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CONTENTS

FOOD PRODUCTION TECHNOLOGY

BIOTECHNOLOGY

FOOD PRODUCTION PROCESSES AND EQUIPMENT

FOOD HYGIENE

STANDARDIZATION, CERTIFICATION, QUALITY AND SAFETY

ECONOMICS

AUTOMATION AND INFORMATIONAL SUPPORT OF TECHNOLOGICAL PROCESSES

GUIDE FOR AUTHORS

Effect of sodium bicarbonate residue
on some characteristics of processed meat products

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Abstract: Using sodium bicarbonate (SB) in cooking meat products is a controversial subject. The aim of this study was to estimate an effect of different SB concentrations on the quality characteristics and organoleptic properties of meat in Kubideh Kebab, an Iranian popular meat product. Ground meat was divided into four groups (a, b, c, and d). After that, SB was added in ratio 0.25, 0.50, 1.00, and 2.00 g/kg respectively. A sample without SB was considered as a blank sample. The Kebab samples were prepared and cooked properly at 350°C for 4–6 minutes and at 450°C for 2–4 minutes. A significant difference was observed in the pH values and the cooking loss between the blank sample and those Kebab samples that were cooked at 350°C and 450°C and pre-treated with SB in the amount of 0.25–2.00 g per 1 kg of meat ($P < 0.05$). The amount of residual bicarbonate ions increased significantly in the cooked Kebab samples at both treatment temperatures in the a-d group in comparison with the blank sample, as well as between the groups ($P = 0.00$). The organoleptic properties did not change in the a-d groups in comparison with the blank sample.

INTRODUCTION

Kebab is a traditional and highly consumed food which is extremely popular in Iran, Anatolia, Central Asia, Russia, and Armenia. It is made from ground lamb, beef, or poultry mixed with chopped onion. To cook Kubideh kebab, one originally had to place meat on a flat stone and smash it with a mallet; modern Kubideh kebabs are made from ground meat barbecued with herbs, tomato and green pepper. Marinating is a traditional technique used to improve the meat quality before thermal treatment. Beef is soaked in marinade (water, salt, and other essential ingredients); alternatively, the marinade can be injected into the meat. Marinating improves meat flavour and prolongs shelf life, since spices and various extracts provide it with antimicrobial and antioxidant properties [1]. Some researches show that nonmeat additives increase the water holding capacity (WHC) of the processed meat [2]. Being a base component of raw meat, water is not a valuable additive for meat products. However, it is usually considered as a nonmeat additive [3]. In general, pH in the isoelectric point of myofibrillar proteins (5.2–5.3) corresponds to the lowest level of water holding capacity. Thus, it is possible to increase the WHC of meat products by accreting the ionic backbone as a result of pH adjustment [4–6].

According to Offer and Trinick [7], it is possible to improve WHC using marinating, since electrostatic repulsion makes beef fibres expand, which allows the added water to penetrate into the myofibrillar network. It is known that some additives are able to enhance the low moisture assimilation of meat products, and sodium chloride and phosphates are among them [1]. Actually, the application of sodium chloride concentrations alignment from 4.6 to 5.8% provides the optimal amplification of myofibrils distension with the optimal damp uptake. It has been universally accepted that sodium chloride alters the solubilization of myofibrillar protein, water absorption, and gelling properties of meat [4–6]. This mechanism of improving the WHC of meat by using sodium chloride was recommended by Offer and Knight [8] and Ruusunen and Puolanne [9]. Moreover, the application of phosphate salts also improves the water retaining and the binding capacities of meat [10]. For instance, by adding 0.3% of phosphate in beef, one can raise its pH, promote the formation of

ionic bonds, as well as magnesium- and calcium-binding proteins with an increase in the solubilization of myosin and actin.

In addition, Xiong et al [11] observed a strong synaeresis between sodium chloride and phosphate in poultry meat. Hence, marinades with sodium chloride and polyphosphates are used to improve various characteristics of muscle-based meat [12, 13–14]. Although it is universally accepted that phosphates affect meat characteristics, some countries banned their use in meat processing [3]. Therefore, phosphates can be replaced some of alternative substances in meat products. Only a few studies focused on the use of bicarbonate to improve the quality of pork [15, 16–17] and poultry [18, 19]. Furthermore, some recent empiric studies actually promote the efficiency of SB (NaHCO$_3$) in pork and poultry products because it can reduce shear force [20–22]. Bicarbonates possess a higher buffering capacity and ionic potency, if compared with phosphates, which explains their efficiency [17]. Unlike sodium chloride and polyphosphates, the basic mechanisms of the SB action remain understudied. The researches mentioned above studied meat marinated with sodium chloride, polyphosphate, and bicarbonate. However, there is a significant gap in data concerning the role of water with the biopolymers injected inside the intra- and extramyofibrillar spaces during marinating.

On the other hand, marinating increases the product yield, reduces the water loss during cooking [23], and raises the tenderness of meat. As a rule, the main ingredients of marinade are sodium chloride and phosphates [23]. Both can improve WHC by increasing the electrostatic repulsion of myofibrillar proteins [23]. Another effective ingredient is SB, as it reduces drip loss and shear force [17, 24–25] and increases the yield of cooked meat [24, 26]. By marinating sirloin and flank in a SB solution, one can achieve a balance between flavour, tenderness, and cost. Generally, the WHC of meat is minimal when its pH is close to the isoelectric point of myofibrillar proteins (about 5.2–5.5). The ionic strength could be steadily increased by adjusting the pH, thus leading to a higher level of WHC in meat products [27]. It is believed that the organoleptic properties of cooked meat, such as flavour, smell, appearance, and palatability, depend not only upon the pH of the muscle tissue and its nutritional status at the time of slaughter but also upon the type of ingredients used in marinating. The cooking loss tended to decrease as SB level went higher: the cooking loss reduced by 1.8% when the concentration of SB was 0.10% per unit. The use of SB did not change the overall appearance of meat, while reducing its hardness [28]. Additionally, SB is an excellent marinating agent: it can be used to process poultry with no phosphates added, which meets the demand and raises the nutritional properties. A recent research showed that the highest marinade performances were achieved when SB was combined with phosphates [24]. The present research focused on evaluating the effect of different concentrations of SB on the quality characteristics and the organoleptic properties of kebab meat cooked at different temperatures.

Table 1. Properties of the lamb meat used for preparing kebab samples

Parameter	Results
Total bacteria, per g	4.2×10^5
Moisture, %	65.4
Protein, %	16.84
Total fat, %	16.6
Total starch, %	trace
Ash, %	1.39

Table 2. Properties of the kebab samples cooked at 350°C and 450°C

Parameter	Results at 350°C	Results at 450°C
Total bacteria, per g	1.1×10^5	10^5
Moisture, %	55.5	55.1
Protein, %	16.52	16.30
Total fat, %	16.40	16.22
Total starch, %	trace	trace
Ash, %	2.3	2.3

STUDY OBJECTS AND METHODS

Chemicals and reagents. Sodium bicarbonate, sodium chloride, nitric acid, and sodium hydroxide were supplied by Merck (Darmstadt, Germany). Distilled and deionized water with 18.0 MΩ specific resistance was prepared by Milli Q Water System (Millipore, Le montsur-Lausanne, Switzerland).

Sampling. To prepare kebab samples, sirloin and mutton flank (13:1, w/w) were pounded and mixed with salt, a diluted saffron solution, grated onion, and black pepper. This mixture was divided into four groups: a, b, c and d. The groups contained 0.25, 0.50, 1.00, and 2.00 g/kg of SB respectively; an individual blank group was provided for the raw and the cooked samples. For each group, five kebab samples were prepared and cooked at two different temperatures: 1) 350–400°C with a long cooking period (4–6 min), the internal temperature of meat being 68–70°C); 2) 450–500°C with a short cooking period at (2–4 min), the internal temperature of meat being 89–91°C.

Sample preparation. Initially, 1.50 g of each raw and cooked Kebab sample were mixed with 10 ml of concentrated nitric acid (65%) and homogenized with a magnetic stirrer for at least 3.0 hours until a completely transparent liquid was obtained. Then the samples were diluted with NaOH 1.0 M and put in an 80 ml (volumetric flask. Finally, the pH of the extracts was adjusted to 8.3–8.7 by NaOH 10 M, and the extract solutions were filtered by a 0.45 μm filter before being injected into the ion chromatograph. These experiments were conducted in three replicates for each sample.

Equipment. A Metrohm 844 UV/Vis compact ion chromatograph was used to identify and determine bicarbonate ions in the extracted samples. A Rehodyne model 7725i injector with a 50 μL loop was used to inject the extracted samples. Chromatographic separations were achieved using an anionic A Supp 8, 5 μm, 4.0 × 150 mm analytical column. A degassed and filtered solution of sodium chloride was used as a mobile phase. Due to this, 10.0 g of sodium chloride was dissolved in some deionized water in a 100 mL volumetric flask and then diluted up to its volume. This

solution was conveyed in the isocratic mode at a flow rate of 1.0 mL/min. All the analyses were carried out at 215 nm, and the ion chromatographic data were acquired and processed using PC and IC Net Ver. 1.1 chromatography manager software.

Determination of pH in the kebab samples. The pH value of meat samples was determined according to the standard method of ISO 2917:1999 [29]. A calibrated digital Metrohm pH meter (model 744) equipped with a combined glass–calomel electrode was used to obtain the pH of meat samples and control the pH of the solutions, as well as to adjust the mobile phase in the chromatographic analysis.

Estimation of organoleptic properties. The hedonic test method was used to estimate the organoleptic properties (smell, flavor, appearance, and palatability) of the kebab samples. The samples were labeled with a random three-digit number and then served to forty panellists. This protocol was used to estimate the smell, appearance, flavour, and palatability of Kubideh kebab using a 9-point hedonic scale.

Cooking loss. The raw meat samples were slightly blotted with paper towels and weighed, then cooked separately at two temperature levels: 350–400°C for 4–6 minutes and 450–500°C for 2–4 minutes. After that, the cooked samples were once again slightly blotted with paper towels and weighed. The cooking loss was calculated as follows:

$$\% \text{ Cooking loss } = \frac{w_1 - w_2}{w_1} \times 100,$$

where w_1 = Weight of raw meat before cooking, and w_2 = Weight of meat after cooking

Chemical and microbial properties of the lamb. A few physicochemical and microbial properties of the lamb used for preparing kebab samples were determined for each sample. The moisture was determined by calculating the meat weight after drying it at 105 ± 2°C. The meat protein content was determined by the Kjeldahl method. The meat fat was analyzed using the Soxhlet apparatus method. The carbohydrates were measured by the starch test. The ash content was determined using a 600°C furnace [30]. Likewise, all the meat samples were analyzed for the total of aerobic mesophilic microorganisms using the aerobic plate count (APC) of colony forming units (CFU) and reported as log CFU per g of meat samples using the Ercolini et al. [31].

Statistical analysis. All the data were analyzed using SPSS software. The significant differences between the treatments were assigned by using the paired samples t-test at a 5% probability level ($P < 0.05$). It was carried out to reveal the difference between two individual parameters. It was performed using statistical SPSS version 16 software.

RESULTS AND DISCUSSION

Chemical and microbial properties of the lamb. Tables 1 and 2 show the main chemical and microbial properties of the lamb. In this study, the total content of the bacteria was studied to ensure that the meat was safe for human consumption and to determine how the pH value was affected by bacteria activity. The total of

bacteria, % moisture, % protein, % total fat, % total starch, and % ash contents were compatible with the national regulations of Iran for meat products.

Physicochemical properties of the marinated raw and cooked samples. All of the treated samples were estimated for pH, cooking loss, and the amount of bicarbonate ions before and after cooking. Tables 3, 4, and 5 show the effects of marinating ingredients and heat treatment. The results revealed that the a-d marinated meat samples had a higher pH value, more bicarbonate ions, and a lower cooking loss compared to the control groups ($P < 0.05$). Petracci et al [29] showed that breast fillets treated with SB and cooked at the maximum heat (80–200°C) had a higher ability to retain water than those treated with phosphate (67.3 vs. 65.7%, $P < 0.05$). Marinating ingredients including sodium chloride accounted for an increase in the solubility of meat proteins as well as an increase in the ionic strength [23]. SB and sodium tripolyphosphate (STPP) increased the number of the ions that reacted with the protein and increased hydration [17, 32].

In addition, a combination of two or more of these ingredients has been reported to result in a lower cooking loss than when they are used individually [26]. It was found that the drip loss correlated with protein solubility increasing the solubility of myofibrillar, sarcoplasmic, and total proteins [28]. In addition, SB produced holes during cooking due to the generation of carbon dioxide leading to a coarser microstructure which could also improve the physical entrapment of water [26].

There was no significant dependence between the SB content and the cooking temperature. The statistical analysis revealed that there was no significant correlation between an increase in the amount of SB from 0.25 to 2.00 g/kg in the meat samples (a-d groups) and the cooking temperature from 350°C to 450°C. The similar was observed for the pH value and the cooking loss. However, the amount of bicarbonate ions decreased when the temperature changed from 350°C to 450°C (Fig. 1).

1 raw samples at 24°C

2 cooked meat at 350°C

3 cooked meat at 450°C
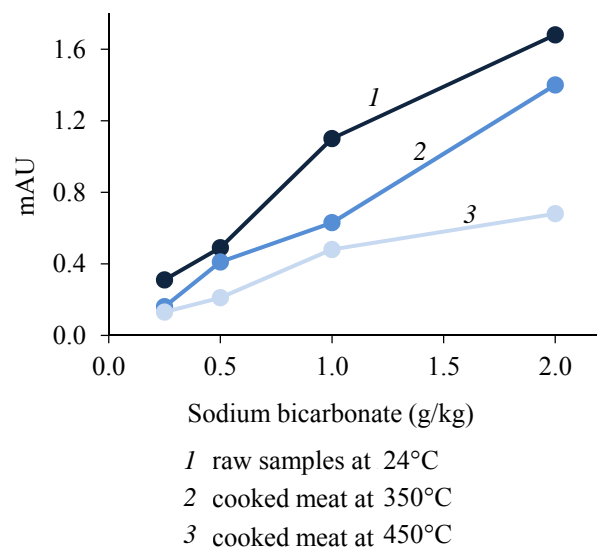
Fig. 1. Mean amount of sodium bicarbonate (as mAU) in the raw and cooked samples at 350°C and 450°C.

Table 3. Physicochemical properties of the marinated raw samples compared to the control sample

pH	Raw samples							
	a		b		c		d	
	mean	P	mean	P	mean	P	mean	P
	6.10 ± 0.00	0.03	6.20 ± 0.00	0.01	6.30 ± 0.00	0.01	6.56 ± 0.05	0.01
Bicarbonate ions, mg/g	0.31 ± 0.00	0.00	0.49 ± 0.00	0.00	1.10 ± 0.01	0.00	1.68 ± 0.00	0.00

Note. a: 0.25 g of sodium bicarbonate per kg meat, b: 0.50 g of sodium bicarbonate per kg meat, c: 1.00 g of sodium bicarbonate per kg meat, d: 2.00 g of sodium bicarbonate per kg meat
The data were represented as the mean ± standard deviation of triplicate tests. The differences between the analyzed physicochemical properties of marinated raw samples compared to the control were statistically significant (P < 0.05).

Table 4. Physicochemical properties of the marinated cooked samples at 350°C compared to the control sample

pH	Cooked samples at 350°C							
	a		b		c		d	
	mean	P	mean	P	mean	P	mean	P
	6.16±0.05	0.01	6.20±0.00	0.01	6.31±0.00	0.00	6.53±0.05	0.00
Cooking loss, %	25.0±0.03	0.03	24.0±0.02	0.00	23.0±0.01	0.00	21.0±0.00	0.00
Bicarbonate ions, mg/g	0.16±0.00	0.00	0.41±0.00	0.00	0.63±0.00	0.00	1.40±0.05	0.00

Note. a: 0.25 g of sodium bicarbonate per kg meat, b: 0.50 g of sodium bicarbonate per kg meat,
c: 1.00 g of sodium bicarbonate per kg meat, d: 2.00 g of sodium bicarbonate per kg meat

Table 5. Physicochemical properties of the marinated cooked samples at 450°C compared to the control sample

pH	Cooked samples at 450°C							
	a		b		c		d	
	mean	P	mean	P	mean	P	mean	P
	6.14 ± 0.03	0.00	6.20 ± 0.00	0.01	6.32 ± 0.01	0.00	6.61 ± 0.01	0.00
Cooking loss, %	27.0 ± 0.00	0.04	27.5 ± 0.04	0.05	25.4 ± 0.04	0.05	21.0 ± 0.02	0.01
Bicarbonate ions, mg/g	0.13 ± 0.00	0.00	0.21 ± 0.00	0.00	0.48 ± 0.00	0.00	0.68 ± 0.00	0.00

Note. a: 0.25 g of sodium bicarbonate per kg meat, b: 0.50g of sodium bicarbonate per kg meat, c: 1.00g of sodium bicarbonate per kg meat, d: 2.00g of sodium bicarbonate per kg meat
The data were represented as the mean ± standard deviation of triplicate tests. The differences between the analyzed physicochemical properties of marinated cooked samples at 450°C compared to the control were statistically significant (P < 0.05).
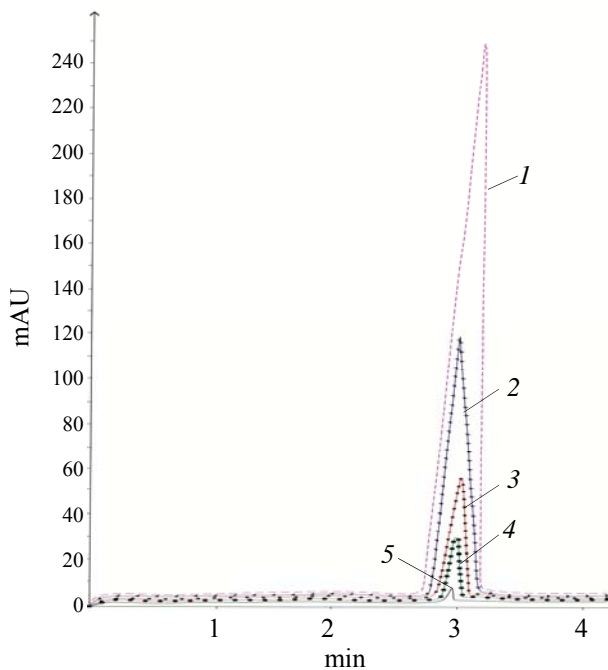
1 – 2.00 gr/kg, *2* –1.00 gr/kg, *3* –0.50 gr/kg,
4 – 0.25gr/kg, *5* – blank

Fig. 2. Ion chromatograms of bicarbonate extracted from the a-d meat samples.

The amount of residual bicarbonate ions increased in cooked kebab samples at two different temperatures when the SB concentration was increased from 0.25 to 2.00 g/kg in meat, if compared to its value in the blank sample, as well as between the groups (*P* = 0.00). In this method, the amount of bicarbonate ions was decreased by increasing temperature from 350°C to 450°C in both the blank and the kebab samples (*P* = 0.00) (Fig 2). While $NaHCO_3$ was heated above 110°C, it was observed that both H_2O and CO_2 underwent some chemical changes. Hence, by increasing the core temperature of the cooked samples from 68–70°C (direct heating at 350°C) to 89–91°C (direct heating at 450°C), the level of bicarbonate ions decreased. The pH of the meat samples treated with bicarbonate did not change after cooking. This result was not in agreement with Sindelar et al, who found that the pH of marinated sow loins with bicarbonate and polyphosphate increased after cooking [33]. These outcomes were most likely because of the essential R groups of the amino acids (histidine) during heating. The second probable reason may refer to the nature of marinade environment, as the alkaline environment may increase the pH value after marinating.

Organoleptic evaluation. The sensory properties of Kubideh kebab were evaluated by 40 panellists according to a 9-point hedonic scale. The organoleptic properties (smell, appearance, flavour,

and palatability properties) did not change when SB was added from 0.25 to 2.00 g/kg of meat, in comparison with the blank group. This differed from the results of a research that showed that Golek chicken marinated with NaCl/STPP/NaHCO$_3$ (Tr6) had the lowest acceptance score compared to other treatments ($P < 0.05$) because of the slight darkening of the meat surface. The darker colour of the meat was probably due to the denaturation of muscle protein after it reacted with NaHCO$_3$. The denaturation resulted in an increase in the reflection and scattering of light and, hence, a paler meat colour [23, 34], as well as in an increase in extracellular water as a result of the marinating process. However, Young & Lyon found no effect of salt and phosphate marinade on meat lightness [14].

CONCLUSION

Marinating lamb meat before processing it into Kubideh kebab affected the quality and the cooking loss of the product. It is the first time that the detection and determination of the residual of bicarbonate ions has been studied in cooked and raw meat. Kubideh kebab cooked at four different SB levels (from 0.25 to 2.00 g/kg) resulted in a high pH value, a high level of biacrbonate ions, and a low cooking loss at different temperatures, while no differences in its organoleptic properties were detected.

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Study of lysate activity to modificate collagene raw materials to use in sausage mixture

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Abstract: In the current conditions of import substitution, the effective use of secondary raw materials in the meat industry is a relevant issue. A significant source of animal proteins is by-products, the yield of which is about 10% of livestock weight. Some by-products, including beef rumen, contain collagen-containing tissues which require modification for tenderization and deodorization. In order to modify rumen tissues, the biotechnological method of treatment with an enzyme solution, lysate, obtained from a whole bovine abomasum was preferred to the known method where enzyme solution is prepared from an abomasal mucosa. The purpose of this project was to study the activity of lysate from a whole bovine abomasum for the modification of rumen tissue to use it in cooked sausage formulations. We have suggested the method of obtaining enzyme solution based on infusing the minced abomasum in a reaction mixture – water, chlorohydric acid, and sodium tripolyphosphate – followed by filtering. The dependence of proteolytic and collagenase activities of the solution obtained from phosphate dose introduced have been studied; it have been revealed that 1.5% of tripolyphosphate is the optimal dose for efficient extraction of enzymes from the whole abomasum. Besides, an effect of the enzyme solution on functional and technological properties of a heat-treated rumen has been studied, and the improvement of hydro- and lipophilic characteristics has been revealed. Paste with modified rumen has been developed and found that the maximum possible dose of rumen for use in cooked sausage from horsemeat is 15%. The color on the cut of sausage developed was identical to that of beef sausage. Thus, paste made on the basis of modified rumen contributes to the formation of functional and technological properties, the stabilization of the color characteristics of the final product, as well as the effective use of basic meat raw materials and the expansion of the range of economy class high-protein sausage production.

Keywords: Modified rumen, cooked sausage, formulation, functional and technological properties, quality

INTRODUCTION

In the current conditions of import substitution, the effective use of secondary raw materials in the meat industry to create natural, protein-containing products of high quality using innovative technological methods is a relevant issue.

A significant source of animal proteins is by-products [1–4], the yield of which is about 10% of livestock weight. Most of by-products are characterized by rather low fat content and increased mass fraction of connective tissue; the latter brings about reduced biological value.

Collagen-containing by-products contain a large amount of connective tissue, which is difficult to digest in humans. However, modern scientific research on processing of collagen-containing raw materials has shown that the use of innovative technologies to modify rumen allows the degree of collagen assimilation to be increased. In addition, collagen, being hard to digest, can act as dietary fiber, improving gastrointestinal tract peristalsis [1, 3, 5, 6].

One of factors limiting the possible effectiveness of by-products use is the specificity of their morphological composition: internal organs are composed of muscular, connective, fatty, and parenchymal tissues. Since various types of raw materials differ in their composition and structure, it requires the use of individual methods for their preliminary processing, which predetermines final product quality as a whole and, in particular, organoleptic indicators and functional and technological properties. Most of the traditional technologies in sausage-canning manufacture, however, include the grouping of secondary protein-containing raw materials in accordance with external morphological

characteristics (fleshy, meat-and-bone, and mucous), heat treatment under high temperatures, and the homogenization of raw materials to destruct connective and cartilaginous tissues, whereas taking into consideration the chemical composition features, organoleptic characteristics, and morphological structure of certain types of secondary raw materials would provide great opportunities for making fundamentally new types of meat products with high quality characteristics.

The low efficiency of using collagen-containing by-products is mainly due to the specificity of chemical and morphological composition and the need for a variety of technological methods aimed both at improving organoleptic characteristics and modifying functional and technological properties, such as water-binding capacity, swelling capacity, water and fat absorbing ability, emulsifying ability, etc.; each type of raw material requires individual methods for their preliminary processing.

The efficiency of using both food and biotechnological potential of collagen-containing by-products is the subject of many studies. Researchers suggest using collagen-containing by-products in formulations of protein-oil emulsions [7], gelatin [8], preparations for zootechnics [9], and meat products [10–12].

Local and foreign researchers suggest various ways of technological processing by-products: physical, chemical, and biotechnological. Rumens, abomasums, stomachs are deodorized by single or multiple cooking in water, broths, spice solutions, milk, whey, weak solutions of organic acids (acetic, ascorbic), or hydrogen peroxide solution; this allows both structural-mechanical and functional-technological properties to be improved.

There are many studies on modification of collagen-containing raw materials by the biotechnological method of processing [13–18]. This method is the most effective but the use of ready-made enzyme preparations in the meat industry is limited due to high cost. Moreover, it is difficult to select an appropriate enzyme which would be exposed to structural proteins of both muscle and connective tissues.

Pepsin, the enzyme preparation isolated from mucous membrane of young calves' abomasums, has proteolytic effect and can be used to soften proteic materials, including collagen-containing ones. The manufacturing process of this enzyme preparation, however, is laborious, since it requires separation of mucous membrane first and then treatment to isolate pepsin. We have suggested a technique for obtaining the enzyme solution from the whole bovine abomasum which can be used to tenderize collagen-containing raw materials.

In connection with the above mentioned, the purpose of the project was to study the activity of lysate from the whole bovine abomasum for modification of rumen tissue to use it in cooked sausage formulations.

STUDY OBJECTS AND METHODS

Experimental research was carried out at the Technology of Meat and Canned Products Department Laboratory of the East-Siberian State University of Technology and Management (Ulan Ude, Russia).

The subjects of the research were bovine abomasum, rumen, sausage mixture, and cooked sausage.

In the previous study we suggested [19] a method of obtaining an enzymatic solution, lysate, from a whole bovine abomasum which contains a mixture of proteolytic enzymes. The lysate was obtained by infusing minced abomasum in a reaction mixture containing water, chlorohydric acid, and sodium polyphosphate. In order to create optimal conditions for activating pepsinogen by adjusting a dose of hydrochloric acid, the reaction mixture acidity was maintained as large as 2.0. The pepsin solution was prepared according to the traditional technique for pepsin isolation from mucous membranes, but whole rumen was used instead of mucous membrane due to difficulty in separating it. This technique is presented in Fig. 1.

The enzyme solution for rumen fermentation must be used within six hours at the storage temperature 2–4°C.

| Reception of abomasums frozen (storage at from -18°C till -20°C no longer than 6 months) | Preparation of reaction mixture with pH 2.0 (chlorohydric acid with $\rho = 1.19$ g/sm^3, sodium polyphosphate, water) |

↓

Defrosting of abomasums at 20–25°C for 4–5 hours

↓

Mincing of abomasums (holes diameter is 2–3 mm)

↓

Mixing with the reaction solution (1.0 kg of abomasums, 2.6 l of the solution)

↓

Infusion (at 31–34°C for 7–8 hours)

↓

Filtering

↓

Quality evaluation of lysate

Fig. 1. Technological scheme of pepsin solution preparation from whole bovine abomasums.

In the course of the experiments, organoleptic indicators were determined by organoleptic tasting, protein mass fraction by the Kjeldahl method, fat mass fraction by the Soxhlet method, water content by drying to constant weight, and mineral substances content by ashing. Functional and technological properties, such as water-holding capacity (WHC), fat-holding capacity (FHC), and thermal stability, were determined by the VNIMMP method [20], moisture binding capacity by Grau-Hamm method, and critical shear stress with a penetrometer. The number of pigments was studied with a photoelectric colorimeter based on the optical density of colored solutions. The mass fraction of common salt was determined in water extract by Mohr's method, residual sodium nitrite content by the photometric method, which is based on the measuring of color intensity formed as a result of interaction between nitrite with sulfonylamide and N-(1-naphthyl)-ethylenediamine dihydrochloride in a protein-free filtrate. Collagen content was determined from the amount of oxyproline (GOST R 50207-92) multiplied by a factor of 8.07 [21].

In order to study lysate proteolytic activity, the method of Ganguly and Bhaler with some modification was used: casein agar was substituted by myosin one as a substrate [22]. The substrate was poured into Petri dishes, holes with a diameter of 12 mm were made in the solidified medium, after that the enzyme solution was poured for incubating. The diameter of the precipitate zone determined solution activity (in mm).

