colonies *Staphylococcus spp.* on Blood agar.

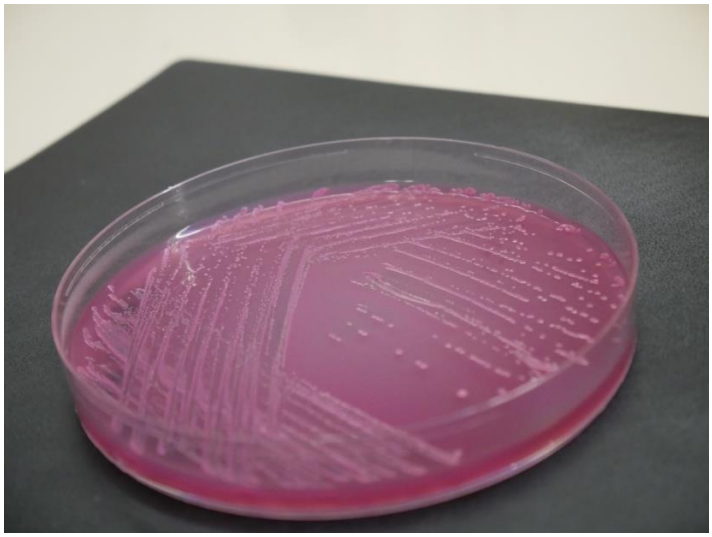

Figure 9 Bacterial colonies *E.coli* on McConkey agar.

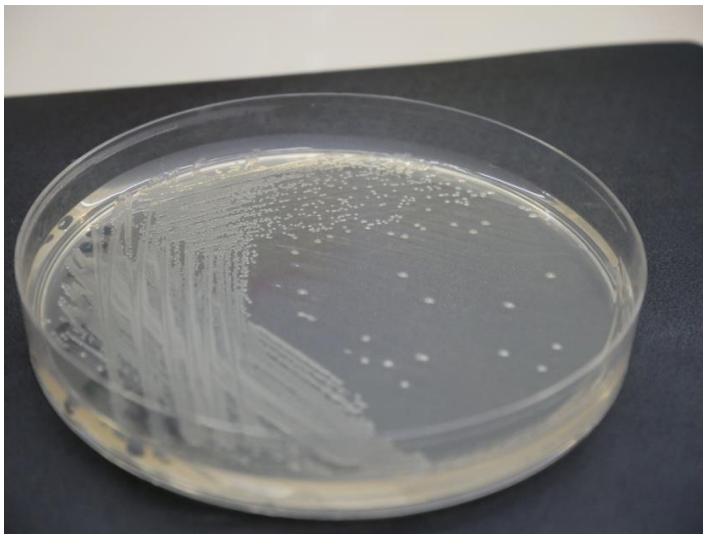

Figure 10 Bacterial colonies *E.coli* on Muller-Hinton agar.

Figure 11 Bacterial colonies *of E. coli* on Blood agar.

Studies conducted in Germany on dairy farms out of 751 clinical cases of cow mastitis indicate the spread of bacterial pathogens of mastitis *Staphylococcus aureus* – 10.0%, *Streptococcus uberis* – 8.5% and coliforms, mainly Escherichia coli, were isolated in 10.2% **[22]**. Studies in France have shown that 707 positive isolates of mammary gland secretions taken from cows with clinical mastitis *S. aureus* occurred in 15.8% of cases,

S. uberis in 22.1%, and *E. coli* 16.0% **[23]**, **[36]**. Studies conducted in Sweden **[24]** out of 743 isolates from 669 cows with a clinical mastitis showed that *S. aureus*, *S. uberis*, and *E. coli* made were 28.4%, 15.2%, and 21.9%, respectively. In the Netherlands out of 438 mammary gland secretion samples from cows with subclinical mammary isolates, *S. aureus* was detected in 18.0%, and *S. uberis* in 9.6% of cases **[25]**, **[37]**.

Studies conducted in Mexico showed that 20 different types of yeast were identified in 282 (25.75%) secret samples **[26]**, **[38]**.

According to the results of our studies, bovine mastitis caused by yeast was detected in 5 (1.4%) isolates of the total number of diagnosed pathogens. The data obtained by us are consistent with the results of research by other authors **[4]**.

Based on the results of the obtained bacteriological studies, the sensitivity of isolated mastitis pathogens to antimicrobial substances was determined (Table 2 – 4).

Studies conducted in Brazil show that out of 89 isolates of *Str. agalactiae*high sensitivity was to Ceftiofur, enrofloxacin, ampicillin, gentamicin, and lincomycin, and the isolates were resistant to neomycin and tetracycline **[27]**, **[39]**. In Germany, studies of milk from cows with mastitis show that this isolate was resistant to Sulfatrimethoprim 50.5%, tetracycline 46.2%, and erythromycin 15.4% **[28]**. As the results of our study show, the isolation of isolated *Str. agalactiae* showed a high level of sensitivity to amoxicillin in 73 (94.8%) isolates, Amoxicillin/claulanic acid in 71 (92.2%) samples. Moderately sensitive to Rifampicin in 65 (84.4%), Ampicillin in 64 (83.1%), Ceftiofur in 61 (77.2%), lincomycin, Cloxacillin, Bacitracin in 61 (77.2%) isolates, to Cephalexin in 55 (71.4%) and Oxytetracycline in 39 (50.6%) isolates. Weakly sensitive to Trimethoprim/sulfamethoxazole, Gentamicin in 36 (46.7%) isolates, Enrofloxacin in 22 (28.5%), Tylosin in 17 (22%), Tilmicosin in 21 (27.3%), to Danofloxacin in 14 (18.2%), to Marbofloxacin in 13 (16.9%), to Spiramycin in 12 (15.5%) isolates, Neomycin and Streptomycin in 6 (7.8%) isolates.

Studies conducted on farms in Ukraine show that isolate *S. aureus* was sensitive to Gentamicin in 77.97% **[29]**, and in 70% of isolates, *S. aureus* – was resistant to Ampicillin, Oxacillin, and Tetracycline **[32]**, **[40]**. Our study shows that *staphylococcus aureus* was highly sensitive to Gentamicin in 59 (100%) isolates, Ceftiofur in 58 (98.3%), Rifampicin in 57 (96.6%), to Cloxacillin in 56 (94.9%), to Cephalexin in 54 (91.5%) isolates. Moderately sensitive to Bacitracin in 54 (86.4%) isolates, to Trimethoprim/sulfamethoxazole in 49 (83%), to Amoxicillin/claulanic acidenrofloxacin in 48 (81.3%) isolates, to Amoxicillin in 40 (67.8%), to Oxytetracycline in 39 (66.1%), to Neomycin in 38 (64.4%), to Lincomycin in 33 (55.95%) isolates. The isolates were weakly sensitive to ampicillin in 28 (47.4%) isolates, Danofloxacin in 25 (42.4%), Tilmicosin in 24 (40.7%), Streptomycin in 22 (37.3%), Marbofloxacin in 18 (30.5%), Tylosin in 10 (16.9%) and Spiramycin in 4 (6.8%) isolates.

Studies of secretions from sick cows with mastitis in Algeria demonstrate the sensitivity of isolate*d staphylococcus spp* to gentamicin and Neomycin **[30]**, which coincides with the results of our studies, which showed that isolate*d staphylococcus spp*, which showed high sensitivity to Rifampicin in 30 (90.9%) isolates, to Amoxicillin/Claulanic acid, to Enrofloxacin, Ceftiofur, Gentamicin – in 29 (87.9%) isolates, to Cloxacillin, Bacitracin, Cephalexin – in 27 (81.8%) isolates, to Neomycin in 24 (72.7%), to Ampicillin in 23 (69.7%), to Amoxicillin in 22 (66.7%), to Oxytetracycline in 21 (63.6%), to Trimethoprim in 19 (57.6%). Moderately sensitive and weakly sensitive were to Streptomycin in 16 (48.5%) isolates, Lincomycin and Tilmicosin in 14 (42.4%) isolates, Marbofloxacin in 12 (36.3%), Danofloxacin in 10 (30.3%), to Tylosin in 6 (18.2%), to Spiramycin in 4 (12.1%) isolates.

Studies of milk from sick cows for the clinical form of mastitis on farms in Bangladesh have shown high resistance of Escherichia coli to Amoxicillin, Ampicillin, and Tetracycline **[31]**, **[41]**. Studies conducted in Canada indicate that this isolate was insensitive to Streptomycin, Tetracycline, Ampicillin, and Colistin, but showed sensitivity to Ciprofloxacin and Gentamicin **[33]**, **[42]**. According to the authors **[32]**, more than 60% of isolates *of E. coli* showed resistance to Oxacillin and Sulfamethoxazole-trimethoprim.

Our research has shown that *E. coli* showed high sensitivity to Ceftiofur, which is consistent with the results of the researchers **[34]**, **[43]**. and Gentamicin – in 27 (100%) isolates, to Enrofloxacin and Oxytetracycline – in 25 (92.5%) isolates. The medium-sensitive was isolating to Amoxicillin/Claulanic acid and Ampicillin – in 24 (88.8%) isolates, to Danofloxacin in 20 (74%), to Trimethoprim/sulfamethoxazole in 19 (70.3%), to Amoxicillin and Marbofloxacin – in 18 (66.6%), to Streptomycin in 6 (22.2%), to Cephalexin in 5 (18.5%)and Neomycin in 4 (14.8%) isolates. Highly resistant isolate *E. coli* was to Lincomycin, Cloxacillin, Tylosin, Bacitracin, Spiramycin, Tilmicosin, and Rifampicin.

Isolates*Corynebacterium spp* were highly sensitive to Gentamicin and Rifampicin in 23 (100%) isolates, to Ampicillin in 22 (95.6%) isolates, to Ceftiofur, Amoxicillin, and Bacitracin in 21 (91.3%) samples. Medium-sensitive isolates turned out to be Amoxicillin/claulanic acid, Lincomycin. Cephalexin – in 20 (86.9%) isolates, Enrofloxacin in 18 (78.3%), Oxytetracycline in 17 (73.9%) isolates, to Streptomycin, Marbofloxacin and

Tilmicosin – in 14 (60.9%) isolates, to Danofloxacin and Tylosin – in 13 (56.5%) isolates. Low sensitivity of the isolates was shown to Cloxacillin and Neomycin – in 11 (47.8%), Spiramycin in 9 (39.1%), and Trimethoprim/sulfamethoxazole in 4 (17.3%) isolates.

Table 2 Sensitivity of isolated mastitis pathogens to antimicrobial substances.

it. no.	Antibiotic	*Streptoccocus agalactiae*		*Staphylococcus aureus*		*Staphylococcus* spp.		*E. coli*		*Corynebacterium* spp.	
		n	***%***	***n***	***%***	***n***	***%***	***n***	***%***	***n***	***%***
1	Amoxicillin (25 µg/disc)	73	94.8	40	67.8	22	66.7	18	66.6	21	91.3
2	Amoxicillin+Cl.acid (30µg/disc)	71	92.2	48	81.3	29	87.9	24	88.8	20	86.9
3	Enrofloxacine (10 µg/disc)	22	28.5	48	81.3	29	87.9	25	92.5	18	78.3
4	Streptomycin (10 µg/disc)	6	7.8	22	37.3	16	48.5	6	22.2	14	60.9
5	Trimethoprim/ Sulfamethoxazole (25µg/disc)	36	46.7	49	83	19	57.6	19	70.3	4	17.3
6	Oxytetracycline (30 µg/disc)	39	50.6	39	66.1	21	63.6	25	92.5	17	73.9
7	Ceftiofur (30 mcg)	61	77.2	58	98.3	29	87.9	27	100	21	91.3
8	Ampicillin (10 µg/disc)	64	83.1	28	47.4	23	69.7	24	88.8	22	95.6
9	Gentamicin (10 µg/disc)	36	46.7	59	100	29	87.9	27	100	23	100
10	Neomycin (30 µg/disc)	6	7.8	38	64.4	24	72.7	4	14.8	11	47.8
11	Lincomycin (15 µg/disc)	61	77.2	33	55.9	14	42.4	0	0	20	86.9
12	Cloxacillin (5 µg/disc)	61	77.2	56	94.9	27	81.8	0	0	11	47.8
13	Tylosin (30µg/disc)	17	22	10	16.9	6	18.2	0	0	13	56.5
14	Bacitracin (0.04 µg/disc)	61	77.2	51	86.4	27	81.8	0	0	21	91.3
15	Cephalexin (30 µg/disc)	55	71.4	54	91.5	27	81.8	5	18.5	20	86.9
16	Danofloxacin (5 µg/disc)	14	18.2	25	42.4	10	30.3	20	74	13	56.5
17	Spiramycin (100 µg/disc)	12	15.5	4	6.8	4	12.1	0	0	9	39.1
18	Marbofloxacin (5 µg/disc)	13	16.9	18	30.5	12	36.3	18	66.6	14	60.9
19	Tilmicosin (15 µg/disc)	21	27.3	24	40.7	14	42.4	0	0	14	60.9
20	Rifampicin (5 µg/disc)	65	84.4	57	96.6	30	90.9	0	0	23	100

The results presented in Table 3 showed that *Streptococcus spp* showed high sensitivity to the following antimicrobial substances: Ceftiofur in 18 (90%) isolates, Ampicillin, and Bacitracin – 17 (85%) isolates. Average sensitivity was to Amoxicillin in 16 (80%) isolates, Rifampicin in 15 (75%) isolates, Amoxicillin/claulanic acid, Gentamicin – in 14 (70%) isolates, Cephalexin in 13 (65%), Cloxacillin in 12 (60%) isolates. Low sensitivity was to Enrofloxacin, and trimethoprim/sulfamethoxazole – 9 (45%) isolates, lincomycin in 8 (40%), Tilmicosin in 7 (35%), Danofloxacin in 6 (30%), to oxytetracycline in 4 (20%), to Marbofloxacin in 3 (15%) isolates, to Streptomycin, Spiramycin, Neomycin, and Tylosin-only in 2 (10%) isolates. However, previous studies conducted in Poland show that the highest resistance of the bacterium of the genus *Streptococcus spp* was to Gentamicin, Kanamycin, and Tetracycline. In contrast, the highest sensitivity was observed to Penicillin, Enrofloxacin, and Marbofloxacin **[35]**, **[44]**.

The high sensitivity of isolate*d streptococcus dysgalactiae* was to Ceftiofur and Bacitracin – 18 (100%) isolate, to Cloxacillin – 17 (94.4%) isolates. Medium-sensitive of isolates were Cephalexin in 16 (88.9%) isolates, Amoxicillin/claulanic acid in 5 (83.3%), Ampicillin in 14 (77.8%) isolates, Rifampicin and Lincomycin -13 (72.2%) isolates, to Trimethoprim/sulfamethoxazole and Enrofloxacin – 12 (66.6%) isolates, to Gentamicin-

9 (50%) isolates. Low sensitivity was shown to Amoxicillin in 8 (44.4%), Tilmicosin 6 (33.3%), Marbofloxacin 5 (27.8%), Danofloxacin 4 (22.2%), Spiramycin 3 (16.6%), to Neomycin 2 (11.1%) isolates and was almost resistant to Tylosin, Streptomycin, and Oxytetracycline – only 1 (5.5%) isolate.

Streptococcus parauberis was insensitive to Spiramycin, Marbofloxacin, and Tilmicosin but was sensitive to Bacitracin 12 (85.7%), Amoxicillin 11 (78.6%) isolates, Ampicillin and Rifampicin 10 (71.4%) isolates, to Amoxicillin/claulanic acid, Ceftiofur, Cloxacillin and Cephalexin 9 (64.3%) isolate, to Trimethoprim/sulfamethoxazole 7 (50%) isolates and weakly sensitive – to Enrofloxacin 6 (42.8%), Gentamicin 5 (35.7%), Lincomycin 3 (21.4%) isolates, Streptomycin, Oxytetracycline, and neomycin – 2 (14.2%) isolates, to Tylosin and Danofloxacin only 1 (7.1%) isolate.

Bacteria *Trueperella pyogenes* showed high sensitivity to Amoxicillin, Ceftiofur, rifampicin-10 (100%) isolates, Amoxicillin/claulanic acid, Cephalexin, Ampicillin – 9 (90%) isolates, medium sensitivity to Enrofloxacin, Lincomycin – showed 8 (80%) isolates, to Gentamicin, Bacitracin, Marbofloxacin, Cloxacillin – showed 7 (70%) isolates, to Tilmicosin 6 (60%), Oxytetracycline 5 (50%) isolates. Low sensitivity to the following antibiotics: Trimethoprim/sulfamethoxazole 4 (40%) isolates, Tylosin, Danofloxacin, Spiramycin – 3 (30%) isolates, Streptomycin 2 (20%) isolates, insensitive to Neomycin. Recent studies **[45]**, **[46]** show that most isolates *T. pyogenes*, were highly sensitive to Amoxicillin, Ampicillin, Gentamicin, and Ceftiofur. At the same time, a high level of resistance was observed to Trimethoprim/sulfamethoxazole and Tylosin, which coincides with our research results.

Isolates*Bacillus spp* were highly sensitive to Enrofloxacin 10 (100%) isolates, Rifampicin, Ceftiofur, and Ampicillin 9 (90%) isolates, and were moderately sensitive to Amoxicillin, Trimethoprim/sulfamethoxazole, Gentamicin, Cephalexin, Tilmicosin 8 (80%) isolates, Amoxicillin/claulanic acid and Oxytetracycline 7 (70%) isolates, Neomycin, Streptomycin, and Cloxacillin – 6 (60%) isolates. They were weakly sensitive to Lincomycin, and Bacitracin 4 (40%) isolate, to Danofloxacin 3 (30%), Tylosin 2 (20%) isolate, and Spiramycin 1 (10%) isolates and generally not sensitive *to Bacillus spp.* was to Marbofloxacin.

The study presented in table 4 shows that the highest sensitivity of *Streptococcus uberis* showed (100%) isolates to Ampicillin Ceftiofuria 7. It was moderately sensitive to Amoxicillin, Cloxacillin, Bacitracin, Cephalexin, and Rifampicin – 6 (85.7%) isolates and Oxytetracycline 4 (57.1%) isolates. Hypersensitive was to Amoxicillin / claulanic acid, and Danofloxacin 3 (42.2%) isolates, Gentamicin and Marbofloxacin only 2 (28.6%) isolates. Once isolate*d streptococcus uberis* was sensitive to Enrofloxacin, Trimethoprim/sulfamethoxazole, Lincomycin, Tylosin, and Spiramycin, which is 14.2%, respectively, and resistant to Streptomycin, Neomycin, and Tylmycosin.

All isolates *of Klebsiella pneumoniae* we have selected demonstrated high sensitivity to only one antimicrobial substance– Gentamicin 6 (100%) isolates. The average sensitivity was up to Amoxicillin/claulanic acid 5 (83.3%) isolates, weakly sensitive to Ceftiofur 2 (33.3%) isolates, once the isolate showed sensitivity to Enrofloxacin, Trimethoprim/sulfamethoxazole, Oxytetracycline, Danofloxacin, Marbofloxacin, which is 16.7%, and showed high resistance to Amoxicillin, Streptomycin, Ampicillin, Neomycin, Lincomycin, Cloxacillin, Tylosin, Bacitracin, Cephalexin, Spiramycin, Tilmicosin, and Rifampicin.

Isolates *Klebsiella terrigen*ous was highly sensitive to Trimethoprim/sulfamethoxazole 4 (100%), Oxytetracycline 4 (100%), Gentamicin 4 (100%), Ceftiofur 4 (100%), medium-sensitive to Amoxicillin/claulanic acid 3 (75%), Enrofloxacin 3 (75%), to Danofloxacin 3 (75%), to Marbofloxacin 3 (75%), to Cephalexin 2 (50%) and Streptomycin 1 (25%) and insensitive to Amoxicillin, Ampicillin, Neomycin, Lincomycin, Cloxacillin, Tylosin, Bacitracin, Spiramycin, Tilmicosin, and Rifampicin.

Table 3 Sensitivity of isolated mastitis pathogens to antimicrobial substances.

It. no.	Antibiotic	*Streptococcus spp.*		*Streptococcus dysgalactiae*		*Streptoccocus parauberis*		*Trueperella pyogenes*		*Bacillus spp.*	
		n	***%***	***n***	***%***	***n***	***%***	***n***	***%***	***n***	***%***
1	**Amoxicillin (25 µg/disc)**	16	80	8	44.4	11	78.6	10	100	8	80
2	**Amoxicillin+Cl.acid (30 µg/disc)**	14	70	15	83.3	9	64.3	9	90	7	70
3	**Enrofloxacine (10 µg/disc)**	9	45	12	66.6	6	42.8	8	80	10	100
4	**Streptomycin (10 µg/disc)**	2	10	1	5.5	2	14.2	2	20	6	60
5	**Trimethoprim/ Sulfamethoxazole (25µg/disc)**	9	45	12	66.6	7	50	4	40	8	80
6	**Oxytetracycline (30 µg/disc)**	4	20	1	5.5	2	14.2	5	50	7	70
7	**Ceftiofur (30 mcg)**	18	90	18	100	9	64.3	10	100	9	90
8	**Ampicillin (10 µg/disc)**	17	85	14	77.8	10	71.4	9	90	9	90

Table 3 Cont.

It. no.	Antibiotic	*Streptococcus spp.*		*Streptococcus dysgalactiae*		*Streptoccocus parauberis*		*Trueperella pyogenes*		*Bacillus spp.*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
9	**Gentamicin (10 µg/disc)**	14	70	9	50	5	35.7	7	70	8	80
10	**Neomycin (30 µg/disc)**	2	10	2	11.1	2	14.2	0	0	6	60
11	**Lincomycin (15 µg/disc)**	8	40	13	72.2	3	21.4	8	80	4	40
12	**Cloxacillin (5 µg/disc)**	12	60	17	94.4	9	64.3	7	70	5	50
13	**Tylosin (30 µg/disc)**	2	10	1	5.5	1	7.1	3	30	2	20
14	**Bacitracin (0.04 µg/disc)**	17	85	18	100	12	85.7	7	70	4	40
15	**Cephalexin (30 µg/disc)**	13	65	16	88.9	9	64.3	9	90	8	80
16	**Danofloxacin (5 µg/disc)**	6	30	4	22.2	1	7.1	3	30	3	30
17	**Spiramycin (100 µg/disc)**	2	10	3	16.6	0	0	3	30	1	10
18	**Marbofloxacin (5 µg/disc)**	3	15	5	27.8	0	0	7	70	0	0
19	**Tilmicosin (15 µg/disc)**	7	35	6	33.3	0	0	6	60	8	80
20	**Rifampicin (5 µg/disc)**	15	75	13	72.2	10	71.4	10	100	9	90

Enterobacteriaceae bacteria family showed a high sensitivity to Enrofloxacin, Trimethoprim/sulfamethoxazole, and Gentamicin-4 (100%) isolates. Average sensitivity was shown to Danofloxacin and Marbofloxacin – 3 (75%) isolates, Bacitracin and Oxytetracycline – 2 (50%) isolates. Once, they were sensitive to Amoxicillin, Ampicillin, Neomycin, Cloxacillin, Cephalexin, Tilmicosin, and rifampicin, which is 25%. Amoxicillin / claulanic acid, Streptomycin, Ceftiofur, Lincomycin, Tylosin, and Spiramycin were highly resistant.

Only 3 isolates *of Streptococcus Bovis* were isolated during the study of mammary glands secretions, which showed 100% sensitivity to Amoxicillin, Amoxicillin / claulanic acid, Ceftiofur, Ampicillin, Gentamicin, Tylosin, Cephalexin, and Rifampicin. The average sensitivity was to Trimethoprim/sulfamethoxazole, Cloxacillin, Bacitracin, Danofloxacin, and Tilmicosin – 2 (66.7%) isolates. Once, they were sensitive to Oxytetracycline, and Lincomycin, 33.3 %, respectively, and were highly resistant to Enrofloxacin, Streptomycin, Neomycin, Spiramycin, and Marbofloxacin.