The collagenase activity of the lysate was studied by spectrophotometric method [23] where collagen was used as a substrate. Collagen was obtained from the Achilles tendon of cattle by removal of extraneous proteins by precipitation, further repeated centrifugation, washing, and drying the collagen resulted. Collagenase activity was determined as follows: 5 ml of the lysate was added to 20 mg of collagen and incubated at 37°C for 18 hours. In addition, two control samples were prepared: in the first one, the substrate was incubated with the lysate heated at 60°C for 2 minutes to inactivate enzymes, while the second sample was prepared as the experimental one, but without incubation. The samples were cooled, filtered, and an equal volume of biuret reagent was introduced into. The experimental and the first control samples were measured with the green filter of a colorimeter against the second control sample. The collagenase activity was determined from an extinction value (E); to convert it to percentage of protein dissolved, the standard data presented in Table 1 were used.

Denaturation temperature was measured by using a device with accordance to GOST 32078-2013 [24]. The amino acid composition of proteins was determined with an amino acid analyzer.

Experiments were carried out in three replications.

RESULTS AND DISCUSSION

Researchers working in this field are developing food with modified collagen from hide, splits, connective tissue, etc. It has been established that collagen has properties of dietary fiber and exerts favorable effect on intestinal tract peristalsis and beneficial microflora function.

We have conducted research on modification of collagen-containing rumen properties by the biotechnological method using enzyme solution obtained from a whole abomasum.

The technological scheme of preparing the enzyme solution is represented in Fig. 1. We used sodium polyphosphate (SP) to improve an efficiency of pepsinogen extraction. In order to prevent the process of enzymes extraction from an influence of the medium pH, the latter had the constant value (2.0) due to introduction of chlorohydric acid solution. When adding 0.5% of sodium polyphosphate to the reaction mixture volume, pH was 2.02, and 2% of sodium polyphosphate led to pH = 2.1.

Sodium tripolyphosphate promotes the loosening of the epithelial layer of the abomasum, thereby assisting enzymes release into the solution, since the epithelium is a loose connective tissue with a touch of reticular one.

In the meat industry, introduction of sodium tripolyphosphate in amount of 1–2% is sufficient to increase hydrophilic activity of muscle proteins. Proteolytic and collagenase activities of enzyme solutions without SP and with 1.0, 1.5, and 2.0% of SP were investigated. The enzyme solution obtained from mucous membrane of bovine abomasum was used as a control sample, while experimental samples were prepared from whole abomasum. This method of obtaining the enzyme solution eliminates the difficult process of mucous membrane separation. Fig. 2 shows the change in the proteolytic activity of the solution as a function of a SP dose.

The data in Fig. 2 indicate that addition of sodium polyphosphate increases the proteolytic activity of the solution, for instance, 1.0% of SP raises the proteolytic activity by 18.5% however its level remains below the control value. Introduction of SP in amounts of 1.5 and 2.0% increases the proteolytic activity up to 18.4–18.9 mm that is 4.0–6.0% higher than in the control sample. This must have been due to an increase in pepsinogen concentration, which indirectly confirms that SP improves enzyme extraction.

Further, the collagenase activity of the enzyme solution was analyzed (Fig. 3).

According to the data in Fig.3, SP increases both proteolytic and collagenase activities of the lysate. Introduction of SP in the amount of 1.5 increases the collagenase activity of the pepsin solution up to 29.93%, which is close to that for the control sample (30.16%). A further increase in SP dose up to 2.0% increases the lysate collagenase activity slightly. Probably, SP facilitates a breakage of bonds between polypeptide chains and fibers separation in the abomasum structure, which assist the process of pepsinogen molecules release.

Table 1. Dependence of extinction value on protein cleavage degree

Indicators	Values				
Extinction value, E	0.174	0.115	0.088	0.062	0.029
Collagen dissolved, %	54.51	36.00	27.81	23.71	11.60
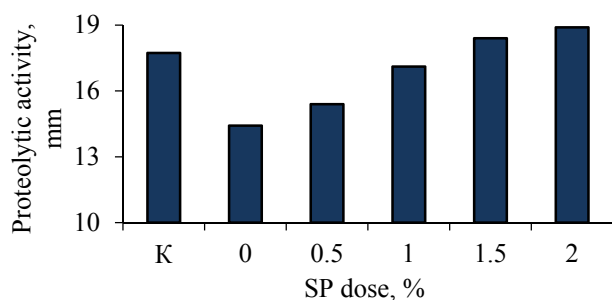
Fig. 2. Change in proteolytic activity of enzyme solution containing SP.
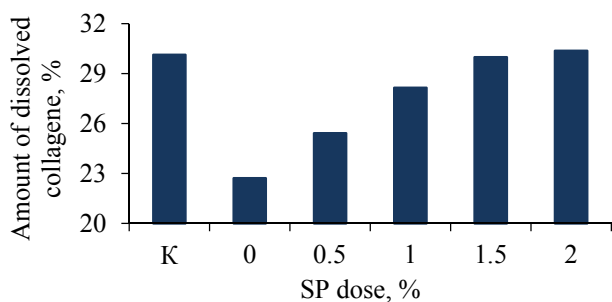
Fig. 3. Change of collagenase activity of enzyme solution containing SP.

Based on the data obtained, the optimal SP dose was accepted to be 1.5%, since it ensures high proteolytic and collagenase activities of the solution. Table 2 shows qualitative indicators of the lysate with SP obtained by using the technological scheme suggested.

The findings from Table 1 show that the pepsin solution obtained from the whole abomasum is similar in appearance to that obtained from the mucous membrane and has high enzyme activity.

On the next stage, experiments on modification of rumen properties with the enzyme solution obtained

were carried out. As known, rumen tissue contains about 7% of collagen and 0.5% of elastin. Collagen and elastin fibers are able to stabilize rumen connective tissue and to give it high mechanical properties. For this experiment, we used prepared rumen meeting the requirements of the documentary standard. Chemical composition and technological properties of bovine rumen were analyzed (Table 3).

The data indicate that the total protein content in rumen is as high as 15.2% which is almost equal to that in muscle tissue; the protein fraction of rumen, however, contains about 65% of collagen.

According to the results of rumen technological parameters analysis, rumen has low water-binding capacity (55.4%) and high shearing force value (6.8×10^2 Pa); this is due to high mechanical characteristics of collagen and elastic fibers, which impart an increased hardness to rumen tissue.

Denaturation temperature is an important indicator of collagen which characterizes its structural changes; for bovine rumen its value is 66.3°C. At this temperature, collagen bundles diminish to the maximum, bend, and intra- and intermolecular interactions in collagen structure tend to weaken. This made it necessary to study the modification process of rumen tissue after a short-term heat treatment, which retards the contamination process.

For modification, rumen was held in the enzyme solution prepared from the bovine abomasum. For this, 30% of the enzyme solution was added to minced rumen and the fermentation process was carried out at 35–36°C for 12–14 hours. This duration was chosen based on the fact that pepsin hydrolyzes proteins and peptides far slower than other proteinases do [23]. In addition, pH was reduced to 5.3–5.5, since at pH 5.6 and higher an inhibitor inactivates enzymes; moreover, acidic conditions contribute to microorganism growth inhibition.

Table 2. Qualitative indicators of enzyme solution

Indicators	Enzyme solution (lysate) obtained from	
	Mucous membrane of bovine abomasum (control)	Whole bovine abomasum (experimental)
Color	Light-beige	Beige
Texture	Slime	Slime
Aroma	Specific	Specific
Proteolytic activity, mm	17.81	18.42
Collagenase activity(amount of dissolved collagen, %)	30.21	29.81

Table 3. Chemical composition and technological properties of bovine rumen

Organoleptic indicators	Characteristics	Chemical composition	Value	Technological properties	Value
Appearance	Fat-free, cut up, separated from mucous membrane, washed, with no dark spots	Mass fraction of protein, % – collagen, %	15.2 ± 1.0 9.6 ± 0.5	Water-binding capacity, %	55.4 ± 1.0
Color	Yellowish-white, with grayish tint	Mass fraction of fats, %	4.9 ± 0.4	Shearing force, $\times 10^2$ Pa	6.8 ± 0.2
Aroma	Typical of good by-products, typical of rumen, without foreign smell	Mass fraction of mineral substances, %	1.4 ± 0.1	Denaturation temperature, °C	66.3 ± 2.0
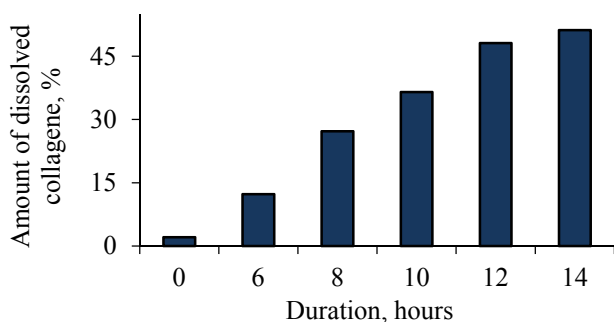
Fig. 4. Effect of holding duration of rumen samples in enzyme solution on amount of collagen dissolved.
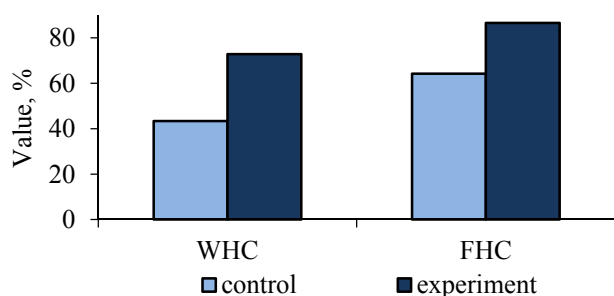
Fig. 5. Functional and technological indicators of rumen.
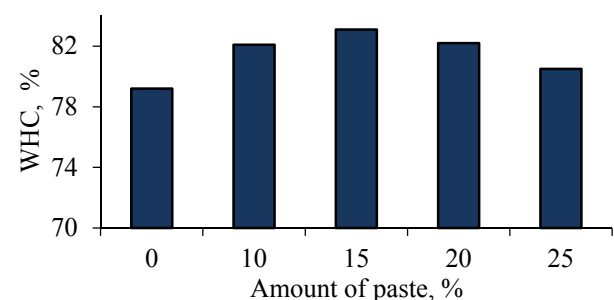
Fig. 6. Effect of paste on water-holding capacity of horse mince.
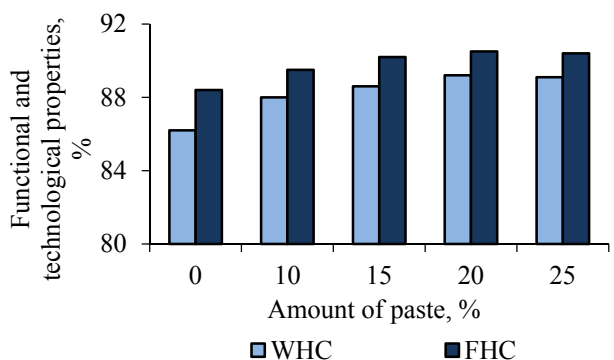
Fig. 7. Water-holding capacity change as a function of amount of paste.

In order to analyze the modification process, an effect of the enzyme solution obtained on the amount of collagen dissolved at various holding duration of rumen samples in the enzyme solution was studied (Fig. 4).

Table 4. Characteristics of paste based on modified rumen

Organoleptic indicators	Value	Physical and chemical indicators	Values
Appearance	Grey-white color	Mass fraction of water, %	79.8 ± 1.4
Texture	Pasty	Mass fraction of proteins, %	13.4 ± 0.7
Aroma	Flavorless	Mass fraction of ash, %	1.2 ± 0.1
Taste	Tasteless	Mass fraction of fats, %	5.4 ± 0.2

The results in Fig. 4 demonstrate a considerable increase of dissolved collagen amount with an increase of the holding duration of the substrate in the enzyme solution, which can indicate the increase of collagen fragmentation degree. Since after 12 hours of holding changes in the studied indicator was insignificant, the optimal time was 12 hours. It should be noted that pepsin is able to destroy hydrogen and disulfide bonds between polypeptide chains that leads to formation of "open" forms of protein molecules. As a result of modification, morphological changes in muscle, collagen and elastin fibers can occur which contribute to improve rumen properties. In order to prove this, functional and technological characteristics of rumen tissue modified were studied (Fig. 5).

The results demonstrated in Fig. 5 show a favorable effect of holding rumen in the enzyme solution on improvement of water- and fat-holding capacities which speaks for modification of collagen-containing rumen properties by treatment with pepsin solution obtained from the whole bovine abomasum. Undoubtedly, the factors that contribute to improvement of functional and technological properties of rumen are changes in the structure of collagen and unstriped and cross-striped muscle fibers of rumen with formation of active functional hydro- and lipophilic groups.

Modified rumen was then finely minced and diffused until paste was obtained. Table 4 represents qualitative characteristics of the paste based on modified bovine rumen.

The next stage was to find a possibility to include the resulting paste in the formulation of horse cooked sausage. In order to determine the optimal amount of the paste, the functional and technological properties of horse mince with 10, 15, 20, and 25% of the paste were analyzed. Fig. 6 illustrates the effect of the paste amount on the water-holding capacity of the mince.

The data in Fig. 4 show that 15% of the paste increases meat hydrophility by 3–4%. Further increase of the paste amount makes the mince water-logged therefore WHC declines.

The indicators of water- and fat-holding capacities of the mince after heat treatment were now studied (Fig. 7).

The results indicate improvement of functional and technological properties of the heat-treated mince with increasing amount of the paste. The paste in amount of 25% increases WHC and FHC by 3% and 2%,

respectively. Denaturation of modified collagen fibers also improves the functional and technological properties, since gelling agents are able hold additional water molecules. Collagen play a critical part in this process, but the role of unstriped and cross-striped muscle fibers, which contribute to active functional hydro- and lipophilic groups, is also significant.

Further, critical shear stress, a parameter which characterizes rheological properties of the system, was studied (Fig. 8).

According to the results from Fig. 8, the critical shear stress of the mince is 450 Pa. Introduction of the paste in amounts of 10% and 15% causes the decrease in critical share stress by 2.3% and 4.9%, respectively; with the increase of the paste amount, the magnitude decreases markedly (by 11–13%) due to the mince watering.

Further, sausages with spices and various amounts of the paste were made according to the formulation of 2nd grade cooked horse sausage; the content of nitroso pigments, which provide for forming the color and appearance of the product, was studied. The reason for using horse sausage was the fact that the paste contains a small amount of muscle tissue which is able to react with sodium nitrite to form nitroso pigments, and horse meat contains more the pigments than beef and pork do. Fig. 9 illustrates the change in nitroso pigments content in horse sausage samples with the paste in amounts of 10, 15, 20, and 25% in comparison with that in control samples of pork, beef, and horse sausages.

The data in Fig. 9 indicate that the paste affects nitroso pigments content, since the rate and the efficiency of their formation depend on myoglobin and oxymyoglobin content in the mince. Myoglobin content is 72.8% in pork sausage, which is light pink in color, 79.3% in beef sausage, and 84.4% in horse sausage, which is dark red in color. The values obtained are consistent with pigments content in these meats. Introduction of the paste caused discoloration of the product as a result of depigmentation. In the experimental sample containing the paste in amount of 15%, color is identical to that of beef sausage, and color of the sample with 20% of the paste is similar to that of pork sausage.

Based on the research conducted, the amount of the paste was established to provide optimal functional and technological properties and color of the product: the paste in amount of 15% was used in the formulation of the cooked sausage instead of 10% of horse meat and 5% of fat. The formulation of the cooked sausage named "Zaigraevskaya" with 15% of the paste based on modified rumen was developed (Table 5).

As seen from Figure 10, organoleptic parameters of the experimental sample are similar to those of the control one.

Quality parameters of the cooked horse sausage with the paste are represented in Table 6.

Since cooked sausages are a mass-consumption product, a priority target on their manufacturing is to keep the balance between proteins and fats, as well as between water and dry matter contents.

Immediately after manufacturing, quality inspection by all parameters of control and experimental samples was performed.

Seven experts participated in sensory analysis of sausages developed; their evaluations were entered in a tasting sheet. The tasting evaluation data were recorded in the form of scores (using nine-point scale method) and description of each indicator by members of the taste panel.
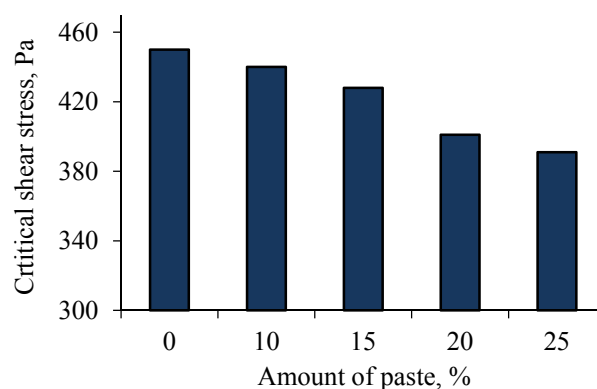
Fig. 8. Effect of amount of paste based on modified rumen on critical share stress.
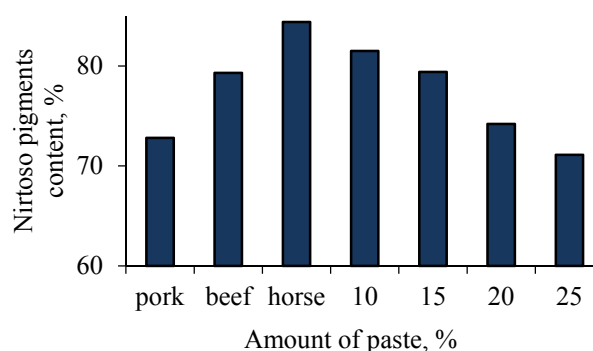
Fig. 9. Nitroso pigments content in cooked horse sausage samples with paste based on modified rumen.

Table 5. Formulation of cooked sausage "Zaigraevskaya"

Raw materials	Amount of raw, kg (per 100 kg of raw)	
	Horse cooked sausage control	Cooked sausage "Zaigraevskaya" experiment
1st grade trimmed horse meat	83.0	73.0
Fat	15.0	10.0
Starch	2.0	–
Paste based on modified rumen	–	15.0
Soya protein	–	2.0
Total amount	100.0	100.0
Salt	2.5	2.5
Sodium nitrite	0.005	0.005
Sugar	0.1	0.1
Black pepper	0.05	0.05
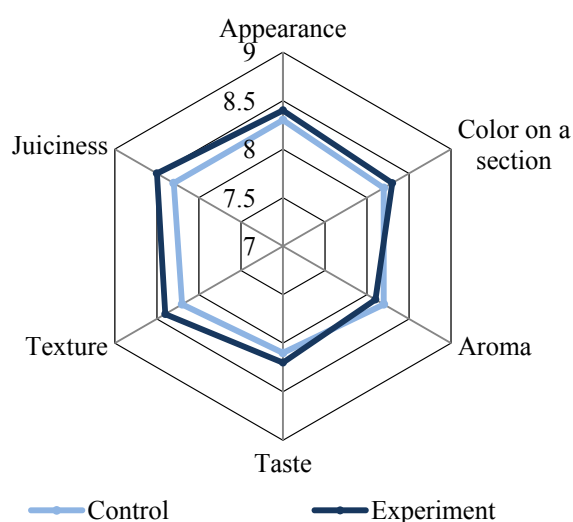
Fig. 10. Profilogram of organoleptic evaluation of cooked horse sausage "Zaigraevskaya" with paste based on modified rumen.

Table 6. Quality parameters of cooked horse sausage "Zaigraevskaya" with paste based on modified rumen

Parameter	Characteristics	Physical and chemical parameters	Value
Appearance	Clean dry surface	Mass fraction of fat, %	16.2 ± 0.5
Texture	Elastic	Mass fraction of protein, %	17.5 ± 0.6
Color and appearance on a cut	Pink, mince is mixed uniformly	Mass fraction of salt, %	2.4 ± 0.3
Aroma and taste	Typical of this product, no foreign taste and odor, with spicy flavor, properly salted	Mass fraction of sodium nitrite, %	0.002 ± 0.0001

Fig. 10 demonstrates tasting sheets data.

The data in Table 10 show that the chemical composition and organoleptic parameters of experimental samples conform to requirements GOST R 52196-2003 for cooked sausages of grades A and B according to which the mass fraction of fat is no more than 20–30%, protein – no less than 10–12%, salt – up to 2.4%, and sodium nitrite – up to 0.005%.

CONCLUSION

As a result of the research, a beneficial impact of the enzyme solution obtained from a whole bovine abomasums with added sodium tripolyphosphate on tough rumen tissue has been established. It has been determined that the optimal time for holding rumen in the pepsin solution is 12 hours. Biotechnological treatment of rumen tissue by holding it in the solution for 12 hours at the temperature of 35–36°C facilitates the improvement of functional and technological characteristics and the increase of collagene fragmentation degree. Based on fine rumen tissue, paste with high organoleptic and technological properties was developed. The paste was used in the formulation of cooked horse sausage. The maximum possible amount of the paste from modified rumen which contributes to form hydrophilic and structural and mechanical characteristics of the mince has been established (15%) to use it in the formulation. It has been revealed that horse sausage with 15% of the paste is similar in color to beef sausage. Thus introduction of the paste based on modified rumen into horse sausage composition provides the formation of functional and technological properties and the stabilization of organoleptic parameters of the final product as well as the efficient use of basic meat raw materials and the expansion of the range of the economy-class high-protein sausage production.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

FUNDING

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Prospects for using pine nut products in the dairy industry

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Abstract: Functional products are currently attracting a lot of research interest. Modern people's diet does not satisfy their need for nutrients, vitamins and minerals, and functional products can make it more balanced. In particular, our diet is lacking in protein. This paper discusses the prospects for enriching dairy products with plant protein derived from pine nuts and their products. Pine nut paste, fat-free milk and oil cake are a valuable source of fatty acids, vitamins, and microelements. The protein, lipid, vitamin, and mineral content of these products makes them suitable for combining with milk. Their water-holding and fat-emulsifying capacities allow their use as stabilizers and emulsifiers. Siberian pine nuts grow wild in the Kemerovo Region, which makes their use as a raw material economically feasible. The article introduces a number of functional dairy products enriched with pine nut products, such as cheese, ice cream, and cottage cheese. Further, it describes the production process and the products' nutritional value. The chemical composition of new types of dairy products shows that using pine nut oil cake, fat-free flour, paste and oil enriches them with plant proteins, vegetable fats, vitamins as well as macro- and microelements. Replacing dairy raw materials with plants does not reduce the nutritional value of new dairy products. Dairy foods are rich in protein, fat, and minerals. The vitamin content of new dairy products with functional ingredients is similar to that of dairy-based products. Moreover, using functional products (pine nut oil cake, fat-free flour, paste and oil) enriches new types of dairy products with tocopherols.

Keywords: Pine nut processing, functional foods, dairy products, dietary supplements, pine nut paste, pine nut oil cake, fat-free pine nut flour

INTRODUCTION

The 21st century has significantly changed our lifestyle in many ways. Such factors as adverse environmental conditions, physical inactivity, and high mental stress lead to a rapid destruction of vitamins and minerals in the human body. The majority of Russian people suffer from malnutrition for social and economic reasons: most accessible are high-calorie foods with a low nutritional value.

Modern diet is characterised by a high proportion of industrially produced foods. As a result of a curing process, such products lose a considerable part of biologically active components – vitamins, minerals, some amino acids, and other substances that play a significant role in the body's metabolism. The deficiency of such essential nutrients weakens the body's defences against adverse environmental factors, contributes to chronic fatigue, and decreases mental and physical performance [1].

On the other hand, there is a growing interest in balanced nutrition and an increased demand for products that not only satisfy hunger, but also minimize the harmful effects of the environmental and stress factors. This is due to the rising cost of medical care and raised consumer awareness of the principles of healthy eating. Another reason is a recent increase in life expectancy and the desire of older people to keep healthy [2]. The modern food industry, with its research and production facilities, is able to meet this growing demand for healthy eating and produce a wide range of foods with improved composition, properties, and functions.

Modern principles of healthy eating highlight a need for low-calorie and low-cholesterol foods, as well

as special natural supplements, preferably of plant origin. Functional products are a current global trend, since nutrition problems are common for the whole world, not only Russia. Foreign studies claim that developing new eating habits is a prerequisite for improving people's health [3].

In Russia, providing the population with affordable and high-quality products is part of the state agenda [4]. "The Russian policy of healthy eating" underlines a need to provide various social groups with balanced functional foods. This paper looks at developing functional foods based on raw milk with the use of pine nut products as one of the ways to improve nutrition in Russia.

STUDY OBJECTS AND METHODS

Our objects of study included:
– Siberian pine seed kernel products, such as pine nut oil, pine nut paste, pine nut oil cake, and fat-free pine nut flour;
– low-fat cottage cheese (GOST R 51074-2003);
– skimmed milk powder (GOST R 52791-2007); and
– dairy prototypes, such as processed cheese, cottage cheese and plant mixture, ice cream, and mayonnaise.

The following studies were conducted with the following methods to determine the nutritional value:
(1) The fractional composition of proteins – by the Yermakov method, sequential extraction of proteins with a 10% aqueous NaCl solution, a 0.2-normal NaOH solution, and a 70% alcohol solution, followed by albumin and globulin separation by dialysis. To measure the amount of glutelins, a co-extraction of prolamins and glutelins with a 0.2% solution of NaOH was carried out after the extraction of albumins and globulins, followed by precipitation of glutelin at pH 10 in the presence of NaCl.
(2) The amino acid composition – by ion-exchange chromatography with an automated amino acid analyser AAA-339M after the preliminary hydrolysis of proteins and fractionation of amino acids.
(3) The fatty acid composition of primary and end products – by chromatography.
(4) The mass fraction of fat – by extracting it with a mixture of chloroform and ethyl alcohol in the Soxhlet apparatus, followed by removing the solvent, drying at $103 \pm 2°C$, and weighing (GOST 23042).
(5) The amount of retinol – by a colorimetric method, vitamin B_1 (thiamine) and vitamin B_2 (riboflavin) – by a fluorimetric method, and vitamin B_3 (pantothenic acid) – according to GOST 50929.
(6) The amount of vitamin E – by a photocolorimetric method.
(7) The content of mineral substances – according to GOST 26176-84 and MU 01-19/47-92.
(8) The mass fraction of macro- and microelements – by the atomic absorption spectrophotometry according to GOST 30178-96.
(9) Functional and technological properties of pine nut oil cake and fat-free flour, namely their water absorption (swelling) and fat absorption capacity as an amount of water (or fat) held by the sample when soaked in a free state without mechanical load (centrifugation) – by weighing the sample before and after watering.

(10) The fat-emulsifying capacity was a relation between the emulsion layer volume and the total mixture volume. The water-holding capacity was a relation between the amount of water bound by the protein product and its weight after drying at 105°C for 4 hours.
(11) The titratable acidity of the end products was measured according to GOST 3624-92.
(12) The organoleptic evaluation of the end products was conducted according to GOST 8756.1 in the following order:
– appearance: shape, surface, uniformity, absence/presence of impurities;
– colour vs. typical colour of a given product;
– aroma: typical features, aroma balance, a so-called 'bouquet', absence/presence of foreign odours;
– consistency: homogeneity, absence/presence of solid particles;
– taste vs. typical taste of a given product.
(13) The caloric value was based on the actual content of protein, fat, and carbohydrates in the dry protein product, given that 1 g of fat is equivalent to 9.3 kcal, 1 g of protein – 4.1 kcal, and 1 g of carbohydrates – 3.75 kcal.
(14) All the experiments were performed three times. The data were processed by standard methods of mathematical statistics. The homogeneity of the sampling effects was checked using the Student's t-test. The differences between the means were considered significant when the confidence interval was less than 5% ($p \le 0.05$).

Functional foods. In recent years, great efforts have been made to return food its health benefits. Modern nutrition science calls for new types of products or functional foods containing ingredients that benefit human health and increase its resistance to disease. Functional foods can improve many physiological processes in the human body.

One of the first functional food projects started in Japan in 1984, resulting in as many as 100 functional foods produced in 1987. Soon, the concept of foods for specified health use (FOSHU) took off in many other parts of the world. It is estimated that in the next few years the market of functional foods will exceed 30% of all foods sold in Europe.

The problem of functional food production attracts a lot of research efforts both in Russia and abroad [5–8]. The Functional Food Centre (FFC) defines functional foods as natural or specially processed products containing biologically active components in an amount sufficient for preventing disease and improving health [9]. Functional foods are widely used along with ordinary foods by both healthy and sick people [5, 10–12].