Table 4 Sensitivity of isolated mastitis pathogens to antimicrobial substances.

It. no.	Antibiotic	*Streptococcus uberis*		*Klebsiella pneumoniae*		*Enterobacte-riaceae*		*Klebsiella terrigenous*		*Streptoc-cocus bovis*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
1	**Amoxicillin (25 µg/disc)**	6	85.7	0	0	1	25	0	0	3	100
2	**Amoxicillin+Cl.acid (30 µg/disc)**	3	42.8	5	83.3	0	0	3	75	3	100
3	**Enrofloxacine (10 µg/disc)**	1	14.2	1	16.7	4	100	3	75	0	0
4	**Streptomycin (10 µg/disc)**	0	0	0	0	0	0	1	25	0	0
5	**Trimethoprim/ Sulfamethoxazole (25µg/disc)**	1	14.2	1	16.7	4	100	4	100	2	66.7
6	**Oxytetracycline (30 µg/disc)**	4	57.1	1	16.7	2	50	4	100	1	33.3
7	**Ceftiofur (30 mcg)**	7	100	2	33.3	0	0	4	100	3	100
8	**Ampicillin (10 µg/disc)**	7	100	0	0	1	25	0	0	3	100
9	**Gentamicin (10 µg/disc)**	2	28.6	6	100	4	100	4	100	3	100
10	**Neomycin (30 µg/disc)**	0	0	0	0	1	25	0	0	0	0
11	**Lincomycin (15 µg/disc)**	1	14.2	0	0	0	0	0	0	1	33.3
12	**Cloxacillin (5 µg/disc)**	6	85.7	0	0	1	25	0	0	2	66.7
13	**Tylosin (30µg/disc)**	1	14.2	0	0	0	0	0	0	3	100
14	**Bacitracin (0,04 µg/disc)**	6	85.7	0	0	2	50	0	0	2	66.7
15	**Cephalexin (30 µg/disc)**	6	85.7	0	0	1	25	2	50	3	100
16	**Danofloxacin (5 µg/disc)**	3	42.8	1	16.7	3	75	3	75	2	66.7
17	**Spiramycin (100 µg/disc)**	1	14.2	0	0	0	0	0	0	0	0
18	**Marbofloxacin (5 µg/disc)**	2	28.6	1	16.7	3	75	3	75	0	0
19	**Tilmicosin (15 µg/disc)**	0	0	0	0	1	25	0	0	2	66.7
20	**Rifampicin (5 µg/disc)**	6	85.7	0	0	1	25	0	0	3	100

As can be seen from the results of the study shown in Table 5, most of the isolated isolates were sensitive to Ceftiofur – 86.3%, Amoxicillin/claulanic acid – 76.6%, Rifampicin – 75.6%.

Table 5 Distribution of the total number of isolated mastitis pathogens by sensitivity to various antimicrobial substances.

It. no.	Antibiotic	The total number of isolates obtained	Number of sensitive isolates	% sensitive isolates
1	**Amoxicillin (25 µg/disc)**		237	74.1
2	**Amoxicillin+Cl.acid (30 µg/disc)**		245	76.6
3	**Enrofloxacine (10 µg/disc)**		196	61.3
4	**Streptomycin (10 µg/disc)**		78	24.4
5	**Trimethoprim/ Sulfamethoxazole (25 µg/disc)**		179	55.9
6	**Oxytetracycline (30 µg/disc)**		172	53.8
7	**Ceftiofur (30 mcg)**		276	86.3
8	**Ampicillin (10 µg/disc)**		231	72.2
9	**Gentamicin (10 µg/disc)**		236	73.6
10	**Neomycin (30 µg/disc)**	320	96	30.0
11	**Lincomycin (15 µg/disc)**		166	51.9
12	**Cloxacillin (5 µg/disc)**		214	66.9
13	**Tylosin (30 µg/disc)**		59	18.4
14	**Bacitracin (0,04 µg/disc)**		228	71.3
15	**Cephalexin (30 µg/disc)**		228	71.3
16	**Danofloxacin (5 µg/disc)**		111	34.7
17	**Spiramycin (100 µg/disc)**		39	12.2
18	**Marbofloxacin (5 µg/disc)**		99	30.9
19	**Tilmicosin (15 µg/disc)**		103	32.2
20	**Rifampicin(5µg/disc)**		242	75.6

Weak sensitivity of 320 isolated isolates was shown to Spiramycin (Spiramycin) – 12.2%, Tylosin – 18.4%, Streptomycin – 24.4%, neomycin – 30%, Marbofloxacin – 30.9%, Tilmicosin – 32.2%, Danofloxacin – 34.7%.

A significant percentage (74-51.9%) of the obtained isolates were sensitive (in descending order) to Amoxicillin – 74%, Gentamicin – 73.6%, Ampicillin – 72.2% and Bacitracin – 71.3% and Cephalexin – 71.3%, Cloxacillin – 66.9%, Enrofloxacin – 61.3%, Trimethoprim/sulfamethoxazole – 55.9%, Oxytetracycline – 53.8% and Lincomycin – 51.9%.

CONCLUSION

Contagious pathogens of cow mastitis are diagnosed in 57.5% of isolates from the total number of 24.1%, *Staphylococcus aureus* 18.4%, *Corynebacterium spp*. 7.2%, *Streptococcus dysgalactiae* 5.6%, *and Streptococcus uberis* 2.2% were most often detected.

Environmental pathogens make up 42.5% of all isolated isolates, most bacteria are Gram-positive microflora. In particular, streptococci 11.5% (*Streptococcus spp*. 6.2%, *Str. parauberis* 4.4%, *Str. bovis* 0.9%), *Staphylococcus spp*. 10.3 %. The landscape of Gram-negative microflora is 20.6%, among which the most significant percentage belongs to *E. coli* 8.4% and *Klebsiella pneumoniae* 1.9%.

Mastitis caused by fungi (yeast) accounts for more than 1.4% of the total number of diagnosed mastitis pathogens.

A significant percentage of the obtained isolates showed sensitivity (in descending order) to Ceftiofur, Amoxicillin/claulanic acid, Rifampicin, Amoxicillin, Gentamicin, Ampicillin, Bacitracin, Cephalexin, Cloxacillin, Enrofloxacin, Trimethoprim/sulfamethoxazole, Oxytetracycline, Lincomycin. The least sensitive isolates were Spiramycin, Tylosin, Streptomycin, Neomycin, Marbofloxacin, Tilmicosin, and Danofloxacin.

The prospect of further research will be to improve the analysis of the sensitivity of pathogens to antimicrobial substances and establish the terms of care and flow to the quality indicators of milk.

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The production of the innovative craft cheese "Anchan"

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ABSTRACT

The analysis of regional raw materials for producing craft cheese "Anchan" and studies of raw milk for its physical and chemical properties and technological indicators. Milk samples were pasteurized in the laboratory at a temperature of 80 °C for 10 seconds. Anchan was added to the milk for colour. Next, the milk before coagulation was heated in a pasteurization boiler by heating with saturated steam 36 – 38 °C. The enzyme 4 mL per 100 kg of milk and 4 mL of black cornflower extract was added to the prepared milk to improve milk coagulation and the formation of a dense cheese clot. Strains of probiotic cultures were selected for Anchan. The composition of the main complex yeast of mesophilic lactococci acid and aroma-forming cultures, namely *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. Lactococcus lactis subsp. diacetilactis*, *Leuconostoc lactis*. As an additional leaven used thermophilic lactic acid sticks of the species *Lactobacillus acidophilus* (incoherent race to obtain a new taste of craft cheese. Using these ingredients reduced fermentation time by 8 – 10 minutes. Closing the skin of the cheese by watering the cheese heats with hot water (50 – 55 °C). Marking, packaging, transportation and storage were carried out per the craft product's specifications for the craft producer. The following criteria were used for optimizing the technological process of Anchan cheese production: temperature treatment of milk, amount of added water for whey deoxidation and amount of salt in cheese. as a result of previous research.

Keywords: craft cheese, milk production, probiotic culture, cornflower, temperature treatment, Anchan

INTRODUCTION

Ukraine is traditionally famous for its range of hard cheeses, a small amount of soft cheeses, processed cheeses and cheeses with fillings, while European countries, in particular France and Italy, are traditionally proud of the sophistication of the range of soft cheeses. Hard cheeses have high nutritional and energy value due to their composition, the presence of essential and non-essential amino acids, vitamins of different groups, and mineral salts of calcium, phosphorus and others **[1]**. The concentration of proteins and fats determines the nutritional value of the food. Different product varieties contain 15 – 27% protein and 20 – 32% fat per 100 grams. The energy value of 100 g of cheese is up to 450 kcal. Hard rennet cheeses with different cooking technology are highly nutritious products. The manufacturing technology differs in the enzymatic fermentation of the milk mixture, including different processing temperatures. The difference is also the temperature and maturation of the cheese in the chambers **[2]**. There are more than 2,500 varieties of cheese in the world by name, region of production and composition of raw materials. Statistically, the quantitative composition has no limits because cheese is a work of nature, human hands and time **[3]**. The works of Ukrainian scientists raise the issue of the functioning of the cheese market and highlight the solution to this problem through the use of both secondary raw materials and innovative processing technologies **[4]**, **[5]**.

The State Statistics Service of Ukraine 2021 provides the production of chees in the amount of 15,326 tons, including fresh unfermented cheese (unripe and unripened, including whey cheese and cottage cheese) amounted to 5,589 tons; sour milk cheese and baby food products 488 tons; grated cheese, powdered, blue and other unmelted cheese (excluding fresh cheese, whey cheese and sour milk cheese) 6,688 t; processed cheese (except grated or powdered) 2601 tons. In August 2018, Ukraine produced 17,125 tons of cheese, including fresh unfermented cheese (unripe and unripe, including whey cheese and cottage cheese) is 5,766 tons; of grated cheese, powdered, blue and other unmelted cheese (except fresh cheese, whey cheese and cottage cheese) 8293 t; processed cheese (except grated or powdered) 2442 t.

Long-standing traditions of cheese-making in Ukraine can be traced in the works of modern cheese producers, such as Ukrainets Agro (Cheese Garden brand), Bilozgar (Ukrainian cheese), and Bimol LLC. Possibilities and problems of adaptation of European craft cheese-making to Ukrainian, particularly Vinnytsia, conditions in Podillya from local, regional raw materials are analyzed.

The introduction of craft production in Ukraine has an imperfect control system, unavailability of loans, licensing, limited financial resources, certification, high costs of economic activity, outdated production technologies, and differences in regional prices. Therefore, analyzing problems and prospects of craft cheese production in Vinnytsia is important in the context of Podillya as a region with a developed cheese industry and quality, safe product.

Ukraine has many tasks - not to be afraid of innovations, new standards, and implementation of regulations. Cheese and craft cheese factories are experiencing an innovative boom: they do not live in a separate reality but are part of the global life of global industry because in 2021, in Ukraine will be organized a global craft cheese competition – World Cheese Awards **[2]**. Great prospects for the transformation of quantity into quality and safety of finished cheeses is the task of the technologist to experiment, the opportunity to assess themselves against the product, without worrying about their individuality.

We have proposed the production of natural craft cheese called "Anchan". It is based on the basic features of the technology (pasteurization temperature), low temperature of the second heating and the method of coagulation of milk with the introduction of extract – anchan and black cornflower, which was used to form a cheese clot craft hard cheeses and the use of milk of 2nd and 3rd grades.

The purpose of writing this work is the craft development of Anchan cheese technology based on the laboratory of food production VTEI KNTEU. The laboratory is certified in the quality management system (certificate No UA.80050.063 QMS-21 recertified from 21.06.2021). The article covers the assessment of the composition and quality of milk obtained in Podolia - regional raw materials.

Scientific Hypothesis

In the development of craft technology of Anchan cheese, unique plants were used: Black Cornflower and Anchan. The application was carried out with pasteurized extracts – to form a cheese clot. Strains of probiotic cultures (including acidophilic bacillus) were selected for the aromatic component.

In the technology, we have used the degree of transition of dry matter (SR) into cheese, which depends on seasonal fluctuations in the chemical composition of milk, which are related to the region and seasonality.

MATERIAL AND METHODOLOGY

Samples

We used milk as a raw material that meets the quality standard of DSTU 3662:2018 "Cow's raw milk. Specifications". The black cornflower extract was obtained at the Ladyzhyn factory of bio- and enzyme preparations "Enzym" (Vinnytsia region, Ladyzhyn). The plant raw material of the grass "Anchan" (Clitoria ternatea) is taken from the assortment of the "Svitchayu" company.

Chemicals

When working on the material of the article, we used high-quality chemicals purchased from IKF-Service Plus (Ukraine), distributor.

Animals and Biological Material

In this study, raw materials from the Podillia region were used for the production of craft cheese.

Instruments

The research used a Bond milk analyzer (120 seconds with a printer, Bulgaria), Somatos "Scan" (Bulgaria), an electric stove ESPERANZA EKH008 (Germany), a dry-air thermostat "MICROmed" TS-80 (Bulgaria), an AD130 pH meter ADWA (Bulgaria).

ADS60 hygrometer scales (AXIS, England), Orbita laboratory centrifuge (Ukraine), Lambda 25 spectrophotometer (PerkinElmer Ltd., USA).

The additional and basic leaven of the State Research Enterprise of Bacterial Ferments TIMM, rennet. We proposed to use the flowers of the clitoris of the trifoliate plant with the original name Anchan. In our work, we proposed using a milk-clotting enzyme as an enzyme, namely a plant coagulant - black cornflower *Centurea* spp. Cornflower is a black plant that contains excess cell sap, which helps form clots. The enzyme preparation "Maxiren" from DSM Food Specialties (Netherlands), which is chymosin obtained from special strains of milk yeast *Kluyveromyces lactis*, is also used as a milk coagulant.

Laboratory Methods

Indicators of raw materials and "Anchan" cheese were determined using microbiological, biochemical and physico-chemical generally accepted standard methods of analysis, outlined in the relevant standards and instructions for microbiological and technical-chemical control.

Description of the Experiment

Number of samples analyzed: 20 samples.

Number of repeated analyses: all biochemical procedures were conducted in triplicate.

Number of experiment replication: 2 times.

Design of the experiment:

For the production of "Anchan" craft cheese, we used raw milk that came from the farm to the Lytinsky dairy plant (Vinnytsia region). In fact, milk is not always stable and standard (Table 3). Therefore, the quality of milk pasteurization was checked. Experimental milk samples were processed at (81 ±1) OS with exposure at 20 – 25 C (option II) and ultraraumizocomperia (UVT) (Figure 7). To reduce contamination, the process was carried out in a FJ 15 raw boiler, the pasteurization efficiency reached 99.99%. (Table 4). During the experiment, we did not violate the technological scheme. Our research is about improving the structure and color of cheese. The recipe includes raw materials for the production of calcium chloride, a synthetic drug containing 27% of calcium. The active substance is calcium chloride. The black cornflower Centurea spp and the flowers of the clitoris plant with the original name Anchan for color were used as an enzyme for fermentation. Introduction of ingredients-extract from plants. To determine the parameters, the extract was centrifuged for 10 min (obtaining the extract). During the experiment, the following were controlled: acidity of cheese, moisture content in cheese (W), salt concentration (N), amount of added water in %, antioxidant activity, polyphenols and flavonoids. The obtained data were expressed in mg of standard compound per gram of dry weight (DW).

The cheese production raw material is calcium chloride, a synthetic preparation containing 27% calcium. The active substance is calcium chloride (Figure 1).

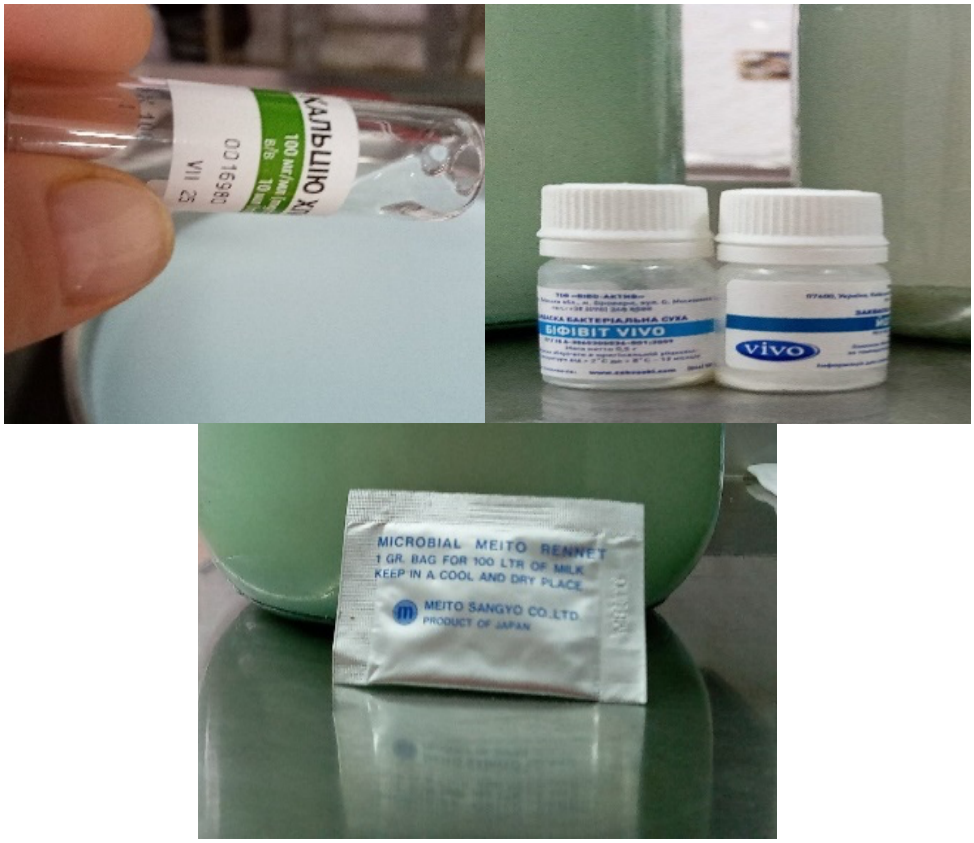

Figure 1 Ingredients of craft cheese.

Figure 2 "Black Cornflower" and "Anchan".

Table 1 Research methods.

Indicator	The principle of the research method
Sampling of raw milk and preparing them for analysis	For GOST 26809-86
Mass fraction of fat, %	Gerber acid method according to GOST 5867-90
Mass fraction of protein, %	According to GOST 2579-90 and GOST 23327-78
Active acidity (pH), (units)	Potentiometric method according to GOST 26781-85
Titrated acidity, °T	Titrometric method according to GOST 3624-92
Density, g/cm^3	Areometric method according to GOST 3625-84
Degree of purity of milk, group	Filtering
Sampling for microbiological analysis	For GOST 26668-85
The amount of acidophilic bacilli, %	The method of limiting dilutions in sterile skim milk at a temperature of 43 °C
QMAFANM, kuo/cm^3	According to GOST 9225-84 and GOST 10444.12-88
The composition of free amino acids, %	Using the amino analyzer "Bio-tronik LC 2000" after treatment with a solution of sulfosalicylic acid
Fractional protein composition of beverages, %	Polyacrylamide gel electrophoresis method (Lemley method in modification with introduction of urea gel)

Statistical analysis

Primary processing of experimental data was carried out with the help of a package of application programs for statistical analysis using criteria. Statistical criteria were used: Cochran's criterion – to assess the homogeneity of variance, Studen's criterion – to assess the significance of the calculated coefficients, Fisher's criterion - to assess the adequacy of the obtained equations. A package of application programs for experiment planning and optimization was used to optimise the parameters of technological processes. Significant differences (p <0.05) between means were assessed using ANOVA and the Tukey–Kramer test. Correlation coefficients were calculated using Statistica software version 13.0 (StatSoft, Tulsa, OK, USA). The research program is included in the DFE-24-1 experiment planning matrix. Experiments were performed in triplicate.

RESULTS AND DISCUSSION

The article presents the craft development of Anchan cheese **[6]**. The expediency and relevance of using regional milk raw materials for production are described. Milk was selected and researched by seasons and regional suppliers for selection by quality indicators (Table 2). Changes in the chemical composition of milk and microbial contamination depending on the type of ownership of the supplier and changes in milk quality depending on the season were statistically processed and studied **[7]**, **[8]**. The use of strains of microorganisms is proposed. Temperature regimes for safe product have been determined. Modes of milk pasteurization in the production of craft cheese. **[9]**. The expediency and relevance of using extracts of black cornflower herbal coagulant – black cornflower *Centurea* spp. and Anchan extract to obtain a specific new taste of cheese. Organoleptic evaluation of Anchan cheese is shown in Table 4.

Craft cheese was presented **[10]** – with extracts of black cornflower *Centurea* spp. and Anchan extract (42nd) **[7]**. The chemical composition of the cheese is enriched with yeasts of mesophilic lactococci. The composition includes acid- and aroma-forming cultures of *Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. Lactococcus lactis subsp. diacetilactis, Leuconostoc lactis*, thermophilic lactic acid bacilli of the species *Lactobacillus acidophilus* (non-viscous race). The main and additional bacteria of starting cultures **[11]** lactobacilli from several different sources **[12]** have broad antimicrobial activity.

Table 2 Organoleptic evaluation of cheeses after maturation at different temperature regimes, (points).

Organoleptic indicators	Options for temperature modes of maturation		
	1	2	3
Taste and smell	38.0	39.0	36.5
Consistence	24.0	24.0	23.5
Drawing	10.0	10.0	10.0
Overall rating	92.0	93.0	90.0

Figure 3 Appearance of craft cheese "Anchan".

All over the world, the task is to expand the range and improve the quality, biological value, safety, taste and range of certain foods **[44]**. Therefore, developing craft technology for cheese production using regional raw materials and extracts of black cornflower "I Anchan" will be a very promising task for craft production **[6]**. We present the organoleptic studies results of the prototype craft cheese "Anchan". The samples were predicted to mature at different temperatures.

The taste and smell of variants 1 and 2, compared to variants 3, were purer and more pronounced, and the texture was not very soft, more typical of hard cheese. Organoleptic evaluation of both individual indicators and the overall organoleptic evaluation of cheeses of the first two variants of maturation was higher. The lowest in the main indicator of organoleptic evaluation of cheese "taste and smell", which primarily depends on the

cheese grade, were cheeses of option 3, which had a variety of unpleasant tastes.

The high saturated fat content of butter has been criticized for many years from a health perspective, and this, combined with recommendations to reduce the total amount of fat in the human diet, has led to a growing interest in low-fat products. Mixed fat spreads containing a mixture of milk fat and vegetable oil (more often rapeseed) are presented on the market. Many of these foods seem to lack the texture, mouthfeel, and flavor of full-fat foods. However, consumer interest in low-fat products is steadily growing, and in the future, quality will undoubtedly be achieved due to the results of numerous studies in this direction **[13]**, **[14]**, **[15]**, **[16]**. The addition of oil shows little effect on the physicochemical characteristics, and consumer evaluation highlighted that all fresh cheeses were considered acceptable, although cheeses with linseed oil and raspberry oil were most appreciated. High-protein milkshakes, powders, milk drinks, smoothies, and fortified dairy products are some examples of commercial milk-based beverages with additional health benefits in the world **[17]**, **[18]**.

Prediction of the content of minerals, fatty acids (FA) and cholesterol in cheese samples was carried out based on the results of near-infrared transmission spectroscopy studies **[19]**. When determining the influence of iodine content on dairy raw materials, we relied on a large number of studies conducted in this area **[20]**, **[21]**, **[22], [23]**.