The technology for developing functional foods is based on the modification of traditional foods to increase their content of useful ingredients and biologically active components. Functional foods are characterized by a high content of nutrients that our diet is usually lacking in, namely amino acids, fatty acids, vitamins and minerals, as well as dietary fibre.

Functional foods acquire beneficial properties due to their complex composition, which depends on the formulators' ability to adequately combine various ingredients. In other words, the beneficial properties of

one ingredient should not be neutralized by those of another.

The goal is to increase the content of valuable nutrients to a level consistent with the physiological norms of their consumption. The content of enriching ingredients in functional foods ranges from 10 to 50% of the average daily requirement. Their quantity depends on how well they can preserve their properties during production or storage so that it does not get lower than the recommended level at the end of the storage period.

A high content of vital nutrients is not the only benefit of functional foods. They are meant to regulate the proportion of harmful substances in the body and produce a positive effect on the immune system, metabolism, and other physiological processes [6, 13–15]. This role is performed by vitamins, minerals, and antioxidants.

The development of foods containing functionally interrelated nutrients of different nature and structure should be based on reliable information about their physiological effect on the metabolic and regulatory functions of the organism, taking into account their synergistic and integrated action.

Unlike traditional products, functional foods enriched with vital nutrients can meet the body's needs in them without leading to excessive eating.

One of the main stages in the development of a functional product is a justifiable selection of functional ingredients that create new properties related to the product's ability to exert a physiological effect.

The potential ability of functional ingredients to change the taste, aroma and texture of the finished product plays an important role here. However, new foods should be as appealing to the consumer as conventional ones and have the same organoleptic properties. The new taste, aroma, and texture should be consistent with most consumers' cultural traditions and eating habits.

To produce new functional foods with a desired structure, as well as technological and consumer characteristics (including storage stability and affordability), the developers need to use additional ingredients that improve their appearance and flavour. Functional foods should be as good as traditional ones, and this is the task that modern nutrition science is able to fulfil.

The technology for developing functional foods makes it possible to regulate their caloric content, which is a very important issue for those people who have a high calorie intake, with the resulting health implications. As a rule, functional foods have a reduced calorie content alongside an increased content of nutrients that our diet is deficient in.

Functional food production is a science-based sector, and studying the impact of nutrition on human health is currently one of the most promising areas of food science [16]. Experimental research in healthy nutrition confirms the beneficial effect of functional foods on the body. In particular, our diet can affect the following aspects of our health:
– immunity;
– the cardiovascular system;
– blood cholesterol level;
– intestinal microflora and the gastrointestinal tract;
– blood glucose level;
– arterial pressure;
– minerals assimilation degree; and
– osteogenesis [17].

Functional foods have a number of major functions, in particular they 1) make up for the deficiency of biologically active components identified as a result of studying the actual diet that people in a particular region live on; 2) help our organs and systems maintain their normal functions due to a high content of essential vitamins and minerals; 3) reduce the risk of disease by making the diet more balanced; and 4) create a favourable dietary background by enhancing beneficial microflora to ensure sufficient absorption of nutrients ingested with food.

Researchers divide functional ingredients into the following main groups: dietary fibre, vitamins, antioxidants (tocopherols), minerals, and polyunsaturated fatty acids [13, 14]. Below are their key characteristics.

Dietary fibres are polysaccharides that can be structural (cellulose, hemi-cellulose, and pectin substances) and non-structural (gums, mucus). Not digested or absorbed by the body, they regulate the physiological and biochemical processes in the digestive organs and improve the digestibility of food nutrients [18, 19]. The most common food fibres are cellulose, pectins (glycanogalacturonans), slimes, and gums.

Cellulose is the most common plant homopolysaccharide and the main structural component of the plant cell membrane. Its major physiological effect lies in its ability to bind water (up to 0.4 g water per 1 kg fibre). Cellulose largely texturizes food and is almost indigestible in the intestine: its digestibility ranges from 6 to 23%, depending on its origin, content in the diet, and the nature of processing.

Microcrystalline cellulose, carboxymethylcellulose, and methylcellulose are widely used in food production. Hemicelluloses are a group of high-molecular polysaccharides which, unlike cellulose, are easily dissolvable in water and aqueous solutions of alkalis, and are able to hydrolyse in aqueous solutions of acids. They are the second largest component of plant cell walls, after cellulose. One of their most important functions in food is that they are able to retain water and bind cations. Also, as dietary fibres, they are part of the indigestible bulk, which is extremely important for intestinal motility. Finally, hemicelluloses help to remove bile acids and lower blood cholesterol.

Pectic substances (glycanogalacturonans) are biopolymers that make up the cell wall and act as a structuring agent. Pectin is often used as a gelling agent and a stabilizer. One of the most important properties of pectic substances is their ability to interact with heavy metal ions and radionuclides, which makes them a valuable additive in food production for health-promoting purposes. The prophylactic dose of pectin, recommended by the Food and Agriculture Organization (FAO) and the World Health

Organization (WHO), is 2–4 g per day or 8–10 g per day for those working in adverse conditions.

Slimes and gums are a group of complex unstructured polysaccharides, including water-soluble carbohydrates forming viscous and sticky solutions. Gum substances have a structure similar to hemicelluloses, but a lower molecular weight. Gums are widely used in food production as binders, thickeners, emulsifiers, and stabilizers owing to their valuable properties of swelling, increased viscosity and stickiness. Arabic gum and locust bean gum are among the most commonly used gums in the food industry.

It is a well-known fact that dietary fibre deficiency is a risk factor for a number of diseases, including colon cancer, irritable bowel syndrome, hypomotor dyskinesia of the colon, diverticula, cholelithiasis, obesity, atherosclerosis, ischemic heart disease, diabetes, varicose dilatation of veins, etc. The FAO and the WHO point to a low consumption of dietary fibre almost in all the countries.

Vitamins are low-molecular biologically active compounds that take part in all essential functions of the body and are therefore vital to man. They are involved in metabolism and provide protection against harmful environmental factors and diseases. Since the human body is not able to synthesize vitamins or store them for future use, they must be regularly supplied from outside. However, the standard daily diet cannot provide the content and variety of essential vitamins that meet the physiological needs.

Many vitamins combine with proteins and form enzymes, which makes them especially important in nutrition. As a rule, vitamins are coenzymes, i.e. active groups of enzyme systems. Lack of vitamins can delay the formation of enzymes and cause impaired digestion, which in turn leads to metabolic disorders.

There are a number of natural compounds that can be converted to vitamins by metabolic processes. Such substances are called provitamins. For example, carotenoids are provitamins of vitamin A, sterols are provitamins of the D group, and nicotinic acid is a provitamin of nicotinamide (vitamin B_5).

Vitamin B_1 (thiamine), a coenzyme of several enzymes, is involved in metabolic processes and energy metabolism, necessary for the normal functioning of the nervous system. Vitamin B_1 is found in baker's and brewer's yeast, soybean, rice bran, pine nuts, and buckwheat. Liver and kidneys are the sources of vitamin B_1 among animal products. In addition, this vitamin is synthesized by certain types of bacteria. Vitamin B_2 (riboflavin) is involved in the metabolism of carbohydrates, fats and proteins, and is necessary for the normal functioning of the visual organs. Pine nut kernels are rich in vitamin B_2.

Antioxidants. Tocopherol (vitamin E) is the most important intracellular antioxidant that protects fats and other easily oxidizable compounds from oxidation. Also, vitamin E is a carrier of electrons in redox reactions and is necessary for normal metabolism in the muscle tissue. Lack of this vitamin leads to muscle atrophy and also affects the vascular and nervous tissues. Approximately 20–30 mg of tocopherol comes with food; however, only 50% or less is absorbed in the intestine [14]. Pine nut products are a possible source of tocopherol: 30 g of pine nut kernels can satisfy a daily human need for this substance.

Minerals. The human body contains about 3 kg of various mineral substances, including 81 out of 92 naturally occurring chemical elements. Many elements are contained in a bound state, in the form of mineral salts, complex compounds ions and organic substances. Twelve elements (C, O, H, N, Ca, Mg, Na, K, S, P, F, and Cl) are structural, since they constitute 99% of the body's elemental composition. Fifteen microelements (such as Fe, J, Cu, Zn, Cr, Mo, Ni, V, Se, etc) are essential for vital activities, despite their low content.

Minerals are constituent elements of proteins; they are involved in the biosynthesis of nucleic acids as well as carbohydrate and lipid metabolisms. They also perform a catalytic function, activate and regulate enzymes; they are involved in the biosynthesis of vitamins and in the synthesis of hormones, affecting their activity. Although minerals do not have the energy value (like proteins, fats or carbohydrate), they need to come in regularly. Their main source is food, although the standard daily diet does not contain all the necessary minerals.

Calcium is the most abundant of all the minerals in the human body (1–2 kg), 98–99% of it contained in the bone and cartilage tissues. Also, calcium participates in the transmission of nerve impulses, promotes blood clotting, and affects the work of the heart muscle. Dietary calcium mainly comes from dairy products, of which there should be plenty. However, there may be certain problems with its absorption, e.g. in a diet rich in fats.

Phosphorus is a biological companion of calcium. The human body contains 600–900 g of this element, of which 90% is found in the bone tissue. As part of adenosine triphosphoric acid (ATP), phosphorus is involved in different metabolic processes, including energy metabolism. Phosphorus metabolism is closely related to calcium metabolism; therefore, the consumption of these two substances must be balanced.

Magnesium is involved in enzymatic, carbohydrate and phosphorus metabolism. This element is important for the normal functioning of the nervous and muscular tissues. The need for magnesium increases in older people who are prone to hypertension and have elevated levels of cholesterol in the blood.

Magnesium enters the body mainly with food. The richest source of magnesium is plant products, such as cereals, legumes, bran, pine kernels, and vegetables. Magnesium is a calcium antagonist; therefore, it is important to consume them separately and maintain their balance in the diet.

Sodium is an important extracellular and intracellular element. It is involved in maintaining a constant volume of fluid in the body and in maintaining an acid-base balance. Sodium is important for the nerve reactions, and its content directly affects the state of the nervous system. The main source of sodium is table salt, whose consumption rate is 8–12 g per person. Excessive salt intake, however, is harmful to the body.

Potassium is an intracellular element that regulates the acid-base balance in blood. It participates in the transmission of nerve impulses and regulates certain

enzymes. The human body contains 160–250 g of potassium, of which 98% is intracellular. One of its most important functions is to maintain the potential forming on the cell membrane and regulate osmotic pressure in the cells. In some physiological processes, potassium acts as a sodium antagonist, and its increased concentration leads to the release of sodium from the body.

Potassium deficiency impairs the functioning of the neuromuscular and cardiovascular systems and manifests itself in depression. Its excess, however, can cause sudden cardiac arrest. The daily need for potassium ions ranges from 2.5 to 5.0 g. Normal metabolism requires the 1:2 ratio of potassium and sodium in the diet.

In addition to the main "structural" elements, all organisms contain microelements (or trace elements), whose concentration is 0.001–0.00001%, and ultramicroelements, with a concentration of 0.00001% or less. Fifteen of those elements (iron, iodine, copper, zinc, cobalt, chromium, molybdenum, nickel, vanadium, selenium, manganese, arsenic, fluorine, silicon, and lithium) are considered essential, or vital.

Trace elements play a very important role in the human body. They perform a structural function, as components of hard and soft tissues (silicon, strontium, fluorine, and aluminium). However, their main role is to contribute to all the physiological functions of the body.

Trace elements ensure the most important functions of proteins. As structural elements of protein substances, they take part in the biosynthesis of nucleic acids and thus affect the functioning of the genetic apparatus, and cell growth and division. Also, they are involved in the formation of high-molecular compounds (nucleoproteins and lipoproteins) and enhance phospholipids, which are important for nervous activity.

Trace elements are involved in carbohydrate metabolism, affecting insulin activity, and in lipid metabolism, which is important in the prevention of obesity and fatty infiltration of internal organs.

Additionally, trace elements perform a catalytic function as metalloenzyme components or enzyme activators and regulators, and participate in the biosynthesis of vitamins, contributing to their absorption and use by the body.

Further, microelements are involved in the synthesis of hormones and other biologically active substances (kinins, serotonin and vasopressin) and mediators, affecting their activity.

It is worth noting the relation between microelements and malignant growth processes. On the one hand, an excess of some microelements and a deficiency of others can predispose the organism to the development of tumours; on the other hand, some microelements have a strong antitumor effect. Moreover, some trace elements (silver, mercury and manganese) have antimicrobial action and others have an anti-stress effect.

However, industrial development and the use of fertilizers contribute to an excessive intake of various trace elements, which can harm the human body. At the same time, trace elements can also have an antitoxic effect by increasing the immunobiological reactivity of the body.

To sum up, both the deficiency and excess of some trace elements can affect the vital functions of the body.

Iron is necessary for the formation of haemoglobin and a number of enzymes, taking part in the transport of oxygen: 80% of iron contained in the human body is a component of haemoglobin. Iron deficiency leads to anaemia and a number of other diseases. Due to unbalanced nutrition, every fifth person in the world suffers from iron deficiency.

Zinc is predominantly found in the muscles, red blood cells, plasma, prostate gland, spermatozoa, and is a component of the insulin hormone. Also, zinc is a part of metalloenzymes involved in various metabolic processes, including the synthesis and breakdown of carbohydrates and fats. It is important for the functioning of a number of enzymes that ensure the normal operation of the pituitary gland and the pancreas. Zinc takes part in the synthesis of proteins and nucleic acids; it is important for stabilizing the structure of DNA, RNA and ribosomes and is involved in the translation process. Thus, zinc affects the functioning of the genetic apparatus, cell growth and division, keratogenesis, osteogenesis, and reproductive function, and contributes to the immune response and wound healing. Finally, zinc affects the production of behavioural reflexes, brain development, and flavour perception.

Manganese is mainly found in bones, liver and kidneys. Its major biological property is in its influence on the bone tissue. Further, manganese has a significant effect on growth, reproduction, immunity, and metabolism. Its biological role is also associated with osteogenesis processes and the metabolism of proteins, carbohydrates, and mineral salts. Manganese activates redox processes and has a positive effect on the blood-forming organs. It takes part in carbohydrate metabolism (ensuring normal insulin secretion and increasing glycolytic activity), lipid metabolism (preventing the deposition of fat in the liver) and in the synthesis of cholesterol. Finally, it is a component of a number of enzymes.

Lipids containing polyunsaturated higher fatty acids. The recommended fat content in the diet is 90–100 g per day, with 30% of this intake coming from vegetable oils. Polyunsaturated fatty acids should comprise about 10% of the total fat content. If there is a pathology of lipid metabolism, the ratio of polyunsaturated to saturated acids should be 5:1. In the nutrition of elderly people and patients with cardiovascular diseases, this ratio should be 2:1 [20]. Polyunsaturated fatty acids are components of cell membranes and other structural elements of tissues. They contribute to the regulation of cell metabolism and platelet aggregation, affect the exchange of cholesterol, participate in the metabolism of group B vitamins, and increase the body's resistance. A deficiency of polyunsaturated fatty acids increases the risk of cardiovascular diseases, leads to the disruption of normal kidney function, and reduces the body's resistance.

According to the monitoring data, Russian people consume plenty of oils containing ω-6 fatty acids (sunflower, soybean and corn oils) and almost no oils rich in ω-3 fatty acids (linseed, rapeseed, camelina, mustard, and hemp oils).

Obviously, enriching traditional foods with functional ingredients has a direct impact on human health. It should be noted that functional foods are not drugs: they are products of mass consumption and often part of a regular diet. In the structure of modern nutrition, functional foods occupy an intermediate position between ordinary and medical foods. Ordinary foods are chosen according to one's eating habits and income to satisfy hunger. Medical foods are prescribed by the doctor as part of a therapeutic diet to be consumed during the period of therapy or medication. Functional foods agree with standard eating habits and, at the same time, help maintain health. Unlike drugs, they have no contraindications for prolonged use.

Healthy people use functional foods to prevent disease. Groups of people in need of functional foods include children of different ages, elderly people, workers in hazardous industries, polar explorers, people living in environmentally unfriendly regions, etc. The composition of multicomponent functional foods may vary according to consumers' differentiated needs. By changing the formula, we can develop specialized baby foods or dietary foods.

Functional foods must meet the following requirements:
– have scientifically-proven beneficial properties;
– have a daily intake rate established by experts;
– possess specific physico-chemical characteristics and precise methods for their measurement;
– have no negative impact on the nutritional value;
– be used as ordinary foods and have similar consumer characteristics; and
– contain natural ingredients [9, 13, 21].

Functional food production requires compliance with hygienic requirements established for each ingredient, as well as the finished product. In terms of quality and safety, functional foods must:
– guarantee safety for prolonged use;
– produce a physiological effect on the body, when consumed daily in moderate amounts;
– be easily and fully digestible; and
– have their biochemical composition and biomedical properties complying with the so-called 'life formula', in terms of both essential substances and their balanced content.

Functional products must be adapted to the Russian food market and be compatible with the local staple foods. It is expected that their organoleptic properties will be what local consumers are accustomed to. In the long term, regular use of functional foods should lead to a remarkable improvement in the structure of everyday nutrition.

The Kemerovo Technological Institute of Food Industry (Kemerovo State University) has developed a concept of producing new generation foods from complex dairy-based raw materials with the use of Siberian pine nuts and their products to give them functional properties.

According to the concept's biomedical aspects, functional foods will:
– make up for the deficiency of essential nutrients in people's diet and meet their physiological needs for nutrients;
– increase the body's resistance to adverse environmental conditions with biologically active substances contained in Siberian pine seeds; and
– contribute to the regulation of the body's organs and systems.

The development of foods with Siberian pine nut products is governed by the following principles:
– natural origin, diversity, availability, stability of physico-chemical properties, and environmental safety of ingredients;
– compliance of the composition with the requirements of modern nutrition science;
– ensuring consumer appeal through flavour, colour, texture, and other properties;
– simplicity of production process without long setup times or changeovers;
– improved efficiency of enterprises processing Siberian pine seeds; and
– diversification of functional dairy products [17].

Protein content in the diet. According to the principle of rational nutrition, the ratio of proteins, fats and carbohydrates in the human diet should be 1:1.2:4. Proteins that enter the body with food are broken down into amino acids, essential and non-essential, during the digestion process. To provide the body with a sufficient amount of essential and non-essential amino acids, the diet should include both animal and plant proteins, whose most beneficial ratio is 1:1 [22].

Protein is an indispensable nutrient and a component of all our organs. Proteins make up 15–20% of human body weight, while the normal proportion of fats and carbohydrates is 5–6% or less [18]. The need for proteins lies in their functions: in particular, they are involved in metabolic processes and are the basis of such vital substances as enzymes, hormones, and antibodies.

The human need for protein can vary under a number of factors, but its average adult intake should be 1 g per 1 kg of body weight to ensure the normal functioning of all vital systems. Protein deficiency disrupts metabolism and lowers immunity. Since proteins are part of enzymes, their deficiency reduces enzymatic activity. Also, lack of protein affects the functioning of the musculoskeletal, cardiovascular, respiratory and other systems.

The biological value of protein coming with food depends on the amount and balance of amino acids contained in it. Although it used to be thought that amino acids were only important for the synthesis of proteins, and their ratio had to correspond to DNA and RNA codes, now researchers are increasingly discovering new amino acid functions and their special influence on certain types of the body's vital activities. Almost all amino acids are transformed into essential biochemical substances that have an important effect on human health.

Amino acids have a beneficial effect on the brain, restore the liver and kidneys functions, and are highly

effective in parenteral nutrition, especially during intensive care. Also, they improve the cardiovascular system and the hematopoietic function of the bone marrow. By accelerating the adaptation of the heart muscles to increasing loads, amino acids contribute to better sports performance.

The most important amino acids for human health are arginine, aspartic acid, glutamine, proline, threonine, lysine, cystine, methionine, valine, leucine, and isoleucine. The best source of essential amino acids are products of animal origin (eggs, milk, meat, and fish). Non-essential amino acids are contained in vegetable proteins coming from legumes, grains, cereals, and oil plants.

Nutrition studies have revealed a deficiency of plant and animal proteins in the Russian diet and a general imbalance between them. Therefore, one of the priorities of the Russian food industry is to enrich people's diet with essential proteins and enhance the nutritional and physiological value of foods.

Making up the deficiency requires two simultaneous measures: 1) increase total protein intake and 2) improve the quality of proteins consumed by the population. It is also important to combine animal and plant proteins in such everyday foods as milk, bread, sour cream, and others. This is one of the most promising directions of the Russian food industry and the development of functional foods [23, 24].

To compensate for the lack of protein, functional products are based on milk, dairy whey, or eggs. They may also use plant proteins from secondary raw materials (e.g. oil cake). Plant proteins have a high nutritional value and are therefore used in the development of combined animal- and plant-based functional foods.

There are four main groups of functional foods currently popular in different parts of the world: grain-based, dairy-based and fat-based foods, as well as non-alcoholic drinks. These four groups can become a good foundation for functional nutrition [25].

Dairy products account for 65% of the modern functional foods market. The Russian market of functional dairy products is growing by 25–30% per year, three times as fast as the entire dairy market in Russia. However, this is not the only reason why functional dairy products play such an important role in the human diet.

Nutritionists recommend daily consumption of dairy products, as they contain most of the nutrients we need and are a valuable source of proteins, fats, carbohydrates, calcium, phosphorus, and other minerals. Also, dairy products contain a number of vitamins and trace elements that are well balanced and easily digestible [23].

However, the fatty acid composition of milk fat, despite the presence of arachidonic and linoleic essential acids, is not well balanced for adults. The content of linoleic acid in it is too low against a high content of saturated fatty acids. It is a fact that a low content of linoleic acid is not good for the body's metabolic processes [25]. Therefore, the dairy industry is actively developing new foods with a complex composition of fats combining milk fat with vegetable oils and their modifications.

Quite promising is a combination of dairy products with plant-based ingredients, such as cereals, vegetables, fruits, and berries. They are a valuable source of dietary fibre (cellulose and pectin), vitamins and minerals. This opens up broad possibilities for the regulation of the amino acid and vitamin composition of the combined foods.

Plant raw materials. Using plant materials in the technology of dairy products is already a steady trend. Plant-based components can be rich in not only protein, but also in other functional ingredients, including polyunsaturated fatty acids with high biological activity. Functional foods enriched with polyunsaturated fatty acids have a beneficial effect on the nervous, immune and cardiovascular systems, helping maintain normal levels of cholesterol and triacylglycerols in the blood.

Plant ingredients have a multifunctional nature: they contain a wide range of biologically active substances, often in high concentrations. These include amino acids, polyunsaturated fatty acids, vitamins, minerals, dietary fibre, etc. Using plant materials makes new products highly balanced in terms of amino acids, fatty acids, minerals, and vitamins. Cereals stand out among other plant ingredients because of a high content of insoluble dietary fibre, various vitamins, and calcium.

According to published research, the most promising types of protein-containing raw materials in Russia are legumes: lentils, peas, beans, and chickpeas. Also popular are grains, mainly in the form of extruded products, and secondary raw materials, including bran. Finally, plant protein comes from oil-bearing plants of the legume family (soybean, peanut), Asteraceae (sunflower, safflower), Malvaceae (cotton), and Cruciferae families (rapeseed, mustard, winter cress, and sesame) [26, 27].

Another promising source of proteins might be unconventional types of oil-containing raw materials, such as tomato pomace (canning industry waste), grape seeds (winemaking industry waste), and corn germ (flour-milling and starch production waste). Grape seeds, for example, contain up to 10–12% of oil, 21% of protein, 45% of carbohydrates, and 12% of tonic substances. There is also increased interest in some species of plants, in particular amaranth, lupine, and flax, whose industrial use is expected in the near future [26]. Grain crops and wild plants play a major role in solving the problem of food protein deficiency.

Nuts, especially pine nuts, are another valuable source of plant materials. A distinctive feature of pine nut kernels is that they are highly variable in composition depending on the geochemical conditions of Siberian pine habitat, environmental factors, the quality and degree of maturity, their storage conditions, method of shelling, and other factors.

Until recently, only oil was considered the main component of Siberian pine seeds and it was mainly used in the cosmetic industry, but also in medicinal and preventative nutrition. However, Siberian pine nuts are also a source of valuable nutrients and therefore they can be successfully used in the food industry.

Pine nut oil cake and flour are rich in plant protein and have a balanced composition of amino acid, which

makes them a good alternative to soy protein. Pine nuts also contain significant amounts of polyunsaturated fatty acids whose content in the finished product is regulated by pine nut oil used as a functional ingredient.

Pine nuts have certain advantages over other types of plant materials. For example, oilseeds and their products (oil cake and oil meal) contain not only a large amount of plant protein, but also some harmful substances. Sunflower seeds contain a lot of polyphenolic compounds (chlorogenic acid). Rapeseed, mustard and other plants of the Cruciferae family have a high content of phytic and erucic acids. Proteins obtained by processing pine nuts, however, do not contain any undesirable substances and can be used in food production.

An important factor is that the proteins of milk and pine nut kernels are complementary in terms of limiting amino acids. Their compatibility has great advantages for enriching the diet.

Pine nut proteins are characterized by relatively high solubility: an average content of soluble proteins is 71.5%. Also, they contain all essential amino acids, including isoleucine, lysine, and tryptophan, and some non-essential amino acids, such as arginine, proline, glutamic, and aspartic acids. Siberian pine nut proteins are of exceptional biological value and, due to their amino acid composition, they can be successfully used in food enrichment.

This combination of amino acids suggests cholesterol-lowering properties; therefore, pine nuts could be recommended as a medicinal or preventative product in the treatment of cardiovascular diseases.

The lipid composition of pine nuts is characterized by a high content of linoleic and γ-linolenic acids. Linoleic acid plays an important role in lipid metabolism. It is converted into arachidonic acid in the presence of group B vitamins and tocopherols, which also come from pine nuts. The physiological effect of γ-linolenic acid is determined by its transformation into prostaglandins of the first type, which regulate the body's numerous cellular and tissue functions. In particular, they contribute to platelet concentration, vessels contraction and expansion, improved immune system, and increased endurance [15].

Pine nut kernels contain significant amounts of minerals, such as phosphorus, potassium, magnesium, and iron. Like any nuts, pine nuts have a low content of calcium; however, this deficiency is easy to compensate for by using pine nuts as an ingredient in multicomponent functional foods. It seems quite promising to combine pine nuts with foods rich in calcium, such as dairy products.

Pine kernels are richer in vitamins than any other nuts. Also, they have a high content of tocopherols, which makes them biologically active and easy to store.

The concentration of toxic substances in pine nuts does not exceed the permissible level, which makes them absolutely safe for consumption. Pine nuts can be used fresh or processed, with no restrictions as to processing methods.

Research evidence suggests that pine nuts can be classified as a multifunctional food material with a high content of technologically significant components [15].

Given their high nutritional value, pine nut products can be used by all people regardless of their age or physiological state, as well as in treatment and prevention of disease. This type of wild plant is optimal for the production of functional foods of high nutritional value.

Economic aspects. Modern food industry has an increased interest in the use of biologically active substances from natural plant materials, including wild plants. Scientists are seeking new sources of raw materials to satisfy a growing demand for multi-component functional products. To ensure their biological value, raw materials should have a good capacity to synthesise biologically active substances.

Siberia is home to a large number of plants with beneficial properties. Many of them have a high content of biologically active substances. Coniferous species are of remarkable value for the medical and cosmetic industries, while Siberian pine is of great interest to the food industry. Siberian pine nuts are popular all over the world due to their nutritional value, and their gathering has been a common trade in Russia for a long time.

Siberian pine, which plays an important role in the plant community, has an enormous habitat, stretching from the north-east of the European part of Russia to Eastern Siberia. Most of the habitat is taiga, located far from large cities. This area is not treated with pesticides, herbicides or chemical fertilizers and is least affected by dust and gas emissions from industrial enterprises. Pine nut harvesting areas are geographically isolated from the sources of technogenic pollution and have a relatively low level of toxic elements, radionuclides and pesticides, ensuring the safety of raw materials and foods [28, 29].

It should be noted that most of the pine forests in Kuzbass (Kemerovo Region) are located in remote mountainous and lowland areas, away from transport routes and other infrastructure facilities. Pine nuts harvesting covers a relatively small part of the forests spread out near the villages along the river banks and some other accessible areas. Altogether, the harvesting area accounts for no more than 3.9% of all Siberian pine forests, or approximately 0.11% of the region's total forestry [30].

According to the statistics, the Kemerovo Region harvested 143 tons of Siberian pine seeds in 2006, which is only 5.8% of the potential yield [30]. The nut harvesting area in Kuzbass covers 7,100 hectares, which is less than 3.8% of the total pine forestry in the region.