Functional foods containing dietary fiber (DF), prebiotics, probiotics, and synbiotics are known to be associated with various health benefits. DFs containing edible carbohydrates and closely related compounds resistant to digestion in the human small intestine with complete or partial fermentation in the large intestine can be classified as water-soluble and water-insoluble **[24]**. Prebiotic properties depend on molecular weight, composition of monosaccharides and type of glycosidic binding. Prebiotic substances stimulate the growth of bifidobacteria and lactobacillus species – bacteria that are considered beneficial for health **[25]**, **[26]**, **[27]**, **[28]**. In particular, the use of S. carnosus strain No. 5304 is effective for denitrification of milk with high nitrate content in the technology of production of fermented milk products **[29]**.

Lactobacillus rhamnosus and L. delbrueckii are known to have broad antimicrobial activity and L. rhamnosus isolate was found to be presented with a survival percentage of 6.9% at pH 4.5 and 5.1% at pH 2.0) and L. rhamnosus (5.7% at pH 4.5 and 4.9% at pH 2.0) is tolerated by an acidic environment, Lactobacillus spp. has an antimicrobial effect **[30-37]**.

The use of spirulina in the production of dairy products leads to a 29.56% increase in the amount of Lactobacillus acidophilus, a 20% reduction in fermentation time and the total amount of probiotics. Spirulina probiotic yogurt was found to be acceptable to consumers as assessed by an affective consumer test **[38-41]**.

For the production of craft cheese, we studied the chemical composition of milk in the farm; the correspondence of fat and protein content in milk was checked; changes in the value of the ratio between fat and protein content as a basis for the normalization of milk for craft cheese. The milk quality indicators obtained during the quarter statistically processed and studied the change in the chemical composition of milk and microbial contamination depending on the type of ownership of the supplier and changes in milk quality depending on the season.

Samples of milk clots were obtained at different temperatures of pasteurized milk. Samples were examined for the moisture-retaining ability of the clot (HEI).

The data show a certain dependence of the obtained rennet clots on the temperature of pasteurization of raw milk and the duration of exposure at the temperatures used. With an increase in the pasteurization temperature of milk from 65 to 75 °C, the university of the obtained clots increases by almost 10%, with a further increase in temperature – for every next 10 °C, the university of rennet clots increases by 5%. The effect can be explained by profound changes in the properties of milk proteins, especially whey, which occur during heat treatment.

The data obtained correlate with the results of studies of other scientists who have analyzed the impact of high -temperature treatment on dairy raw materials and qualitative indicators of finished cheese **[43]**.

The results of the study of protein, fat and DMSR in milk from different suppliers during the year are shown in Figure 4, Figure 5 and Figure 6.

At the same time, it should be noted that the protein content of milk obtained from farms throughout the year is higher than 3.0%, and in the third quarter even exceeds this Figure, which almost meets the requirements for milk intended for hard cheese. The fat content of milk obtained from farms in the first, third and fourth quarters was above 3.6%.

The lowest amount of fat – 3.3% and protein 2.97% contained milk received from private farms in the second quarter. Reducing the amount of these components in milk, especially protein, is accompanied by a decrease in cheese yield. The content of lactose and minerals in milk during the lactation period of animals is practically unchanged.

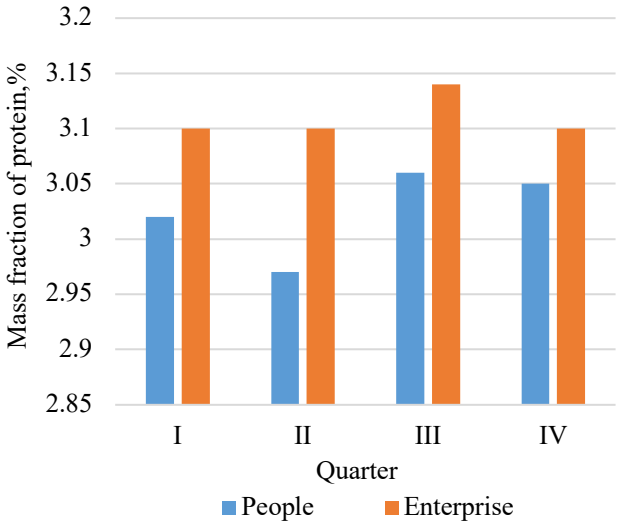

Figure 4 Changes in protein content in milk depending on the type of farm and period of the year.

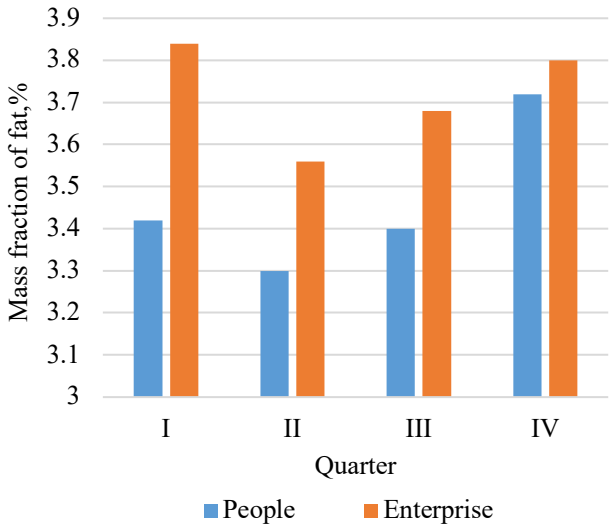

Figure 5 Changes in fat content in milk depending on the type of farm and period of the year.

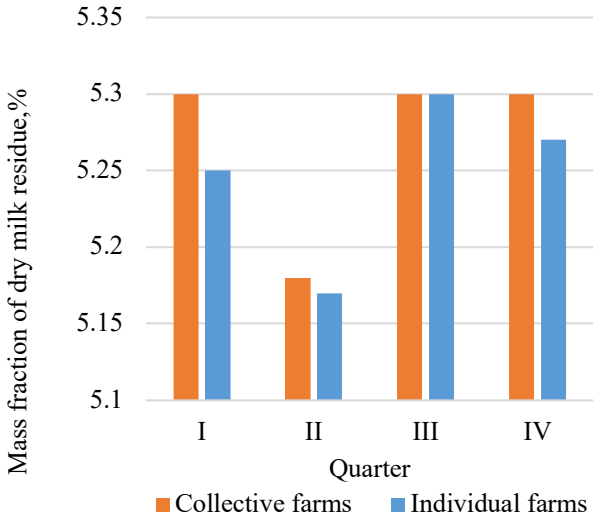

Figure 6 Change in the content of DMSR in milk depending on the type of management and period of the year.

Table 3 Characteristics of milk depending on the type of supplier and season (n = 3, $p \geq 0.95$).

Indexes	Farms (quarter)				Private farms (quarter)			
	I	II	III	IV	I	II	III	IV
Density, kg/m^3	1032	1030	1032	1032	1030	1028	1032	1029
Titrated acidity, °T	18	17	18	18	18	17	18	17
Active acidity (pH)	6.68	6.58	6.80	6.70	6.66	6.52	6.76	6.68
Degree of purity, group	I	I	I	I	I	I	I	I
QMAFAnM	II	II	II	II	II	II	II	II
Rennet-fermentation test, class	II	II	II	II	II	II	II	II
Coagulation of milk (according to Dylanian), type	II	II	II	II	II	II	II	II
Number of somatic cells, thousand/cm^3	320	450	300	410	460	495	380	478
Bacterial contamination, CFU thousand/cm^3	280	380	310	350	360	390	360	380
Inhibitors of growth of fermenting microflora	Not found				Not found			

Table 4 Bacterial contamination of raw milk before and after high-temperature treatment (n = 3, $p \geq 0.95$).

Milk suppliers	QMAFAnM (t = 6 °C) for 24 h CFU/cm^3	Raw milk Pasteurization mode	QMAFAnM, CFU/cm^3	Efficiency of pasteurization,%
Farms (n = 10)	920 ±41	(73 ±1 °C) with exposure 25 c (control)	20 ±1.3	99.35
Private farms (n = 18)	95900 ±9100	(73 ±1 °C) with exposure 25 c (control)	188 ±13.76	99.81
Farms (n = 10)	920 ±41	(81 ±1 °C) with exposure 25 c	16 ±1.4	99.88
Private farms (n = 18)	95900 ±9100	(81 ±1 °C) with exposure 25 c	97 ±7.76	99.94
Farms (n = 10)	920 ±41	(120 ±5) °C with exposure 3 – 5 c	0	100.0
Private farms (n = 18)	95900 ±9100	(120 ±5) °C with exposure 3 – 5 c	12 ±0.63	99.99

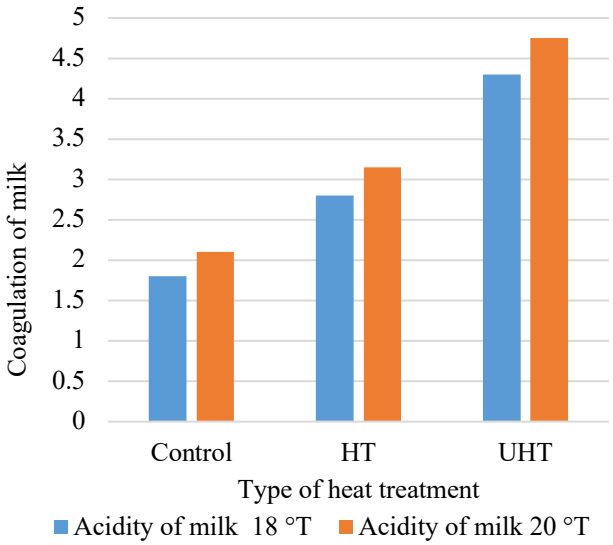

Figure 7 Dependence of milk coagulation on the type of heat treatment and the amount of rennet enzyme and extract of "Black Cornflower" in the presence of 20 g of calcium chloride.

Table 5 Characteristics and storage of ingredients.

Name the ingredient for fermentation	Manufacturer	Composition	View
Cornflower black-ball plant extract	Ladyzhyn Plant of Bio- and Enzyme Preparations “Enzyme” (Vinnytsia Region, Ladyzhyn). Podillya	Enzyme preparation with different mechanism of action based on bacteria and microscopic fungi	Enzyme preparation
Calcium chloride	PJSC "Biopharma" (Kyiv). Biopharma Company	Synthetic preparation	White powder, may be granules
Enzymatic preparation "Maxiren"	DSM Food Specialties (Netherlands),	Chymosin obtained from special strains of milk yeast *Kluyveromyces lactis* enzyme	Brown powder, with odor, natural strain of *Penicillium canescens*
Basic/Additional	TIMM State Research Enterprise of Bacterial Yeasts Ukraine, Kyiv. www.ddpbz.com.ua	*Lactobacillus acidophilus* (incoherent race)	BZ "ANV" cocci present sticks of different lengths

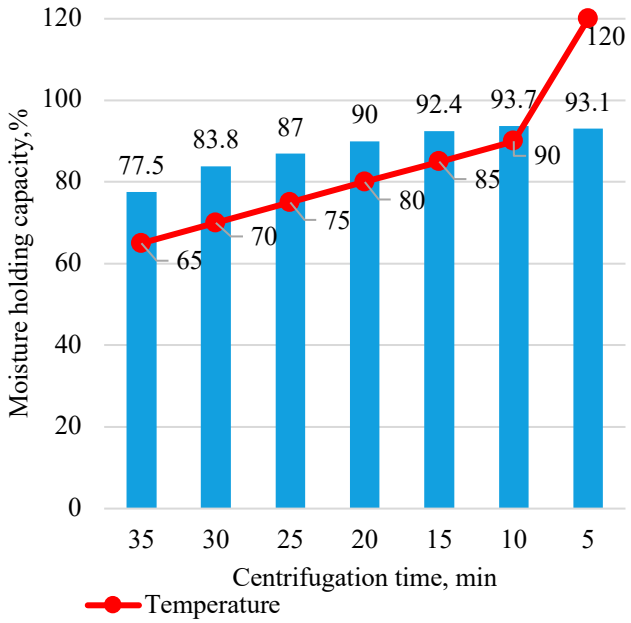

Figure 8 Dependence of moisture-retaining capacity of rennet clot on the mode of pasteurization of raw milk.

The main independent parameters that change and significantly affect the initial optimal indicators of the technological process in the production of hard rennet cheeses were determined: the temperature of the second heating – x1 (T), the amount of added deoxidation water – x2 (V), salt concentration in cheese – x3 (N). The mass fraction of moisture determined the initial optimization parameters – y (W) and soluble nitrogen – y (P) content.

In the production of cheese, the temperature of the second heating was chosen in the range from 40 to 42 °C, the amount of added water – from 5 to 15%, the mass fraction of salt in the cheese – from 1.3 to 2.5%.

As a parameter with a fixed value, we chose the duration of salting cheese in brine – 2 days.

The research was performed according to the matrix of experimental planning following the plan of small factorial experiment DFE-24-1.

The scheme of the experiment consists of three stages of the multifactorial experiment of the production of "Anchan" cheese. At first, doses of the main components (black cornflower, anchan, water, salt) and their effect

on the process of fermentation and ripening of cheese were experimentally studied. At the second stage, the total content of polyphenols in plant extracts of black cornflower and anchan grass was determined.

At the third stage, the parameters of the ready-made "Anchan" cheese were determined: the mass fraction of moisture in the cheese was determined by the express method in the drying cabinet and the arbitration method according to GOST 3626-73; the mass fraction of sodium chloride without ashing of the product according to GOST 3627-81 and the mass fraction of protein – by the Kjeldahl method according to GOST 25170-90. Active acidity was determined electrometrically on a pH meter with a measurement error of 0.05 units. pH according to GOST 26781-85. The mass fraction of fat is following GOST 5867-69.

The implementation of a multifactorial experiment made it possible to evaluate the influence of the second heating temperature of 40 °C, the amount of added water in the amount of 10% and the level of salting of cheese within the limit of the concentration of salt in the finished product of 2.3% on the quality of ripened cheese and to link the above-mentioned factors into a mathematical model. Experiments on the introduction of components were carried out in triplicate.

The milk was processed at a temperature of 71 ±1 °C with a holding time of 20 – 22 seconds. The raw material was cooled to 32 – 34 °C and the components were added: a 40% calcium chloride solution at the rate of 25 g of anhydrous salt per 100 kg of milk, bacterial starter from pure cultures of specially selected DVS microorganisms, as well as plant corn enzyme - 0.5 kg/t and extract of anchan grass – 0.5 kg/t.

The duration of milk coagulation was 25 minutes. For "Anchan" cheese, milk curdled at a temperature of 40 °C. For the action of the plant lactic enzyme, the optimal pH value is 5.9 – 6.0.

Converting dimensionless variables xi into independent values, we obtained the following equation

$$W = 348.4 – 13.96 \cdot T – 2.16 \cdot V – 14.68 \cdot N + 16 \cdot T2 + 0.053 \cdot T \cdot V + 0.33T \cdot N + 0.97 \cdot V \cdot N – 0.024 \cdot T \cdot V \cdot N$$

The obtained equation adequately describes the process of cheese production in the given intervals of changes in the most influential factors, which we established as a result of previous studies. The obtained results of the dependence of the moisture content in the product on technological factors are shown in Figure 9.

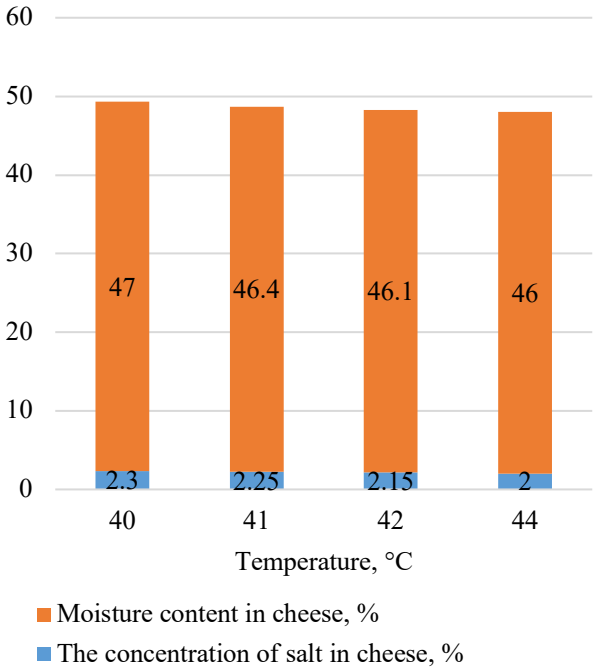

Figure 9 Dependence of moisture content in cheese (W) on the salt concentration (N) at the temperature of the second heating (T) at the amount of added water 15%.

The graphs show that in the variant where the temperature of the second heating was 40 °C, the mass fraction of moisture in the cheese is in the range from 46.5% to 46.8%. The addition 5 to 15% water for the deoxidation of whey affects the moisture content of cheeses insignificantly. The main criterion that actively influences the conditions of development of the microflora in cheese is the mass fraction of salt in the water contained in the cheese.

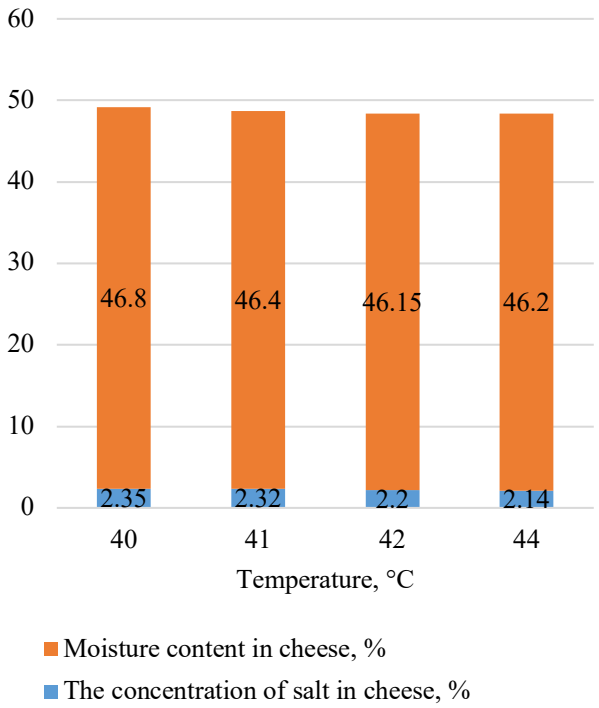

Figure 10 Dependence of moisture content in cheese (W) on salt concentration (N) at the second heating temperature (T) and the amount of added water 10%.

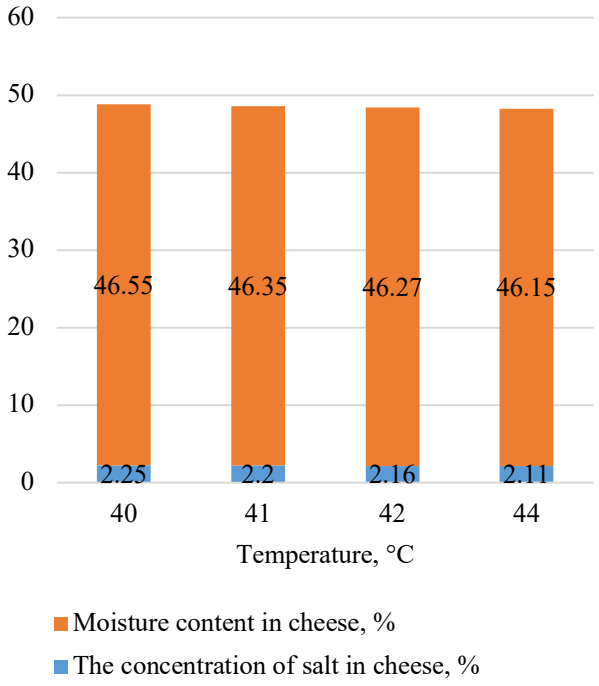

Figure 11 Dependence of moisture content in cheese (W) on salt concentration (N) at the second heating temperature (T) and the amount of added water 5%.

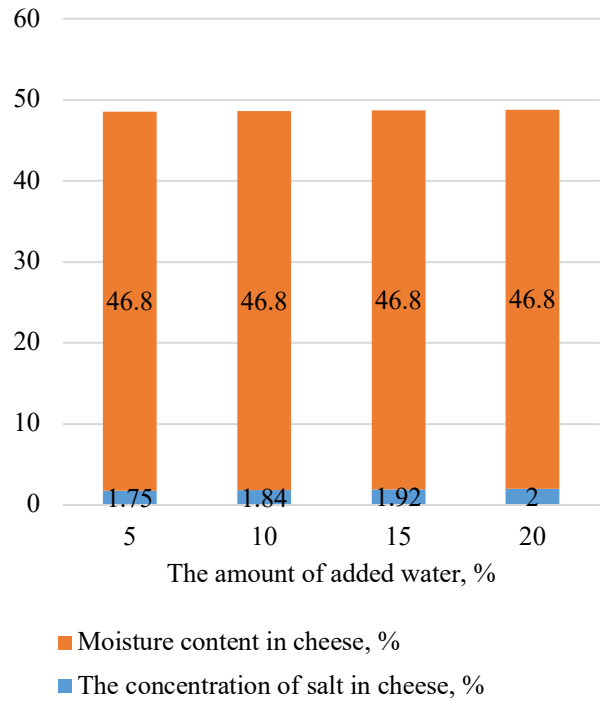

Figure 12 Dependence of moisture content in cheese (W) on the amount of added water (V), and salt concentration (N) at the temperature of the second heating 41 °C.

CONCLUSION

As a result of this work, we can conclude that the increase in ripening temperatures of cheeses is accompanied by an increase in lactic acid microflora, especially lactic acid bacteria, and, as a consequence – an increase in protein proteolysis and fat lipolysis. However, increasing the content of hydrolysis products of proteins and fats does not always give cheeses high organoleptic properties. It was found that the most acceptable temperature regime of maturation to obtain a product with high organoleptic characteristics is a step temperature regime, namely, 10 – 12 °C during the first 10 days of maturation and 14 – 16 °C until the end of maturation.

Our proposed method of production of hard rennet cheeses with low temperature of the second heating, in comparison with the existing method of production of cheese "Litinsky", which we used as a control, improves the quality of the finished product by reducing bacterial contamination of raw milk and prevent defects in the product maturation process.

Studies of microbiological, biochemical and physicochemical parameters of raw butter and semi-finished products in the production of fermented milk drinks were carried out by generally accepted methods of analysis, which are set out in relevant standards and guidelines for microbiological and techno-chemical control of fermented milk products. literature. Table 1 shows the research methods.

We used regional raw materials to produce craft cheese "Anchan" and researched raw milk for its physical and chemical properties and technological parameters. Test milk samples were pasteurized in the laboratory at a temperature of 80 °C for 10 seconds. Anchan was added to the milk for color. Next, the milk before coagulation was heated in a pasteurization boiler by heating with saturated steam 36 – 38 °C. The enzyme 4 mL per 100 kg of milk and 4 mL of black cornflower extract was added to the prepared milk to improve milk coagulation and the formation of a dense cheese clot. Strains of probiotic cultures were selected for Anchan. The composition of the main complex yeast of mesophilic lactococci acid and aroma-forming cultures, *Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. Lactococcus lactis subsp. diacetilactis, Leuconostoc lactis*. As an additional leaven used thermophilic lactic acid sticks of the species *Lactobacillus acidophilus* (a non-viscous race to obtain a new taste of craft cheese. When using these ingredients, the fermentation time is reduced by 8 – 10 minutes. Processing of cheese grain, and removal of whey was carried out according to the general technology. We have proposed technology for closing the cheese skin by pouring hot cheese heads (t = 50 – 55 °C). Marking, packaging, transportation and storage were carried out according to the craft product's technical conditions for the craft product.