Biologically, however, Kuzbass is one of the most promising regions for the nut industry [29]. It is a sustainable area of Siberian pine productivity.

Siberian pine nut crops are known to be uneven, with high-yielding years alternating with low-yielding years, which has certain implications for harvesting and production planning. However, Kuzbass does not have such dramatic leaps in seed productivity as some other regions; moreover, it has the most frequent increased yields – one in every 3–5 years. This can be explained by the optimal correspondence between the rhythm of physiological and biochemical processes in Siberian pine, on the one hand, and external conditions, on the other. The average annual biological yield of Siberian pine nuts in the Kemerovo Region is 50 kg per

hectare. To sum up, the region has good forest resources to develop the nut industry and organize the production of pine nut oil and other useful products.

On the other hand, in view of a difficult environmental situation in the Kemerovo Region, the task of providing the population with balanced nutritional foods acquires a special importance. In this regard, the use of unconventional plant materials, in particular Siberian pine seeds, seems to be quite a promising direction for the food industry. Firstly, it ensures sustainable use of natural resources. Secondly, it is a new source of food raw materials. Finally, it expands the range of foods for general and functional purposes.

The fact that Siberian pine grows wild in remote areas makes pine nuts quite an expensive product. Therefore, it is important to ensure the maximum yield of high quality products. The technological properties of Siberian pine seeds depend not only on their genetic features, as well as the climatic and geographic conditions of their habitat, but also on the conditions of their post-harvest treatment and storage.

Siberian pine seeds are biologically unequal. Small seeds (with a diameter of 7 mm or less) have a lower technological quality (high acid number, low content of crude lipids and protein), compared to large seeds.

The post-harvest ripening of pine seeds also depends on their size. Large seeds display an increased oil content, a decreased acid number with a relatively high lipase activity, and a reduced peroxide number with a stable lipoxygenase activity. Large seeds ripen longer than small ones, which makes it possible to control this process by technological means in order to increase the oil yield from the seeds and improve its quality.

At constant temperature and relative humidity, different seed tissues absorb a different amount of moisture from the air. Nut shells have a higher water-absorbing capacity than kernels or seeds. This creates favourable conditions for the development of microorganisms on the seed surface.

Because of sorption hysteresis, seeds may retain a different content of moisture even after prolonged storage in a dry state, which must be taken into account when establishing the optimal conditions for post-harvest seed treatment, drying and storage.

Drying significantly reduces the content of mould and bacterial flora. Dry seeds display a poor development of microflora and a limited number and variety of microorganisms, which contributes to longer storage. When stored in favourable conditions, the content of oil in the kernel, as well as its acid and peroxide numbers, remains almost unchanged.

Observing the optimal storage conditions for dried seeds (temperature: 4 ± 2°C; relative humidity of air: 70 ± 5%) maintains their quality throughout the year. Also, optimal storage contributes to nuts maturation, increasing the content of oil in the kernels and thus improving their quality [17].

RESULTS AND DISCUSSION

Pine nut kernels and their products are used as ingredients in the production of pine nut milk, dairy products, bakery and confectionery, alcoholic drinks, medicinal and preventative drinks, dietary supplements, hygiene products, soap, toothpaste, and cosmetic creams.

Pine nut oil is the main end-product of processed kernels. It has a mild nutty aroma, light amber colour, and a slightly bitter taste. The quality of pine nut oil depends on the quality of raw materials, post-harvest treatment and storage, as well as methods and modes of extraction. Cold pressed oil has the finest flavour and nutritional qualities. The combination of essential and biologically active substances in pine nut oil opens up good prospects for its use as a dietary oil and as a biologically active additive in dairy products with a pronounced therapeutic effect.

Pine nut oil is produced by pressing or extraction. Pressed oil is used for dietary, cosmetic, medicinal, and preventative purposes. Extracted oil has a dietary use after refining.

Modern technologies allow us to diversify and increase the output of target products. They help make pine kernels processing more cost-effective and create new ingredients for functional foods, for example, pine nut paste, oil cake and fat-free flour.

Cakes and meals made from oil plants, which are classified as secondary raw materials, are important additional ingredients in food production. They are a source of high-grade protein, easily digestible carbohydrates, vitamins, and minerals. Flour obtained by extracting lipids from fat-containing material (cake or meal) is the cheapest and most accessible form of plant protein.

Pine nut products are recognized as safe and easily producible materials that can be used as complex enriching agents in the production of functional foods due to their nutritional value and functional properties.

Pine nut paste. Siberian pine seeds are introduced into complex dairy products in the ground form. A disintegrator is used as a fine grinder to produce a homogeneous, plastic and viscous paste, resistant to separating. The product has a light cream colour, a sweet and slightly astringent taste, and an intense nutty aroma.

Pine nut paste has the same organoleptic characteristics as its raw material, Siberian pine seed kernels, but a more intense taste and aroma. Its chemical composition is almost identical to that of the raw material. However, it has a lower amount of fibre and ash and a higher content of protein and fat due to the partial separation of the kernel shells.

Mechanical treatment does not affect the amino acid composition of pine nuts. However, the destruction of the kernel cellular structures and a high degree of fineness increase the protein digestibility coefficient, making proteins more accessible to digestive enzymes. The high fineness of pine nut paste also contributes to its uniform distribution in the product enriched.

Pine nut oil cake. Pine nut oil cake is a light cream-coloured powder without any foreign inclusions that has a light aroma, characteristic of pine nut kernels, and a sweetish taste. Its main components are proteins, lipids, carbohydrates, and minerals. The oil cake protein includes 19 amino acids, at least 40% of which are essential. Among the deficient amino acids are leucine, methionine and phenylalanine.

Oil cake carbohydrates, which are of great nutritional and functional importance, affect the taste and the

digestibility of end-products. The vitamin and mineral value of pine oil cake depends both on the chemical composition of the processed pine nuts and on the residual oil content after pressing. Pine oil cake has an increased content of phosphorus and magnesium. Like pine kernels, it is characterized by a low calcium content and a pronounced predominance of potassium over sodium. Calcium deficiency can be compensated by combining pine oil cake with calcium-rich dairy products.

The vitamin value of pine nut oil cake is in its content of water-soluble B vitamins (B_1, B_2, B_3, B_5, and B_6), and tocopherols. Vitamins B_1, B_3, and B_5 are present in significant amounts, while the content of thiamine and tocopherols is lower than in the nut kernels. Tocopherols perform an antioxidant function, contributing to the product preservation. However, it is recommended to store both the oil cake and the products containing it at a low temperature to ensure minimal loss of vitamin E.

Pine nut flour. Pine nut fat-free flour is a light cream-coloured powder with a subtle aroma, characteristic of pine nut kernels, and a sweetish taste. Its major chemical components are proteins and carbohydrates, resulting in its classification as protein- and carbohydrate-rich raw material with a high content of mineral substances. Table 1 shows the amino acid composition of pine nut flour.

Fat-free pine nut flour has at least 40% of essential amino acids. Its well-balanced protein has a high content of lysine, which is deficient in cereals, and arginine, as well as glutamic acid. However, we should also note the presence of a limiting amino acid, leucine.

The protein digestibility coefficient of pine nut flour averages 78.5%, which is common for plant materials. However, it is a few times lower than that of pine nuts, possibly due to the protein denaturation as a result of heating when extracting oil. The carbohydrate composition of pine nut flour is characterized by a high content of sucrose and starch. In addition, the product is rich in dietary fibres, mainly cellulose and pentosans. Fat-free pine nut flour is also rich in B vitamins and minerals (excluding calcium).

The most important functional and technological properties of plant-based supplements are those resulting from the interaction of proteins and water, namely, hydration, swelling, solubility, viscosity, thickening, water- and fat-holding capacity, as well as emulsifying and foaming capacities. Good swelling capacity of pine nut flour and oil cake, and their ability to absorb and retain moisture, are important for the product consistency.

Fig. 1 shows the degree of increase in the volume of pine nut oil cake and fat-free flour during hydration depending on temperature.

Both products are characterized by the maximum degree of swelling at a temperature of 75–80°C. Pine nut flour has a greater swelling capacity and is more hydrophilic than pine nut oil cake.

The ability to bind and firmly hold fat is another important characteristic of a raw material intended as a protein fortifier in the production of fat-containing dairy and emulsion products.

Pine nut oil cake and fat-free flour have good fat-holding and fat-emulsifying capacities, which can be maximized by regulating the time of mixing, as well as the temperature and duration of emulsification.

Table 1. The amino acid composition of pine nut flour compared to ideal protein

Amino acid	Ideal protein (FAO/WHO)		Pine nut flour	
	g/100 g protein	Score, %	g/100 g protein	Score, %
Valin	5.0	100	5.20	104.00
Isoleucine	4.0	100	5.00	125.00
Leucine	7.0	100	5.86	83.70
Lysine	5.5	100	6.40	116.36
Methionine + Cystine	3.5	100	3.50	100.00
Threonine	4.0	100	4.00	100.00
Tryptophan	1.0	100	2.40	240.00
Phenylalanine + Tyrosine	6.0	100	7.90	131.66
Total	36.0	100	40.26	
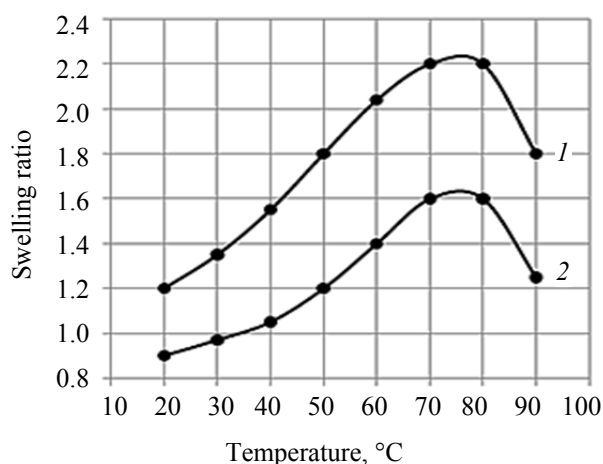
(a)
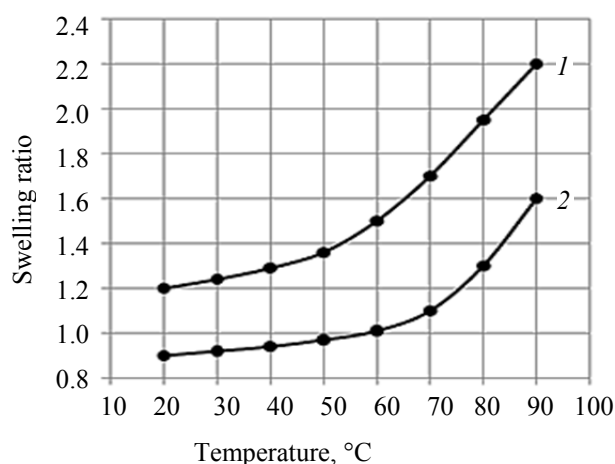
(b)

Fig. 1. The degree of increase in the volume of fat-free pine nut flour *1 –* and oil cake *2 –* when swelling in water (a) and milk (b).

Table 2. The fat-holding capacity of pine nut oil cake and fat-free flour depending on temperature

Type of additive	Temperature, °C			
	20 ± 2	40 ± 2	60 ± 2	80 ± 2
Pine nut oil cake	150 ± 3	160 ± 3	175 ± 3	193 ± 2
Fat-free pine nut flour	205 ± 2	240 ± 5	252 ± 3	285 ± 3

According to a study of their functional properties, pine nut oil cake and fat-free flour can be used as emulsifiers and stabilizers. Therefore, they can be used in the production of emulsion products, such as cheeses, sauces, paste-like dairy products, and creams. In particular, we could recommend their use in the production of foods whose technological properties can be improved by binding fat or water.

The chemical composition of pine nut oil cake and fat-free flour and their functional and technological properties justify their wide use as a protein and mineral supplement to increase the nutritional and biological value of functional foods. Adding fat-free pine nut flour and pine nut oil to dairy products (for example, ice cream) creates a high-quality functional product that contains easily digestible proteins (of milk and flour) with a balanced amino acid composition, essential fatty acids, important bio-elements (magnesium, zinc, iron, and iodine), and vitamins (E and B group).

It is possible to add a small amount of pine nut oil to butter to balance the lipid composition of the end-product by changing the proportion of saturated, monounsaturated and polyunsaturated fatty acids. Butter in this case is produced in the traditional way.

In general, pine nut oil cake and fat-free flour are well compatible with food materials, including dairy products, and can be used as a stabilizer of protein-fat emulsions (sauces, pastes, and creams).

A study of cultivating lactic acid microorganisms on the basis of milk and pine nut material revealed good prospects for combining pine nut oil cake with dairy materials. Such a combination is expected to expand the range of low-calorie dairy products with an increased nutritional and biological value.

Adding pine nut oil cake or fat-free flour to dairy materials significantly changes the content of proteins, fats and minerals. Introducing oil cake increases their content by 10.0%, 8.0%, and 7.4% respectively per each percent of the added plant component. When pine nut flour is added, the protein content increases by 13.3% and minerals by 9.0%, but the mass fraction of fat, on the contrary, decreases on average by 1.2% per each percent of the added component.

Changes in the composition affect the properties of milk. In particular, titratable acidity of milk and plant mixtures is higher than that of milk and is directly proportional to the mass fraction of the plant component. Active acidity of milk and plant mixtures is weakly dependent on the amount of the protein component. However, it decreases with an increase in the amount of pine nut oil cake or fat-free flour. This process can be explained by the ongoing restructuring of proteins and accumulation of alkaline groups [31].

The process of ripening in milk and plant mixtures is different from ripening in the milk medium. Adding pine nut products to milk activates the lactic acid process, reducing the time of ripening and increasing the rate of lactic acid accumulation. The reproduction of microorganisms becomes more intense, which affects the ripening process. Active reproduction of lactic acid bacteria changes the properties of the product, increasing the average size of casein particles and the number of peptides and free amino acids [32].

A combination of pine nut oil cake with a dairy base is also used to produce cottage cheese and yogurt. The mixture is pasteurized, cooled and fermented with lactic acid bacteria. The use of oil cake as an active additive intensifies the process of ripening, reducing its length and improving the quality of curd.

Pine nut meal is used in the production of fermented milk products. Due to a significant content of poly-and oligosaccharides, it is used as a prebiotic in the production of bifidus-containing fermented milk products. Pine nut milk can be used as a base for preparing a fermented product obtained either from pine nuts or from pine nut oil cake and water or buttermilk in the ratio of 1:15 to 15:1. A small amount of skimmed milk powder can be added to the formulation, from 0.5 to 3.0% of the mixture weight [17]. The use of pine nut products can significantly reduce the amount of expensive raw milk and produce foods that are rich in plant proteins and have good consumer appeal.

In general, pine nut oil cake and fat-free flour are dry, light, compact and easily stored products with low transportation costs. When used in traditional food production, they do not require any special equipment or capital investment. Thus, the use of these ingredients does not increase production costs.

To sum up, the benefits listed above make the use of pine nut paste, oil, oil cake and fat-free flour highly desirable in the production of functional dairy products, especially fermented milk products.

Table 3. The composition and properties of milk and plant mixtures with pine nut oil cake

Indicator	Cow milk	Mass fraction of pine nut oil cake, %				
		1	2	3	4	5
Mass fraction of dry substances, % including:	$11.00 ± 0.10^a$	$11.70 ± 0.10^a$	$12.36 ± 0.10^a$	$13.51 ± 0.10^a$	$14.30 ± 0.10^a$	$15.12 ± 0.10^a$
protein	$3.0 ± 0.1^b$	$3.3 ± 0.1^b$	$3.6 ± 0.1^b$	$3.9 ± 0.1^b$	$4.2 ± 0.1^b$	$4.5 ± 0.1^b$
fat	$2.50 ± 0.05^c$	$2.70 ± 0.05^c$	$2.90 ± 0.05^c$	$3.10 ± 0.05^c$	$3.40 ± 0.05^c$	$3.60 ± 0.05^c$
carbohydrates	$4.70 ± 0.10^b$	$4.98 ± 0.10^b$	$5.27 ± 0.10^b$	$5.55 ± 0.10^b$	$5.84 ± 0.10^b$	$6.12 ± 0.10^b$
ash	$0.67 ± 0.02^d$	$0.72 ± 0.02^d$	$0.76 ± 0.02^d$	$0.81 ± 0.02^d$	$0.85 ± 0.02^d$	$0.9 ± 0.02^d$
Titratable acidity, °T	$18.0 ± 0.3^a$	$18.5 ± 0.2^a$	$19.0 ± 0.3^a$	$21.0 ± 0.3^a$	$23.0 ± 0.3^a$	$25.0 ± 0.2^a$
Active acidity, pH unit	6.60	6.63	6.68	6.72	6.75	6.80

Note. The values followed by the same letter in the table do not differ significantly (p-value < 0.05).

At the next stage, we studied possible uses of pine nut products in dairy products.

Processed cheese. Recent years have seen intensified research efforts to develop new types of soft cheese products, since they are more cost-effective in production than hard cheeses, they do not require maturation and have a high nutritional value. Processed cheese is a very popular product, relatively inexpensive but highly nutritional. Its production process allows producers to satisfy various tastes, even when there is a shortage of raw materials.

Processed cheese production technology offers ample opportunities for diversifying its composition. Functional additives in processed cheese production include fruits, berries, vegetables, marine products, vegetable fats, and other components [33, 34].

Production of dairy products using pine nut oil cake and fat-free flour is based on the joint precipitation of animal and plant proteins. Introducing pine nut products into milk leads to changes in the protein and fatty phases, carbohydrate and salt composition, significantly increasing the content of proteins and minerals. Pine nut oil cake increases the content of lipids, while pine nut flour produces the opposite effect.

The titratable acidity of milk and plant mixtures is higher than that of milk, and is directly proportional to the mass fraction of the plant component. The active acidity, on the contrary, is inversely proportional to the amount of pine nut oil cake and fat-free flour.

Introduction of protein supplements (pine nut oil cake and fat-free flour) into milk changes its organoleptic and technological properties. In particular, milk acquires a nutty aroma and taste which intensify as the amount of the protein component increases. The colour of the milk changes from white to light cream.

An increase in the protein component (pine nut oil cake and fat-free flour) reduces rennet coagulability and curd synergistic ability, prolonging the duration of renneting. Titratable acidity of whey decreases, while its active acidity increases, changing the quality of curd. Less whey is released and the curd becomes moister. When a milk and plant mixture is used, the curds are softer than those obtained by coagulating milk. This effect is produced by the hydration properties of pine nut oil cake and fat-free flour.

The release of whey during curd synersis decreases to a greater extent when pine nut oil is introduced, rather than fat-free flour. Unlike the latter, pine nut oil cake contains lipids, which leads to an increased amount of fat in the milk and plant mixture. Fats slow down the release of whey. In general, larger amounts of pine nut oil cake or fat-free flour in milk and plant mixtures contribute to increased curd yield. The dependence is almost linear: the more oil cake (or fat-free flour) is used, the greater the curd yield. This occurs due to increased protein content and curd moisture, lowering the cost of production.

The ripening of dairy and plant mixtures containing pine nut oil cake or fat-free flour is accompanied by an increased number of microorganisms, higher titratable acidity and lower active acidity, as well as improved coagulability and synersis capacity. The clotting time gets shorter. Thus, the use of pine nut products not only enriches the end-product with useful substances, but also optimizes the production process.

The process of making a cheese product is also influenced by a number of technological factors, such as the temperature of curd processing and cheese forming, the duration of self-pressing, and the method of salting, maturation and storage. These factors were studied to determine their optimal values [17].

It was established that raising the curd-processing temperature decreases the mass fraction of moisture and salt in the cheese and increases the active acidity of the cheese mass. Changes in the cheese composition caused by the differences in the curd processing temperature from 35 to 39°C affect the organoleptic properties of the product. The optimal curd-processing temperature, which significantly improves the organoleptic properties, is 37 ± 1°C.

The optimal duration of self-pressing was established as 16 hours. A series of experiments with varying methods of salting confirmed that the best method was curd salting.

The optimal duration of cheese product ripening was established as 24 hours. This period ensures good organoleptic characteristics of the cheese product and makes it ready for consumption. A longer period of ripening only leads to insignificant changes in organoleptic characteristics [17].

Pine nut paste is also used in the production of processed cheese. Replacing milk with pine nut paste is not equivalent in terms of protein content, since the mass fraction of protein in pine nut paste is lower than in animal products. However, pine nut paste has a balanced amino acid composition with all the essential amino acids. Thus, using pine nut paste to replace only part of dairy ingredients can ensure a similar biological value in processed cheese products.

The main component of pine nut paste are lipids, in particular, triacylglycerols. Compared to milk fat, pine nut oil contains 10 times as much linoleic acid and more than 20% of biologically valuable γ-linolenic acid, which is completely absent in animal fats. The use of pine nut paste in processed cheese production can significantly change the fatty acid composition of the product's fat phase and increase its biological value.

Experimental data show that the complete replacement of animal fats with pine nut products has a negative effect on the organoleptic indicators. The optimal amount is 5 to 10% pine nut paste replacing some part of butter and skimmed milk powder.

Processed cheese has a moderate cheesy flavour and a light nutty aroma, a moderately firm texture and a light cream colour.

Several studies were conducted to assess the influence of major process factors (such as melting temperature and a mass fraction of paste and fat) on the organoleptic, structural and technical properties of processed cheeses with pine nut paste, and to identify optimal indicators. They showed that the best flavour and aroma were achieved at a melting temperature of 75–85°C, a paste mass fraction of 8.75 to 10.0%, and a fat mass fraction of 40 to 45%.

Table 4. The organoleptic indicators of processed cheese

Amount of additive	Flavour and aroma		Texture		Colour		Total score
	Characteristic	Score	Characteristic	Score	Characteristic	Score	
Control	Moderate cheesy	14	Moderately firm	8	Light cream	2	28
5.0	Moderate cheesy	14	Moderately firm	8	Light cream	2	28
10.0	Moderate cheesy and nutty	15	Moderately firm	8	Light cream	2	29
15.0	Moderate cheesy, nutty, and slightly bitter	13	Slightly sticky	7	Light cream	2	26

Table 5. The organoleptic properties of ice-cream with fat-free pine nut flour

Number of sample	Degree of replacement, %	Organoleptic assessment, score			
		Colour and appearance	Flavour and aroma	Texture	Total score
1	1.0	5.0	5.0	3.0	13.0
2	1.5	5.0	4.5	3.0	12.5
3	2.0	5.0	5.9	3.0	13.9
4	2.5	5.0	6.0	2.9	13.9
5	3.0	5.0	5.8	2.8	13.6
6	3.5	4.8	5.8	2.6	13.2
7	4.0	4.0	5.3	2.4	11.7

A study of the temperature-texture dependency established the optimal temperature as 75°C. Increasing a mass fraction of pine nut paste up to 7.5–12.5% made the texture more plastic and softer. Its fraction of 8.75–10.0% added a light pine nut flavour to the product, improving its organoleptic properties compared to the control cheese.

Finally, the optimal dosage of pine nut paste was established as 8.75–10.0%, both in terms of the quality and biological value.

The stage of entering pine nut paste into the cheese base affects the fatty acid composition of the end product. Prolonged heat exposure leads to the formation of free fatty acids as a result of partial hydrolysis. At the same time, low molecular weight fatty acids are partially distilled off with steam. Therefore, it is recommended to minimize the heating time of the fat phase, which includes pine nut paste, and introduce the latter 5–7 minutes before the end of melting. In this case, the product texture will meet all consumer requirements.

Cottage cheese and plant products. Pine nut paste is also used in the production of cottage cheese and plant foods based on low-fat cottage cheese. Functionally, pine nut paste enriches the product with polyunsaturated fatty acids, plant protein, minerals (Mg, Fe, and Zn), vitamins, and antioxidants.

Pine nut paste affects the rheological characteristics of the end-product. First, cottage cheese has a coagulation and condensation structure with a high moisture content, both in the free and in the bound state. Free moisture affects the mobility of macromolecular protein bodies and dissolves low molecular weight compounds. Ultimately, free moisture affects the ductility. Pine nut paste reduces the moisture content of the cottage cheese and plant product and, as a result, increases its viscosity.

In the experimental study, the mass fraction of pine nut paste in the cottage cheese and plant mixture ranged from 5 to 30%. The samples with a paste content of 15 and 20% showed the best organoleptic characteristics.

The cottage cheese and plant foods made with pine nut products contain 5–8 times as much linoleic acid and 10–20 times as many polyunsaturated fatty acids as ordinary 9%–fat cottage cheese.

Pine nut paste enriches cottage cheese with manganese, which the latter contains in negligible amounts, as well as potassium, magnesium, phosphorus, iron, and zinc.

Ice cream. Ice cream is less often modified to improve its nutritional properties, compared to other dairy products. Nevertheless, there is a need for ice cream consistent with the principles of healthy eating. For this, ice cream needs to have a reduced calorie content, a lower fat and sugar content, and be enriched with vitamins, minerals, and biologically active substances [7, 8]. Such functional ingredients as pine nut oil and fat-free flour can increase the nutritional and biological value of ice cream by enriching it with essential amino acids, polyunsaturated fatty acids, vitamins, and mineral elements. Fat-free pine nut flour also has good stabilizing and emulsifying properties and can improve the texture and appearance of ice cream.

Due to its high nutritional and biological value, fat-free pine nut flour can be used instead of skimmed milk powder. It can also improve ice cream overrun. The optimum mass fraction of fat-free pine nut flour is 2.5 to 3.5%, while pine nut oil can replace 10–30% of cream butter. These amounts of pine nut products give ice cream a pleasant milky and nutty flavour and aroma.

The use of pine nut oil and fat-free flour in ice cream production does not impair the quality of the end-product, but it allows producers to use less raw milk and reduce production costs. The combination of animal and plant proteins makes ice cream more resistant to melting and improves its structure and texture. New types of ice cream enriched with pine nut oil and fat-free flour are characterized by high organoleptic scores.

It was established that homogenized cream mixtures where milk fat was replaced with pine nut oil had 1.3 times as many fat globules up to 1.5 μm in

size, compared to milk fat mixtures. Ice cream mixtures with smaller fat droplets had a larger number of small bubbles forming during the freezing process that are resistant to mechanical stress and do not burst during mechanical processing in the freezer, compared to large ones.

Replacing milk fat with plant oil (pine nut oil) in the range of 10–30% of the total fat phase has a positive effect on the organoleptic characteristics of ice cream, giving it a pine nut flavour and aroma.

After studying the effect of fat-free pine nut flour, pine nut oil and stabilizer doses on ice cream flavour, aroma and overrun, we established their optimum dosage as 2.8–3.4%, 20–25%, and 1.5% respectively, ensuring good organoleptic, structural, and mechanical properties of the end-product.

The use of unconventional raw materials in ice cream production (namely fat-free pine nut flour and pine nut oil) affects the quality and technological properties of liquid cream mixtures. A study of their homogenisation modes showed that the process of homogenisation changed the structure and properties of protein substances. In particular, the size of casein micelles decreased, and some of them broke up into submicelles, which were adsorbed on the surface of fat globules. A higher degree of the protein phase dispersion (total surface) led to an increase in surface charge and enhanced the hydration properties. Milk proteins spread on the surface of fat globules and protected them from sticking, which prevented fat from settling in the process of mixture maturation and storage and improved overrun, with no grains of butter forming during freezing.

The homogenization of liquid ice cream mixtures was carried out in laboratory conditions at different temperatures – 70, 80 and 90°C – and a constant pressure of 12.5 MPa. It was found that introducing 2.8–3.4% of fat-free pine nut flour instead of skimmed milk powder did not reduce the quality of the homogenized mixtures.

Since the use of pine nut flour increased the mass fraction of protein in the mixture, its dispersion rose from 76.4 to 89%, while the degree of homogenization decreased from 9.5 to 6.5%. The presence of plant proteins contributed to the formation of strong and stable fat globule membranes.

According to the experiments, 80°C was the optimum temperature of mixture homogenization that ensured a high-quality product. At this temperature, the average size of fat globules was no more than 1.5 μm, and there was an increase in the kinetic stability of the fat phase.

The pressure of homogenization is another factor affecting the emulsion stability. Studies showed that 12.5 MPa was the optimum pressure [17].

A study was conducted to measure the impact of freezing modes on the quality of ice cream containing pine nut flour and oil. Freezing leads to the formation of ice crystals and a structure of the product. The size and shape of ice crystals depends on the freezing rate, the mixture composition, the mass fraction of bound moisture, overrun, and the size of air bubbles. The presence of fat-free pine nut flour has a positive effect on the overrun. An increase in the mass fraction of pine

nut flour from 3.0 to 3.4 improves the overrun by 12.0%. Moreover, a combination of animal and plant proteins makes ice cream more resistant to melting.