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The mathematical model of drying melon pulp by the convective method

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ABSTRACT
Melon is a dessert loved by many, captivating with its thick aroma and delicate honey taste. The juicy, fragrant pulp is not only delicious but also very useful for dietary purposes, with a therapeutic effect on diseases of the liver and kidneys, anaemia, rheumatism and cardiovascular disorders. This storehouse of vitamins is especially rich in potassium and iron salts, pectins, fibre, easily digestible sugars, proteins, starch and other elements necessary for health. This article presents the results of a study of the Myrzachulskaya melon variety and establishes the optimal parameters for drying the pulp, pre-treating melons with 99.5% ethanol before drying. Twenty drying experiments were carried out, in which the parameters of the operating variables, namely temperature, air velocity and sample size, were varied according to the compiled mathematical processing planning matrix. Drying caused a decrease in biologically active compounds, affecting some antioxidant properties (vitamin C content, total phenol content and antioxidant capacity) of melon pulp. As a result, the optimal parameters were established, at which samples of dried melon pulp showed insignificant losses (up to 1%) in the total content of phenolic compounds, carotenoids and ascorbic acid. The optimal parameters for drying melon fruits are a temperature of 55 °C, a drying time of 11 h and a slice thickness of not more than 0.5 cm.

Keywords: drying, melon, convective drying, phenols, mathematical modelling

INTRODUCTION
Consumer demand for high-quality food products is growing, driving the food industry to look for new food processing technologies. New food products must meet consumer demands for healthy and nutritious food. Thus, the food industry is looking for ways to preserve and increase the nutritional value of foods, which can be achieved in several ways, such as adding functional compounds and vitamins to the product, as well as improving food processing to avoid the loss of nutritional qualities. New industrial processes may need to fulfil some of these requirements. As such, techniques that can preserve as much of the natural vitamins as possible or even increase their availability and minimize the appearance of unwanted degradation products are of great industrial interest **[1]**. Natural antioxidants are essential, especially in fruits and vegetables, because of their proven ability to prevent the effects of oxidative stress. Redox imbalances in the body can cause severe damage to tissues, proteins, enzymes and genetic material such as DNA and RNA **[2]**. The antioxidant capacity of food is related to vitamins or phenolic compounds **[3]**. Melon is high in antioxidants, including choline, zeaxanthin and beta-carotene. The carotenoid zeaxanthin is a natural pigment. Under the rays of the sun in any living organism, including in the human retina, free radicals are formed. These particles damage the structures of the eye. The function of carotenoids is to protect the eyes from such damage. Thus, they play a protective role concerning the eyes and reduce the damage from macular degeneration. Melon flavonoids protect against breast and colon cancer **[4]**. Vitamins are particularly important because they play an important role in many important reactions, and any lack of vitamins can lead to serious illness **[5]**. Melons are high in vitamin C, an antioxidant with strong anti-inflammatory properties. Vitamin C strengthens the immune system, increases resistance to infectious and viral diseases, accelerates recovery processes, prevents cell damage during oxidation and activates the production of interferon and antibodies **[6]**. The shelf life of melons is considered short after harvest, not exceeding 1 or 2 weeks under normal conditions, or special cooling equipment such as cold stores is needed, leading to an increase in marketing costs **[7]**. Fresh food can be considered a matrix consisting of carbohydrates, proteins, fats, water and components dissolved in water. In fresh products, the molecular mobility of the compounds in the aqueous phase

is high and therefore, they are susceptible to chemical, enzymatic, microbiological and physical degradation **[8]**. This way, the melon can be dried to preserve a portion of the product that will be easily consumed or exported, providing extended shelf life, lighter shipping weight and less storage space **[9]**. Drying fresh food reduces the water content and, therefore, the concentration of dissolved components such as sugars **[8]**. Drying is a process widely used to increase the shelf life of melons by reducing the moisture content and has many benefits such as higher concentration of nutrients due to water loss; higher stability at room temperature; inhibition of the action of microorganisms; protection against enzymatic and oxidative degradation; ease of transportation and storage; and the reduction of post-harvest losses to obtain new forms of consumption and reduce the cost of transportation and storage. Removing water from fruits and vegetables by drying is one of the oldest forms of food preservation known to humans and is the most important process for maintaining nutritional value **[10]**, **[11]**. Therefore, consumers demand processed products that retain their original characteristics to a greater extent. From an industry perspective, this requires the development of operations that minimize the adverse effects of the process **[12]**.

Modelling of the air-drying process is well known and has been presented in most of the studies on drying fruit with warm air **[13]**, **[14]**, **[15]**, and there is little data on mass transfer coefficients that are required for the optimization of the industrial dehydration process. Optimization is selecting the best alternative from a given group of alternatives for a particular process. This requires a relation describing the potential alternatives of the process and a criterion for determining which of the alternatives is the best. An appropriate experimental design is fundamental to allow the researcher to explore the process under investigation and successfully lead to its optimization, obtaining a maximum or minimum, if they exist, or to determine the area in the general space of factors in which certain desirable operating conditions are satisfied **[16]**, **[17]**.

However, a decrease in the quality of dried products is often observed since most traditional technologies use high temperatures in the drying process. Processing may also result in undesirable changes in appearance and a change in the natural flavour and colour.

Appearance and a complete colour change are important physical properties of dehydrated fruits. It is important to visually evaluate dehydrated fruits because consumers primarily judge the quality of a product by its appearance and colour. An incorrect colour or a significant change in appearance will cause the consumer to reject the product **[18]**. The colour change after dehydration occurs because fruits contain high amounts of reducing sugars such as glucose, sucrose, fructose and carbohydrates. These reducing sugars can undergo a Maillard reaction through the intervention of amino compounds during drying **[19]**. The Maillard reaction is reported to occur after air drying at a high temperature and with a long duration **[20]**. In addition, an enzymatic reaction can cause dehydrated fruits to turn brown or darker due to the oxidation of phenols to o-quinones (brown pigment or melanins) **[21]**.

Colour is a sensory parameter widely used to justify consumer acceptance of dehydrated fruit. Fruit subjected to the convection drying process suffers from loss of quality in terms of colour, taste (taste and aroma) and texture, while rehydration is often poor. The main problems are hull hardening (formation of a hard outer shell) and shrinkage. In recent years, the main goal of the research has been to improve the quality retention of dried products (rehydration capacity, etc.) by changing the process conditions and pre-treatment **[22]**. Therefore, dehydration technology should focus on producing dried products with little or no loss of flavour characteristics. Research is encouraged to optimize process conditions and apply pre-treatment to minimize the above changes **[23]**.

In recent years, many studies have been published on using ultrasound as a pre-treatment for drying **[24]**. They reported changes in the structure of melon cells caused by ultrasound. However, unlike osmotic dehydration, cell destruction was not observed, and microscopic channels appeared in the cell structure, which could be the reason for the increase in the water diffusion coefficient. When studying the use of ultrasonic or vacuum pre-treatment with sucrose solutions and distilled water concerning the drying efficiency of melon slices, a reduction in drying time was observed when pre-treating samples. Dried melon pre-treated using ultrasound and vacuum showed a lower total carotenoid loss, softer texture, better colour retention and good sensory acceptance **[25]**. However, we note that it is still necessary to understand, describe and improve the mechanisms for using ultrasound, which also depends on the processing conditions used. The authors emphasized the importance of evaluating its effect on food components to decide if its use is an advantage **[26]**.

Another little-explored alternative is to use an ethanol solution pre-treatment before convective drying. Several papers have reported an increase in drying speed in this case. In this sense, pre-treatment with ethanol gave interesting results. A balance of structural and compositional changes, such as changes in cell wall thickness and removal of air from intercellular spaces, may be responsible for improving process and product properties **[27]**. Alcohol is harmless to humans and leaves no residue after drying. It can replace water as the ultrasonic propagation medium, which will greatly improve the final food product's drying process and quality **[28]**.

In addition, during pre-treatment, a mixture with water is formed when ethanol is introduced into the sample. Then, during drying, the efficient evaporation of ethanol contributes to mechanisms that speed up the drying

process, such as the Marangoni effect **[29]**. The use of ethanol pre-treatment during convection drying has also shown very positive results in terms of reduced drying time and improved product properties such as rehydration, texture, nutrient retention and availability **[30]**, **[31]**, **[32]**.

The use of ethanol as a drying pre-treatment and its effect on the quality of dried melon has never been studied. Therefore, the present study was aimed at evaluating the effect of using ethanol solutions at various concentrations as a pre-treatment, whether or not associated with the use of ultrasound and vacuum pulse, in the drying of melons, and also to test the effect of pre-treatment on several quality parameters, such as ascorbic acid content, total phenol content, total carotenoid content and colour. The results will be important for understanding drying processes and useful for developing appropriate drying and pre-treatment conditions.

Scientific Hypothesis

Improving the quality of dried melon will depend on the mode and technology of drying. The drying temperature will have a significant effect on the vitamin C content in drying melon pulp.

MATERIAL AND METHODOLOGY

Samples

For the study, melons of the Myrzachulskaya (Torpedo) variety were taken. Melons harvested in summer 2021 were provided by a farm (Shymkent, Kazakhstan) for research. The tests were carried out with melons with a soluble solids content of 10 to 12 °Brix (measured with a PAL-α refractometer). The average initial humidity was 9.61 ±0.08 kg water/kg dry matter.

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Animals and Biological Material

Animal and biological materials were not used in this study.

Instruments

We used an electric multi-cutter Moulinex DJ-905832 (Moulinex, France), an electric dryer Kitfort KT-1921 (Kitfort, China).

Laboratory Methods

The following indicators of raw materials and the resulting product were studied in work: the content of ascorbic acid (vitamin C) according to GOST 24556-89, the content of carotenoids according to GOST 54058-2010, organoleptic indicators according to GOST 1750-86. For the study of raw materials and finished products, standard conventional chemical and organoleptic methods were used.

Description of the Experiment

Preparation of raw material

The melons were cleaned on apparatus developed for cleaning gourds (developed in the Astana branch of the Kazakh Research Institute of Processing and Food Industry LLP, Nur-Sultan, Republic of Kazakhstan) **[33]**. Further, the peeled melons were subjected to deseeding by cutting the fruit in half with a knife. For drying, the pulp was cut into slices of various thicknesses (Table 1) on a Moulinex DJ-905832 electric multi-cutter. After that, the sliced melon pulp segments were soaked in absolute ethanol (99.5%) **[34]**.

Drying process

Drying was carried out in a Kitfort KT-1921 electric dryer (Figure 1). The operating principle of the dryer is based on the principle of convective drying **[35]**. The KT-1921 dryer has a temperature range from 35 to 75 °C and is equipped with a timer for up to 24 h. Slices of melon fruits were laid out on a mesh baking sheet, avoiding contact with each other to avoid sticking due to the release of sugars during the drying process.

Figure 1 Kitfort KT-1921 dryer for vegetables and fruits.

Drying (xeroanabiosis) of products is one of the oldest preservation methods. It limits the growth and development of microorganisms with minimum moisture content in dried products. Microorganisms do not develop in products with a moisture content of 4 – 30%. For products with a large mass fraction of sugars and other water-soluble substances, in which the concentration in solutions during drying increases significantly and the osmotic pressure increases, dehydration can be carried out up to 13 – 20% of moisture.

Drawing up plans for full-factor drying experiments and building a model

The primary stage in constructing a mathematical model is coding intervals and levels of parameter variation. The coding of intervals and levels of variation of input factors is compiled for the characteristics of the origin of raw materials. To obtain a mathematical model of the process of drying melon pulp, which is a regression equation, a second-order rotatable plan (Box plan) was used; the number of factors x was 3, the number of experiments was more than 20, the number of experiments at the zero point was 6, and the number of equation coefficients was 10. As a mathematical apparatus, we used mathematical and statistical methods; the resulting system of regression equations models the relationship of the most preferred optimality criterion with the rest. The main criterion for the process of drying melon fruits is the moisture content of the finished product (y), influenced by the following factors: drying temperature (T, °C), drying time (t, min) and slice thickness (L, cm); the above factors determine specific production terms. Therefore, it is advisable to adjust the system of regression equations following these factors.

The regression equation has the form (1):

$$y_1 = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (1)$$

Coding intervals and levels of variation of input factors for drying melon fruits are presented in Table 1. An experiment planning matrix is presented in Table 2.

Table 1 Coding of intervals and levels of variation of input factors.

Factors		Levels of variation					Variation
Natural	**Coding**	**−1.68**	**−1**	**0**	**+1**	**+1.68**	**intervals**
T, °C	x_1	51.6	55	60	65	68.4	5
t, min	x_2	9.3	10	11	12	12.6	1
L, cm	x_3	0.1	0.3	0.5	0.7	0.8	0.68

Table 2 Matrix for rotatable planning of experimental studies of the process of drying the melon pulp.

No.	Encoded values			Natural values		
	x_1	x_2	x_3	T, ˚C	t, min	L, cm
1	2	3	4	5	6	7
1	−	−	−	55	10	0.3
2	−	−	+	55	10	0.7
3	−	+	−	55	12	0.3
4	−	+	+	55	12	0.7
5	+	−	−	65	10	0.3
6	+	−	+	65	10	0.7
7	+	+	−	65	12	0.3
8	+	+	+	65	12	0.7
9	−1.68	0	0	51.6	11	0.5
10	+1.68	0	0	68.4	11	0.5
11	0	−1.68	0	50	9.3	0.5
12	0	+1.68	0	50	12.6	0.5
13	0	0	−1.68	50	11	0.1
14	0	0	+1.68	50	11	0.8
15	0	0	0	50	11	0.5
16	0	0	0	50	11	0.5
17	0	0	0	50	11	0.5
18	0	0	0	50	11	0.5
19	0	0	0	50	11	0.5
20	0	0	0	50	11	0.5

Quality research

All studies of the quality indicators of dried melon fruits were carried out in triplicate for fresh and processed samples: moisture content, ascorbic acid content, total phenol content, total carotenoid content and colour (to preserve the presentation).

Humidity was determined gravimetrically in an oven at 105 °C for 24 h **[36]**. The results are expressed as a percentage (%).

The ascorbic acid content was determined according to the AOAC method **[36]**, based on the reduction of 2,6-dichlorophenol-indophenol with ascorbic acid. The results are expressed as dry matter (mg ascorbic acid/100 g dry matter).

The total phenolic content of the extracts was measured based on the Folin–Ciocalteu reagent as described by Singleton et al. **[37]**. The reaction mixture contained 0.5 mL of the phenolic extract, 2.5 mL of the Folin–Ciocalteu reagent (Sigma-Aldrich, Germany) and 2 mL of sodium carbonate (4 g/100 g). Then the mixture was left in the dark for 2 h at room temperature. The absorbance of the sample was determined at 760 nm using an aqueous solution of gallic acid (5 – 100 μg/mL) as a standard. The results are expressed as mg gallic acid equivalents (EAA)/g sample (on a dry weight basis).

Total carotenoids were quantified based on the method described by Rodriguez-Amaya et al. **[38]**. Briefly, extraction was carried out with acetone, followed by separation and dilution in petroleum ether; finally, the absorbance was measured at 470 nm. Some precautions were taken against degradation or alteration of the pigment, such as protection from light and high temperatures and using a short analysis time. The results are expressed as μg carotenoids/g dry matter.

Organoleptic indicators determined the colour and presentation of dried melon fruits.

Number of samples analyzed: We analyzed 2 samples.

Number of repeated analyses: All tests were performed in triplicate.

Number of experiment replication: 2 times.

Statistical analysis

The data were analysed using MS Excel for Windows version 10 Pro, 2010, and a second-order rotatable plan (Box plan) was also used. In the analysis process, absolute and relative statistical indicators, and tabular and graphical methods for presenting the results were used.

RESULTS AND DISCUSSION

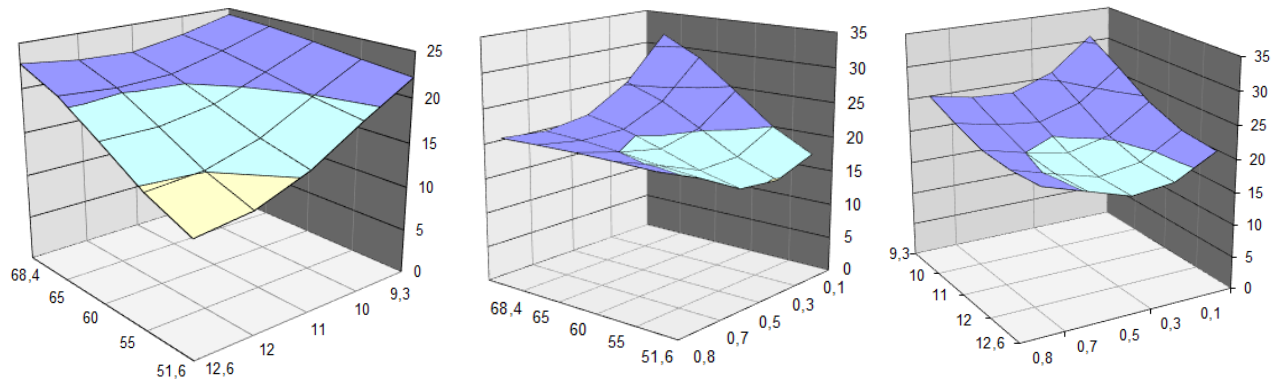
Studies were carried out to achieve the best values of melon pulp drying parameters. A number of experimental studies were carried out to determine *y* for indicators with different ratios of input parameters *x*. The results are shown in Table 3.

Table 3 Data from experimental studies of the process of drying the melon pulp.

No.	Encoded values			Natural values			Experimental values
	x_1	x_2	x_3	T, °C	t, min	L, cm	*y*, %
1	2	3	4	5	6	7	8
1	−	−	−	55	10	0.3	17
2	−	−	+	55	10	0.7	35
3	−	+	−	55	12	0.3	21
4	−	+	+	55	12	0.7	30
5	+	−	−	65	10	0.3	15
6	+	−	+	65	10	0.7	17
7	+	+	−	65	12	0.3	11
8	+	+	+	65	12	0.7	26
9	−1.68	0	0	51.6	11	0.5	15
10	+1.68	0	0	68.4	11	0.5	20
11	0	−1.68	0	50	9.3	0.5	14
12	0	+1.68	0	50	12.6	0.5	25
13	0	0	−1.68	50	11	0.1	34
14	0	0	+1.68	50	11	0.8	9
15	0	0	0	50	11	0.5	19
16	0	0	0	50	11	0.5	18
17	0	0	0	50	11	0.5	19
18	0	0	0	50	11	0.5	18
19	0	0	0	50	11	0.5	20
20	0	0	0	50	11	0.5	19

Table 3 shows that 20 experiments were carried out. The discrepancy between the results at the zero point was ±0.1.

The mathematical model of drying melon pulp is shown in Figure 2.



a b c

Figure 2 Dependence of input parameters: a – dependence of temperature and drying time; b – dependence of temperature and thickness of slices; c – dependence of drying time and slice thickness.

Figure 2 shows that drying proceeds correctly if the rate of evaporation of moisture from the surface of the product is equal to the rate of moisture transfer inside it. At a higher evaporation rate, that is, with an increase in temperature, a crust forms on the product's surface to be dried, slowing down the drying process; with slow evaporation, the product is steamed. The drying process was intensified by increasing the evaporation surface, for which the melon pulp was cut into slices with a thickness in the range of 0.1 to 0.8 cm.

Thus, when the temperature rose above 55 °C, the final product had a low moisture content; at a temperature below this value, pieces of melon pulp were not dried to the full extent, regardless of the thickness of the slices into which melon was cut.

The sample size, namely the surface to volume ratio, can also significantly impact the drying process: the drying time is significantly reduced as the size is reduced, which facilitates the processing of small products when possible.

It is easy to see that the planning matrix is orthogonal with linearly independent column vectors; hence the diagonality of the matrix and the system of equations is normal, and thus the mutual independence of the estimates of the coefficients of the regression equation. Then the regression equation for drying melon pulp with the optimal importance y has the form (2):

$$y = 16.0437 - 0.31097x_1 - 25.8639x_2 + 13.25491x_3 - 0.15x_1x_2 + 0.125x_3 - 2.50000x_2x_3 + 0.00283x_1^2 + 0.63492x_2^2 - 33.5131x_3^2 \quad (2)$$

Thus, the optimum drying parameters fall on a point with a temperature of 55 °C and a drying time of 11 h, and slices of melon pulp must be cut to a thickness of no more than 0.5 cm. At these parameters, a moisture content of 20% is achieved.

Melon samples were dried to a moisture content of 0.25 kg H_2O/kg dry weight (20%, wet basis) (Figure 3) and then subjected to quality analysis.

Figure 3 Dried samples of Myrzachulskaya (Torpeda) melon.

The average values of the assessed quality parameters are shown in Table 4. There was a significant decrease (total phenol up to 14.3%, carotenoids up to 24.9%, ascorbic acid up to 57.9%) in the values of these parameters for all dried samples compared to fresh melon, indicating a possible connection with thermal decomposition. The type of pre-treatment and the concentration of ethanol also influenced the results.

Table 4 Physico-chemical characteristics of fresh and dried melon samples.

No.	Melon samples	Total phenols (mg GAE/g DM)	Carotenoids (µg/g DM)	Ascorbic acid (mg/100 g DM)	Colour
1	Fresh	3.5	138.1	185.3	White
2	Dried	0.5	34.4	107.2	White with a yellowish tint

Table 4 shows that the total phenol content of the dried samples was significantly reduced (up to 14.3%) compared to the fresh melon samples, from 3.5 to 0.5 mg GAE/g DM, respectively. The amount of carotenoids in the formulation decreased from 138.1 to 34.4 µg/g DM. Ascorbic acid turned out to be the most resistant in a dried sample of melon pulp; its content decreased from 185.3 to 107.2 mg/100g DM.

Phenolic compounds. In the dried sample, a decrease in the concentration of total phenolic compounds (TPC) was observed. The decrease in these phytochemicals may be due to their being sensitive to high temperatures and thus may be affected by the drying process, resulting in a decrease in their content and antioxidant capacity **[39]**. Also, polyphenol oxidase's enzymatic oxidation occurs during the decomposition of phenol and convective drying **[40]**. Thus, the result was a lower phenol content in the pre-treated dried melon. In addition, morphological changes may occur.

Carotenoids. In total carotenoids, the decrease was due to exposure to a higher temperature (50 °C) and longer processing time, as these pigments are very unstable and subject to degradation or isomerization **[41]**. In addition, carotenoids are fat-soluble molecules (non-polar) and soluble in organic solvents such as ethanol but insoluble in water (polar), which may explain the lower retention of carotenoids when a 100% ethanol solution was used for pre-treatment compared to water-ethanol solution (50%) and untreated dried samples.

Vitamin C. A decrease in the content of ascorbic acid after drying was noted. Degradation is also strongly influenced by the characteristics of the drying process, most of the content being lost due to heat and the presence of oxygen **[42]**. Also, degradation is associated with the destruction of the internal structure and the release of nutrients during drying. Natural plant materials are often well organized into cellular compartments, where nutrients and other components (sugar, starch and protein) are located in natural cellular compartments. However, the cell wall also becomes a factor that controls nutrient bioavailability. The cell structure's physical state regulates the components' release, mass transfer, availability and biochemical stability.

Colour. There was no significant difference (up to 3 – 5%) in colour determination between dried and fresh samples. Fresh slices of melon pulp were white, while dried slices were white with a yellowish tint.

CONCLUSION

By constructing a mathematical model, the optimal parameters for drying the pulp of the Myrzachulskaya melon variety were determined. The optimization criterion was aimed at achieving an optimal moisture content of 20% (to achieve a solids content of 80%) without losing consumer properties, that is, maintaining the main nutrients and presentation. So, as a result of optimization, the optimal parameters for drying melon fruits were determined: temperature 55 °C, drying time 11 h and slice thickness not more than 0.5 cm. The qualitative indicators obtained for dried melon samples show that phenolic compounds and carotenoids are sensitive to high temperatures. In the convective drying of melon pulp, their content decreased significantly, phenols by 7 times and carotenoids by 4 times. However, the content of ascorbic acid underwent minor losses of only 1.7 times comparing fresh and dried samples. Ascorbic acid, in turn, prevents the harmful effects of free radicals and fights oxidative processes, which allows dried melon slices to be stored for even longer.