In addition, when using pine nut flour and oil, producers can economise on milk raw materials (skimmed milk powder and butter) and therefore reduce production costs.

Mayonnaise. A current trend is to produce medium- and low-calorie mayonnaise that does not contain cholesterol and is enriched with vitamins and biologically active substances [12, 35–38].

Unlike high-calorie mayonnaise, medium- and low-calorie mayonnaise needs emulsifiers and stabilizers. However, the choice of emulsifying and stabilizing agents should provide a high physiological value of the product. Natural plant-based emulsifiers are the safest and the most active biologically. Partial or complete replacement of egg powder, the traditional emulsifier, with plant materials will also reduce the cholesterol level in the end-product.

In particular, mayonnaise emulsions can be based on a combination of skimmed milk powder and fat-free pine nut flour in the ratio of 2.6:1.0, respectively. This optimum ratio ensures a high content of protein and essential amino acids, including limiting amino acids, methionine and cystine. Adding fat-free pine nut flour to skimmed milk powder enriches the milk and plant mixture with potassium, magnesium, phosphorus, iron, zinc, and manganese.

The milk and plant mixture has a high solubility and a fat emulsifying capacity. According to the equations analysis, the maximum stability of the emulsion, high viscosity and maximum organoleptic scores were observed in those mayonnaise samples which had the following composition: 30.0–35% fat, 1.5–2.0% egg powder, 12–14% dry milk and plant mixture (3.4–3.8% fat-free pine nut flour) [17].

The final stage of the study aimed to determine the nutritional value of dairy foods with pine nut products, which is the key quality criterium.

The chemical composition analysis of the new dairy products containing wild plant materials (Siberian pine seeds and their products) showed that pine nut oil cake, fat-free flour, paste, and pine nut oil enriched the products with plant protein, vegetable oil, polysaccharides (starch, fibre, and pentosans), vitamins, and macro- and microelements. Replacing raw milk with plant materials does not reduce the nutritional value of new dairy products, which have a high content of protein, fat and minerals.

The use of pine nut oil cake, fat-free flour and paste as a component of fermented milk products supplements animal proteins with plant proteins.

A comparative analysis of the amino acid composition showed that adding pine nut oil cake or fat-free flour to soft cheese products did not reduce their nutritional value. These plant ingredients led to a slight decrease in essential amino acids, compared to the control sample, but increased the amount of sulphur-containing amino acid, which limits dairy product proteins, and tryptophan.

According to the quality assessment, the proteins of processed cheese with pine nut paste have a well-balanced content of essential amino acids and a high

biological value. The enrichment of cottage cheese with pine nut paste leads to a slight decrease in essential amino acids and, at the same time, an increase in isoleucine and leucine. Also, the product acquires some non-essential amino acids (arginine, aspartic and glutamic acids), compared to the control sample.

Ice cream and dietetic mayonnaise enriched with fat-free pine nut flour have a well-balanced content of essential amino acids (over 100% score). Cottage cheese enriched with pine nut paste acquires a few times as much vitamin E, 5–8 times as much linoleic acid, and 10–20 times as much polyunsaturated fatty acids as ordinary 9%-fat cottage cheese.

Further, functional ingredients (pine nut oil cake, fat-free flour, paste and oil) enrich new types of dairy products with tocopherols.

All the dairy products with pine nut additives described in the paper have a biologically balanced amino acid composition. Combinations of animal and plant proteins are easily digestible and have a beneficial effect on the maintenance of human nitrogen balance. The products are adapted to the Russian food market; they are compatible with the main foods and do not change their organoleptic properties, familiar to every person.

It was also established that pine nut products did not reduce the shelf life of new dairy foods. The use of pine nut oil cake and fat-free flour did not affect the microbiological indicators during storage. Storing processed cheese at a temperature of $(4 \pm 2)°C$ for 7 days led to no changes in organoleptic characteristics (taste, aroma, texture, and appearance). Storing a 9%-fat cottage cheese and plant product at a temperature of $(4 \pm 2)°C$ maintained its organoleptic characteristics for 15 days. The microbiological indicators of liquid ice cream mixtures remained within permissible limits for 48 hours at a temperature of $(4 \pm 2)°C$.

Thus, pine nut products used as biologically active ingredients in functional foods do not reduce their shelf life, compared to traditional dairy products.

CONCLUSIONS

Siberian pine seeds and their products are promising plant raw materials, with a wide range of physiological properties, a unique biochemical composition and a set of biologically active substances. Always comprising a large proportion of the Siberian food balance, pine nuts have not lost their value or local appeal.

Modern processing technologies are able to increase the output of pine nut oil and expand the range of by-products. Pine nuts are a raw material for producing pine nut paste, oil cake and fat-free flour, which are used as supplements in the food industry.

Siberian pine seeds (pine nuts) contain extremely valuable substances, such as fats rich in polyunsaturated fatty acids, proteins with a well-balanced amino acid composition, soluble and insoluble carbohydrates, minerals (phosphorus, magnesium, iron, zinc, and iodine), B vitamins, and tocopherols. Their high content of functional proteins, polyunsaturated fatty acids, vitamins, and biocomponents makes them a good ingredient for dairy products. Siberian pine seeds and their products (pine

nut oil, oil cake and meal) are widely used in the production of dairy and fermented milk products, namely, cottage cheese, sour cream, kefir, yogurt, and desserts, including whipped milk drinks, confectionery creams, cheese and others.

Dairy products enriched with Siberian pine nut ingredients have a high nutritional and biological value due to their unique biochemical composition. The synergistic effect of dairy and plant raw materials is manifested in increased digestibility of new dairy products, improved physico-chemical and organoleptic indicators, inhibited development of undesirable microflora, and a longer shelf life.

The nutritional and biological value of dairy products containing pine nut oil, oil cake or meal improves as a result of enriching the finished product with plant proteins with a balanced amino acid composition, polyunsaturated fatty acids, minerals (magnesium, iron, and iodine), vitamins, and ballast substances (cellulose, hemicellulose, and pentosans).

Adding a plant component containing over 60% of oil changes the ratio between monounsaturated and polyunsaturated fats and enriches the dairy product with vitamin E, essential amino acids, and minerals.

Given their valuable properties and accessibility, Siberian pine nuts and their products are used to create a variety of new functional foods. The development of competitive multi-component foods with beneficial functional properties seems to be a promising direction in the current environmental, social and economic situation.

The chemical analysis of new dairy products showed that adding pine nut oil cake, fat-free flour, paste or oil to their formula enriched them with plant protein, vegetable oil, polysaccharides (starch, fibre, and pentosans), vitamins, and macro- and microelements. Replacing raw milk with plant materials did not reduce the nutritional value of new dairy products. They had a high proportion of protein, fat and minerals and were as rich in vitamins as their dairy-based counterparts. Also, the use of functional ingredients (pine nut oil cake, fat-free flour, paste and oil) enriched new dairy foods with tocopherols.

Thus, including functional dairy foods enriched with Siberian pine nut products in the daily diet ensures efficient nutrition of cells and has a comprehensive therapeutic and health-promoting effect on the human body in the long run. Finally, the development of functional dairy-based products with pine nut ingredients is worthwhile both technologically and economically.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Microstructure and cooking quality of barley-enriched pasta produced at different process parameters

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Abstract: Pasta is one of the most popular meals in the world. It is affordable, easy to combine with other foods and easy to cook. Unfortunately, pasta is energy-rich and nutrient-poor. Whole-wheat pasta is somewhat better in nutritional quality, but further improvements may be made. One option is to add different raw materials and specific nutritive components (vitamins, polyphenols, fiber, protein, etc.) to semolina. However, this approach changes its physico-chemical properties, e.g. cooking loss, texture, etc., which cannot be disregarded. The current research investigates possibilities for production of barley-enriched pasta with acceptable cooking qualities. To ensure the beneficial health effects of β-glucan, β-glucan-rich barley was selected asa starting material. Pasta enriched with 10–50% β-glucan-rich barley flour was produced in the mini-press and the laboratory extruder and then dried at low, medium and high temperature regimes. Colour, cooking quality and microstructure of the enriched pasta were investigated to determine its acceptability. The research showed that barley-enriched pasta of good cooking quality might be produced by selecting an optimal combination of suitable production parameters for forming and drying.

Keywords: Barley, cooking quality, drying regimes, extrusion, pasta

INTRODUCTION

Pasta is very popular, yet nutrition-poor. Traditionally, it is made from durum wheat semolina, and it is a good source of low glycaemic index carbohydrates [1]. It is affordable, easily to cook and convenient, which makes it a staple food both in low-income and high-income countries. Since consumers' awareness regarding food quality and its health impact is rising, current food industry, including pasta producers, is forced to seek solutions for healthier products with quality and taste similar to the ones consumers are used to.

Recently, health benefits of β-glucan have gained much attention both in scientific and consumer community [2]. In 2009 and 2011 [3–4], the European Food Safety Authority (EFSA) published scientific opinion regarding the beneficial effect of β-glucan on cholesterol level and postprandial glucose in blood. Primarily, barley used to be grown for brewery and animal feed, but it is a rich source of β-glucan. Thanks to the novel understanding of health effects of β-glucan, crops rich in β-glucan are now produced for human consumption [5]. Among barley varieties, hull-less barley is recognized for its superior nutritional quality [6].

There have been a number of research that dealt with the influence of flour, flour fractions and β-glucan enriched flours on pasta quality [7–10]. They showed that addition of fibre, starch and/or other flours led to 'dilution' of gluten and resulted in reduced cooking quality. Hence, pasta manufacturers have to find an appropriate solution to overcome this problem. Pressure applied during pasta forming process and the drying temperature influence its texture properties and cooking quality. For instance, higher drying temperature would result in increased hardness of pasta due to a more pronounced protein denaturation, resulting in a more compact gluten network [11–13]. The aim of the present research was to enrich durum wheat pasta with β-glucan-rich barley flour and to define the influence of pasta forming process and drying temperature on product features.

STUDY OBJECTS AND METHODS

The durum semolina was produced by Sgambaro, Italy, and the hull-less barley Osvit (harvest 2015) was kindly provided by Agricultural Institute Osijek. The hull-less barley was milled in a laboratory mill (IKA WERKE 10.1, Staufen, Germany) with a 1 mm sieve. The durum semolina had 11.06% moisture, 1.07% d. m.

minerals, 11.77% d. m. proteins, 1.11% d. m. fat and 70.36% d. m. starch. The barley flour contained 10.14% moisture, 2.32% d. m. minerals, 14.11% d. m. proteins, 2.42% d. m. fat and 54.72% d. m. starch, and was used as a source of β-glucan (it contained 5.16% β-glucan). For pasta formulations, tap water (40 ± 2°C) was used.

Pasta preparation. Control pasta sample was produced from semolina, without barley flour (BF) addition. For barley-enriched pasta, samples with 10, 20, 30, 40 and 50% of BF were prepared.

For pasta produced in the laboratory pasta press Fimar MPF2.5N (Lineapasta, Cittadella (PD), Italy), dry ingredients were added directly into the press, and calculated amount of tap water was added through the opening in the press with stirring to obtain a dough with 36% moisture. After the water was added, the dough was stirred for 15 min, at which point the press was turned on to pasta formation process. At the exit of the press, a fettuccine die was placed to cut pasta into specified length. Finally, the pasta was put on perforated drying plates.

In case of the pasta produced in the laboratory extruder, mixtures were prepared in the laboratory mixer. Dry ingredients were added to the mixing bowl of the laboratory mixer (Kenwood KMM020, JVCKenwood, Uithoorn, Netherlands), and tap water was added with stirring until 34% moisture was reached. After the water was added, stirring continued for further 10 min, at which point the dough was placed into a plastic bag and conditioned for 30 min to ensure that moisture spread evenly. Extrusion was performed in the laboratory single-screw extruder 19/20 DN (Brabender, Duisburg, Germany) with the following parameters: screw 3:1, temperature regime 35/40°C, cooling with water in last section, just before the die, 7 × 2 mm fettuccine die coated with teflon. The extruded pasta was cut by hand and put on perforated drying plates. The moistures of the samples were set according to a preliminary research, where optimal water quantity had been investigated.

The fresh pasta was dried in Climacell 111 chamber (MMM GmbH, Munchen, Germany), with pre-drying at 40°C and air moisture 60% for 35 min. The main drying was performed at one of the following conditions: 50°C/air moisture 70%/450 min; 70°C/air moisture 70%/240 min; 90°C/air moisture 70%/120 min. The specified drying time was determined during a preliminary research of the time necessary for pasta moisture to sink below 13.5% at the end of the process. After drying, the pasta was conditioned at the ambient temperature, packed into plastic bags and stored in the refrigerator at 4°C before analysis.

Colour determination. The colour of the samples was measured by Konica Minolta CR-400 chromameter (Konica Minolta, Japan) after calibration of the apparatus on the white calibration tile. Five fettuccini were placed closely together on a white mat, and their colour was measured in 5 replicates in CIELab* system, where L denoted lightness (0 is black and 100 white), a – redness (positive values)/greenness (negative values) and b – yellowness (positive values)/blueness (negative values).

Total colour change ΔE was calculated according to equation (1) in relation to the control sample (without barley flour):

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}, \quad (1)$$

where L, a, b represent values for the sample and L_0, a_a, b_0 – values for the control sample.

Cooking quality. *The optimal cooking time* and cooking loss were determined in two parallels [14]. Briefly, 10 g of pasta was added to 200 mL of boiling water, and after 5 min loss of white core was monitored every 30 sec by squeezing pasta between two glass tiles.

The cooking loss was determined after cooking pasta for optimal cooking time. Briefly, cooking water and the water used to wash pasta were collected and dried at 115°C until the constant mass was reached, and the cooking loss *(CL)* was calculated according to Eq. 2:

$$CL\ (\%) = (\text{weight of dry residue/weight of dry pasta}) \times 100. \quad (2)$$

The water absorption index and *the swelling index* were determined in two parallels [4]. Briefly, after being cooked for an optimal period of time, the pasta was washed over a colander, drained, weighed and dried at 105°C to constant mass. The water absorption index (*WAI*) was calculated according to Eq. 3:

$$WAI\ (\%) = [(\text{mass of cooked pasta} - \text{mass of dried pasta})/\text{mass of dried pasta}] \times 100 \quad (3)$$

and swelling index (*SI*) – according to Eq. 4:

$$SI\ (\text{g H}_2\text{O/g dried pasta}) = (\text{mass of cooked pasta} - \text{mass of pasta after drying})/\text{mass of pasta after drying}. \quad (4)$$

Microstructure of pasta. The microstructure of the selected pasta samples was determined by Scanning Electron Microscope JSM 7000F (Jeol USA Inc., Peabody MA, USA) at 1000 × magnification. The cooked samples were freeze-dried prior to analysis.

Statistical analysis. Statistical analysis was performed using Statistica® 12 (StatSoft Inc, USA), by Main effects ANOVA and Fisher's LSD at p < 0.05.

RESULTS AND DISCUSSION

The colour of pasta samples is shown in Table 1. Barley flour addition resulted in a darker surface of the fresh pasta, expressed as reduction of L^* values (eg. from 78.78 ± 1.54 for fresh control sample to 71.72 ± 0.22 for fresh sample with 50% barley flour produced in the extruder), with more pronounced differences for samples produced in the laboratory pasta press (from 75.72 ± 0.39 to 67.88 ± 0.31, respectively). a^* values increased, indicating increase of red component, while b^* values decreased (decreased yellow component) proportionally to barley flour addition, again, with more pronounced effects for samples produced in the press. Compared to the samples with the same proportion of barley flour produced in the extruder, the fresh samples produced in the press had lower L^* and b^* and higher a^* values, probably due to higher moisture. Total colour change ΔE increased with the increase of barley flour proportion regardless of process used.

Table 1. Colour of pasta samples measured in CIELab* system, with total colour difference ΔE calculated in relation to corresponding sample without barley flour

Barley flour (%)	Extruder L*	a*	b*	ΔE	Press L*	a*	b*	ΔE
				Fresh pasta				
0	78.78 ± 1.54[e]	-3.96 ± 0.11[a]	35.40 ± 0.48[f]		75.72 ± 0.39[e]	-3.35 ± 0.13[a]	28.35 ± 0.45[e]	
10	77.07 ± 0.26[d]	-2.14 ± 0.19[b]	33.16 ± 0.52[e]	03.35	72.26 ± 0.16[d]	-0.93 ± 0.15[b]	24.13 ± 0.43[d]	05.97
20	74.90 ± 0.55[c]	-0.60 ± 0.14[c]	30.85 ± 0.53[d]	06.86	71.47 ± 0.20[c]	-0.34 ± 0.20[c]	23.12 ± 0.19[c]	07.38
30	73.92 ± 0.49[b]	0.48 ± 0.15[d]	28.98 ± 0.54[c]	09.20	68.87 ± 0.38[b]	0.82 ± 0.16[d]	22.67 ± 0.26[c]	09.83
40	72.32 ± 0.33[a]	1.42 ± 0.16[e]	26.94 ± 0.43[b]	11.92	68.20 ± 0.45[a]	1.50 ± 0.23[e]	21.04 ± 0.66[b]	11.56
50	71.72 ± 0.22[a]	2.02 ± 0.08[f]	25.01 ± 0.61[a]	13.91	67.88 ± 0.31[a]	1.81 ± 0.08[f]	19.46 ± 0.25[a]	12.93
				Dried pasta				
	50°C L*	a*	b*	ΔE	**50°C** L*	a*	b*	ΔE
0	73.29 ± 0.27[f]	-2.47 ± 0.21[a]	20.50 ± 0.63[d]		75.38 ± 0.40[f]	-3.33 ± 0.26[a]	26.28 ± 0.54[e]	
10	72.20 ± 0.24[e]	-1.98 ± 0.12[b]	20.09 ± 0.60[d]	01.26	71.69 ± 0.80[e]	-1.92 ± 0.51[b]	25.96 ± 0.28[e]	03.96
20	71.40 ± 0.31[d]	-0.86 ± 0.22[c]	18.84 ± 0.24[c]	02.99	65.91 ± 0.53[d]	0.48 ± 0.30[c]	23.44 ± 0.19[d]	10.59
30	69.21 ± 0.39[c]	1.30 ± 0.13[d]	18.19 ± 0.13[b]	06.02	63.30 ± 0.47[c]	1.55 ± 0.27[d]	22.38 ± 0.46[c]	13.59
40	67.76 ± 0.36[b]	1.92 ± 0.11[e]	18.03 ± 0.06[ab]	07.47	60.37 ± 0.35[b]	3.15 ± 0.19[e]	21.34 ± 0.37[b]	17.08
50	66.52 ± 0.41[a]	2.71 ± 0.22[f]	17.50 ± 0.37[a]	09.03	58.97 ± 0.30[a]	3.52 ± 0.26[e]	19.75 ± 0.26[a]	18.94
	70°C L*	a*	b*	ΔE	**70°C** L*	a*	b*	ΔE
0	74.47 ± 0.30[f]	-3.03 ± 0.31[a]	21.72 ± 0.35[f]		75.28 ± 0.26[e]	-3.39 ± 0.17[a]	29.34 ± 0.78[e]	
10	73.73 ± 0.28[e]	-2.18 ± 0.18[b]	20.55 ± 0.30[e]	01.62	66.10 ± 0.59[d]	1.09 ± 0.28[b]	25.52 ± 0.50[d]	10.91
20	72.53 ± 0.31[d]	-1.42 ± 0.27[c]	19.56 ± 0.44[d]	03.32	65.98 ± 0.13[d]	1.37 ± 0.32[bc]	23.44 ± 0.35[c]	12.00
30	71.43 ± 0.35[c]	-0.98 ± 0.14[d]	18.68 ± 0.29[c]	04.76	65.12 ± 0.43[c]	1.68 ± 0.14[c]	23.10 ± 0.33[c]	12.96
40	68.42 ± 0.29[b]	0.60 ± 0.30[e]	18.15 ± 0.29[b]	07.91	58.96 ± 0.14[b]	3.84 ± 0.21[d]	22.20 ± 0.28[b]	19.23
50	66.52 ± 0.33[a]	2.40 ± 0.29[f]	17.11 ± 0.04[a]	10.66	57.80 ± 0.35[a]	4.46 ± 0.35[e]	21.46 ± 0.40[a]	20.73
	90°C L*	a*	b*	ΔE	**90°C** L*	a*	b*	ΔE
0	69.46 ± 0.31[f]	-1.92 ± 0.21[a]	24.33 ± 0.45[f]		72.52 ± 0.37[f]	-2.49 ± 0.12[a]	31.35 ± 0.21[f]	
10	68.19 ± 0.22[e]	-1.12 ± 0.07[b]	23.26 ± 0.28[e]	01.83	70.36 ± 0.39[e]	-0.59 ± 0.27[b]	28.38 ± 0.37[e]	04.13
20	66.74 ± 0.42[d]	-0.20 ± 0.06[c]	22.00 ± 0.40[d]	03.97	67.33 ± 0.36[d]	1.53 ± 0.34[c]	27.25 ± 0.24[d]	07.74
30	65.98 ± 0.09[c]	1.08 ± 0.08[d]	20.54 ± 0.53[c]	05.95	65.47 ± 0.43[c]	2.14 ± 0.24[d]	23.47 ± 0.31[c]	11.54
40	65.30 ± 0.28[b]	2.08 ± 0.05[e]	19.53 ± 0.21[b]	07.50	62.26 ± 0.41[b]	3.52 ± 0.34[e]	23.05 ± 0.14[b]	14.50
50	64.58 ± 0.32[a]	3.12 ± 0.10[f]	17.70 ± 0.49[a]	09.65	61.44 ± 0.31[a]	4.63 ± 0.37[f]	22.26 ± 0.16[a]	16.01
				Cooked pasta				
	50°C L*	a*	b*	ΔE	**50°C** L*	a*	b*	ΔE
0	76.31 ± 0.82[f]	-3.54 ± 0.24[a]	19.90 ± 0.95[b]		76.85 ± 0.76[d]	-4.29 ± 0.25[a]	25.34 ± 2.24[c]	
10	75.35 ± 0.60[e]	-2.72 ± 0.27[b]	19.19 ± 0.49[b]	01.46	72.63 ± 0.73[c]	-2.07 ± 0.40[b]	25.51 ± 1.64[c]	04.77
20	74.57 ± 0.67[d]	-1.30 ± 0.38[c]	17.65 ± 0.90[a]	03.62	69.29 ± 1.33[b]	-0.38 ± 0.14[c]	22.17 ± 0.73[b]	09.09
30	73.20 ± 0.29[c]	0.06 ± 0.60[d]	17.34 ± 0.05[a]	05.41	67.66 ± 1.88[b]	0.91 ± 0.60[d]	21.98 ± 0.84[b]	11.09
40	72.08 ± 0.19[b]	1.13 ± 0.47[e]	17.05 ± 0.04[a]	06.92	62.94 ± 1.74[a]	2.68 ± 0.59[e]	20.16 ± 1.32[a]	16.40
50	70.49 ± 0.53[a]	2.15 ± 0.28[f]	16.91 ± 0.06[a]	08.67	62.45 ± 2.09[a]	2.61 ± 0.53[e]	18.51 ± 0.16[a]	17.37
	70°C L*	a*	b*	ΔE	**70°C** L*	a*	b*	ΔE
0	76.52 ± 0.86[d]	-3.51 ± 0.04[a]	19.97 ± 1.56[d]		76.53 ± 0.41[c]	-4.13 ± 0.19[a]	28.43 ± 2.26[d]	
10	76.30 ± 0.09[cd]	-3.22 ± 0.10[b]	19.53 ± 0.11[cd]	00.57	67.71 ± 2.35[b]	0.37 ± 1.09[b]	23.88 ± 1.39[c]	10.89
20	75.85 ± 0.13[c]	-2.07 ± 0.08[c]	18.89 ± 0.10[bc]	01.92	67.03 ± 2.41[b]	0.89 ± 1.14[b]	23.05 ± 1.85[bc]	12.01
30	75.11 ± 0.10[b]	-1.16 ± 0.07[d]	18.02 ± 0.07[ab]	03.63	67.30 ± 1.14[b]	1.07 ± 0.54[b]	22.08 ± 0.85[abc]	12.34
40	74.94 ± 0.08[b]	-0.18 ± 0.08[e]	17.58 ± 0.07[a]	04.39	60.49 ± 2.24[a]	3.28 ± 0.74[c]	21.42 ± 0.88[ab]	19.01
50	74.21 ± 0.09[a]	1.22 ± 0.07[f]	17.11 ± 0.04[a]	05.99	60.32 ± 1.56[a]	3.68 ± 0.25[c]	20.06 ± 1.75[a]	19.84
	90°C L*	a*	b*	ΔE	**90°C** L*	a*	b*	ΔE
0	72.56 ± 1.64[c]	-1.92 ± 0.21[a]	23.15 ± 1.33[d]		74.07 ± 0.41[e]	-4.01 ± 0.30[a]	30.35 ± 0.46[c]	
10	72.31 ± 0.44[c]	-1.12 ± 0.07[b]	21.17 ± 0.17[c]	2.15	72.29 ± 1.32[d]	-0.89 ± 0.43[b]	25.84 ± 1.85[b]	5.77
20	71.99 ± 0.09[c]	-0.20 ± 0.06[c]	20.67 ± 0.32[c]	3.06	68.50 ± 1.17[c]	0.98 ± 0.46[c]	26.11 ± 1.28[b]	8.59
30	70.50 ± 0.46[b]	1.08 ± 0.08[d]	19.34 ± 0.20[b]	5.26	66.54 ± 1.16[b]	1.83 ± 0.48[d]	21.22 ± 0.60[a]	13.20
40	70.01 ± 0.17[ab]	2.08 ± 0.05[e]	18.46 ± 0.40[a]	6.67	63.36 ± 1.40[a]	3.01 ± 0.52[e]	22.21 ± 0.98[a]	15.18
50	69.23 ± 0.18[a]	3.12 ± 0.10[f]	18.12 ± 0.10[a]	7.96	63.00 ± 1.58[a]	3.29 ± 0.51[e]	21.12 ± 1.18[a]	16.15

Note. Data are presented as mean value ± standard deviation (n = 5)
Different letters in the same column for appropriate drying temperature represent statistically significant difference (p ≤ 0.05)

Addition of barley flour reflected in the decrease of $L*$ and $b*$ values, as well as in the increase of $a*$ values in the dried samples. When comparing drying conditions, the brightest samples were obtained in the medium temperature regime (70°C), followed by samples dried at the low- (50°C) and high temperature (90°C) regimes. There was no statistically significant difference between colour parameters $L*$ and $a*$ of the samples dried at low- and high temperature regime, as shown in Fig. 1. However, $b*$ values significantly increased following the increase of drying temperature. These phenomena may be ascribed to Maillard reactions during drying at elevated temperatures [15]. Drying at 70°C was much shorter than drying at 50°C, resulting in a similar advancement of Maillard reactions, unlike drying at 90°C, where the high temperature had a much more pronounced influence on the reaction progress. The trend of influence of barley flour addition on the colour of cooked pasta was similar to the dried samples, with a slightly larger statistical significance (Fig. 1).

Only trained sensory analysts can perceive the colour difference between the dried control sample and the dried sample with 10% barley flour produced in the extruder, regardless the drying temperature [16]. Addition of 40% and 50% of barley flour resulted in a large colour difference, easily perceived by ordinary people. When samples were produced in the pasta press, an obvious colour difference was perceived already at 20% barley flour, and a further increase in barley flour content made the colour difference extreme [16]. This can also be ascribed to higher initial moisture of the samples, which enabled a higher extent of browning reactions, such as Maillard reactions.

When considering barley content regardless of production and drying processes, it is evident that it has a significant influence on pasta colour (Fig. 1). Production process also produced a visible effect, which cannot be said about the drying temperature, except for high temperature drying, in which case statistical significance is evident (Fig 1).

The optimal cooking time is shown in Table 2. Barley addition did not significantly influence the optimal cooking time (F-test, p-value = 0.018) (Fig. 2), as opposed to pasta enriched with quinoa flour [17] and amaranth [18], in which case the optimal cooking time was significantly reduced. However, both quinoa and amaranth contain less fibre than barley examined in this research, and when a part of semolina was replaced with carob fibre, it did not influence the optimal cooking time significantly [19].

The press-made pasta had a shorter cooking time than the extruder-made samples regardless the barley content and drying temperature (Table 2, Fig. 2). This can be ascribed to lower mechanical energy applied during production in the press, which results in a less compact product that needs less time to fully gelatinise [20].