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Nutritive, chemical, and technological properties of liver paté formulated with beef offal, sheep tail fat and liquorice, and ginger root

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ABSTRACT

The present study investigated the incorporation of sheep tail fat, beef heart, kidneys, and herbal ingredients (grounded licorice and ginger root, pumpkin, carrots, and onions) into liver paté formulations. Four types of liver paté were prepared: control sample containing only liver and butter; experimental sample S1 – paté with sheep tail fat (5%), ground dried licorice root (1%), and ginger (2%); experimental sample S2 – paté with sheep tail fat (8%), ground dried licorice root (2%) and ginger (3%); experimental sample S3 – paté with sheep tail fat (10%), ground dried liquorice root (3%) and ginger (4%). Inclusion of the ingredients mentioned above in the paté recipe did not cause significant changes in the mass fraction of table salt and protein (p >0.05) and conversely, significantly increased the moisture content, carbohydrates, fat, and be-ta-carotene in the test sample (p <0.05). The number of amino acids in the experimental samples decreased except for arginine. Among the experimental samples, the highest content of amino acids (18 g/100g) and essential amino acids (8.89 g/100g) was detected in S1. The results of determining the fatty acid composition showed significant changes in the composition of experimental samples compared with the control. The total content of saturated acids in the experimental samples decreased while the content of polyunsaturated and monounsaturated fatty acids increased (p <0.05). Textural characteristics, such as hardness, cohesiveness, and adhesiveness in the test sample, have changed significantly (p <0.05). However, the paste mass's elasticity (springiness) and stickiness were almost the same for the control and experimental samples. The introduction of the ingredients mentioned above in the experimental samples increased the pH and water-binding capacity) values, which suggests an increase in juiciness. The conducted studies have confirmed the prospects for improving the chemical composition without deterioration of the consistency and structure of the finished product.

Keywords: paté, sheep tail fat, heart, kidneys, liquorice root, ginger root

INTRODUCTION

Patés are the most widely used and common type of meat product. Liver patés mainly consist of liver and fat components. Still, they could be diverse on the intended and applied packaging (in sausage casing, in the form of canned food), the use and type of raw materials (with liver, various types of meat and offal, herbs, and natural spices) **[1]**, **[2]**. However, specific features of liver paté, such as the high concentration of fat and low content of antioxidants in the composition, cause their high sensitivity to oxidation of fats **[3]**, **[4]**. In this regard, paté producers use preservatives such as sodium nitrite and synthetic antioxidants (butylhydroxytoluene, butyl hydroxyanisole), and in most cases, their inclusion possesses negative consequences **[5]**. For example, studies conducted by Schulze et al. **[6]** and data from the annual report of the World Health Organization (WHO) **[7]** noted the negative effect of synthetic preservatives used in meat products, as well as high risks to human health from excessive consumption of meat products: high content of certain saturated fatty acids, oxidation of fatty acids during heat treatment, etc. The preservatives mentioned above are causes of several types of cancer, cardiovascular disease, diabetes, and so on. According to **[8]**, preservatives such as nitrite and its derivatives in foods can cause cancer in humans. According to the research results of **[9]**, the use of synthetic antioxidants in meat products can be harmful to the human body. All the factors mentioned above have become the basis for the search for new, safer ways to decrease the oxidation processes in meat products with high-fat content. One of the

possible promising solutions is the use of herbal antioxidant supplements. The main benefit of these additives is their high vitamin, mineral, fiber, and low-fat content **[10]**, **[11]**. It is possible to design the final finished product content knowing the vegetable and animal origin raw material's nutrient content. According to several scientific works, a synergistic effect could be achieved by combining different types of raw materials in the production of meat products **[12]**, **[13]**, **[14]**, **[15]**. These implementations also could solve environmental-food problems. Licorice root is known for its immunomodulatory effects against many diseases. It is used in pharmaceutics to treat respiratory diseases – asthma, pharyngitis, infection, malaria, soothe abdominal pain, peptic ulcers and insomnia **[16]**, **[17]**. The main biologically active component in licorice root is glycyrrhizic acid. In smaller amounts, it contains flavonoids, iso-flavanoids, chalcones, coumarins, triterpenoids, sterols, starch, sucrose and glucose, lignans, amino acids, amines, gum, volatile oils **[18]**, **[19]**. Ginger root is also known in the folk medicine of Asia, especially in Chinese medicine, in the folk medicine of West Africa. It has a positive effect on inflammatory diseases: coughs, colds, and rheumatoid arthritis in stomach diseases: dyspepsia, colic, gastroparesis, etc. **[20]**, **[21]**, **[22]**. The analysis of publications shows that ginger and licorice root is mainly considered by scientists in food products to prolong shelf life **[23]**, **[24]**, **[25]**, **[26]** and to enrich the composition of prepared foods **[27]**, **28]**, **29]**. However, research data on licorice root and ginger in meat products are minimal **[23]**, **[25]**, **[30]**, **[31]**. Due to the growing environmental problems of waste processing in the last decades, the issue of including components such as brains, a combination of the liver of various animals and birds, offal, blood, bones, and other animal by-products into the composition of traditional patés, which has become more relevant in the modern industry **[32]**, **[33]**, **[34]**. Another interest is the use of low-demand by-products, which are sent to animal feed production and are practically not processed for food purposes. However, animal by-products have a high nutritional and biological value **[35]**, **[36]**. Considering that in Kazakhstan, based on its natural and climatic features, sheep breeding has been historically developed, including sheep of indigenous breeds with a different sheep tail fat – a post-slaughter raw material, the processing of which is acute and whose weight reaches up to 40 kg for some breeds. The share of fat-tailed sheep is 70% of the total number of sheep in the Republic of Kazakhstan **[37]**. Their breeding has long been predetermined by climatic and economic conditions, as well as the national traditions of the indigenous population. They are famous for their unsurpassed precocity and adaptability to specific local, often extreme par-atypical environmental conditions in certain regions, where it is practically impossible to conduct other branches of the agricultural sector **[37]**. However, with mass breeding, only meat and skin are in demand and processing. Traditionally, lard and butter are used as fat components in liver paté recipes. Still, in countries with a predominant number of Muslims, this product does not meet demand due to the inclusion of non-halal ingredients. As stated by Unsal et al., there are practically no studies on the beneficial properties of chicken fat as a food ingredient **[38]**. Sheep tail fats are mainly included in the production of fermented Turkish sausages, such as foreign and barbecue sausages, which are prepared from beef, lamb, and goat meat with the addition of sheep tail fat **[39]**, **[40]**, as well as for cooking traditional meat products Gudid and Khabib in Tunisia, Algeria, and Morocco **[41]**. The main obstacle to the use of tail fat in the composition of food products is the presence of a specific taste and smell. We considered the possibility of neutralizing them by frying local types of vegetables (carrots, pumpkins, and onions) on them.

This work aimed to study and evaluate the use of sheep tail fat, beef heart, kidneys, and herbal ingredients (pumpkin, licorice, and ginger root) to replace the liver and butter in the patés partially paté.

Scientific Hypothesis

The scientific hypothesis consists in increasing the nutritional value of meat paté using by-products (liver, heart, kidney) and vegetable raw materials (pumpkin, carrots, onions, ground licorice root, ginger root).

MATERIAL AND METHODOLOGY

Experimental studies were conducted jointly with Nazarbayev University (Nur-Sultan, Republic of Kazakhstan), Kazakh National Agrarian Research University (Almaty, Republic of Kazakh-stan), and Almaty Technological University (Almaty, Republic of Kazakhstan).

Samples

Raw materials: Liver and beef offal (heart and kidney) and sheep tail fat were kindly delivered from the LLP "Aigerim Enterprise" (South Kazakhstan region, Republic of Kazakhstan) immediately after slaughtering. Pumpkin, carrots, onions, ground licorice and ginger root, butter (72.5% fat), and spices were purchased from the local Magnum grocery store (Almaty city, Republic of Kazakhstan).

Paté samples: We have manufactured three kind of paté represents the experimental sample and one control sample.

Chemicals

Potassium hydroxide (Labor Farma Limited Liability Partnership, Kazakhstan). n-hexane (VWR International, France). Sodium Acetate, CH_3COONa (Chemistry and Technology Company, Kazakhstan). Toluene (Labor Farma Limited Liability Partnership, Kazakhstan). Sodium methoxide (Labor Farma Limited Liability Partnership, Kazakhstan). Sulfuric acid, H_2SO_4 (Labor Farma Limited Liability Partnership, Kazakhstan).

Instruments

MX-50 weight moisture meter (LTD A&D Co, Japan). High-performance liquid chromatography SHIMADZU LC-20 Prominence HPLC (Japan). Gas chromatography (GC-Agilent 7890B, Agilent Technologies, Santa-Clara, California, USA). Texture Analyzer (Model Brookfield CT3, AMETEK, Berwyn, PA, USA). pH meter HI 99163 instrument (Hanna Instruments Inc., UK).

Description of the Experiment

Paté preparation technology: Four paté samples were prepared with different sheep fat tail fat contents and plant ingredients. The control paté sample included beef liver and butter, while in the experimental samples, the liver was partially replaced by offal meat (heart, kidney) and plant ingredients. Sheep fat tails partially replaced butter in the experimental samples. So, in the first experimental sample (S1), 21% liver and 8% of butter were replaced by offal meat (8%), sheep tail fat (5%), ground dried liquorice root (1%), and ginger (2%) and vegetables (pumpkin 5%, carrot 4%, onion 4%). In the second experimental sample (S2), 26% of liver and 8% of butter were replaced by offal meat (8%), sheep tail fat (8%), ground dried liquorice root (2%), and ginger (3%) and vegetables (pumpkin 5%, carrot 4%, onion 4%). In the third experimental sample (S3), 30% of liver and 8% of butter were replaced by offal meat (8%), sheep tail fat (10%), ground dried liquorice root (3%), and ginger (4%) and vegetables (pumpkin 5%, carrot 4%, onion 4%) (Table 1). Paté was manufactured in the meat processing workshop at the Kazakh Food Processing and Industry Research Institute. For each formulation, 8 cans of paté were produced. The net weight of one can of paté was 330 g. The technological scheme of paté production is shown in Figure 1. Liver and offal meat were cleaned of skin, blood vessels, bile ducts, and other inclusions. Then soaked in chilled water for 10 – 15 minutes, cut, and blanched in hot water for 15 minutes. Then liver and offal meat were minced in a meat grinder (the diameter of the plate is 2 – 3 mm). Pumpkin, carrots, and onions are peeled, cut, and sautéed in sheep tail fat for 10 – 15 minutes. Liquorice and ginger root was crushed on a grinder (CHANGI, Singapore), and sifted twice through a sieve with a diameter of 1 mm. The ingredients were weighed according to the recipe and then mixed and homogenized on a cutting machine L5-FKM (Voronezh, Russia). After the paste mass was filled into cylindrical tin cans (diameter 72.8 mm, height 95 mm), rolled up with tin lids on a manual seamer MZ04 (Russia), and dyed by sterilization in an autoclave “Malysh Nerzh” (Russia) at a pressure of 0.25 MPa and a sterilization temperature of 117 °C. The finished patés were cooled to ambient temperature and stored at 4 °C until analysis. Obtained meat paté was a homogeneous light brown mass with a smeared consistency and the typical flavour and aroma of meat paté.

Table 1 Control and experimental samples formulation.

Name of raw materials and materials	Control	Experimental sample		
		S1	S2	S3
Main raw material, kg per 100 kg of unsalted raw material				
Blanched beef liver	65	44	39	35
Blanched beef heart	-	5	5	5
Blanched beef kidneys	-	3	3	3
Butter (72.5% fat)	10	2	2	2
Bouillon from cooking beef offal, unfiltered	25	25	25	25
Fat tail fat, melted	-	5	8	10
Sautéed pumpkin	-	5	5	5
Browned carrots	-	4	4	4
Sauteed onion	-	4	4	4
Ground licorice root	-	1	2	3
Ground ginger root	-	2	3	4
Spices and materials, g per 100 kg of raw materials				
Ground black cumin	-	0.16	0.16	0.16
Ground nutmeg	-	0.5	0.5	0.5
Ground turmeric	-	0.16	0.16	0.16
Ground black pepper	0.1	0.1	0.1	0.1
Ground marjoram	-	0.5	0.5	0.5
Salt iodized	0.1	0.1	0.1	0.1

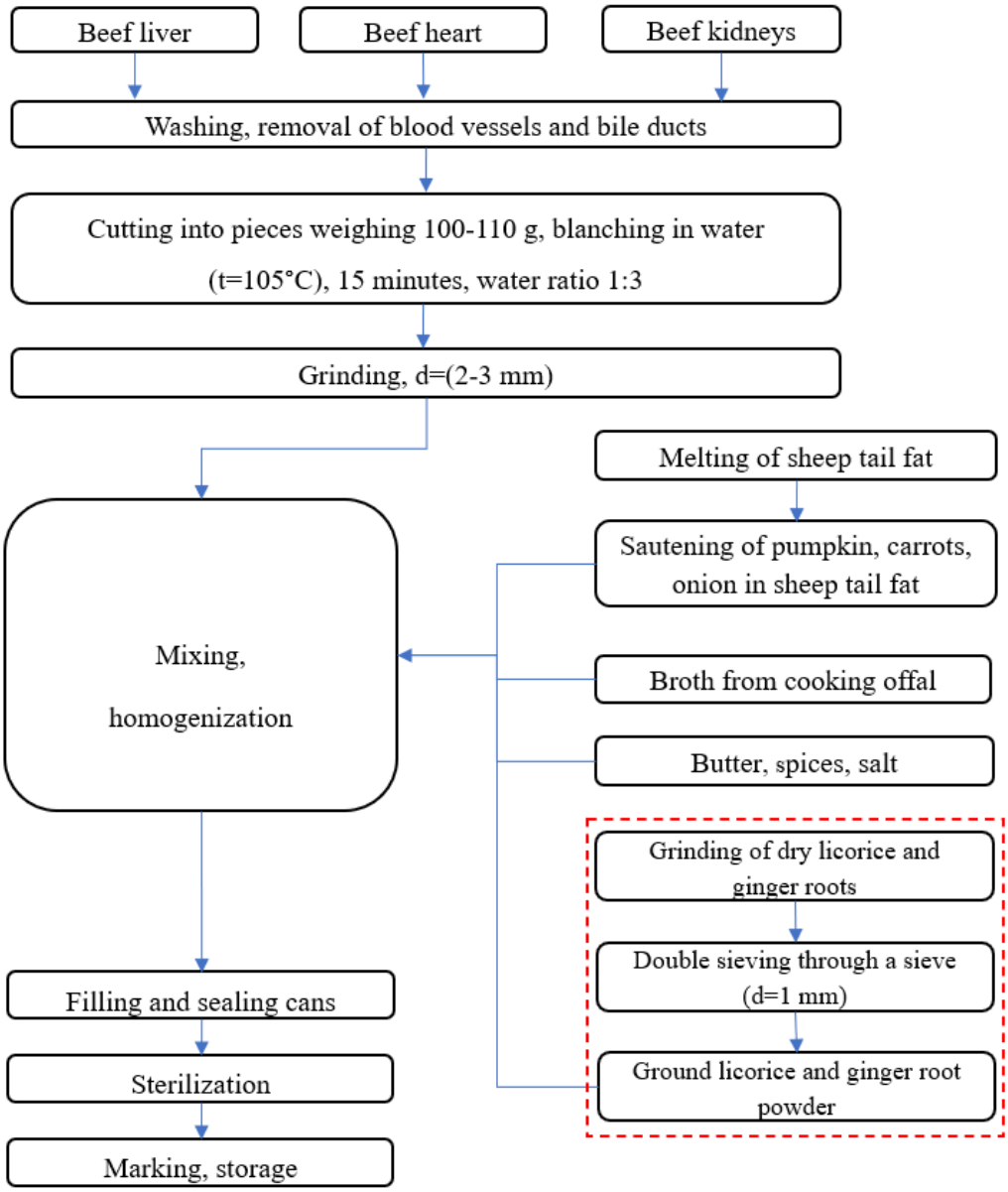

Figure 1 Technological scheme for preparing liver paté.

Laboratory methods

Determination of chemical composition: The moisture content in the meat was determined using an MX-50 weight moisture meter (LTD A&D Co, Japan). All samples for determination of moisture content were weighed in 5 g and evenly distributed inside the instrument cup. The determination of fat, ash, and protein were determined by the methods previously described in **[42]**. The sodium chloride in the paté was determined according to the Volhard method **[43]**. The content of beta-carotene was determined by the method described in **[44]** using beta-carotene standards (Sigma Chemical, USA). Carotenoids were extracted from samples according to the described Yang technique **[45]**. The determination was carried out according to the calibration curves of standard solutions.

Determination of total cholesterol: The total cholesterol content was determined according to the method described in **[46]**. An ethanol solution of potassium hydroxide was added to 2 g of the paté sample from each batch, and then cholesterol was extracted with n-hexane. Then the cholesterol was separated from the rest of the solution, and its content was determined by high-performance liquid chromatography (HPLC).

Determination of amino acid composition: Amino acids were determined using SHIMADZU LC-20 Prominence HPLC (Japan) with fluorimetric and spectrophotometric detectors. We used a chromatographic column 25 cm x 4.6 mm SUPELCO C18, with a diameter of 5 µm (USA), including a pre-column to protect the main column from foreign impurities. The HPLC analysis was based on the method **[33]**. Chromatographic analysis was performed in the eluent gradient mode at a 1.2 mL/min flow rate and a column thermostat temperature of 40 °C. The measurement was carried out on a reverse phase column with fluorimetric and

spectrophotometric detectors at 246 and 260 nm wavelengths using acid hydrolysis and modification of amino acids with a solution of phenylisothiocyanate in isopropanol to obtain phenylthiohydantoines. A 6.0 mm solution of CH_3COONa with a pH of 5.5 (component A), a 1% solution of isopropanol in acetonitrile (component B), and a 6.0 mM solution of CH_3COONa with a pH of 4.05 (component C) were used as the mobile phase. Samples of amino acids produced by Sigma Aldrich (Germany) were used as standards.

Determination of fatty acid composition: The fatty acids were obtained using the protocol described by Bly and Dyer, with a modification proposed by Barros et al. Once the fatty acids were extracted, these compounds were transesterified according to the method described by Dominguez et al. some changes **[46]**. Briefly, 1 mL of toluene was used to dissolve 20 mg of fat before mixing with 2 mL of 0.5 n sodium methoxide solution in a test tube. This mixture was stirred for 10 seconds and kept for 15 minutes at room temperature. After that, 4 mL of 10% was added to the mixture, which was stirred for several seconds, and a methanol solution of H_2SO_4 was added. The mixture was then stirred again for a few seconds after adding 2 mL of saturated sodium bicarbonate solution. Subsequently, fatty acid methyl esters (FAMEs) were separated by adding 1 mL of hexane to a test tube and stirring for 10 seconds. Finally, the vapours were transferred to the appropriate vial.

The vapours were separated and quantified using gas chromatography (GC-Agilent 7890B, Agilent Technologies, Santa-Clara, California, USA) and a PAL RTC-120 autosampler a flame ionization detector (FID). The injection was carried out in the separation mode (1:50) with 1 µL, the injector was maintained at a temperature of 260 °C, and the total flow rate was set to 64.2 mL/min.

Separation was carried out in a capillary column of fused silica SP-2560-100M (inner diameter 0.25 mm, film thickness 0.25 microns; Supelco Inc., Bellefonte, Pennsylvania, USA). The chosen gas carrier was helium, with a 1.2 mL/min flow rate. Pressure at the head of the column was set at 42.135 psi. The chromatography conditions were set as follows: the initial temperature of the furnace was 140 °C (sustained for 4 minutes), the first rise at 5 °C/min to 190 °C, the second rise at 2 °C/min to 210 °C (sustained for 4 minutes), the third rise at a speed of 1 °C/min to 220 °C and the fourth ascent at a speed of 3 °C/min to a final temperature of 235 °C (maintained for 7 minutes). The operating pressure in the FID was set as follows: temperature 260 °C, H2 flow rate 35 mL/min, air 350 mL/min, and recharge flow rate 15 mL/min. The total time of chromatographic analysis was 50 minutes.

MassHunter GC/MS Acquisition B.07.05.2479 software (Agilent Technologies, Santa Clara, California, USA) was used for equipment management and data collection. Data analysis was carried out in the MassHunter Quantitative Analysis B.07.01 software. Authenticated standards (FAME Mix-37 components, docosapentaenoic acid, trans-vaccinoic acid, cisvaccienoic acid, and CLA) were used to identify fatty acid methyl esters by comparing storage times. The results were expressed in g/100g of the total amount of identified fatty acids.

Texture Profile Analysis (TPA): Analysis was performed on a Texture Analyzer (Model Brookfield CT3, AMETEK, Berwyn, PA, USA). The hardness index of the sample was recognized as compression peak loading. For the indicator of cohesiveness, the inclination of the ratio A2/A1 were used. A2 is the zone under compression shock of the second cycle. A1 is the zone under compression shock of the first cycle. If the sample structure was destroyed under the action of the first compression, this ratio was considered equal to zero. If the product's structure was practically not damaged under the influence of compression, this ratio was equal to one. Springiness is an indicator that reflects the ability of the sample to return to its original shape after compression. The adhesiveness value expresses the bonding ability and is calculated as the area under the negative peak when the sensor returns after the first compression. Stickiness (gumminess) was calculated according to the method described in **[47]**. The Brookfield texture analyzer's operation principle is based on measuring the appearance of a stationary tool on a sample, a portable stage (TA-RT-KIT), vertically according to a given velocity law. The device's design consists of a control unit, a measuring head, and a set of interchangeable tools and fixtures. From each batch of paté, a sample of the same size was cut (cubic, 10 mm on each side) and placed in aluminum cylinders. The test was performed in two cycles of sample compression at a speed of 5 mm/s and a sample temperature of 20 – 25 °C, until the samples were compressed to 75% of the initial temperature.

Energy value of paté: The energy value was determined based on the value of the main three nutrients (protein, fat, and carbohydrates) in the product's composition. The energy value of the control and experimental samples were determined according to equation (3):

$$E = 4\,(P+C)+9F \tag{3}$$

Where:

E is an energy cell, kcal/100g; P – protein content, g; F – fat content, g; C – carbohydrate content, g; 4 – protein and carbohydrate calorie index; 9 – fat calorie index.

Water-binding capacity: Water-binding capacity (WBC) was identified by the method proposed by Grau and Hamm using filter paper and a weighted press **[48]**. The weight of the test sample was 0.3 g.

pH determination: The paté's active acidity (pH) patéwas determined by the potentiometric method. The twice ground sample was mixed with distilled water in a ratio of 1:10, followed by stirring on a magnetic stirrer for 30 minutes. The pH was determined using an HI 99163 instrument (Hanna Instruments Inc.).

Sensory analysis. Sensory analysis of samples was carried out according to the Interstate standard GOST 33741-2015 "Canned meat and meat-containing. Methods for determining organoleptic characteristics, net weight and mass fraction of components". Sensory analysis of control and experimental samples of patés was carried out using the profile method. The following indicators were considered: taste, odor, and consistency. In connection with the addition of different components, the following descriptors were selected: taste (sweet, pumpkin, carrot, salty, sharp, spicy, fatty, liver); color (brown-gray, gray); smell (sharp liver, sharp fatty, specific, pronounced); consistency (delicate, smeary, dry, loose, stiff, watery, fibrous). The intensity of each descriptor was evaluated on a scale from 0 to 5 (if no expression of any characteristic was observed, the intensity was evaluated as zero). After statistical processing, the results were obtained, according to which the profiles were designed.

Number of samples analyzed: Three experimental batches of samples (six cans each) and one control batch (six cans each) of patés were analyzed.

Number of repeated analyses: Each study was carried out three times, with the number of samples being twenty-four, which amounted to seventy-two analyses.

Number of experiment replication: The study was repeated three times, with the experimental data processed using mathematical statistics methods.