When considering the influence of drying regimes, it is evident that pasta dried at low-temperature regime requires longer cooking time than the one dried at medium- and high temperature, and there is no statistical difference between the latter two (F-test, p-value < 0.001) (Table 2, Fig. 2). There was no difference in cooking time between the semolina pasta dried at low- and high temperature regimes [20], while "the optimum cooking time for spaghetti samples decreased as the drying temperature profile decreased" [21]. Padalino et al. [22] stated that "higher temperatures induced cross-link density of both protein and starch, decreasing water diffusion". The present research differs from that performed by Padalino et al. in the aspect of barley flour added, which probably interfered with the effect of temperature on protein cross-linking.

Unlike cooking time, barley flour proved to be an important factor when it comes to cooking loss (F-test, p-value < 0.001) (Table 2, Fig. 2), increasing it proportionally, with minor exceptions. Addition of barley flour caused formation of a weaker and discontinuous protein network, reducing its ability to hold dry matter during cooking, and starch leached into the surrounding water [5]. Similar results were reported for pasta with barley β-glucan [7], with oat β-glucan [21] and oat flour [23]. On the contrary, β-glucan does not influence cooking loss significantly [7].

The extruder-produced samples had a smaller cooking loss than their press-made counterparts, probably because a larger pressure during extrusion in the extruder results in a more compact protein network that holds dry matter better during cooking (F-test, p-value < 0.001).

The increase of drying temperature resulted in the decrease of cooking loss (F-test, p-value < 0.001) (Table 2, Fig. 2). The same trend for pasta made from semolina was reported, which can be explained by protein denaturation and formation of stronger protein network at higher temperatures [24], and by the fact that "the increase in drying temperature (90°C vs. 55°C) promoted the covalent aggregation of proteins in pasta, enhancing their resilience and reducing their cooking loss, without altering the degree of protein hydrolysis" [25].

The water absorption index decreased slightly, although not significantly, after adding barley flour (F-test, p-value = 0.250) (Table 2, Fig. 2) [9]. Although high fibre content should theoretically raise the hydration, Aravind et al. [9] assumed that β-glucan competed for water with gluten and starch during mixing and therefore did not have significant influence on water absorption during cooking [9]. On the contrary, an increase of the water absorption index after addition of different fibres was reported; however, the authors also noticed that some types of fibres influenced it to a lesser degree [26]. They ascribed it to the particle size and the structural difference between the fibres.
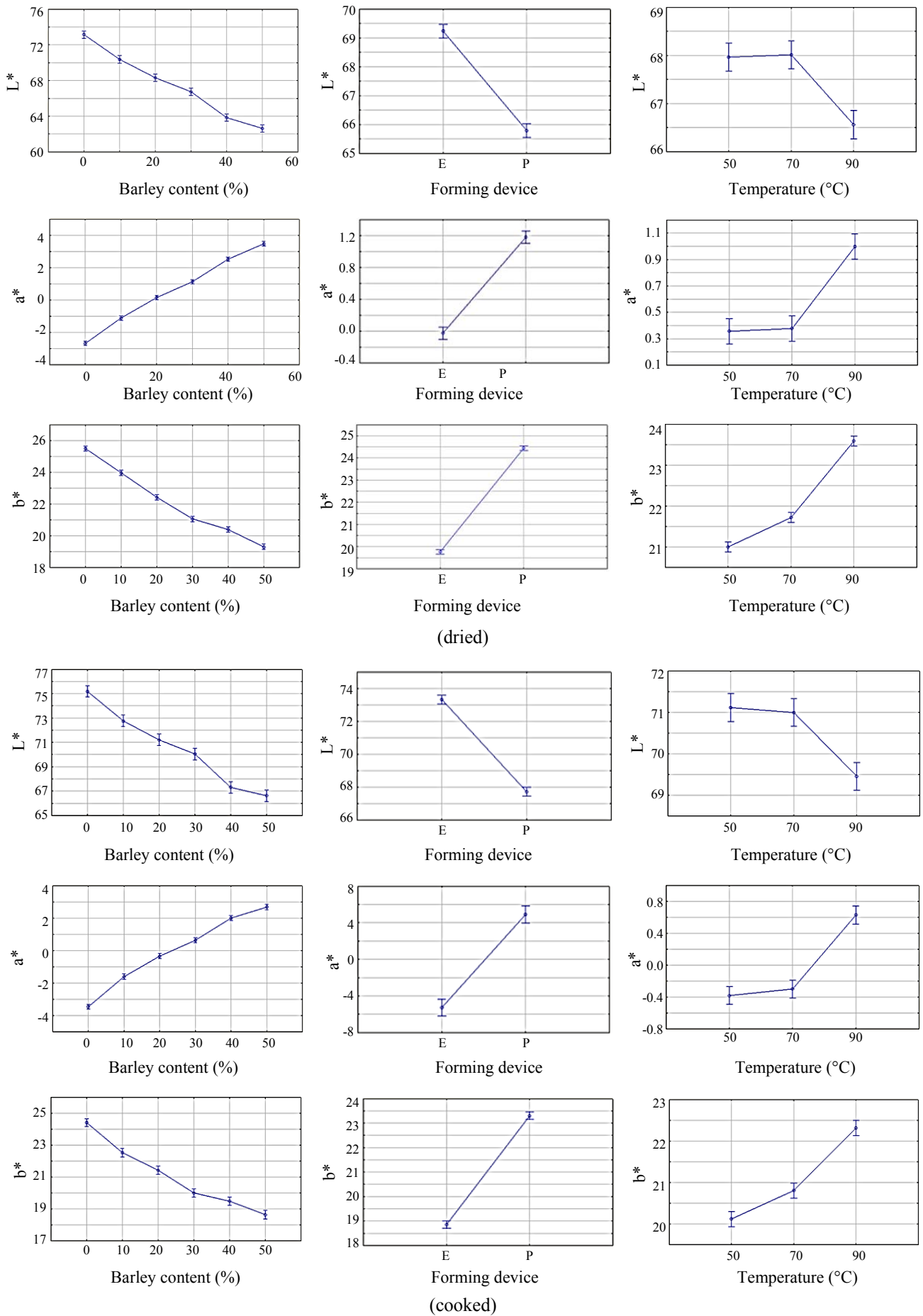
Fig. 1. Statistical influence of barley flour content, forming device (E – extruder, P – press) and drying temperature on colour parameters of dried and cooked pasta. Interval for each value represents statistical error.
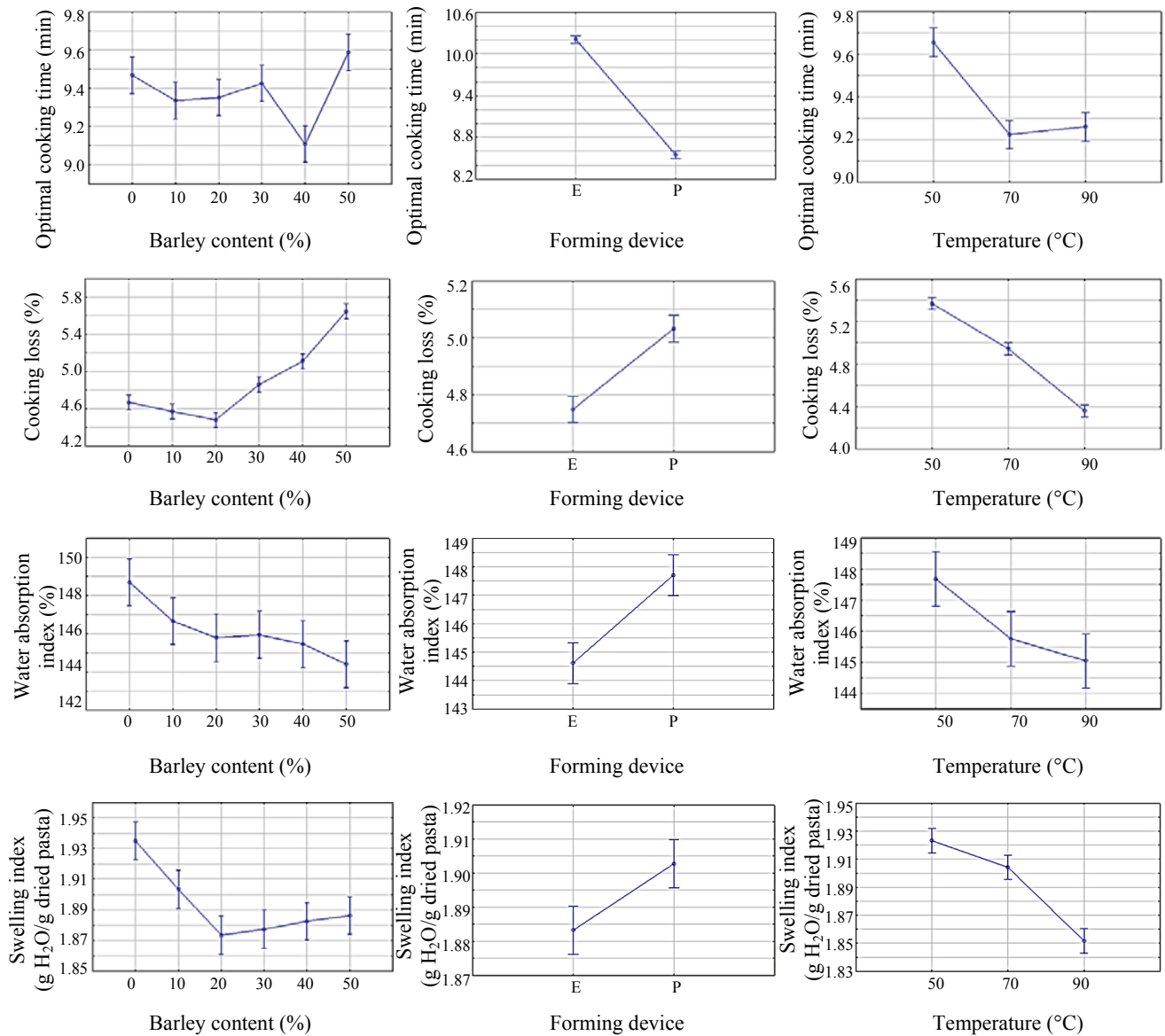
Fig. 2. Statistical influence of barley flour content, forming device (E – extruder, P – press) and drying temperature on pasta cooking quality. Interval for each value represents statistical error.

The water absorption index of the press-made pasta is evidently larger than the extruder-made counterparts (F-test, p-value = 0.003) (Fig. 2). As it has already been mentioned in regard to cooking time, the pasta production in the extruder resulted in formation of a more compact network, which hindered water molecules penetration, whereas in the case of the press-made pasta, protein network is weaker and water penetrates more easily.

The swelling index generally followed the same trend as the water absorption index and was consistent with the previous research [9, 27]. It is worth specifying that only addition of 10 and 20% barley flour had a significant influence on swelling reduction. A further increase of barley content slightly raised it, but with no statistical significance (Fig 2).

The microstructures of selected dried and cooked pasta samples are shown in Fig. 3–5.

The microstructure of the samples without barley flour revealed that combination of extrusion with medium and high drying temperature influenced starch granules (Fig. 3 A, C, E), causing their disruption and starch leakage, gelatinisation and interactions (starch-protein, starch-lipid, starch-starch complexes). In the press-made samples, starch granule disruption was reduced significantly even when high drying temperature was applied (Fig. 3 B, D, F), but granules did swell partially. In the samples with 50% barley flour the same trend was observed: the extruded samples dried at high temperatures had fewer untacked starch granules, and the press-made samples demonstrated partially swollen granules (Fig. 4).

Table 2. Cooking quality of pasta with addition of 10–50% barley flour, produced in the extruder and in the mini-press, dried at 50, 70 and 90°C

Barley flour (%)	Extruder			Press		
	optimal cooking time (min)					
	50°C	70°C	90°C	50°C	70°C	90°C
0	10.17 ± 0.10^{bc}	10.17 ± 0.15^{c}	10.33 ± 0.06^{ab}	9.44 ± 0.05^{a}	8.39 ± 0.04^{b}	8.31 ± 0.01^{c}
10	10.14 ± 0.05^{bc}	10.45 ± 0.05^{a}	10.20 ± 0.26^{ab}	9.46 ± 0.05^{a}	7.52 ± 0.05^{d}	8.25 ± 0.05^{c}
20	10.28 ± 0.09^{ab}	10.18 ± 0.08^{bc}	10.23 ± 0.38^{b}	9.25 ± 0.05^{ab}	8.27 ± 0.06^{b}	8.29 ± 0.09^{c}
30	10.11 ± 0.09^{c}	10.11 ± 0.11^{c}	10.30 ± 0.53^{ab}	9.18 ± 0.28^{c}	8.28 ± 0.03^{b}	9.48 ± 0.03^{a}
40	10.38 ± 0.08^{a}	10.34 ± 0.08^{ab}	10.14 ± 0.09^{ab}	8.45 ± 0.06^{d}	8.25 ± 0.30^{c}	7.48 ± 0.08^{d}
50	10.40 ± 0.10^{a}	10.27 ± 0.03^{bc}	10.37 ± 0.12^{a}	9.09 ± 0.03^{b}	9.27 ± 0.28^{a}	8.55 ± 0.05^{b}
	cooking loss (%)					
	50°C	70°C	90°C	50°C	70°C	90°C
0	4.85 ± 0.19^{c}	4.36 ± 0.41^{cd}	3.95 ± 0.23^{b}	5.09 ± 0.11^{c}	5.02 ± 0.05^{c}	4.75 ± 0.19^{b}
10	4.84 ± 0.22^{c}	4.32 ± 0.21^{d}	4.01 ± 0.23^{b}	5.16 ± 0.06^{c}	4.79 ± 0.16^{c}	4.31 ± 0.06^{d}
20	5.18 ± 0.02^{bc}	4.03 ± 0.23^{d}	4.00 ± 0.07^{b}	5.26 ± 0.15^{c}	4.16 ± 0.06^{d}	4.26 ± 0.10^{d}
30	5.30 ± 0.21^{bc}	4.03 ± 0.04^{bc}	4.30 ± 0.20^{ab}	5.32 ± 0.04^{c}	5.00 ± 0.15^{c}	4.39 ± 0.13^{cd}
40	5.62 ± 0.23^{ab}	5.11 ± 0.12^{b}	4.30 ± 0.23^{ab}	5.79 ± 0.15^{b}	5.41 ± 0.11^{b}	4.61 ± 0.08^{bc}
50	5.90 ± 0.22^{a}	6.15 ± 0.12^{a}	4.50 ± 0.06^{a}	6.09 ± 0.14^{a}	6.12 ± 0.14^{a}	5.11 ± 0.12^{a}
	water absorption index (%)					
	50°C	70°C	90°C	50°C	70°C	90°C
0	148.87 ± 1.67^{c}	147.45 ± 0.10^{c}	154.24 ± 7.32^{b}	151.82 ± 1.38^{bc}	144.76 ± 5.37^{a}	145.05 ± 2.34^{a}
10	142.67 ± 1.63^{ab}	155.48 ± 3.60^{d}	141.65 ± 1.03^{a}	151.73 ± 0.21^{bc}	145.07 ± 2.97^{a}	143.36 ± 1.83^{a}
20	142.66 ± 4.11^{ab}	146.09 ± 2.65^{bc}	147.37 ± 0.11^{ab}	149.65 ± 0.63^{a}	145.66 ± 0.36^{a}	143.31 ± 0.46^{a}
30	146.23 ± 2.67^{bc}	142.03 ± 0.08^{ab}	143.45 ± 2.95^{a}	151.60 ± 0.43^{b}	146.05 ± 0.74^{a}	146.30 ± 1.93^{a}
40	139.02 ± 2.06^{a}	143.03 ± 0.21^{bc}	144.00 ± 0.04^{a}	152.68 ± 0.32^{bc}	147.16 ± 4.44^{a}	146.88 ± 1.73^{a}
50	141.70 ± 2.06^{ab}	137.57 ± 2.47^{a}	139.53 ± 1.06^{a}	153.50 ± 1.00^{c}	148.72 ± 0.96^{a}	145.42 ± 2.10^{a}
	swelling index (g H_2O/g dried pasta)					
	50°C	70°C	90°C	50°C	70°C	90°C
0	1.95 ± 0.01^{b}	1.91 ± 0.02^{b}	1.92 ± 0.09^{b}	1.96 ± 0.02^{d}	1.93 ± 0.00^{b}	1.92 ± 0.04^{b}
10	1.89 ± 0.01^{a}	2.03 ± 0.07^{c}	1.78 ± 0.00^{a}	1.93 ± 0.00^{bcd}	1.91 ± 0.01^{ab}	1.88 ± 0.01^{ab}
20	1.90 ± 0.03^{ab}	1.89 ± 0.03^{ab}	1.87 ± 0.01^{ab}	1.89 ± 0.01^{a}	1.87 ± 0.04^{a}	1.83 ± 0.03^{a}
30	1.92 ± 0.04^{ab}	1.86 ± 0.01^{ab}	1.82 ± 0.03^{a}	1.92 ± 0.01^{b}	1.89 ± 0.01^{ab}	1.85 ± 0.01^{ab}
40	1.89 ± 0.01^{a}	1.89 ± 0.00^{ab}	1.84 ± 0.01^{ab}	1.93 ± 0.01^{bc}	1.92 ± 0.01^{b}	1.82 ± 0.02^{a}
50	1.93 ± 0.02^{ab}	1.82 ± 0.02^{a}	1.78 ± 0.00^{a}	1.95 ± 0.02^{cd}	1.93 ± 0.01^{b}	1.90 ± 0.04^{b}

Note. Data are presented as mean value ± standard deviation (n = 2)
Different letters in the same column for appropriate drying temperature represent statistically significant difference ($p \leq 0.05$)

(a)	(b)	(c)

(d)	(e)	(f)
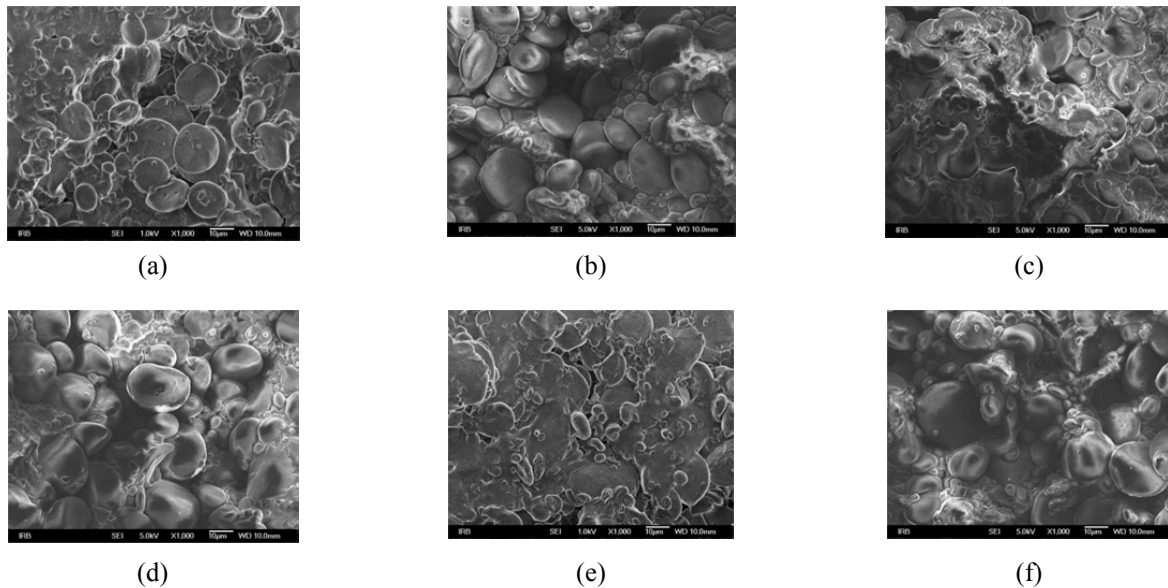
Fig. 3. SEM microstructure (1000 ×) of dried pasta produced in the extruder (a, c, e) and in the press (b, d, f) without barley flour, dried at 50°C (a, b), 70°C (c, d) and 90°C (e, f).
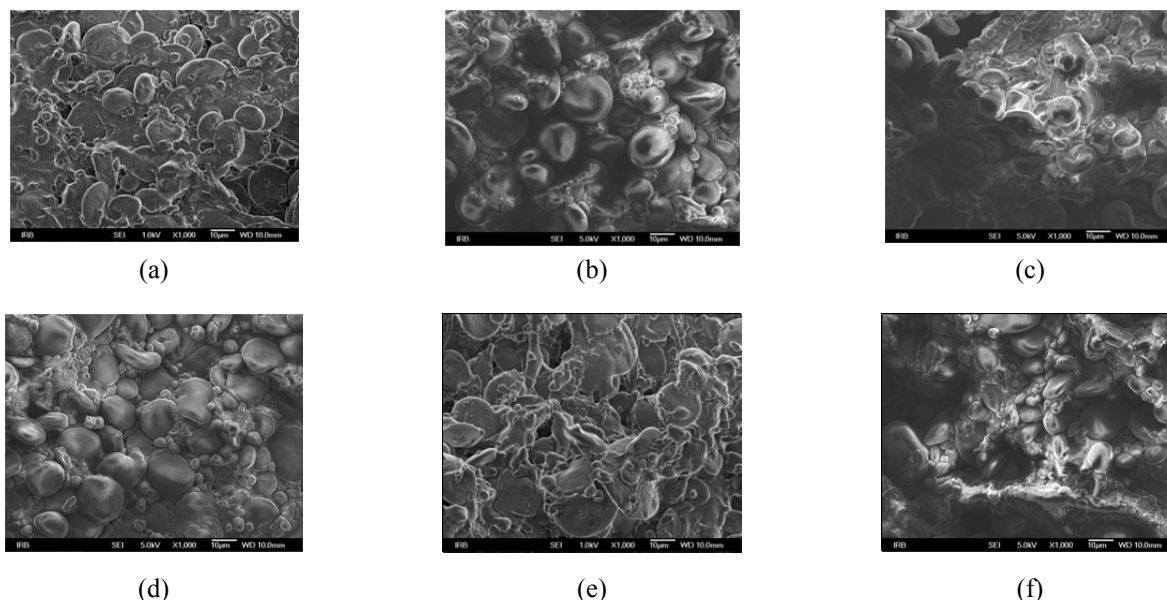
Fig. 4. SEM microstructure (1000 ×) of dried pasta with 50% barley flour, produced in the extruder (a, c, e) and in the the press (b, d, f), dried at 50°C (a, b), 70°C (c, d) and 90°C (e, f).
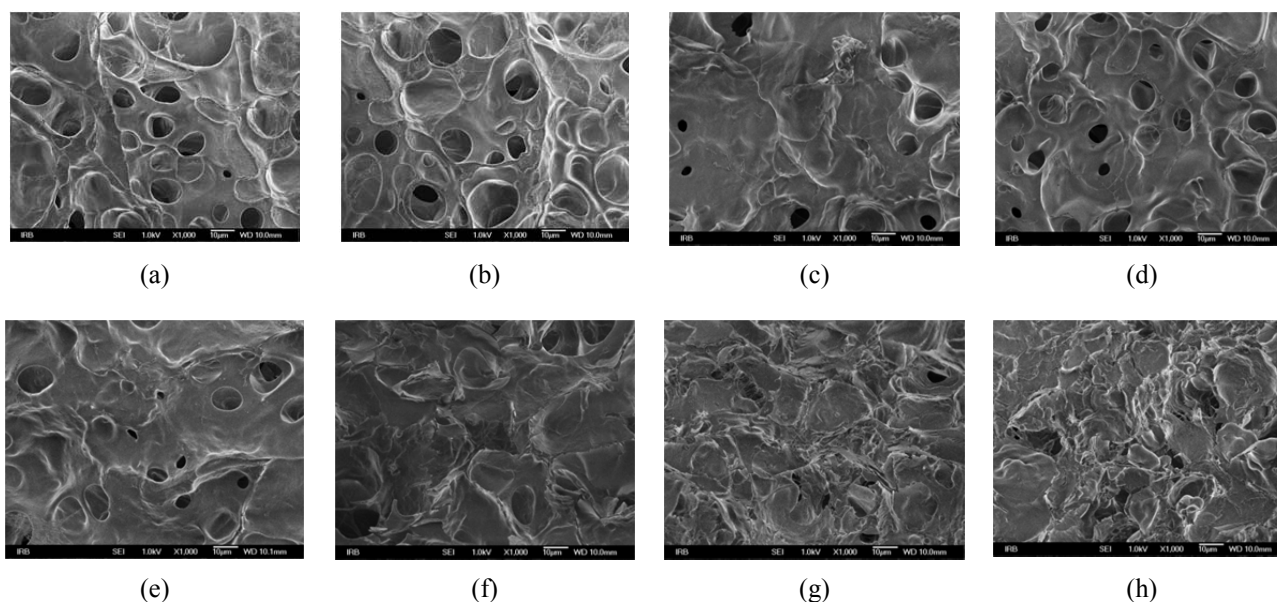
Fig. 5. SEM microstructure (1000 ×) of cooked pasta without barley flour produced in the extruder (a, c) and in the press (b, d), dried at 50°C (a, b) and 90°C (c, d), and the cooked pasta with 50% barley flour produced in the extruder (e, g) and in the press (f, h), dried at 50°C (e, f) and 90°C (g, h).

All micrographs revealed that starch granules were incorporated in the protein network to a lesser extent in the press-made pasta, and this influenced cooking time, absorption and swelling. Gelatinised starch complexes with lipids and proteins rather easily, and formation of compounds requires far more energy. Starch in granules starts to gelatinise during cooking, and the afore-mentioned complexes do not influence cooking time that much [19].

During cooking, starch gelatinises completely, and a unique network of starch and proteins is formed (Fig. 5). The micrographs in Fig. 5 show orifices where starch has leached. In the samples dried at 50°C, the number of thus formed cavities was larger compared to the samples dried at 90°C, and the extruder-made

samples had fewer cavities than the press-made samples, all of which can be linked to cooking loss.

CONCLUSION

This research aimed to explore the potentiality of barley-enriched pasta production with acceptable physical properties important for consumer acceptance. Two processes for production and different drying regimes were investigated in order to tackle the problem of "diluted" gluten network and its effect on cooking loss. Although addition of barley flour reduced the cooking quality of pasta, thus increasing the cooking loss, it can be successfully applied as a source of polyphenols and β-glucan in pasta. The pasta produced in the extruder required a longer time to cook, but had a

lower cooking loss than in case of the pasta produced in the laboratory press, and medium- and high drying temperature regimes resulted in a shorter cooking time and a lower cooking loss. The results obtained in this research show that a proper combination of production conditions (pressure and temperature during the extrusion process) and drying conditions can compensate for gluten network "dilution" due to addition of barley flour. Further research is needed to establish the optimal combination of barley flour content and production conditions to obtain nutritionally valuable pasta of optimal physical properties.

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Optimisation of a process for cocoa-based vermicelli

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Abstract: Due to its health promoting properties owing to a high phenolic content and sensory acceptability, cocoa has gained interest as an additive of choice in many food products. The purpose of this study was to incorporate cocoa powder (CP) in vermicelli. Different proportions of cocoa powder (5, 10, 15 and 20%) were prepared by mixing it into a blend of wheat flour and rice flour (60:40) as base ingredients. The quality parameters, including nutritional characteristics, antioxidant activity, cooking and functional properties, and sensory acceptability, were studied. The nutritional profiling showed a significant ($p < 0.05$) increase in the protein, fat, ash, and carbohydrate alongside a significant decrease in the moisture content. Similarly, an antioxidant activity increased significantly at $p < 0.05$, with the increase of cocoa powder concentration. It can be concluded that vermicelli with the 10% cocoa powder incorporated was the best treatment since it was rated as the highest in overall acceptability compared to the other formulations. The bulk density, cooked weight, cooking time, gruel solid loss, and water absorption capacity of samples with 10% cocoa powder were 0.714 g/cm^3, 11.56 g, 7.21 min, 0.47 g/100 g, and 146%, respectively. The energy value of the optimised cocoa-based vermicelli was 375 kcal/100g of sample.

INTRODUCTION

Cocoa beans nave relatively high polyphenolic content and high antioxidant activity. Flavanol monomers, epicatechin and catechin, along with the procyanidins, are the major phenolic phytochemicals present in cocoa [1]. Cocoa and its various products have become an area of interest as they have health-stimulating properties. Cocoa and its derivative products, Such as dark chocolate, cocoa liquor, and cocoa powder, showed remarkable changes in suppressing atherosclerotic lesions and increased dermal blood flow. The phenolic properties of cocoa, especially flavonoids, help in suppressing the multiplication of human breast cancer cells [2]. Cocoa consumption improves the lipid profile, insulin sensitivity, and blood pressure [3]. Cocoa powder is added in the preparation of *shrikhand* [4], biscuits [3], and cakes [5]; however, no study has been reported for its incorporation in vermicelli.

Vermicelli is one of the most popular instant food products. Vermicelli comes in the category of extruded products and is prepared from the whole or refined wheat flour. It is a snack food item liked by all age groups and groups with changing lifestyles. Instant food items have gained popularity and have become the source of mass consumption [6]. Vermicelli is basically judged by uniformity, cooking, as well as eating quality. Cooking, sensory, and nutritional qualities are major attributes of vermicelli.

The present study was therefore undertaken to formulate vermicelli enriched with cocoa powder, using wheat flour and rice flour as the base material. Physical, antioxidant, cooking, and sensory properties were studied.

STUDY OBJECTS AND METHODS

Materials. Cocoa powder (Weikfield premium cocoa powder, Loni, Pune, Maharashtra, India) was procured from the local market of Phagwara, Punjab. The commercial wheat flour and rice flour were procured from the flour mill of a local market of Phagwara, Punjab.

Methods. Preparation of the blend. A preliminary investigation was carried out by preparing base flours in different ratios of wheat flour and rice flour, of which 60 : 40 proportion ensured proper binding during the cocoa incorporation. This blend became the base flour. The base flour was mixed with cocoa powder in different proportions to make the treatments. The vermicelli samples were prepared using 95:5 (CP_1), 90:10 (CP_2), 85:15 (CP_3), and 80:20 (CP_4) proportions of the base flour and cocoa powder, respectively; the base flour was used as a control. The flours were sieved using a sieving machine (Flour Tech Engineers Pvt. Ltd., Faridabad, India) with particle sizes of 63, 80, 100, 125? and 140 μm and 200 mm diameter sieves. The flours with the moisture content of 5% were stored in zip sealed polyethylene pouches (Wellworth Packers Pvt. Ltd., Delhi, India) until further use.

Vermicelli preparation. The standard vermicelli preparation process described by Devi et al. [7] was adapted. On the basis of the texture and consistency of the pre-experiment sample, 5 different compositions with the blend of rice and wheat flours were prepared for experiments. Hard dough was prepared by mixing wheat flour, rice flour, and cocoa powder in different compositions (0, 5, 10, 15, and 20%). Distilled water (60 ml) was added slowly and kneaded into a homogenous soft dough. The dough was prepared using a traditional cold extruder (Siddhmurti kitchen press, India) The obtained vermicelli samples were dried at 60 ± 2°C for an hour in a tray drier; the dried vermicelli was packed in zip pouches for further use. The process flow chart for the preparation of vermicelli is shown in Fig. 1.

Nutritional profiling of cocoa vermicelli. The nutritional profiling of the optimised vermicelli, which includes moisture, carbohydrates, protein, total minerals (ash), and fat content, was determined by standard AOAC procedures [8]. The energy value was determined by Bomb Calorimeter, Labtronics, Model LT-1160 (Bombay Scientific, Ambala, Haryana, India).

Antioxidant activity. Antioxidant activity (free radical scavenging activity) was measured using the method described by Rasane et al. [9]. DPPH (2, 2-diphenyl-1-picrylhydrazyl) was used as a source of free radicals. The concentration of the DPPH solution used was 90 μol/L, the DPPH solution was diluted in 2.9 ml of ethanol, and 0.1 ml of the extracts at various concentrations was added. After shaking the mixture well, it was kept for 60 min in dark and then the absorbance was measured at 517 nm against the blank. The antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity (\%)} = \frac{A_{control} - A_{Sample}}{A_{control}} * 100,$$

where

$A_{control}$ = Absorption of the DPPH solution

A_{sample} = Absorption of the sample

Cooking and functional properties of cooked vermicelli. The bulk density was calculated by transferring 10 g of the sample into 50 ml of the graduated measuring cylinder. The sample was packed with gentle tapping the cylinder on the flat table at least for 10 times from a height of 5 cm. The volume of the sample was recorded by using the formula given by AOAC 2000 [8]:

$$\text{Bulk density} \left(\frac{g}{ml}\right) = \frac{weight\ of\ the\ sample}{volume\ of\ the\ sample\ after\ tapping}$$

The water absorption was calculated by using the methods described by [10]. The formula used was:

$$WAC = \frac{Weight\ of\ centrifuge\ tube + weight\ of\ sediment - weight\ of\ empty\ tube}{Weight\ of\ sample}.$$

Cooking time was estimated using the method described by Singh et al. [10]. A sample of 10g was cooked in 200 ml of boiling water until the disappearance of white core and judged by squeezing it between two slides. The resulted time was noted as cooking time. Cooking loss was calculated by using the method described by Aydin and Gocmen [11]. The water uptake percentage was determined by using the method described by Ma et al. [12]. The difference in the cooked and uncooked samples of vermicelli was calculated and expressed as the weight of the uncooked sample in percentage.

Sensory evaluation of noodles. The vermicelli samples (formulated and control) were cooked in milk with a small amount of sugar. The prepared vermicelli was analysed for its sensory properties by 50 semi-trained panellists (25 men and 25 women at the ages of 20–35 years). The parameters used were colour and appearance, flavour (taste and aroma), texture, and overall acceptability. Nine point hedonic scale was used for sensory evaluation of the vermicelli samples. The samples freshly cooked in milk and sugar were served in an odourless plastic container, in a separate chamber. The panellists were served with one sample at a time at an interval of 2 min in between two samples. Potable water was also served at room temperature to rinse mouth in between samples.

Statistical Analysis. All the data were obtained in triplicate and presented in mean ± standard deviation. One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range test and the Post hoc test was used to analyse the data using the SPSS 22.0 software (SPSS Italia, Bologna, Italy) at the error level of 5%.
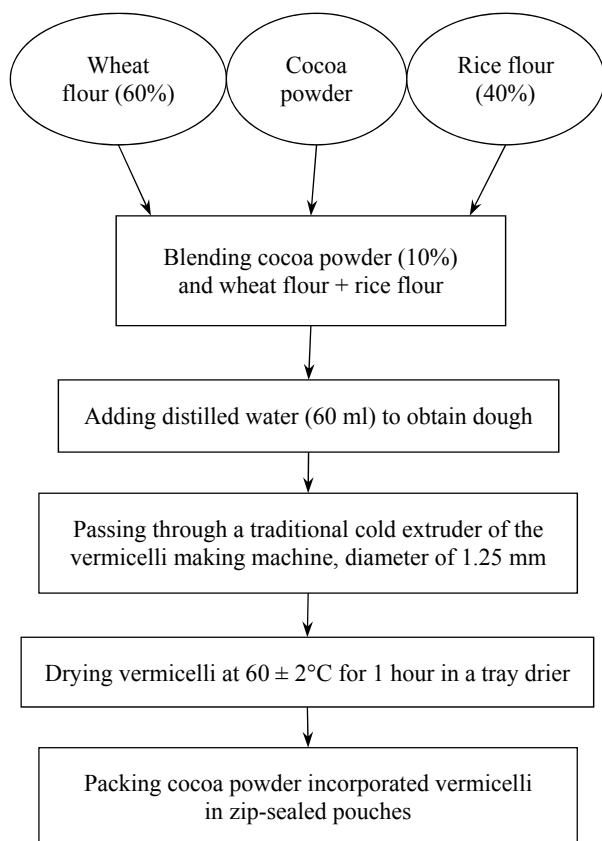
Fig. 1. Process flow chart for the production of cocoa powder vermicelli.

RESULTS AND DISCUSSION

Nutritional profiling of vermicelli. The ap proximate composition of vermicelli with cocoa powder incorporated into the blend is given in Table 1. The mean moisture content of vermicelli incorporated with cocoa powder decreased from 8.78 (100% WRF and 0% CP) to 6.89 (80% WRF and 20% CP) per cent. A low moisture content is a favourable factor in increasing the shelf stability of the product. The protein content increased significantly with the increase in cocoa solids which may be ascribed to the addition of various amounts of cocoa powder to the experimental treatments of vermicelli. The ash content increased significantly ($p < 0.05$), which could be attributed to the high mineral content in the cocoa powder, i.e. 5.7 g/100g [5]. A significant increase in the fat content ranging from 1.34 to 4.06 (80% WRF and 20% CP) per cent was observed. These values are which is less than the values of the fermented cocoa beans, which vary from 10.115 to 12.015% as reported by Ajala and

Ojewande [13]. A low fat content in the diet helps in reducing coronary heart diseases and improves health as stated by Osakebe et al. [14]. The difference method was used to calculate the carbohydrate content of the samples. The carbohydrate content decreased, and a significant difference ($p < 0.05$) was observed in and above 10% cocoa powder incorporation. The energy value ranged from 365 (control) to 385 Kcal/100 g (20% incorporation). There was a significant increase ($p < 0.05$) in the calorific value with the increase in the percentage of cocoa powder. The increase in the calorific value of the developed cocoa-based vermicelli could be attributed to the high content of fat and protein in the cocoa powder [5].

Antioxidant activity. The antioxidant activity was observed in the blend, dry as well as cooked vermicelli, and a significant difference was observed with the increase in the cocoa powder incorporation (Fig. 2). The antioxidant activity ranged from 37 (100% WRF and 0% CP) to 72.96 (80% WRF and 20% CP) per cent in blends, from 30.22 (100% WRF and 0% CP) to 61.07 (80% WRF and 20% CP) in dry vermicelli, and from 17.28 (100 % WRF and 0% CP) to 46.73 (80% WRF and 20% CP) in cooked vermicelli. The antioxidant percentage has increased in all cases. The observed effect of drying and cooking on the antioxidant level is shown in Fig. 2. Similar results were reported by Ajibola et al. [3], in their study of whole-wheat biscuits with cocoa powder and *Moringa oleifera* leaves incorporation. They reported a substantial increase in the antioxidant activity with the incorporation of 5% cocoa powder.

Cooking and functional properties of cocoa vermicelli. The cocoa powder vermicelli was analysed for its cooking properties. Table 2 shows the cooking properties of cocoa incorporated vermicelli. Bulk density is a good physical attribute for determining the mixing quality of particulate matter and helps in indicating the relative amount of packaging material [4]. The moisture content of a product affects the bulk density of the sample. A similar result has been reported by Ajala and Ojewande [13] in their study on drying of fermented cocoa beans. They stated that bulk density of cocoa powder increased with the decrease in a moisture content. The cooked weight decreased significantly (p < 0.05) from 12.02 (control) to 9.93 (5% incorporation) with an increase in the cocoa powder incorporation. This could be due to the decrease in a starch content owing to cocoa powder incorporation, resulting in the reduction of imbibed water.

Table 1. Approximabe composition of blends

Sample Code	Moisture content, %	Ash, %	Protein, %	Fat, %	Carbohydrate,%	Energy value, (Kcal/100g)
Control	8.78 ± 0.14^a	0.94 ± 0.12^a	9.13 ± 0.05^a	1.34 ± 0.01^a	79.81 ± 0.17^a	365 ± 0.10^a
CP$_1$	8.13 ± 0.06^b	1.09 ± 0.19^b	9.58 ± 0.06^b	1.83 ± 0.02^b	79.38 ± 0.10^a	371 ± 0.17^b
CP$_2$	7.97 ± 0.18^c	1.17 ± 0.09^c	9.86 ± 0.11^c	2.40 ± 0.04^c	78.63 ± 0.09^b	375 ± 0.14^c
CP$_3$	7.32 ± 0.17^d	1.51 ± 0.06^d	10.52 ± 0.10^d	3.14 ± 0.08^d	77.43 ± 0.16^c	380 ± 0.21^d
CP$_4$	6.89 ± 0.13^e	1.89 ± 0.15^e	11.21 ± 0.03^e	4.06 ± 0.09^e	75.92 ± 0.08^d	385 ± 0.13^e

Note. CP$_1$: 5% cocoa incorporation, CP$_2$: 10% cocoa incorporation, CP$_3$: 15% cocoa incorporation, CP$_4$: 20% cocoa incorporation.
The values are represented in Mean ± Standard deviation derived for triplicate experiments (n = 3). The values denoted with different superscripts differ significantly at $p < 0.05$ in a column.
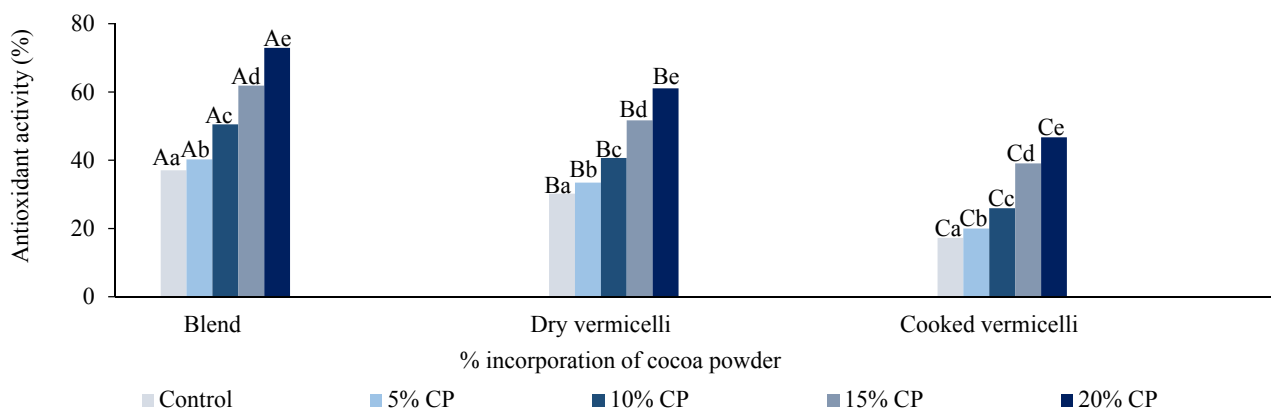
Note. The values are represented in Mean ± Standard deviation derived for triplicate experiments (n = 3). The values denoted with the capital letters differ significantly at $p < 0.05$ in different treatments. The values denoted with small letters differ significantly at $p < 0.05$ in the percentage of incorporated cocoa powder.

Fig. 2. Graphical representation of antioxidant properties of cocoa incorporated vermicelli.

Table 2. Cooking and functional properties of cocoa vermicelli

Sample Code	Cooked weight (g)	Cooking time (min)	Gruel solid loss (g/100g)	Bulk density (g/cm^3)	WAC, %
Control	12.02 ± 0.03^a	6.20 ± 0.05^a	0.18 ± 0.01^a	0.789 ± 0.01^a	121 ± 4.83^a
CP$_1$	11.81 ± 0.02^b	6.80 ± 0.15^b	0.22 ± 0.01^a	0.769 ± 0.01^a	136 ± 3.12^b
CP$_2$	11.56 ± 0.05^c	7.21 ± 0.19^c	0.47 ± 0.04^b	0.714 ± 0.03^b	146 ± 3.60^c
CP$_3$	10.90 ± 0.10^d	8.03 ± 0.20^d	0.62 ± 0.08^c	0.681 ± 0.04^c	176 ± 3.12^d
CP$_4$	09.93 ± 0.06^e	9.17 ± 0.16^e	0.80 ± 0.04^d	0.642 ± 0.02^d	181 ± 4.57^e

Note. CP$_1$ 5% cocoa incorporation, CP$_2$ – 10% cocoa incorporation, CP$_3$ – 15% cocoa incorporation, CP$_4$ – 20% cocoa incorporation. The values are represented in Mean ± Standard deviation derived for triplicate experiments (n = 3). The values denoted with different superscripts differ significantly at $p < 0.05$ in a column.

Table 3. Sensory properties of cocoa vermicelli

Sample	Color & Appearance	Taste	Aroma	Texture	Overall Acceptability
Control	7.33 ± 0.41^a	7.13 ± 0.22^a	6.75 ± 0.04^a	6.98 ± 0.83^a	7.04 ± 0.03^a
CP$_1$	7.00 ± 0.41^c	7.00 ± 0.20^c	6.83 ± 0.30^b	6.88 ± 0.99^a	6.92 ± 0.07^c
CP$_2$	7.44 ± 0.53^b	7.44 ± 0.53^b	7.50 ± 0.53^b	7.50 ± 0.76^a	7.47 ± 0.01^b
CP$_3$	6.89 ± 0.76^d	7.00 ± 0.62^c	6.65 ± 0.41^c	6.18 ± 0.06^b	6.68 ± 0.04^d
CP$_4$	6.33 ± 0.32^e	6.19 ± 0.62^d	6.50 ± 0.51^c	6.00 ± 0.19^c	6.25 ± 0.06^e

Note. CP$_1$ is 5% cocoa incorporation, CP$_2$ – 10% cocoa incorporation, CP$_3$ – 15% cocoa incorporation, CP$_4$ – 20% cocoa incorporation. The values are represented in Mean ± Standard deviation derived for triplicate experiments (n = 3). The values denoted with different superscripts differ significantly at $p < 0.05$ in a column.

The increased water absorption capacity is also an indicator for the increased cooked weight of the formed samples [15]. It is evident from Table 2 that the cooking time increased significantly at $p < 0.05$, with the increase in cocoa powder levels from 6.80 (5% incorporation) to 9.17 min (20% incorporation) while the control vermicelli took 6.20 min to cook. This could be due to the high starch content and a lower gelatinisation thus achieved. It was noted that gruel solid loss of the cooked vermicelli showed a significant increase in all samples with cocoa incorporation (viz. CP$_1$, CP$_3$, CP$_4$, and CP$_5$). The result reported in the present experiments was in agreement with those observed in Ronge et al. [16]. The functional property, water absorption capacity (WAC), is an indicator of the association of water with the product at instances where water is a factor subjected to limitations. It was observed that WAC increased with an increase in cocoa powder proportion. This could be attributed to such properties of a cocoa powder as hygroscopicity, low water activity, and high dietary fibre content as stated by Victor et al. [17].

Sensory properties. The scores of sensory evaluation were attained for various sensory characteristics, such as colour and appearance, taste, aroma, texture, and overall acceptability. All the values obtained are shown in Table 3, for the sensory attributes of the different types of cocoa vermicelli were well accepted by the sensory panellists. The 10% incorporation of cocoa powder was acceptable in all sensory parameters except texture. The sensory score for colour, appearance, and the overall acceptability of the product varied significantly among different samples at $p < 0.05$. The overall acceptability of sample 2 (CP$_2$), which is made of 10% cocoa powder incorporation, is higher compared to the others.

CONCLUSION

About five different treatments with different concentrations of cocoa powder (0, 5, 10, 15, and 20%) were made including control (whole wheat flour). With the increase in cocoa powder levels, the fat, protein, and

ash content of vermicelli also increased significantly ($p < 0.05$). It has been observed that there was a decrease in the moisture content, which is a good indicator for shelf-life. Furthermore, the gruel solid loss and water absorption capacity increase significantly ($p < 0.06$) as the cocoa powder incorporation increases. The high antioxidant property of cocoa powder increases both the nutritional value and the antioxidant activity of vermicelli. The cocoa powder incorporation up to 10% was found to be acceptable. The optimized product with 10% cocoa powder incorporation had 0.714 g/cm^3 bulk density, 11.56 g cooked weight, 7.21 min cooking time, 0.47 g/100 g gruel solid loss, and 146% water absorption capacity. The optimized cocoa-based vermicelli was high in calories with 375 Kcal/100g of the product.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Cherry chemical composition and antioxidant activity under freezing comprehensive relations assessment

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Abstract: Cherry is a successful combination of sugars, acids, attractive color and taste. However, its shelf life is limited and can be prolonged only with the help of new freezing technologies. Therefore, the gool of this work was to investigate changes in component composition of fresh and frozen cherry. The objects of the research were cherries of the varieties of Shpanka and Lotovka. The studies were carried out with cherries grown in the Central region of Ukraine at the Department of Technology of storage and processing of fruits and vegetables at Uman National Horticulture University. For cherries of both varieties were kept in 20% sugar solution with the addition of 4% ascorutin 1% chitosan for 30 minutes, dried with air flow, frozen at –25°C, packed in 0.5 kg plastic bags, and stored at –18°C. For control purposes, nontreated cherries were packed in plastic bags of respective volume. According to the research, preprocessing with 20% sugar solution with the addition of 1% chitosan contributes to preservation of quality and biological value of frozen cherries. Thus antioxidant activity in frozen cherries of Shpanka and Lotovka varieties is 27 and 18 mmol/dm^3, ascorbic acid content – 17.6 and 20 mg/100g. So the indexes of quality of cherries for freezing are interrelated and constitute one correlation pattern in which the major index indicator is the content of dry soluble substance and antioxidant activity.

Keywords: Frozen cherries, tanning and coloring substances, ascorbic acid, antioxidant activity

INTRODUCTION

Cherry is a successful combination of sugars, acids, attractive color and taste. Formation of the chemical composition of fruits, including cherries, depends on the species, variety, degree of ripeness, growing area, weather conditions, etc. To a large extent these change food quality and nutritional value [1–5].

The total sugar content is 6.5–21.5%, of which glucose is 3.8–5.3%, fructose is 3.3–4.4%, and sucrose – 0.8%. Acidic component is 0.7–3.0%, mainly containing malic and citric acids, and in a small amount – succinic, formic, and traces of salicylic acid. Combined with sugars, acids determine the sweet and sour taste of cherries. As for vitamins, the fruit contains vitamin C in the amount of 10–50 mg/100 g, as well as vitamins B_1, B_2, B_9, and PP. Tannins in the amount of 0.05–0.34% provide the tart flavor. However, the number of useful substances is 0.8% [1–3].

Moisture regime, minimum and maximum temperatures at different periods of the tree growth are the main factors that determine the yield of cherries. The ripening conditions of fruits, including cherries,

depend on the cultivation characteristics. For example, warm weather in June and July contributes to early ripening of the berries while low temperatures with heavy rains cause later ripening of fruits [4–6].

Weather conditions, especially before harvesting, affect cultivation of fruits and the yield. Warm weather during the flowering period with temperatures above 16°C promotes the production of good flower pollinate [7].

Temperature lowering during flowering within some years to –2.4°C causes decrease in crop productivity of cherry [8]. High rainfalls reduce productivity due to cracking of the fruit. High daytime temperatures (33°–37°C) during the harvest are unfavourable [7].

Cherries quality has a varietal character and changes during the growing season. Different varieties of cherries display various reactions to change in weather conditions, including hydrothermal coefficient (HTC), especially in the last ripening period of 10–15 days before removal [22].

Increased temperatures and reduced humidity increase the content of soluble dry substances,

including sugars [10, 11]. In years with high temperatures and minimal rainfall during ripening acidity is reduced while in years with a cool and humid ripening period it is, conversely, increased [12].

Rainfalls, temperature and humidity affect accumulation of ascorbic acid. Cool weather with lots of rainfalls is one of the positive factors for accumulation of ascorbic acid [6, 13]. Temperature lowering during fructification increases its content in cherries [10].

The number of tannins in cherries varies depending on weather conditions, but less than the amount of ascorbic acid [13]. The total content of phenolic compounds depends on the temperature of growing fruits. Black cherry grown at a temperature of 25–30°C have a higher content of anthocyanins and phenols. The formation of basic phenols (anthocyanins) observed within the last few weeks before harvest contributes to the antioxidant activity [14].

Antioxidant activity most significantly correlates with the content of total phenolic compounds and anthocyanins, while ascorbic acid significantly impacts the total antioxidant activity [17].

Antioxidants determine the color of fruits and exhibit antiradical and antioxidant activities. However, during processing they are lost. We know about the 88% change of anthocyanins in cherries frozen at −23°C for six months.

Therefore, to reduce the loss of anthocyanins and the antioxidant activity, ways of enriching and adding antioxidants to food products are used. It is possible to extend storage by slowing lipid peroxidation, which is one of the reasons for food quality reduction during processing and storage [16–19].

M.S. Shaheen suggested the use of complex chitosan-fructose to prevent lipid oxidation in meat [20].

However, the period of cherry consumption is limited to the period of maturation, only 10–15 days. And it is possible to continue the period of maturation by using new technologies, in particular freezing. However, at the stage of defrosting of raw materials a significant amount of sugars and acids is lost, thus deteriorating the appearance. So before the freezing process, antimicrobial substances, antioxidants and film-forming substances are used. One of these substances is chitosan. This polysaccharide is a biopolymer that is widely used in agriculture, biotechnology, food industry and has antibacterial and antifungal properties [21–29].

According to the literature, coating based on chitosan and 5% calcium gluconate or chitosan with the addition of 0.2% tocopherol acetate reduces fruit disease and weight loss, delays discoloration, loss of density, and deterioration of texture during storage [30]. So, according to Begon a de Ancas [31], in frozen berries of raspberry treated with a chitosan solution the content of phenolic compounds by the end of the freezing decreased by 14–21%, ascorbic acid by 33–55%.

Also according to Yan Jiang et al. [32], the mixture of 3% chitosan, 0.75% glycerol, 0.25% sapn, 40.5% corn syrup, 3% pectin solution, 88% distilled water was used for pre-freezing blueberries. Thereby loss of density and juice reduced, and product quality improved.

It is also known from the literature that to calculate statistical research results is to use evaluation of appropriate dependency and statistical comparison of the variance/covariance. Correlation matrix, sometimes visualized, using correlation galaxy is among principal components of the analysis [33–38].

The goal set is to investigate changes in the composition of cherry to establish correlations between the components of fresh cherry and frozen cherry treated with chitosan solution [36, 39–42].

STUDY OBJECTS AND METHODS

The objects of research were cherries of the varieties of Shpanka and Lotovka. The studies were carried out during 2015–2016 with cherries grown in the Central region of Ukraine. The variety is zoned in the forest steppe, Polesye and Western Ukraine. For this research, cherry varieties of Shpanka and Lotovka were harvested 2–3 days before the consumer maturity stage. These conditions of cultivation affected the long ripening periods in 2015 and 2016 years.

A significant difference in growing season length of cherries in 2015–2016 (Table 1) was caused by hydrothermal conditions within 15 days of ripening before harvesting. In particular, in the last 15 days of ripening the sum of effective temperatures in 2016 was 143.2°C less than in 2015.

This happened alongside with the amount of precipitation reduction to 12.8 and 30.7 mm and with increase in HTC to 0.4 and 2.1 in 2015 and 2016, respectively.

Obviously, the total rainfall has more influence on cherries than the temperature during the growing season. Specifically, comparing hydrothermal conditions, the sum of effective temperatures during the growing season in 2016 was lower by 166°C to 2015. But rainfall was only 42.8 mm less than in 2016. While rainfall for the 15-day period of maturation in 2015 was 12.8 mm, in 2016 its index was 30.7 mm, respectively HTC 4.1 and 4.8. Thus, fruits ripen more effectively at lower precipitation and lower temperatures (2015).

Reduced amount of effective temperatures and increased precipitation at the final stage prolong the period of ripening.

Table 1. Agroclimatic indexes during the growing season of cherries (According to Uman weather station)

| Year | Growing season | The sum of effective temperatures,°C | | Rainfall, mm | | HTC | |
		growing season	phase of ripening 15 days	growing season	phase of ripening 15 days	growing season	phase of ripening 15 days
2015	89	564.2	286.8	233.9	12.8	4.1	0.4
2016	80	398.2	143.6	191.1	30.7	4.8	2.1

Table 2. The content of some components in the chemical composition of the cherries

Year	Dry soluble substances, %	Acid (in terms of malic), %	Tanning and coloring substances, %	Ascorbic acid, mg/100g	Antioxidant activity mmol/dm^3
			Shpanka		
2015	16.4	1.02	0.66	20.0	26
2016	15.8	0.72	0.68	17.2	28
Average	16.1	0.87	0.67	17.6	27
			Lotovka		
2015	15.2	1.40	1.03	18.0	17
2016	14.8	0.90	1.04	22.0	19
Average	15.0	1.15	1.04	20.0	18
NSD$_{05}$	0.7	0.70	0.50	0.5	1.0

The study of cherry varieties was conducted at the Department of Technology of storage and processing of fruits and vegetables at Uman National Horticulture University during 2015–2016. Cherries were kept in 20% sugar solution with the addition of 4% ascorutin or 20% sugar solution with the addition of 1% chitosan for 30 minutes, dried with air flow and frozen at –25°C, packed in 0.5 kg plastic bags and stored at –18°C. For control purposes, nontreated cherries were packed in plastic bags.

Contents of dry soluble substances in cherries before and after freezing were determined by refractometric method [43], acids by alkali titration (GOST 25555.0-82) [44], the content of tannins and coloring substances – by Neubauer and Leventhal methods [43], ascorbic acid – by iodometric method [43], and antioxidant activity – by FRAP [48]. Weight of the analysis material was 2 kg. The procedure was repeated three times.