Statistical Analysis

The experiments were carried out in triplicate. Standard deviation values are given for all measurements. Differences in the measurements of the experimental and control groups were calculated using analysis of variance (one-way ANOVA) using Tukey's test. The measurement value $p < 0.05$ was taken as reliable.

RESULTS AND DISCUSSION

The chemical composition of the paté

The moisture content of paté samples is considerably influenced by the incorporation of offal meat, sheep tail fat, and plant ingredients (Table 2). The experimental data obtained differ somewhat from the data of Gonzalez et al. on a decrease in moisture content when adding 3% persimmon flour powder **[9]** and from data from Dominguez et al. on a reduction of moisture content when replacing pork fat with fish oil in liver patés **[49]**.

Table 2 Chemical composition of the control and experimental samples of paté.

Indicator	Control sample	Experimental sample		
		S1	S2	S3
Moisture content, %	60.0 ±1.60^{a}	63.5 ±1.59^{b}	63.5 ±1.59^{c}	64.8 ±1.95^{d}
Mass fraction of protein, %	17.42 ±0.95^{a}	17.01 ±1.02^{a}	16.49 ±1.02^{a}	16.38 ±0.99^{b}
Mass fraction of fat, %	12.53 ±0.01^{a}	12.65 ±0.006^{b}	13.06 ±0.015^{c}	13.08 ±0.012^{c}
Mass fraction of carbohydrates, %	0.71 ±0.01^{a}	0.87 ±0.01^{b}	1.12 ±0.01^{c}	1.25 ±0.02^{d}
Mass fraction of table salt, %	1.3 ±0.03^{a}	1.3 ±0.04^{a}	1.3 ±0.04^{a}	1.3 ±0.02^{a}
ß-carotene, mg/100 g	nd	0.67 ±0.01^{a}	1.02 ±0.02^{b}	1.19 ±0.01^{c}

Note: Indicated values: ± standard deviation calculated from three parallel measurements; nd – not detected; a – d values with different letters inside the graph mean a significant difference between different batches ($p < 0.05$).

The protein content is not significantly changed unless in sample S3, lowering up to 16.38% ($p < 0.05$). These results are consistent with the results of Gonzalez et. al. **[9]** about the absence of the effect of 3% addition of persimmon flour of the "Triumph" and "Rojo Brillante" varieties on the protein content in patés and a significant decrease with an increase in the content of persimmon flour. Sánchez-Zapata et al. **[50]** reported no significant differences in protein content when tiger nut fiber was added in an amount of 5 – 15%.

The mass fraction of table salt for all samples remained the same. The mass fraction of fat and carbohydrates increased in proportion to the increase in the content of additives, and the content of beta-carotene in all experimental samples increased significantly (not detected in control) ($p > 0.05$). These data differ from the results of the fat determination of Gonzalez et al. **[9]**, where the fat content has significantly decreased with a 3% addition

for persimmon flour of the "Rojo Brillante" variety. Still, similar behaviour was reported by Dominguez et al. when replacing pork fat with fish oil in liver patés **[49]**.

Studying the total cholesterol content

The total cholesterol content is significantly decreased (p <0.05) in experimental samples of paté compared with the control sample. Within the experimental samples, the cholesterol content is lowered as the added sheep tail fat increases. The results of determining the cholesterol content are shown in Table 3. Our results are combined with the data obtained by Martins and others with partial re-placement of pork fat with oleogels, where the cholesterol content varied in the range of 19.7 – 24.4 mg/100g **[51]**, but differ from the data of Vargas-Ramella and others on the addition of capsulated olive oil to reindeer meat patés, where the cholesterol content varied between 27.8 – 39.2mg/100g **[52]** and from the data of Dominguez et al. **[49]** when replacing pork fat with fish oil (60.35 – 147.11 mg/100g). These differences are related to the difference in the recipe of patés.

Table 3 The total cholesterol content of the control and experimental paté samples.

Indicator	Control Sample	Experimental sample		
		S1	S2	S3
Total cholesterol, mg/100g	24.4 ±0.97^{a}	22.1 ±0.79^{b}	21.6 ±0.79^{c}	20.3 ±0.56^{d}

Note: Indicated values: ± standard deviation calculated from three parallel measurements; a – d values with different letters inside the graph mean a significant difference between different batches (p <0.05).

Studying the amino acid composition

Replacing the liver in the paté with kidneys, heart and herbal supplements from 21% to 30% significantly reduced the content of all amino acids except arginine. Among the experimental samples, the highest indicator in terms of the total number of amino acids (18 g/100g) and the content of essential amino acids (8.89 g/100g) was shown by samples of S1 with 26% liver replacement in the formulation (plant additives (9%), beef heart (5%), kidneys (3%) licorice root powder (1%), ginger root powder (2%) (Figure 2, 3). The results of the amino acid composition of the batch of patés are presented in Table 4.

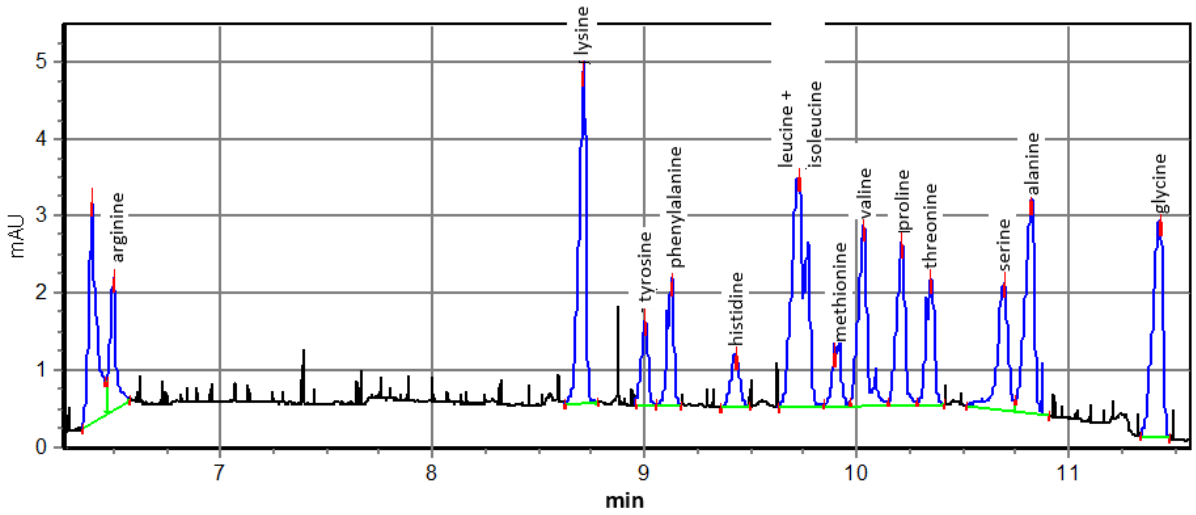

Figure 2 Amino acid composition of control paté sample.

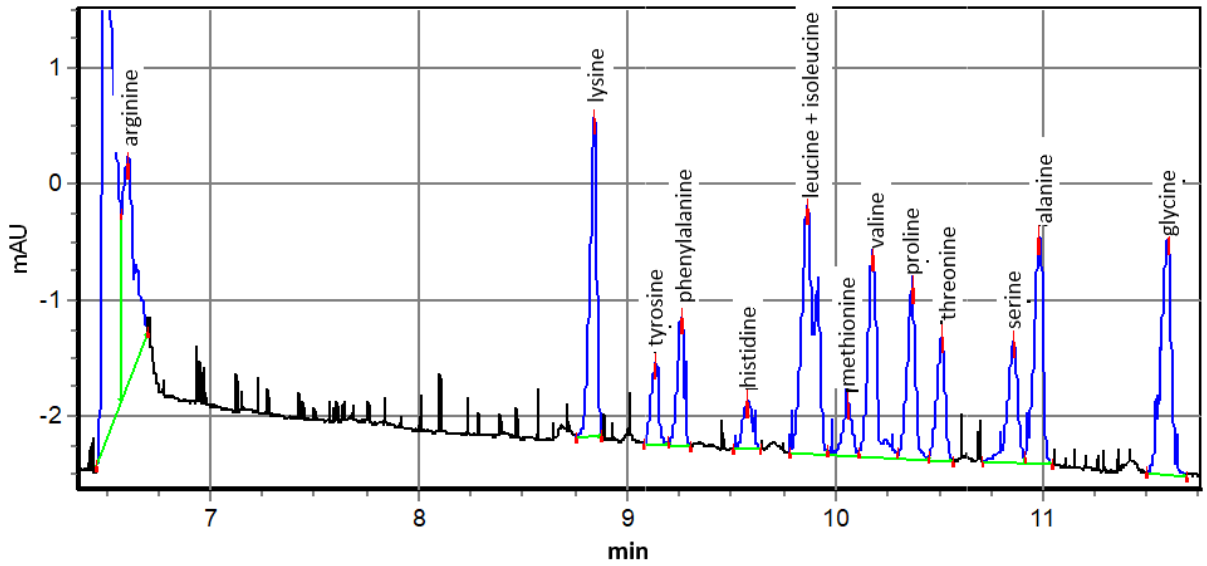

Figure 3 Amino acid composition of experimental paté sample S2.

Table 4 Amino acid composition of control and experimental paté samples (g/100g).

Indicator	Control	Experimental sample		
		S1	S2	S3
Essential Amino acids				
Threonine	1.26 ±0.50^{a}	1.13 ±0.29^{a}	0.87 ±0.35^{b}	0.79 ±0.26^{b}
Lysine	2.15 ±0.73^{a}	2.01 ±0.59^{a}	1.38 ±0.47^{b}	1.25 ±0.34^{b}
Phenylalanine	1.57 ±0.47^{a}	1.43 ±0.36^{a}	1.13 ±0.34^{b}	1.09 ±0.28^{b}
Leucine+isoleucine	2.29 ±0.59^{a}	1.78 ±0.20^{b}	1.56 ±0.41^{c}	1.42 ±0.19^{d}
Methionine	0.90 ±0.30^{a}	0.65 ±0.22^{b}	0.56 ±0.19^{bc}	0.49 ±0.10^{c}
Valine	2.11 ±0.84^{a}	1.89 ±0.74^{b}	1.51 ±0.61^{c}	1.40 ±0.45^{c}
Total essential amino acids	19.3 ±1.51^{a}	8.89 ±0.73^{b}	7.01 ±0.49^{c}	6.44 ±0.75^{c}
Nonessential amino acids				
Tyrosine	1.12 ±0.34^{a}	0.96 ±0.44^{b}	0.82 ±0.25^{c}	0.76 ±0.21^{c}
Histidine	0.81 ±0.40^{a}	0.76 ±0.30^{a}	0.56 ±0.28^{b}	0.45 ±0.14^{b}
Proline	1.61 ±0.42^{a}	1.28 ±0.36^{a}	1.13 ±0.29^{b}	1.09 ±0.20^{b}
Arginine	1.93 ±0.015^{a}	2.56 ±0.79^{b}	3.81 ±1.52^{c}	3.99 ±0.35^{c}
Serine	1.39 ±0.36^{a}	1.06 ±0.29^{b}	0.82 ±0.21^{c}	0.73 ±0.13^{c}
Alanine	1.70 ±0.44^{a}	1.35 ±0.34^{b}	1.13 ±0.29^{c}	1.04 ±0.36^{c}
Glycine	1.43 ±0.49^{a}	1.14 ±0.28^{b}	1.08 ±0.37^{b}	0.98 ±0.28^{c}
Total nonessential amino acids	9.99 ±0.78^{a}	9.11 ±0.65^{b}	9.35 ±0.45^{c}	9.04 ±0.74^{b}
Total amino acids	29.29 ±0.93^{a}	18 ±1.64^{b}	16.36 ±0.95^{c}	15.48 ±1.52^{b}

Studying the fatty acid composition

Changing the composition of the paté, especially replacing butter with sheep tail fat, significantly affected all measured fatty acids, both saturated and unsaturated, except for thymnodonic and erucic acids. All experimental samples of paté showed a significant decrease in the amount of all saturated fatty acids (except stearic acid) in proportion to the increase in the content of sheep tail fat, ground liquorice root, and ginger, and a simultaneous increase in the sum of monounsaturated and polyunsaturated fatty acids. The results of the determination of the fatty acid composition are presented in Table 5.

Table 5 Fatty acid composition of control and experimental paté samples (%).

Fatty acid name	Control sample, % weight	Experimental sample, % weight		
		S1	S2	S3
Saturated fatty acids				
Butyric acid	1.91 ±0.02[a]	1.84 ±0.011[b]	1.79 ±0.01[c]	1.64 ±0.015[d]
Caproic acid	76.81 ±0.017[a]	74.65 ±0.0096[b]	72.69 ±0.0082[c]	70.88 ±0.0126[d]
Caprylic acid	0.76 ±0.0208[a]	0.458 ±0.0021[b]	0.333 ±0.0015[c]	0.208 ±0.0011[d]
Capric acid	0.423 ±0.0015[a]	0.326 ±0.001[b]	0.188 ±0.001[c]	0.096 ±0.0015[d]
Undecylic acid	0.12 ±0.0115[a]	0.085 ±0.0015[b]	0.056 ±0.001[c]	0.031 ±0.0015[d]
Stearic acid	10.016 ±0.0015[a]	11.75 ±0.01[b]	13.09 ±0.0153[c]	14.94 ±0.0153[d]
Lauric acid	0.556 ±0.001[a]	0.419 ±0.0015[b]	0.292 ±0.0015[c]	0.158 ±0.0015[d]
Myristic acid	0.034 ±0.001[a]	0.028 ±0.0011[b]	0.014 ±0.0015[c]	0.006 ±0.001[d]
Pentadecanoic acid	0.189 ±0.0011[a]	0.168 ±0.0015[b]	0.147 ±0.0015[c]	0.131 ±0.0015[d]
Palmitic acid	2.229 ±0.001[a]	2.0961 ±0.014[b]	1.9867 ±0.003[c]	1.7495 ±0.027[d]
Total	93.047 ±0.002[a]	91.8201 ±0.009[b]	90.5867 ±0.003[c]	89.8395±0.01[d]
Monounsaturated fatty acids				
Palmitoleic acid	0.78 ±0.016[a]	0.65 ±0.009[b]	0.5106 ±0.001[c]	0.39 ±0.0153[d]
Myristoleic acid	0.266 ±0.005[a]	0.213 ±0.005[b]	0.1515 ±0.002[c]	0.101 ±0.003[d]
Oleic acid	3.92 ±0.006[a]	4.98 ±0.1001[b]	6.041 ±0.007[c]	7.015 ±0.007[d]
Nervonic acid	0.0093 ±0.003[a]	0.0081 ±0.001[ab]	0.0062 ±0.001[ab]	0.0041 ±0.001[b]
Erucic acid	0.008 ±0.001[a]	0.008 ±0.001[a]	0.01 ±0.001[a]	0.011 ±0.004[a]
Elaidic acid	0.019 ±0.001[a]	0.024 ±0.002[a]	0.029 ±0.001[ab]	0.033 ±0.001[b]
Total	5.0023 ±0.001[a]	5.8831 ±0.002[b]	6.7483 ±0.002[c]	7.5541 ±0.002[d]
Polyunsaturated fatty acid				
Linoleic acid	0.213 ±0.011[a]	0.698 ±0.004[b]	1.394 ±0.008[c]	1.499 ±0.007[d]
Linolenic acid	0.9734 ±0.001[a]	0.7896 ±0.001[b]	0.4016 ±0.002[c]	0.2356 ±0.001[d]
Timnodonic acid	0.021 ±0.008[a]	0.022 ±0.006[a]	0.025 ±0.008[a]	0.026 ±0.005[a]
Arachidonic acid	0.6297 ±0.0014[a]	0.6827 ±0.001[b]	0.7519 ±0.0019[c]	0.7711 ±0.0018[d]
Cervonic acid	0.108 ±0.009[a]	0.096 ±0.007[ab]	0.084 ±0.010[bc]	0.072 ±0.003[c]
Total	1.9451 ±0.001[a]	2.2883 ±0.001[b]	2.6565 ±0.002[c]	2.6037 ±0.001[d]

Note: Indicated values: ± standard deviation calculated from three parallel measurements; a – d values with different letters inside the graph mean a significant difference between different batches (p <0.05).

The highest content of monounsaturated fatty acids (7.5541%) was showed sample S3, and the highest content of polyunsaturated fatty acids (2.6565%) showed sample S2. The growth of monounsaturated fatty acids is connected mainly with the development of oleic acid content, which increased from 3.92% (78% of total PUFAs) in the control sample to 4.98 – 7.015% in the experimental samples (85 – 93% of total PUFAs). The increase of polyunsaturated fatty acids content is connected with the increase of linoleic acid content from 0.213% (11% of PUFA sum) in the control sample to 0.698 – 1.499% in experimental samples (30 – 58% of PUFA sum) and with the increase of arachidonic acid content from 0.6297% (32% of PUFA sum) in control sample to 0.6827 – 0.7711% in experimental samples (28 – 30% of PUFA sum). The highest content of monounsaturated fatty acids (7.5541%) was showed sample S3, and the highest content of polyunsaturated fatty acids (2.6565%) showed sample S2. The growth of monounsaturated fatty acids is connected mainly with the growth of oleic acid content, which increased from 3.92% (78% of total PUFAs) in the control sample to 4.98 – 7.015% in the experimental samples (85 – 93% of total PUFAs). The increase of polyunsaturated fatty acids content is connected with the increase of linoleic acid content from 0.213% (11% of PUFA sum) in the control sample to 0.698 – 1.499% in experimental samples (30 – 58% of PUFA sum) and with the increase of arachidonic acid content from 0.6297% (32% of PUFA sum) in the control sample to 0.6827 – 0.7711% in experimental samples (28 – 30% of PUFA sum).

Similar results of a decrease in saturated fatty acids and an increase in monounsaturated fatty acids were described in **[53]** when studying burger patties with partial replacement of pork fat with sunflower, olive oil, and avocado oil. However, our data differ from Domínguez et al. **[49]** on replacing pork fat in the paté with fish oil at 50% and 75%. These differences may be related to differences in the fatty acid composition due to the different raw materials used.

Profile texture analysis

The profile analysis results were obtained to compare the structure of samples of the control and experimental batches of paté (table 6). Texture profile analysis is about detecting the simulation of chewing processes in the human mouth by subjecting it to cyclic compression **[54]**, **[55]**.

The experimental samples' values of hardness, cohesiveness, and adhesiveness, expressed as a negative value of the curve in the simulation of chewing, were significantly higher than the control (p <0.05). This, in turn, indicates an increase in the hardness of the consistency, the ability to adhere, and the stickiness of the mass of experimental products. In our study, sample S1 and the control showed approximately the same elasticity (springiness) and stickiness (gumminess) (p >0.05), and its a significant variation in samples S2 and S3. A change in the composition of the paté above a certain value in samples S2 and S3 significantly reduced the required energy for crushing the product's structure before swallowing it in the mouth,.

Table 6 Analysis of the texture profile of the control and test samples of the paté.

Indicators	Control sample	Experimental samples		
		S1	S2	S3
Hardness (kg)	2.09 ±0.08	2.18 ±0.05^{b}	2.35 ±0.11^{c}	2.44 ±0.19^{d}
Adhesiveness (g/s)	- 64.05 ±20.05	-64.75 ±14.10^{b}	- 65.02 ±16.60^{c}	66.15 ±10.12^{d}
Spring force (mm)	0.18 ±0.01	0.20 ±0.01^{a}	0.23 ±0.02^{b}	0.25 ±0.05^{b}
Cohesiveness (g)	0.45 ±0.05	0.59 ±0.04^{b}	0.68 ±0.04^{c}	0.74 ±0.03^{d}
Gumminess (g)	927.10 ±31.25	901 ±27.45^{a}	788.63 ±22.03^{b}	625 ±12.04^{b}

Note: Indicated values: ± standard deviation calculated from three parallel measurements; a – d values with different letters inside the graph mean a significant difference between different batches (p <0.05).

The incorporation of additional ingredients may to some extent affect the structural and mechanical characteristics of the paté. Since the paté is a paste-like homogeneous mixture of various ingredients and with the inclusion of new ingredients, not only textural but also physical–chemical characteristics may change **[56]**, **[57]**, **[58]**.

Studying pH, water-binding capacity, and energy value of paté

The calculation of energy value calculation showed that the prototypes showed a significantly higher caloric content compared to the control (p <0.05). This is due to the test sample's higher fat content (4.22% higher) and carbohydrates (57.75% higher). The water-binding capacity (WBC) of meat products varies depending on the structure of the constituent particles and the size of the constituent particles of the dispersed medium of the product **[59]**, **[60]**. Accordingly, the ability of the paste to bind and retain moisture, including the moisture-binding ability, is affected by factors such as the degree of grinding of ingredients, the content of connective tissue in the product, the salt content, the presence and content of binding mass of ingredients (flour, eggs, etc.), etc. A high value of WBC increases meat products' juiciness, tenderness, appearance, and technological properties **[61]**, **[62]**. In our experience, the WBC of the experimental samples significantly increased with the addition of 8% sheep tail fat, 2% and 3% of ground liquorice and ginger root (S2), and 10% sheep tail fat, 3% and 4% of ground liquorice and ginger (S3) compared with the control sample. The results of the pH determination also showed an increase in proportion to the content of tail fat, ground ginger root, and liquorice compared with the control. Oksukhanova obtained similar results and others **[34]**. When replacing beef tripe and melted horse fat with part of the raw materials in the deer meat paste, an increase in WBC and pH values was observed in the experimental samples.

Table 7 Indicators of pH, WBC, and energy values of the control and experimental samples of paté.

Indicators	Control sample	Experimental sample		
		S1	S2	S3
pH	5.90 ±0.11	5.98 ±0.13^{b}	6.15 ±0.09^{c}	6.18 ±0.07^{d}
WBC, %	67.95 ±0.45	68.02 ±1.30^{a}	68.78 ±0.74^{b}	68.85 ±0.89^{b}
Energetic value, kcal/100g	185.29	185.37	187.98	188.68

Note: Indicated values: ± standard deviation calculated from three parallel measurements; a – d values with different letters inside the graph mean a significant difference between different batches (p <0.05), nd – not detected.

Sensory analysis of paté

Figure 4 shows that the flavour profilogram of the samples is distributed unevenly due to differences in taste characteristics. The control sample has a pronounced liver flavour compared to the experimental samples. The pumpkin and carrot flavour and sweetness are slightly evident and interfere with the taste of sheep fat in the experimental samples. Ginger complements and enriches the taste of paté's paté light spicy flavour due to a large amount of essential oils. The taste of liquorice root is hardly perceptible. Figure 5 shows the sharp liver flavour in the control sample. With the introduction of pumpkin, carrots, and spice flavour additives (cumin, nutmeg, turmeric, black pepper, marjoram), the odours of additives interfere with the smell of sheep fat, are respectively manifested. The profiles to characterize the consistency of the control and experimental samples are shown in Figure 6. The consistency profilograms of control and experimental samples are unevenly distributed. The control sample is drier and stiffer. In the experimental samples with an increasing amount of fat and vegetable ingredients, there is a tendency to a more expressed soft and creamy consistency. As a result of sensory analysis of samples of liver paté visible positive changes in taste, consistency, and odour with the introduction of sheep fat and plant ingredients in the recipe.

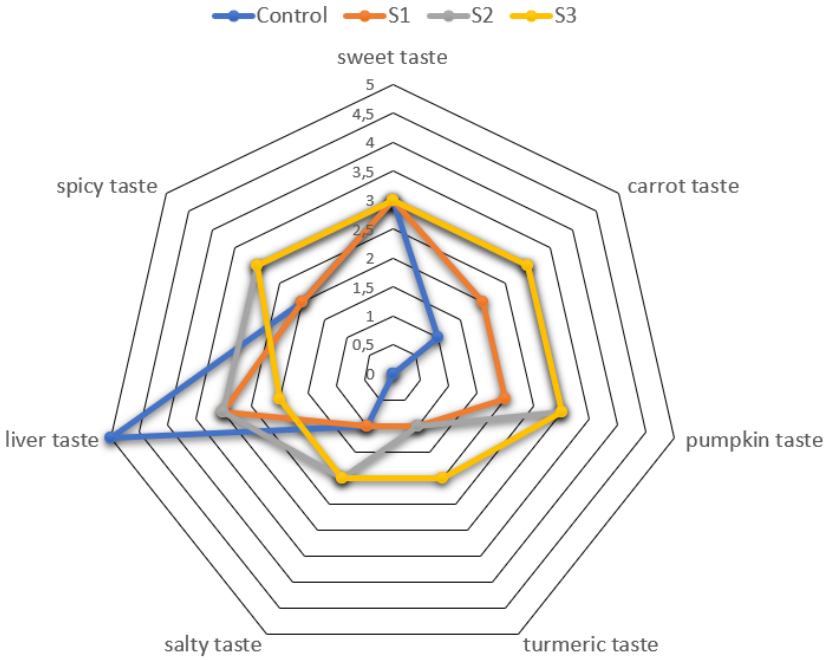

Figure 4 Profiles to characterize the taste sensations of experimental and control samples.

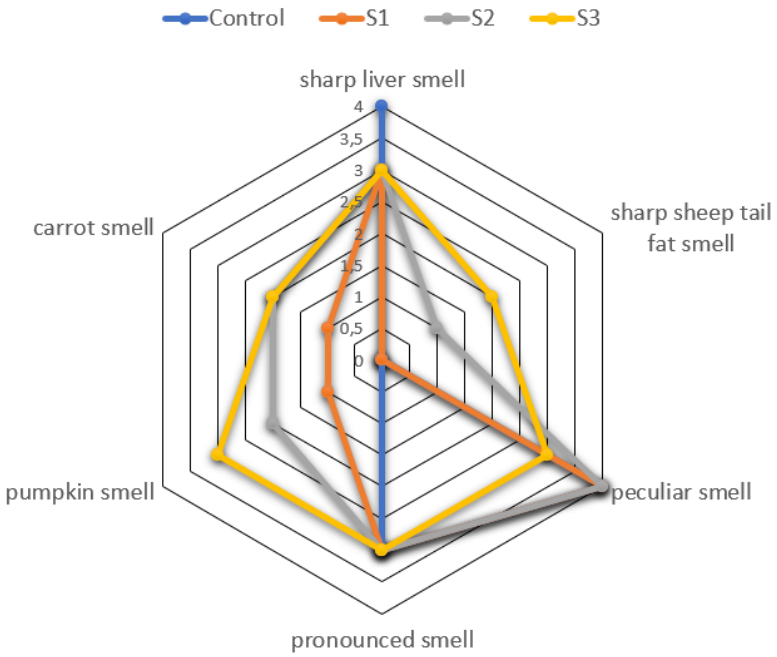

Figure 5 Odor characterization profiles of experimental and control samples.

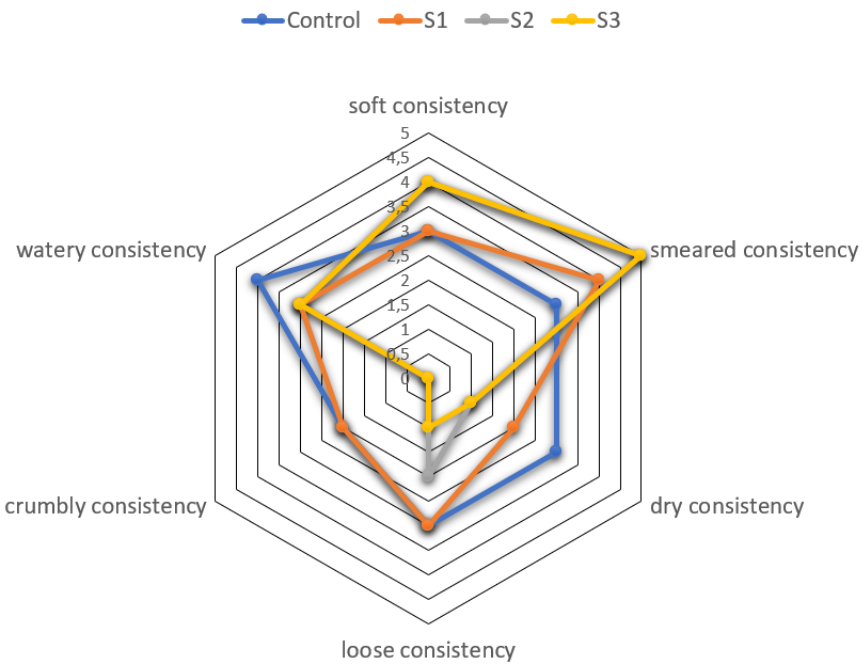

Figure 6 Profiles for consistency characterization of experimental and control samples.

CONCLUSION

Following the principle of minimizing animal slaughter waste, we evaluated the possibility of replacing many ingredients in liver patés with less popular ones: butter with sheep tail fat, liver with beef heart, kidneys, as well as vegetable additives (liquorice root and ginger, pumpkin, carrot, and onion). Including the ingredients mentioned above in the paté recipe did not cause significant changes in the mass fraction of table salt and protein and, conversely, significantly increased the moisture content, carbohydrates, fat, and beta-carotene in the experimental samples (p <0.05). The presence of ß-carotene in the experimental samples is of particular interest, given that in the control sample, this provitamin was not detected at all. The addition of new ingredients in the experimental samples reduced (p <0.05) the amount of amino acids except for arginine and increased the content of fatty acids. Notably, the content of all fatty acids increased significantly (p <0.05) in proportion to the growth of tail fat, ground liquorice root, and ginger. With adding new ingredients of plant and animal origin, hardness, cohesiveness, and adhesiveness have changed significantly (p <0.05). However, stickiness and elasticity (springiness) were almost the same for the control and experimental samples. The introduction of the ingredients mentioned above increased the WBC of the experimental samples, which suggests an increase in the juiciness of the product. A similar effect was found for pH. Sensory analysis showed more expressed soft and creamy consistency in experimental liver pate samples. The use of these ingredients in liver paté demonstrated the possibility of enrichment with ß-carotene, improving the consistency and structure of the product without loss in the quality of the finished product.

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Comparative characteristics of goat milk products in farms of Akmola and North Kazakhstan regions

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ABSTRACT

Providing the population with high-quality products is a priority intention of the government. North Kazakhstan and Akmola regions are the most promising in developing the country's dairy cattle breeding and milk processing. An assessment of the qualitative indicators of milk production and processing in these areas would allow us to assess the dynamics of the development of the country's dairy industry and identify the main problems, so research on this issue is relevant. The study aimed to analyze the qualitative indicators of milk production and processing at the enterprises of the North Kazakhstan and Akmola regions, the factors affecting the quality of dairy products, and the prospects for expanding the range of enterprises. In the study, an InfraXact infrared analyzer was used to determine the quality of haylage in the diet of goats and for goat milk - the CombiFoss FT + analyzer. Generally, the quality of milk on the goat farms "Zeren" and "Tamasha-2050" in terms of fat, protein, lactose, fatty acid composition of milk fat, and somatic cells meets the regulatory requirements. The specificity of the goat farm "Tamasha-2050" is the production of various hard and soft cheese types. Farm "Zeren" is focused on the production scale and a wide range of products (drinking milk, yogurt, kefir, and ice cream). It was concluded that there is a wide choice for the consumer of high-quality dairy products from goat milk in enterprises.

Keywords: goat milk, dairy product, quality, goat farm, Kazakhstan

INTRODUCTION

Recently, there has been a positive trend in dairy goat breeding in Kazakhstan, a profitable segment of the Kazakhstan market. Analyzing the changes that have taken place in the industry, it should be noted that according to the statistics agency of the Republic of Kazakhstan, the number of goats amounted to more than 400 thousand heads. According to statistics, the total number of sheep and goats in all categories of farms as of June 1, 2022, was 28,023.7 thousand heads. Compared to 2021, the increase was already 3.18%, as shown in Table 1.

The livestock of goats by category of agriculture includes large private farms (approximately 70%), agricultural organizations (1%), and peasant farms (29%).

As part of the project for developing the agro-industrial complex of the Kazakhstan Republic for 2021-2025, the volume of state support for the agro-industrial complex in 2022 is 48.1 billion tenges. Support for the development of animal husbandry is carried out in the given areas: to increase the productivity and quality of livestock products and subsidize farm animals. So, for example, funding for the development of animal husbandry is 6.5 billion tenges. Investments and subsidization of processing – 17.2 billion tenges (investments – 16,544.4 million tenges, for subsidizing the costs of processing enterprises – 697.8 million tenges) [1].

Table 1 Number of sheep and goats, thousand heads.

Region	2018	2019	2020	2021	2022 (on June 1)
Akmola	524.2	527.8	537.4	741.0	767.2
North Kazakhstan	384.4	402.7	416.9	615.4	634.9
Aktobe	1,206.1	1,216.9	1,265.1	1,578.6	1,636.9
Almaty	3,798.8	3,847.8	3,926.0	5,121.1	5,392.9
Atyrau	574.1	588.3	598.9	787.1	789.5
West Kazakhstan	1,291.0	1,219.9	1,254.9	1,734.6	1,815.7
Zhambyl	2,837.3	2,922.9	3,091.4	3,881.8	4,080.8
Karaganda	925.0	918.6	943.7	1,441.4	1,500.8
Kostanay	466.0	479.3	490.2	551.3	524.4
Kyzylorda	627.4	689.0	747.0	895.2	926.8
Mangist	399.1	394.7	424.9	610.7	561.5
Pavlodar	537.5	551.2	575.6	752.2	827.6
Turkestan	4,013.6	4,217.6	4,447.6	5,981.2	6,087.9
East Kazakhstan	1,809.7	1,784.1	1,769.8	2,370.7	2,401.9
Nur-Sultan	1.6	1.4	1.0	1.0	1.1
Almaty city	2.4	2.2	1.8	1.9	1.8
Shymkent	102.3	106.3	99.6	93.4	71.8
The Republic of Kazakhstan	19,500.4	19,870.7	20,592.0	27,158.6	28,023.7

Note: Compiled by the authors.

Figure 1 Map of agro-industrial projects in Kazakhstan.

According to the data in Figure 1, the agro-industrial map highlighted areas in which the development investments were made. Thus, in the Akmola region, from 2018 to 2021, the following projects were launched: processing of 1000 tons of oilseeds per day, production of 20 thousand tons of products per year (canned food, steaks, meat delicacies, etc.), the output of 500 tons of trout per year (live/chilled); in the Kostanay region from 2019 to 2020, a project is being implemented to produce 20 thousand tons of meat products per year; in the Almaty region from 2018 to 2021, the production of 60 thousand tons of extruded feed, the storage of grain – 15.0 thousand tons, the output of 1.2 thousand tons of dairy products per year are being implemented. More than 90 farms (29 modern dairy complexes) are in milk production in the North-Kazakhstan region. To increase the available livestock, about 1549 heads of breeding young cattle from Russia and European countries were imported this year. 34 projects worth 48 billion tenges are being implemented in the North Kazakhstan region. 16 dairy farms for 9 thousand heads and two feedlots for 13 thousand places are being built in the region **[2]**.

Akmola region is one of the leading agricultural and industrial regions of the Republic of Kazakhstan **[3]**. As part of the development of dairy farming in 2021, 19 farms for 2.3 thousand heads were created in the Akmola region (Akkolsky – 1 farm for 40 heads, Arshalynsky – expansion of 1 existing farm for 108 heads, Astrakhan – 1 farm for 50 heads was created and expansion of 1 operating farm for 400 heads, Atbasarsky – 1 farm for 300

heads, Birzhan sal district – expansion of 2 existing farms for 210 heads, Bulandinsky – 1 farm for 50 heads, Burabaysky – 2 farms for 348 heads were created and development of 1 operating farm for 40 heads, Ereymentausky – 1 farm for 93 heads, Esilsky – 1 farm for 50 heads, Zhaksynsky – 1 farm for 50 heads, Zerenda – 1 farm for 90 heads, Korgalzhynsky – 1 farm for 50 heads, Sandyktausky – 1 farm for 100 heads, Tselinogradsky – expansion of 1 operating farm for 275 heads, Shortandinsky – expansion of 1 existing farm for 93 heads) **[1]**. The volume of processed milk production amounted to 41 thousand tons, a decrease of 21.4% compared to the same level in 2021, 759 tons of butter were produced with an increase of 3.3%, and cheese and cottage cheese – by 452 tons, a decrease of 42.4%, fermented milk products - 3.5 thousand tons with an increase of 6%. The workload of milk processing enterprises amounted to 59.9% (in 2021 – 76.2%) **[1]**.

Table 2 shows data on milk production in Kazakhstan over the past six years.

Table 2 Milk production in Kazakhstan in 2016-2021 (tons).

Index	2016	2017	2018	2019	2020	2021
Total production of milk	5341647.1	5 503418.3	5 686210.8	5 865087.6	6 051407.7	6 247202.3
cow's milk	5300014.2	5460451.4	5642283.0	5819317.2	6004364.4	6198842.0
mare's milk	25497.0	26600.8	27221.2	27565.3	28424.5	29025.2
goat milk	1486.4	1413.5	1398.3	1374.5	1381.6	1331.0
camel milk	14649.5	14952.6	15308.3	16827.6	17234.1	18022.0

As seen in Table 2, the production of all types of milk is increasing from year to year; at the same time, the production of mare and camel milk is growing, then the gross collection of goat milk remains at almost the same level.

According to the Ministry of Agriculture in Kazakhstan, there is an annual increase in prices for socially significant food products. Table 3 shows the average prices for dairy products as of 2021; for example, for the whole republic: unsalted butter was sold for 2720 per 1 kg, pasteurized milk (2.5%) – 256 per 1 liter, kefir (2.5 %) – 280 per 1 liter, cottage cheese – 1530 per 1 kg.

Table 3 Average prices for socially important food products as of 2021.

Region	Butter unsalted	Pasteurized milk 2.5%, liter	Kefir 2.5%, liter	Cottage cheese
Nur-Sultan	3 154	275	298	2 205
Almaty	3 207	271	321	1 838
Shymkent	2 332	231	244	1 849
Aktau	3 162	-	340	2 045
Aktobe	2 427	245	277	1 358
Atyrau	3 307	-	321	1 348
Zhezkazgan	2 774	297	280	1 822
Kokshetau	2 509	252	312	1 167
Karaganda	3 053	275	249	1 465
Kostanay	2 540	244	267	1 476
Kyzylorda	2 860	-	252	1 460
Uralsk	2 380	241	261	1 335
Ust-Kamenogorsk	2 759	274	352	1 406
Pavlodar	2 489	244	271	1 404
Petropavlovsk	2 433	-	229	1 173
Semei	2 450	274	263	1 504
Taldykorgan	2 767	209	216	1 092
Taraz	2 364	-	248	1 528
Turkestan	2 700	-	288	1 595

Note: Compiled by the authors. Source **[2]**.

Despite high production costs, milk production and processing remain an attractive economic niche. However, for the sustainable development of the dairy goat industry in Kazakhstan, it is necessary to modernize the material and technical base and goat milk processing technologies, create a forage base, and modern methods of storing

dairy and processing secondary raw materials. This study aimed to confirm that goat farms in Akmola and North Kazakhstan produce good quality raw materials, which could be successfully used to create a wide range of dairy products from goat milk.

Given the development of dairy cattle breeding in the regions, it is necessary to accumulate statistical information on domestic experience in the production of goat milk and products from it.

Scientific Hypothesis

The feeding of goats must be optimized using additional feed additives to improve the quality component of raw milk. To reach this goal, we analyzed the quality of feed value of haylage included in the diet of goats and milk on two farms. Such feed's nutritional value provides goats with a maximum of 80% of the norm of feed units per day.

MATERIAL AND METHODOLOGY

This article is a comparative description of the production of goat milk and dairy products, compiled on the example of two goat farms in the Akmola and North Kazakhstan regions. The review includes statistical reports from the Department of Agriculture of Akmola and the North Kazakhstan regions. The analysis of the suitability of Akmola and North Kazakhstan goat milk as raw materials for cheese and dairy products was carried out. Links to studies on the production of dairy products from goat milk worldwide are given. Since product characteristics ultimately depend on milk quality, there is great interest in identifying the fundamental relationship of milk production factors that affect its composition **[3]**. The research aims to improve the quality of production and processing of goat's milk. An analysis of the qualitative indicators of milk and dairy products in the regions and the factors influencing these indicators is relevant for obtaining a general idea of the present state and further development of the dairy industry in the country.

Description of the Experiment

Goat farms "Zeren" and "Tamasha-2050": The breeding farm "Zerenda" produces products from goat milk under the ZEREN trademark and is widely represented in stores in Astana (Nur-Sultan), Karaganda, and Kokshetau. It is the largest goat farm in the Kazakhstan Republic and has more than 1200 breeding goats of Zaanen, Alpine, and Angol-Nubian breeds. The breeding farm "Zerenda" has a complete production cycle, from fodder harvesting to the release of final products, allowing to control of product quality at each stage **[4]**. Farm "Tamasha-2050" – the farm specializes in breeding goats and producing dairy products. Farm "Tamasha-2050" was founded in 2020. Farm "Tamasha" breeds goats of the Saanen breed. At the Tamasha-2050 farm, cheeses are made from milk immediately after pasteurization; classic recipes are used. The cheese factory produces 36 products, including elite cheeses such as parmesan, asiago, cheddar, and montasio **[5]**.

Determination of feed quality: Feed quality was determined according to GOST 32040-2012 "Feedstuffs, compound feeds, feed raw materials. Method for determination of crude protein, crude fiber, crude fat and moisture using spectroscopy in the near-in-infrared region" on an InfraXact infrared analyzer (FOSS Electric, Denmark). It provides fast, accurate, and reliable analysis of feed, grain, and other components (protein, moisture, fat, crude fiber, ash, etc.) both in production and in the laboratory. The study was conducted based on the laboratory of the North Kazakhstan Research Institute of Agriculture LLP, Beskol, Kazakhstan **[6]**.

InfraXact is a spectrometer operating in the near-in-infrared region of the spectrum (NIR, 570-1848 nm); the analysis does not require reagents and solvents. The essence of the principle of operation of InfraXact spectrophotometers is based on comparing two light fluxes: full, taken as 100% reflection, and weakened when reflected from the sample under study. The spectrophotometers are assembled according to a single-beam scheme. The following main components are located in the device body: light source (halogen lamp); monochromator with a movable diffraction grating; focusing optical system; cuvette compartment for placing a cup with a test sample or solution; radiation receivers - a silicon photodiode for the wavelength range of 570-1098 nm and an InGaAs-based detector for the wavelength range of 1100-1848 nm, as well as a power supply system and a communication circuit with a control computer. Management of operating modes, all calibration operations, measurements, and saving results are performed by a specialized computer program "ISIScan," running in a Windows environment **[6]**.

Feed quality was determined according to GOST 32040-2012 Feedstuffs, compound feeds, and feed raw materials. Method for determination of crude protein, crude fiber, crude fat, and moisture using spectroscopy in the near-in-infrared region on an InfraXact infrared analyzer (FOSS Electric, Denmark). While preparing and carrying out measurements in the laboratory, the following conditions were met: ambient temperature – 27 °C, relative air humidity – 52%, supply voltage – 220V, AC frequency 50Hz. The crushed analyzed sample is transferred into a tightly closed container and, after it is cooled to room temperature, is used to take the spectrum. The mass of the laboratory sample was 250 g. Sampling is carried out according to ISO 6498:2012 Animal feeding

stuffs-Guidelines for sample preparation, IDT **[7]**. The device is turned on and brought to the measurement mode following the operating instructions. Taking spectra is as follows: a carefully cleaned cuvette on a special stand included in the IR analyzer kit is tightly filled with a thoroughly mixed analyzed sample using a spatula. Avoid sudden movements and cuvette shaking. It is not allowed to pour the analyzed sample from the vessel, as this leads to gravitational separation of fractions and reduces the accuracy of the determination. Measurements are taken immediately after filling the cuvette. The measurement results are processed automatically. The content of the determined indicators is read from the display of the IR analyzer and can be printed. For the final result of the determination, the arithmetic mean of two parallel determinations is taken, with a confidence level of $p = 0.95$. The result is rounded to the first decimal place **[6]**.

Determination of milk quality: For the determination of milk quality the CombiFoss FT+ milk analyzer was used (North-Kazakhstan Research Institute of Agriculture LLP, Beskol, Kazakhstan). Fossomatic FC is based on flow cytometry technology. This approach is based on the enumeration and characterization of particles and cells. MilkoScan FT+ is based on Fourier IR spectroscopy (FTIR) analysis. It operates in the mid-IR range of 3-10 μm, corresponding to 1000-5000 cm^{-1}. The MilkoScan FT 6000 (Foss Electric) is based on Fourier transform infrared technology and provides a wide range of compositional parameters, including fat, protein, lactose, solids, CN, FFA, urea, and FP depression **[8]**. However, the laboratory is accredited to determine only 3 indicators: the mass fraction of fat and protein, and the content of somatic cells. The analyzed samples were checked for compliance with GOST 32940-2014 "Raw Goat Milk Technical conditions" **[9]**. Milk consumption for analysis: 5 mL. Sample temperature: 37-42 °C. VC (coefficient of variation) – standard (root mean square) deviation of CO, divided by the average value of the measured samples and multiplied by 100%, i.e. for milk with a fat content of 3%, the absolute accuracy will be ±0.03%.

The quality of goat milk products: The physicochemical parameters of goat milk products were obtained according to the data from the official websites of the Zeren and Tamasha-2050 enterprises **[10]**, **[11]**. The energy value of goat milk products was determined according to formula 1:

$$EV = 0.845 \times P \times 4.0 + 0.94 \times F \times 9.0 + 0.956 \times C \times 4.0 + OA \times 3.0 \quad (1)$$

Where:
P – proteins; F – fats; C – carbohydrates; OA – organic acids. Energy values are calculated per 100 g of food and are expressed in kilocalories and kilojoules **[12]**.

Statistical Analysis

The research materials were subjected to statistical processing using the methods of parametric and non-parametric analysis. Accumulation, correction, systematization of initial information, and visualization of the results were performed in Microsoft Office Excel 2016 spreadsheets. Statistical analysis was carried out using the program STATISTICA 13.3 (developer – StatSoft. Inc). In the case of describing quantitative indicators with a normal distribution, the obtained data were combined into variational series, in which the calculation of arithmetic means (M) and standard deviations (SD), boundaries of the 95% confidence interval (95% CI) was carried out.

RESULTS AND DISCUSSION

The diet of goats at the Zerenda farm includes haylage, pasture grass, alfalfa, barley, goat's rue, and rump. The fodder base of the enterprise is 400 hectares of irrigated fields. Since the quality and quantity of feed are of the most significant value for obtaining high-quality milk and other manufactured products, it is necessary to introduce biodiversity into the nutrition of goats. In turn, the Tamasha-2050 farm also grows forage grasses, oats, and barley for animal feed. The basis of food for goats at the farm "Tamasha" is hay, barley straw, barley, and oats. Goats on both farms are kept indoors; their feed consists of haylage (3 kg per animal). Also, for feeding highly productive goats, a grain mixture is used (corn, barley, and oats in equal proportions) **[13]**. The results of the experiment are shown in Table 4. Table 4 shows the nutritional value of haylage, which is the main part of the diet of goats on farms. According to the author **[13]**, goats should receive a nutritional value of 0.70 feed units per day during the grazing period. To ensure intensive development and necessary vital activity, the amount of digestible protein in the diet should be contained at 80 g.

As could be seen from Table 4, the nutritional value of feed LLP "Zeren" provides goats with 71.4% of the norm of feed units per day, while on the Tamasha-2050 farm – only 68.5% of the norm. The amount of digestible protein in the diet of the goats of the farm "Zeren" is – 75.5% of the norm, and the farm Tamasha-2050 – 61.8% of the norm. Thus, the goats of Zeren LLP consumed more haylage in terms of exchange energy than at the Tamasha-2050 farm by 2.93% and dry matter – by 1.54%, respectively.

Table 4 Feed value of haylage included in the diet of goats.

No.	Indicators	Zeren LLP		Tamasha-2050	
		In natural moisture of forage		In natural moisture of forage	
		content, g	content, g	content, %	content, %
1	Dry matter	863. 28	86.33	850.16	85.02
2	Organic matter	785.95	-	767.18	-
3	Humidity	136.72	13.67	149.84	14.98
4	Raw ash	77.34	7.73	82.98	8.30
5	Crude protein	112.41	11.24	92.10	9.21
6	including digestible Protein	60.43	6.04	49.51	4.95
7	Crude fat	25.18	2.52	25.68	2.57
8	Crude fiber	300.35	30.04	297.56	29.76
9	NFE, g incl.	348.01	34.80	351.84	35.18
10	Starch, %	1.10		1.00	
11	Sugar, %	6.70		6.42	
12	Carotene, mg	22.70		20.50	
13	Calcium %,	0.80		1.06	
14	Phosphorus, %	0.25		0.31	
	Nutritional value of forage:				
15	Exchange energy MJ	6.32		6.14	
16	Energy feed units (EFU)	0.63		0.61	
17	Feed unit	0.50		0.48	

Note: Compiled by the authors.

As could be seen from Table 4, the nutritional value of feed LLP "Zeren" provides goats with 71.4% of the norm of feed units per day, while on the Tamasha-2050 farm – only 68.5% of the norm. The amount of digestible protein in the diet of the goats of the farm "Zeren" is – 75.5% of the norm, and the farm Tamasha-2050 – 61.8% of the norm. Thus, the goats of Zeren LLP consumed more haylage in terms of exchange energy than at the Tamasha-2050 farm by 2.93% and dry matter – by 1.54%, respectively.

Goat milk quality

Table 5 presents goat milk's physical and chemical parameters on the farms "Zeren" and "Tamasha-2050".

Table 5 Physical and chemical indicators of goat milk on farms.

Name	Arithmetic mean (M):	Median (Me):	Standard deviation (σ):	The coefficient of variation (Cv), %:	The average error of the arithmetic mean (m):
			Zeren LLP		
Fat	3.76	3. 97	0.1	2.75	0.05
Protein	3.27	3.23	0.05	1.60	0.03
Casein	2.31	2.31	0.01	0.36	0.00
Lactose	3.93	3.93	0.01	0.21	0.00
TS	11.23	11.23	0.02	0 .18	0.01
Cells	137.40	138	2.19	1.59	1.10
MUFA	0.76	0.747	0.02	2.77	0.01
PUFA	0.29	0.285	0.01	3.48	0.01
Saturated FA	2.34	2.352	0.02	0.85	0.01
Total UFA	0.60	0.594	0.02	3.97	0.01

Table 5 Cont.

Name	Arithmetic mean (M):	Median (Me):	Standard deviation (σ):	The coefficient of variation (Cv), %:	The average error of the arithmetic mean (m):
Tamasha-2050					
Fat	3.82	3.83	0.1	2.52	0.05
Protein	3.23	3.23	0.01	0.26	0.00
Casein	2.32	2.32	0.00	0.19	0.00
Lactose	3.94	3.94	0.00	0.00	0.00
TS	11.27	11.27	0.02	0.16	0.01
Cells	135.40	135	1.14	0.84	0.57
MUFA	0.770	0.775	0.01	1.81	0.01
PUFA	0.29	0.288	0.01	2.30	0.00
Saturated FA	2.33	2.342	0.05	1.94	0.02
Total UFA	0.61	0.618	0.01	1.60	0.00

Note: Saturated Fatty Acids (SFA); Monounsaturated Fatty Acids (MUFA); Polyunsaturated Fatty Acids (PUFA); Unsaturated Fatty Acids (UFA). Compiled by the authors.

Table 5 shows that the fat, protein and total unsaturated fatty acids content in goat milk samples did not vary significantly: fat ($p = 0.424$), protein ($p = 0.224$), total UFA ($p = 0.35$); for example, in the milk of the Tamasha-2050 farm, the fat content was, on average, 1.5% higher, while the protein content was less by 1.2%. Total lactose contents in milk samples ranged between 3.92% and 3.93%, $p < 0.01$, casein – between 2.31% and 2.32% ($p < 0.01$) and TS – between 11.23% and 11.27% ($p < 0.01$). In general, the quality characteristics of goat milk in both farms met the requirements of GOST 32940-2014 "Raw Goat Milk Technical conditions" **[8]**.

Tables 6-7 provide data on the physical and chemical parameters of dairy products from goat milk, energy value, types of packaging, and prices for products on the farms "Zeren" and "Tamasha-2050".

Table 6 Physical and chemical parameters of assortment products in the goat farm “Zeren”.

Product’s name	Mass fraction of fat, %	Mass fraction of protein, %	Mass fraction of carbohydrates, %	Energy value, kcal	Package type	Shelf life, days	Weight (L)	Price (tenge)
Natural pasteurized goat milk	3.9-4.0	3.3	4.4	62 kcal/ 259 kJ	Plastic bottle	7	1	1 000
Goat milk	3.9-4.0	3.3	4.4	62 kcal/ 259 kJ	Plastic bottle	7	0.5	600
Yogurt drink 4%	3.9-4.0	3.3	4.3	59 kcal/ 247 kJ	Plastic bottle	14	0.3	750
Yogurt drink – Strawberry 4%	3.9-4.0	3.3	5.2	65 kcal/ 254 kJ	Plastic bottle	14	0.3	850
Yammi ice cream – ice cream	10	3.7	18.4	178 kcal/ 745 kJ	plastic bucket	6 months	0.25	1 200
Yammi ice cream – chocolate	10	3.7	18.4	178 kcal/ 745 kJ	plastic bucket	6 months	0.25	1 200
Kefir 2.5%	2.5	2.8	4.0	49.7 kcal/ 208 kJ	Plastic bottle	14	0.5	750
Kefir 1% (lite)	1	3.0	4.0	36 kcal/ 151 kJ	Plastic bottle	14	0.5	600

Table 6 Continue.

Product's name	Mass fraction of fat, %	Mass fraction of protein, %	Mass fraction of carbohydrates, %	Energy value, kcal	Package type	Shelf life, days	Weight (L)	Price (tenge)
Creamy ice cream	10	3.7	18.4	178 kcal/ 745 kJ	plastic bucket	6 months	0.7	2 550
Chocolate ice cream	10	3.7	18.4	178 kcal/ 745 kJ	plastic bucket	6 months	0.7	2 550
Cottage cheese	1.8	18	1.2	88 kcal/ 370 kJ	PET Packaging	7	0.2	750
Whole goat milk kurt	1	20	17	200 kcal/ 837 kJ	vacuum packaging	15	0.1	750
Homemade sour cream	58	2.5	3.3	556 kcal/ 2322 kJ	plastic cup	10	0.2	900
Butter goat	82.5	0.5	0.5	765 kcal/ 3 202 kJ	PET Packaging	15 to 30	0.1	900
Butter goat	82.5	0.5	0.5	765 kcal/ 3 202 kJ	PET Packaging	15 to 30	0.2	1700
Whey	0.05	0.8	3.5	18.1 kcal/ 76 kJ	Plastic bottle	7	1	450

Note: Compiled by the authors. Source **[10]**.

As could be seen from Table 6, the Zeren enterprise presents a wide range of products supplied on an industrial scale: drinking pasteurized milk (4.0%), kefir (1%, 2.5%), yogurts with and without filling (4%), cream and chocolate ice cream (10%), butter (82.5%), sour cream (58%), whey (0%).

Table 7 Physical and chemical parameters of assortment products in the goat farm Tamasha-2050.

Product's name	Mass fraction of fat, %	Mass fraction of protein, %	Mass fraction of carbohydrates, %	Energy value, kcal	Package type	Shelf life, days	Weight (L)	Price (tenge)
Natural goat milk	3.9-4.3	4.4-4.5	4.2-4.5	68-70 kcal/ 284-294 kJ	Plastic bottle	7	1	700
Sour cream	24	2.9	3.7	247 kcal/ 1034 kJ	PET Packaging	10	0.1	1200
Butter	82	0.6	0.9	748 kcal/ 3131 kJ	PET Packaging	30	0.1	1400
Cottage cheese	9	8	3.5	84 kcal/ 351 kJ	PET Packaging	7	0.1	900
Kurt	0.05	20	16	200 kcal/ 837 kJ	PET Packaging	15	0.1	1000
Cheese candies	0.05	20	25	240 kcal/ 1000 kJ	PET Packaging	15	0.1	1000
Mold (Blue) Cheeses								
Camembert	24	22	0	304 kcal/ 1272 kJ	vacuum packaging	45	0.1	1000
Pouligny St Pierre	28	22	1	330 kcal/ 1381 kJ	vacuum packaging	to 120	0.1	650
Shaurs	30	18	0	342 kcal/ 1431 kJ	vacuum packaging	30	0.1	650
Bree	27	30	0	372 kcal/ 1557 kJ	vacuum packaging	15	0.1	650

Table 7 Continue

Product's name	Mass fraction of fat, %	Mass fraction of protein, %	Mass fraction of carbohydrates, %	Energy value, kcal	Package type	Shelf life, days	Weight (L)	Price (tenge)
Soft cheeses								
Feta	27	15	0	268 kcal/ 1122 kJ	Plastic boxes	15	0.1	650
Burrata	20	17	1.8	255 kcal/ 1060 kJ	plastic cup	4	0.1	900
Suluguni	24	17	0	290 kcal/ 1213 kJ	vacuum packaging	60-120	0.1	650
Brynza	19.8	18.2	0	251 kcal/ 1050 kJ	plastic cup	20	0.1	650
Adyghe	18	16	0	228 kcal/ 954 kJ	vacuum packaging	44	0.1	650
Ricotta	10	13	2.8	142 kcal/ 594 kJ	Plastic boxes	10	0.1	700
Hard cheeses								
Fracket	26	17	1.8	264.2 kcal/ 1105 kJ	vacuum packaging	15	0.1	550
Asiago	26.3	11	1.2	209 kcal/ 875 kJ	vacuum packaging	15	0.1	900
Belper Knolle	25	10	0.8	193.2 kcal/ 808 kJ	Plastic boxes	60	0.1	900
Cachotta	20	25	0	280 kcal/ 1172 kJ	vacuum packaging	90	0.1	900
Gouda	25	26	2	354 kcal/ 1482 kJ	vacuum packaging	30	0.1	900
Maasdam	26.3	26.8	0	342 kcal/ 1433 kJ	vacuum packaging	90	0.1	900
Cheddar	26.8	25.2	0	334 kcal/ 1398 kJ	vacuum packaging	15	0.1	900
Monterey Jack	26	23	0	326 kcal/ 1364 kJ	vacuum packaging	30	0.1	900
Parmesan	23	21	0	281 kcal/ 1176 kJ	vacuum packaging	90	0.1	900
Montasio	32	24	1	388 kcal/ 1642 kJ	vacuum packaging	7	0.1	900
Jarlsburg	28	26	0.3	364 kcal/ 1523 kJ	vacuum packaging	30	0.1	900
Canestarto	29	23	0	362 kcal/ 1115 kJ	vacuum packaging	15	0.1	900
Tilsiter	23.4	24.4	0	308 kcal/ 1281 kJ	vacuum packaging	30	0.1	900
Bulaevsky	25	26	2	354 kcal / 1482 kJ	vacuum packaging	30	0.1	900

Note: Compiled by the authors. Source **[11]**.

As could be seen from Table 7, the Tamasha-2050 enterprise mainly presents a wide range of different types of soft and hard cheeses, as well as blue cheeses.

Prices for goat milk products and raw materials in 2020 averaged: milk, pasteurized normalized (3.2%) 580 tons per 1 liter; sour cream (30%) – 3,125 tons per 1 kg; semi-fat cottage cheese (9%) – 2,679 tons per 1 kg; hard cheeses – 3,795 tons per 1 kg; milk – raw materials – 520 tons per 1 liter; butter – 8500 tons per 1 kg, whey – 300 tons per 1 liter; yogurt – 450 tons per 300 mL **[14]**.

In general, in 2022, all Zeren dairy products from goat milk increased in price, as shown in Table 5. Prices increased for milk (+34.61%), yogurt (+66.66%) and sour cream (+44 .0%), butter (+5.88%) and cottage cheese (+38.88%). The products of the Tamasha-2050 farm, represented mainly by cheeses of various types, similarly increased in price: milk (+34.61%), sour cream (+284%), butter (+64.7%), and cottage cheese (+233 %), as well as fresh and semi-hard cheeses (+ from 71.3 to 137%).

The production of cheese and butter is the most costly; as a result, the price of these types of products is higher and domestic enterprises, due to the high cost of production, are forced to either reduce their production or work for export. At the same time, the Republic of Kazakhstan subsidizes the costs of a dairy processing enterprise for the purchase of milk for the production of butter, hard cheese, and powdered milk (whole). It involves the reimbursement of the difference between the guaranteed purchase price and the purchase price of a unit of processed products, considering the final conversion factors given in the table of products for milk subsidy standards per unit of purchased agricultural products approved by akimats **[15]**.

The norm of subsidies per kg of purchased milk varies depending on the akimat and is for the production of: butter – from 12 to 16 tenges per liter; hard cheese – from 15 to 20 tenge per liter; powdered milk – 15 tenges per liter **[15]**.

In the Republic of Kazakhstan, the share of the subsidy in the total direct costs incurred for purchasing machinery and equipment for a milk processing facility is 30%, and for buying a portable milking machine, 50% **[15]**.

According to Miller et al. (2019) **[16]**, the global goat population continues to grow and is now over one billion. Most of the world dairy goat production and consumption is in Asia, but most organized market for goat milk is found in Europe, especially in France. The European goat sector specializes in milk production, mostly for industrial cheese making, while supporting traditional on-farm manufacturing. Government involvement is significant in sanitary regulation, research, extension, support for local producer organizations, and markets, and ensures safety and quality **[16]**. The study **[17]** of goat farms demonstrated that the extension system suffers from limitations of financial and man-power resources. Farmers have minimal access to scientific advancement in the field of livestock production. Further, the meta-analysis **[17]** revealed that the farmers had knowledge of 47% of technologies that has the potential to mitigate the effects of climate change on goats. The health, feeding, breeding, and housing practices had an adoption level of 31%, 40%, 58% and 70% respectively **[17]**. The results **[18]** show that in Algeria three different farming systems: cluster 1 (pastoral system), cluster 2 (mixed crop-livestock system) and cluster 3 (small herds in zero grazing system).

According to Kisku et al. **[19]**, goat farming projects can be a considerably profitable business for small and marginal farmers. In long run, a farmer may increase flock size depending on market demand for more profit **[19]**. The study of fermented dairy products **[20]** demonstrated that they feature functions are immunomodulatory agents, anti-carcinogenic agents, hypocholesterolemic agents, antioxidants and hypotensive agents.

Caprine milk fat is a reward in short- and medium-chain fatty acids, particularly caprylic and decanoic (capric) acids **[21]**. The fatty acid profile of raw milk samples is shown in Table 4. Therefore Saturated Fatty Acids (SFA) were most prevalent in goat milk samples. A previous study of goat milk identified several aspects of using local milk raw materials in Kazakhstan is little known **[22]**.

Extensive research has shown that goat milk has been an essential part of human nutrition for millennia, partly because of the more remarkable similarity of goat milk to human milk, softer curd formation, a higher proportion of small milk fat globules, and different allergenic properties compared with cow milk. As a result, the need for higher-quality goat milk has increased in recent years. Goat milk and milk products are held to the same high standards for safety and quality that the known in the dairy industry. Furthermore, their compact size (compared with cows) makes them appealing from herd management and milking standpoint. Additionally, physiological differences render unique physical characteristics to goat milk in terms of flavor profile, fat globule size, coagulation properties, and allergenicity, making goat milk the dairy product of choice for many consumers **[23]**.

However, a study investigating advances in goat milk **[24]** offers additional research into methods to sustainably feed goats, responsibly improve productivity, ecologically manage effluents, and creatively utilize goat whey will be essential in managing the global dairy industry.

These experiments confirmed that the quality of raw milk and various types of dairy products from goat milk on the goat farms "Zeren" and "Tamasha-2050" meet regulatory requirements and are presented to the consumer in a wide range. The total solid content, dry matter, and proximate composition of goat milk and feedstuffs from the different farms were determined. The results were analyzed using principal component analysis, as seen in Tables 3-4. The total solid content of goat milk from the "Tamasha-2050" farm had the highest solid content ranging from 11.27% to 11.29%, compared to milk from farm "Zeren." Previous studies find that milk's quality and composition vary according to breed, diet and feeding practices, management system, lactation stage, parity, and animal health **[25]**, **[26]**. These results are consistent with data obtained in earlier studies **[27]**, which found that goat milk had a solid content ranging from 10.95% to 14.63%, milk fat ranging from 2.49% to 7.36%, and

protein contents (4.34%). However, some researchers **[28]** investigated how can naturally increase omega-3 fatty acid content in goat milk using goat nutrition as one of the key factors. In this study, results showed that goat feed supplementation with linseed oil indeed positively changed fatty acid profile of goat milk by increasing α-linolenic acid content **[28]**.

It has been noted **[29]** that goat milk and its derived products have high nutritional value. The study **[30]** revealed that goat milk may be considered as the basis for the creation of a sour-milk product for special dietary consumption. Goat milk has a low content of αs1- αs2- and a high content of β-casein fractions of proteins compared to cow milk **[30]**. To increase their production, it is necessary to increase food safety, improve their flavor, and potentiate their functional value. Several researchers **[31]** attempted to find the advantages of goat milk, the necessary theoretical background, and some details about using prebiotics and/or probiotics in goat milk products. Sumarmono (2022) **[32]** demonstrated that goat milk utilization includes fluid milk for direct consumption; frozen fresh milk; dried or powdered milk; fermented milk products such as yogurt, kefir, including its derivatives such as cosmetics, concentrated yogurt, yogurt cheese, ice cream, shakes; cheeses such as fresh cottage-type cheese, acid-coagulated cheese and mozzarella.

O'Brien, Aryana, Prinyawiwatkul, Ordonez, and Boeneke (2016) cited that many health benefits are associated with traditionally produced kefir; for instance, the usually made kefir exhibited significantly higher counts of bacteria and yeast at each sampling of frozen storage **[33]**. In addition, Domagała, Wszolek, and Dudzińska (2012) reported that probiotic yogurts prepared from goat's milk concentrated via ultrafiltration have better sensory properties and maintain a good texture **[34]**. Goat's milk might also be utilized to produce ice cream with desirable textural properties, good sensory quality, and desirable melting characteristics: commercial raspberry and blackberry fruit sauces and fruit sauce prepared from frozen raspberry were used for the production of ice cream (Açu, Kinik, Yerlikaya, 2017) **[35]**. The sensory characteristics of goat yogurt could be greatly improved by integrating to a culture typical of yogurt starters, Streptococcus thermophilus, Lactobacillus delbrueckii spp. bulgaricus, with cultures of Leuconostoc lactis, as reported by De Santis et al. (2019) **[36]**. The results of this study indicated that adding L. lactis to the traditional yogurt starter improved the sensory characteristics of fermented goat milk. Hadjimbei et al**.** (2020) **[37]** found that goats' milk yogurt fortified with Pistacia atlantica resin extract alone or in combination with Saccharomyces boulardii were produced. Dual supplementation of yoghurt promoted the growth of LAB, enhanced the stability of resin phytochemicals and improved the organoleptic properties. El-Shafei et. al. (2020) **[38]** investigated the impact of supplementing goats' milk with quinoa extracts, in the range of 5, 10 and 15 g/100g on the milk fermentation. The supplementation of goats' milk with quinoa extracts, particularly permeate extract, reduced the fermentation time and enhanced the viability of lactic acid bacteria. Panellists highly accepted the yoghurt that contained quinoa permeate extract **[38].** The study of milk quality demonstrated that **[39]** it depends on chemical parameters (fat and protein content and absence of inhibitory substances), as well as microbial and somatic cells counts, and affects the price of milk.

There are results not only about an investigation of goat milk **[40]**, but also of cheese. The position of goat milk and its products may vary depending on factors such as season, feeding system and heat treatment. Total phenolic compounds concentrations were highest in unpasteurized samples from dry season compared to pasteurized and rainy season: 132.4 ±7.3, 76.5 ±0.77 mg of gallic acid equivalent (GAE)/L for unpasteurized milk and milk whey, respectively, and 363.21 ±2.97 mg GAE/Kg for cheese. Antioxidant capacity for dry season produce was significantly higher (p <0.05) than rainy season produce. Free-range grazing was a food option for producing a higher concentration of phenolic compounds and a higher antioxidant capacity **[40]**. For instance, Bennato et al. **[41]** determined that dietary integration with dried licorice root modified chemical and technological properties of goat cheeses, reducing lipid oxidation during ripening and inducing changes in texture that could improve consumer acceptability.

Results obtained by Vargas-Bello-Pérez et al. **[42]** for consumer attitudes found consumer preferences for small ruminant dairy products, between continents. Among consumers in Italy, Greece, Denmark, and Mexico, the most desirable was fresh cheese, and the preferred product in Spain and Chile was aged cheese. Another significant aspect was that consumption frequency was the highest in Italy, Spain, and Greece. In Chile and Mexico, consumption was limited to 2 to 3 times per month, whereas frequency in Bangladesh was 1 to 2 times per year, or as necessary due to sickness **[42]**.

This study confirms previous findings **[24]** about high production costs and correspondingly inflated prices for goat milk products. However, the development of goat breeding has economic and social significance for all areas of activity. In particular, the production and processing of products will create additional jobs and provide the population with high-quality meat, wool, and dairy products **[43]**. It should be noted that the fundamental condition for effective animal husbandry management is the need for organizational and economic reorganization of all parts of the technological process on new production and technical basis. The modernization of industries involves the creation of large goat-breeding complexes, specialized farms, and inter-farm enterprises, the

introduction of advanced technologies; increasing the efficiency of selection and breeding work; improvement of the forage base; introduction of intensive rearing and fattening of young growth using resource-saving technologies **[43]**. In the future, it is necessary to pay serious attention to increasing the number of goats and creating conditions for maximizing their productivity, as well as the rational use of pastures by goats, observing pasture rotation and load **[43]**. This will increase the gross income of the country's agro-industrial complex and meet the growing demand for medical nutrition for people who cannot tolerate cow's milk, people with weakened immune systems, diseases of the gastrointestinal tract, and diabetics **[43]**. At the same time, some authors **[24]** point out the importance of other measures, such as professional management training and a well-thought-out goat farm management plan.

CONCLUSION

A brief analytical review of production projects launched in Akmola and North Kazakhstan regions, affecting the development of animal husbandry and dairy cattle breeding in the regions, was carried out. Although most goat milk producers are private farms, there are also large goat farms: in the Akmola region – Zerenda LLP, in the North Kazakhstan region – Tamasha-2050 LLP. The results showed a significant difference (p <0.05) on the lactose, caseimilk samples' ns and total solids contents in milk samplebetween the general, in 2022, all dairy products from goat milk from the Zeren farm went up, prices increased for milk (+34.61%), yogurt (+66.66%) and, sour cream (+44.0%), butter (+5.88%), and cottage cheese (+38.88%). The products of the Tamasha-2050 farm similarly increased in price: milk (+34.61%), sour cream (+284%), butter (+64.7%) and cottage cheese (+233%), as well as fresh and semi-hard cheeses (+71.3 to 137%). The diet of the animals complied with the norms; the considered physical and chemical parameters of milk meet the requirements of regulatory documents and, accordingly, could be used for the production of various types of dairy products.

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