By the method of correlation pleiades [39, 45, 46], the correlation between the components of the chemical composition of the cherries was determined. For this:
(1) Using modern statistical analysis software, correlation coefficients were calculated between the set attributes for a specific number of connections, whose number

$$C = N (N–1) 2,$$

where C is the number of connections; N is the number of characters, of = 5 (5 – 1) : 2 = 10.
(2) Correlation matrix of dependencies was built in which correlation coefficients significant at confidence level of p < 0.05 were extracted .
(3) Correlation ring was built in which primary connections centers were defined.
(4) Degree of dependence between the following qualitative characteristics was set:
− CSS is the content of soluble substances, %;
− TA is content of titrated acids (on conversion to malic acid), %;
− AA is ascorbic acid content, mg/100g;
− TC is the content of tannins and coloring substances, %;
AOA is antioxidant activity, mmol/dm^3.
Mathematical processing of data held by Dospyehov B.A. (1979) [47] on the PC using the programs "Excel 2000" and "Statistica".

RESULTS AND DISCUSSION

The chemical composition of the cherry (Table 2, Fig. 1) depends on climatic parameters in the last 15 days of maturation.

A high content of soluble dry matter was observed in the cherries in 2015 (15.2% and 16.4%). High temperatures and almost no rain over the 15 days of ripening contributed to this. The sum of effective temperatures within the 15 days of ripening amounted to 286.8°C which was 143.6°C more than in 2016. Over the 15 days of cherry ripening in 2015 there was little rain – 12.6 mm.

Significant reduction of dry soluble substances (14.8% and 15.8%) was recorded in cherry harvested in the years of 2016 and 2015 which were characterized by a low amount of effective temperatures, especially in the last 15 days of ripening, while rainfall rates amounted to 12.8 and 30.7 mm. This confirms the increasing value of HTC (2.1 and 1.6) in the last 15 days of maturation. As compared to 2015, in 2016 the content of dry soluble substances decreased by 4.0 and 4.2%.

The level of generalized quality indicator of fruits − dried soluble substances − significantly increased by 0.4 and 0.6% with the increase of effective temperatures to 286.8 and 143.6°C and the reduction of rainfall to 12.8 and 30.7 mm in the last 15 days of maturation.

An important component of the chemical composition of the fruits is acids. Their level in cherries is 0.72−1.4%. However, the level of acids depends on environmental indicators. Given that there was no significant difference (1.4%) between the acid content in the cherries harvested in 2015 and 2016, the increase in the fruit harvested in 2015 (1.4%) was ruled by its own laws in synthesis and accumulation.

Dry conditions during ripening and much rain influence acid accumulation and, being a synthetic material, it is rapidly accumulated. Acid content increased by 29–35% in 2016 compared with 2015 yield. Conversely, it decreased significantly in the cherry in 2016 due to the increase in rainfall. The results of research of Kaldmae et al. (2013) [6] and Chernozubenko (1993) [13] confirm that low temperatures and adequate moisture contribute to longer ripening and higher levels of acid accumulation.

Biologically active substances of cherries are represented by polyphenolic substances and ascorbic acid.

(a)

(b)

(c)

Fig. 1. Influence of factors A (variety) and B (harvesting year) on: (a) tanning and coloring substances, (b) ascorbic acid, (c) antioxidant activity of cherry.

The total content of tannins and coloring substances was consistently high averaging 0.66–1.04%. Weather conditions had no significant effect on this indicator.

The content of ascorbic acid in cherries was 17.2–22% mg/100g, changing under the influence of weather conditions during the growing season and the 15-day period of maturation. High concentrations of ascorbic acid reached 22 mg/100g in 2016 and 17.2 mg/100g in 2015. In 2015 and 2016, respectively, the decrease of effective temperatures by 1.1 and 2.3 times exceeds the rainfall by 3.4 and 2.0 times as compared to 2015, HTC reaching 0.4 and 2.1.

In 2015 and 2016 the highest amount of effective temperatures was observed, precipitation was practically unavailable in 2015. Thus, the high content of ascorbic acid in cherries correlated with conditions during ripening. Drought was extreme conditions for cherry in 2015, and the content of ascorbic acid was the lowest – 18.0 mg/100g. This confirms the results of Kaldmae et al. (2013) [6] .

Antioxidant activity of cherries depends on polyphenolic substances and ascorbic acid contents. Antioxidant activity is not only consistently high averaging 17–22 mmol/dm^3.

In order to emphasize the contribution of each factor, they were represented as the proportion of total changes attributable to each factor. For each chemical characteristic, a pie chart was used for displaying the proportion of total change that corresponds to experimental factors and interactions (Figs. 1 and 2).

The impact of each factor is demonstrated in Fig. 1 which shows the influence of the harvesting year and the varieties of the chemical composition of the cherries. The analysis of variance revealed that the main effects – harvesting year and varieties of the phase – indicated highly significant differences ($p < 0.05$) for all chemical characteristics analyzed, as shown in Fig. 1. The influence of various pre-treatment methods was the most effective. The influence of A (variety), B (harvesting year) and AB (interaction) was 13, 29.5 and 52.7% for ascorbic acid; 74.8, 2.6 and 0.4% for antioxidant activity; 10% each for tanning and coloring substances.

For the tanning and coloring substances the main effects were the harvesting year and features of the varieties. As for ascorbic acid, the statistical tests performed showed the influence of the features of the varieties (which explained 13% of the variability) and the importance of the harvesting year (29.5%). Among the most important interactions were the features of the variety, which registered 74.8% of the antioxidant capacity, while the remaining interactions altogether had less than 3%.

The contents of some components of the chemical composition of cherry before and after storage are shown in Table 3. It is seen that content of soluble dry matter in fresh cherry varieties of Shpanka and Lotovka is 16.1 and 15.0%, titrated acids – 0.87 and 1.15%, tanning and coloring substances – 0.67 and 1.04%, and ascorbic acid – 17.6 and 20.0 mg/100g. After six months of freezing, the content of dried soluble substances in processed cherries increased by 2.0–28.0% which was caused by processing in sugar solution with added chitosan or ascorutin and diffusion process.

Table 3. The contents of some components of the chemical composition of cherries

Type of treatment	Dry soluble substances, %	Titrated acid (in terms of malic), %	Tanning and coloring substances, %	Ascorbic acid, mg/100 g	Antioxidant activity, mmol/dm^3
Shpanka Before freezing					
	16.1 ± 0.2	0.87 ± 0.02	0.67 ± 0.02	17.6 ± 0.2	27 ± 2
After freezing:					
Without treatment (control)	16.1 ± 0.3	0.86 ± 0.03	0.51 ± 0.03	11.0 ± 0.2	23 ± 3
20% sugar solution with 4% ascotutin	16.4 ± 0.3	0.80 ± 0.02	0.60 ± 0.02	11.0 ± 0.3	24 ± 2
20% sugar solution with 1% chitosan	20.0 ± 0.2	0.75 ± 0.02	0.65 ± 0.01	17.6 ± 0.2	27 ± 1
Lotovka Before freezing					
	15.0 ± 0.2	1.15 ± 0.02	1.04 ± 0.02	20.0 ± 0.2	18 ± 2
After freezing					
Without treatment (control)	15.0 ± 0.1	1.14 ± 0.02	0.88 ± 0.01	13.3 ± 0.2	14 ± 1
20% sugar solution with 4% ascotutin	15.4 ± 0.2	1.08 ± 0.02	0.96 ± 0.02	13.4 ± 0.3	15 ± 2
20% sugar solution with 1% chitosan	9.2 ± 0.1	1.00 ± 0.03	1.03 ± 0.03	20.0 ± 0.1	18 ± 1
NSD$_{05}$	0.4	0.20	0.40	0.6	0.4

Obviously, the content of titrated acids also changed. In particular, in the control batch as compared to the fresh fruits these contents remained almost unchanged. In the cherries processed by 20% sugar solution with the addition of 4% ascorutin it decreased to 7% and those processed by 20% sugar solution with the addition of 1% chitosan to 13%. Apparently, this is due to osmotic processes and diffusion of solutions.

The content of tannins and coloring substances in frozen cherries decreased slightly. After a six months of freezing, it decreased by 23 and 15% in the control variant of cherry varieties Shpanka and Lotovka while in the processed cherries, compared to the fresh ones, it decreased by 3 and 10%. Obviously, for tanning and coloring substances preservation pre-treatment before freezing is preferable. Confirming the results of research of Scibisz et al. [35], it is shown that the loss of phenolic compounds in cherries after six months of freezing amounted to 20–50%.

The content of ascorbic acid in the control variant, compared to fresh cherries varieties decreased by 37.5 and 37.9 %, except for the cherries processed with 20% solution of sugar with the addition of 1% chitosan, in which the content of ascorbic acid remained the same as in fresh cherries. This confirms the results of Begon a de Ancos' research [31], according to which after freezing the content of ascorbic acid is reduced by 33–55%.

Antioxidant activity of Shpanka and Lotovka cherry varieties is caused by fruit tannins and coloring substances and averages 27 and 18 mmol/dm^3 for fresh cherries, decreasing after freezing by 11−22%. It remained the highest for cherries processed with 20% solution of sugar with the addition of 1% chitosan, which is confirmed by the research of Scibisz et al. [35] stating that freezing does not result in significant losses of antioxidant activity.

The impact of each factor is shown in Fig. 2 that shows the influence of various pre-treatment methods and varieties after freezing. The influence of various pre-treatment methods was the most effective.

(a)

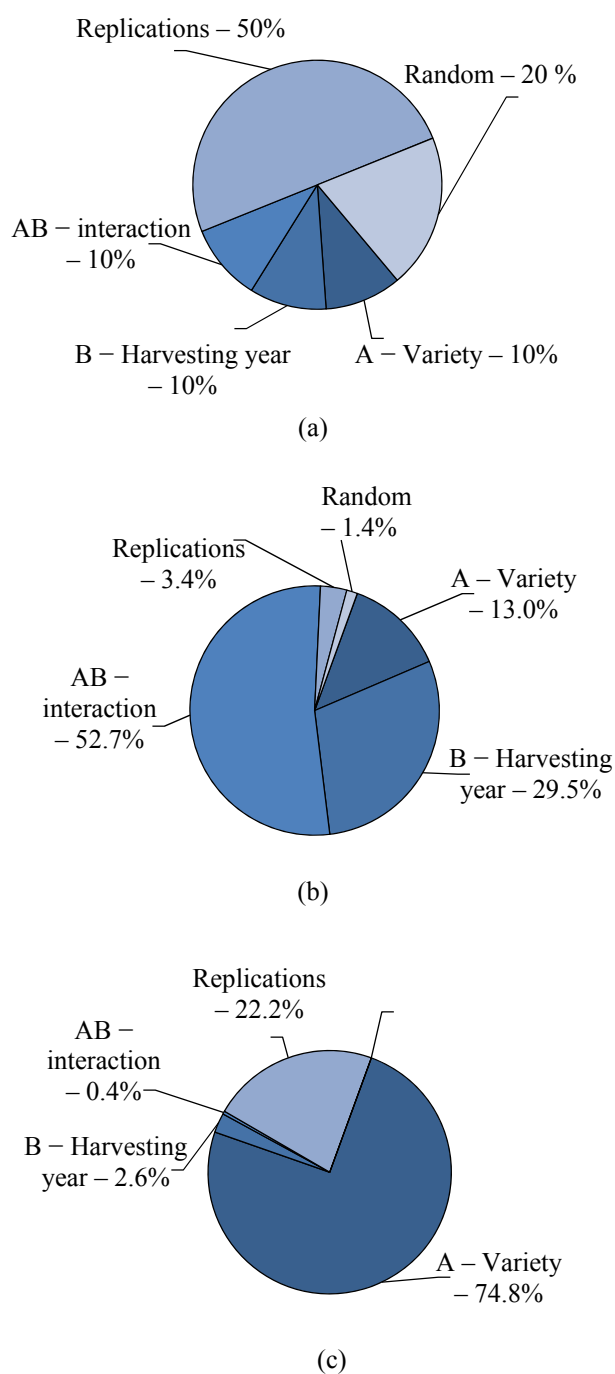(b)

(c)
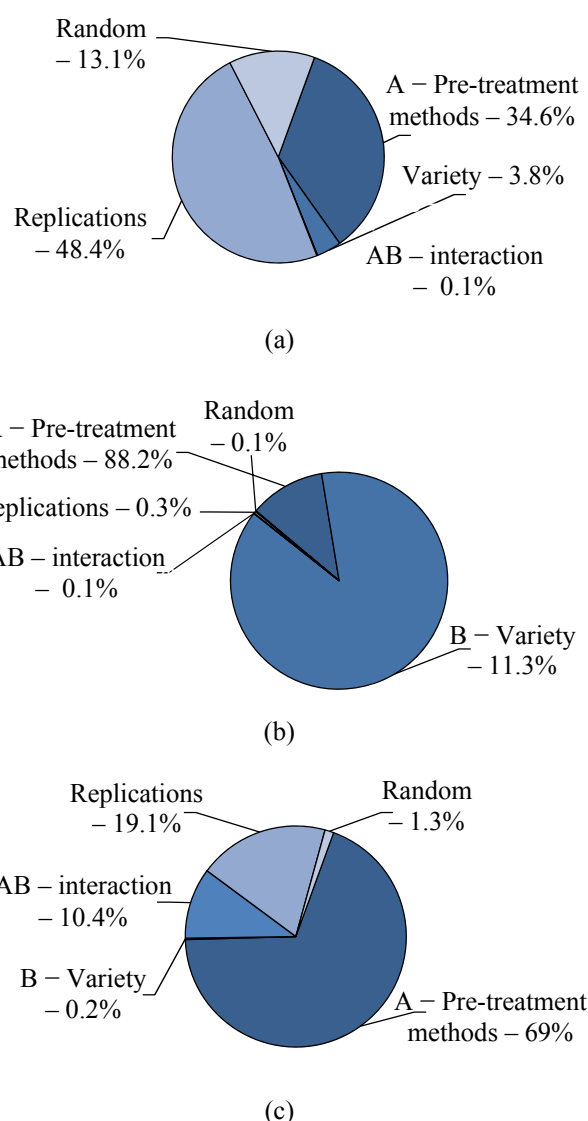
Fig. 2. The share of influence of factors A (pre-treatment methods) and B (variety) on: (a) tanning and coloring substances, (b) ascorbic acid, (c) antioxidant activity of cherry.

Table 4. The matrix of pairwise correlations between some parameters of the chemical composition of frozen cherry

Indicator	Dry soluble substances	Titrated acid	Tanning and coloring substances	Ascorbic acid	Antioxidant activity
			Shpanka		
Dry soluble substances	1	**0.87**	0.40	0.55	0.55
Titrated acid	**0.87**	1	–0.30	–0.21	–0.29
Tanning and coloring substances	0.40	–0.30	1	**0.85**	**0.93**
Ascorbic acid	0.55	–0.21	**0.85**	1	**0.98**
Antioxidant activity	0.55	–0.29	**0.94**	**0.98**	1
			Lotovka		
Dry soluble substances	1	**0.87**	–0.47	0.60	–0.58
Titrated acid	**0.87**	1	–0.37	–0.29	–0.36
Tanning and coloring substances	–0.47	–0.37	1	**0.90**	**0.97**
Ascorbic acid	0.60	–0.29	**0.90**	1	**0.98**
Antioxidant activity	–0.58	–0.36	**0.97**	**0.98**	1

(a)   (b)
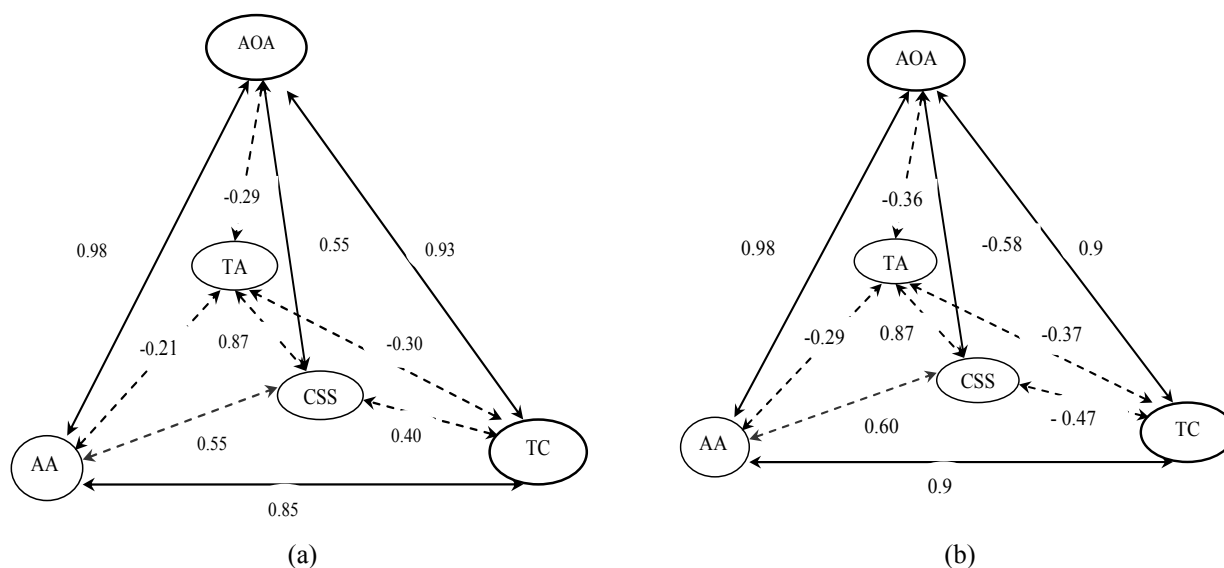
Fig. 3. Correlation galaxy of relationship between the content of dry soluble substances (CSS), titrated acids (TA), tanning and coloring substances (TC), ascorbic acid (AA) and antioxidant activity (AOA) in cherry varieties of (a) Shpanka, (b) Lotovka during freezing.

The analysis of variance revealed that the main effects of the pre-treatment methods and varieties phase indicated significant differences ($p < 0.05$) for all studied chemical characteristics as shown in Fig. 2.

The influence of A (pre-treatment methods), B (variety) and AB (interaction) was 34.6, 3.8 and 0.1% for tanning and coloring substances; 88.2, 11.3 and 0.1% for ascorbic acid; 69.0, 0.2 and 10.4% for antioxidant activity.

Among the most important interactions were the pre-treatment methods which registered 34.6% of the tanning and coloring substances while the remaining interactions altogether had less than 4%.

The experimental factor of the pre-treatment methods explained 88.2% of the total ascorbic acid, indicating, as expected, the high significance of pre-treatment methods on the ascorbic acid content. For the antioxidant capacity the main effects had the pre-treatment methods and varieties. These positions explain 69% and 0.2%, respectively, indicating the particular significance of the pre-treatment methods while the remaining interactions contribute less than 10.4%.

For the determined correlation coefficients of the chemical composition of cherry see Table 4.

To study the relationship between these components of the chemical composition in cherry varieties of Shpanka and Lotovka after freezing, the correlation ring (Fig. 3) was built by means of correlation pleiades [39, 45, 46].

The figure shows the relations of cherry quality indicators. Thus, the content of dry soluble substances has inverse and high dependence on acid content ($r = –0.87$) and average dependence on ascorbic acid ($r = 0.55$ and $r = 0.60$) and tanning and coloring substances ($r = 0.4$ and $r = –0.47$).

And it is natural because mainly acids, tannins and coloring substances and ascorbic acid were involved in the formation of the chemical composition of dry soluble substances. Besides, the content of dry soluble substances is a significant indicator of antioxidant activity.

There is a strong correlation dependence between antioxidant activity, content of tannins and coloring substances ($r = 0.93$; $r = 0.97$), ascorbic acid ($r = 0.98$), and dry soluble substances ($r = 0.55$; $r = −0.58$). Such dependence is apparently connected with processes of deterioration and transformation, which affect cherries during freezing and storage.

CONCLUSION

Thus, the quality indexes of cherries during freezing are interrelated and constitute one correlation galaxy in which the main indicators are the content of dry soluble substances and antioxidant activity.

The content of dry soluble substances is determined by the content of acids, while the content of ascorbic acid, tannins, and coloring substances determines antioxidant activity of raw materials.

According to the research, preprocessing with 20% sugar solution with the addition of 1% chitosan contributes to preservation of the quality and biological value of frozen cherry. Herewith antioxidant activity in frozen cherry varieties of Shpanka and Lotovka is 27 and 18 mmol/dm^3, ascorbic acid content is 17.6 and 20 mg/100g, respectively.

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Prospects of using extremely low doses of physical factors impact in food biotechnology

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Abstract: The paper deals with urgent issues of development of resource-saving methods of biotechnological processes intensification. The main purpose of the work is to show the efficiency of using extremely low doses of physical factors impact in food biotechnology. Acoustic and electric treatment was in various modes. Impact capacity did not exceed 10^{-4} W/kg. The duration of exposure ranged from 5 to 15 min. Barley grains enzymes and lactic acid microorganisms were subjected to treatment. Impact of vibration as a physical factor, its frequency is considered as a priority in controlling growth and biochemical processes in biological objects. Impact frequency in the range of 50–10000 Hz influences the activity of hydrolytic enzymes in bimodal way. It is presented in the article. The coincidence of the frequency ranges of the maximum activity of enzymes in the model reactions with the ones in the grain of barley under the treatment of alternating current and sound is noted. Improvement of all indexes of germinating barley and improvement of the quality of the finished malt were observed in these ranges. Low-intensity acoustic treatment at a frequency of 2000 Hz contributed to an increase in β-galactosidase activity of the *CT-95 Str. thermophilus* strain. Selected *ST-95 Str. thermophilus* strain was used in the starter composition for the experimental sample production of fermented melted milk. The fermentation process intensification was observed. The lactose content of this product is 30% less in comparison with the feedstock. The use of traditional starter without a selected strain can reduce the lactose content by an average of 7.5%. The results of biotechnological processes modification by means of the treatment of enzymes and extremely low doses of physical factors impact used in meat technologies are observed. Thus, the prospects of using low-intensity physical effects of vibration in the development of innovative food technologies are substantiated. It is also promising to use these technologies in making absolutely new food products with different qualities.

Keywords: Food biotechnology, biotransformation, hydrolytic enzymes, barley grain, lactic acid microorganisms, the impact of physical factors

INTRODUCTION

It is believed that physical factors to a large extent influence the life activity of biological objects [1], including biochemical processes in them. In nature, such factors are temperature, light, and pressure. In the food industry there is an approach to use electro-physical methods while processing raw materials and food products. These methods are characterized by the lack of inert work of the equipment, by reduction of duration of processing objects and high efficiency of energy use. The electro-physical factors include the impact of electromagnetic fields (EMF) of high-frequency (HF), ultra-high-frequency (microwave) and infrared (IR) bands, magnetic fields, electro-contact (EC) processing methods, electrostatic processing methods, pulse processing methods, ultrasonic exposure, pulsation and vibration processing methods, the use of laser, ultraviolet (UV) processing, radiation ionization of materials. The choice of impact factor is determined by the goals and objectives. One of the main processing parameters is the power of the applied effect – W. Electrochemical cleaning, electric separation, electroplating, electro-deposition, dispersion, emulsification, homogenization, bringing the food product to readiness using microwave and IR heating are widely used and carried out at relatively high specific power ($W_{s.p.}$ = 1.5–2.0 kW/kg) [2, 3].

In the works of scientific schools (at the head of academicians Devyatkov N. D. and Rogov I. A.) the bio effects such as changes in cell morphology,

metabolic processes, enzyme activity, growth rate, increase or decrease in survival, change in functional processes in biological objects are highlighted. These effects become manifested when living systems are subject to EMF [3, 4]. To implement the biological effects of physical factors, an important parameter is the dose of exposure. Numerous experiments described in articles show that biological objects react to physical impact even at the minimum applied power and significant effects are observed at a very low-intensity exposure. In scientific works this issue is given much less attention than to the thermal effects. The interpretation of the results of the biological effects of physical factors is diverse, due to the complex structure of biological systems both containing a large number of negative feedbacks that can support homeostasis and trying to neutralize the external influence [5–7]. As a rule, bio effects are shown at $W_{s.p.} \leq 10^{-3}$ W/cm^2, when increase in temperature of the object by more than one degree is excluded, and these effects do not depend on the intensity of the impact up to those $W_{s.p.}$ values at which the thermal effect becomes noticeable [3]. At this stage of development of science and technology, scientists are forming a new scientific approach to the use of ultra-small doses of various physical and chemical effects to intensify the life of biological systems [8–15].

The purpose of this work is to show the efficiency of extremely low doses of different physical factors impact on biochemical processes in biological objects and to justify the prospects of using these effects while processing food raw materials and obtaining food with improved quality.

STUDY OBJECTS AND METHODS

Barley grain as the main biological object was taken for germination to malt. The significance of this object for research is due to the value of its biochemical composition: amino acid-balanced protein (8–12%), starch (62–68%), rough fiber (up to 5.2%), minerals (2.9%), lipids (2.4%), ashy substances, mucus, enzymes, different vitamins. Barley sprouts are also valuable, and have healing properties due to the activity of vitamins (C, B$_{12}$, K and provitamin A) in their composition, as well as the content of trace elements (Zn, Cu, Mn, Fe, etc.). In addition, barley is one of the main products for obtaining malt and necessary for the production of whiskey and beer. That fact says about the importance of the obtained scientific results for practical purpose. A promising scientific approach to the food industry is the use of barley malt in the production of various starchy food, confectionery, bakery and culinary products. Barley varieties ("BIOS", "Ryadovoy", "Scarlet", "Odessa-115") with different germination capacity were used in the research. The object of the study was also malt obtained from the barley of above mentioned varieties.

To model enzyme systems, the following enzyme preparations were used in the scientific research:
(1) "Amilosubtilin G 10X", having amylase activity; the starch is applied as a substrate.

(2) "LAMINEX®BG Glukanase Complex", which has cytolytic activity; wet mass of WF 200 wheat fiber is applied as a substrate.
(3) "Kazarin and bovine Pepsin", which has proteolytic activity; the sodium Caseinate is used as a substrate.

The objects of the experiments on finding out impact of physical factors of low intensity on the properties of microorganisms were strains of thermophilic lactic streptococcus (*Streptococcus thermophilus*) from the collection of microorganisms of the Moscow State University of Food Production, registered in the all-Russian collection of industrial microorganisms (*ST-9 Str. thermophlus, ST-13 Str. thermophlus* (VKPM V-9647), *ST-14 Str. thermophlus, ST-138 Str. thermophlus* and *ST-95 Str. thermophlus* (VKPM V-7985)). These cultures are producers of the enzyme β-galactosidase and are widely used in starter cultures for many types of dairy products such as curds and sour cream, various types of sour milk, yogurt, pro- and synbiotic products.

Barley was germinated in funnels in accordance with the laboratory version of the industrial malting process. It includes that is soaking for 4 hours then air pause for 16–18 hours, the next stage was soaking again for 4 hours and then germination in a humid atmosphere for 5 days. In experimental germination the physical factors impact on the grain both after the first and after the second soaking was carried out. The methods widely used in the technology of producing malt and beer were applied for analysis of barley growth and the activity of hydrolytic enzymes [16]. The ability of grain germination ($E_{ability}$), amylolytic activity (AA), proteolytic activity (PA) and the degree of grain endosperm dissolution (DD), which varies by the combined action of proteolytic and cytolytic enzymes, were determined. $E_{ability}$ was estimated as a percentage of the proportion of seeds sprouted on the fifth day. The value of AA was determined by the Windisch-Kolbach method, the value of PA – by the Anson method, DD – refractometrically. Different indexes of malt obtained in the experimental germination were evaluated in relation to the same indexes obtained in the control germination. Indexes of the control sample were taken as 100%.

In studying the impact of physical factors of low intensity on the life of thermophilic lactic streptococci, the following methods of identification, control and application of probiotic and lactic microorganisms were used [17, 18]:
– Control of the strain and starter cultures for purity was performed by microscopy of the stained smear of bacterial suspension.
– Quantitative accounting of lactic acid microorganisms was carried out in accordance with GOST 10444.11-89 immediately after application of the ferment (zero point) and after fermentation made by limit dilutions in sterile physiological solution. The nutrient medium was sterilized skim milk.
– Active acidity (pH) of dairy raw materials and fermented baked milk was determined according to GOST 26781-85 using pH meter (pH meter mark-150 MA) with the help of instructions attached to it.

– Titratable acidity of raw milk and fermented baked milk was determined according to GOST R 54669-2011 by titration with sodium hydroxide solution.

– The rate of fermentation was determined by fixing the time of clot formation, which characterized the activity of curdling milk by the studied ferment.

– Water-holding capacity of the clots generated in the fermented baked milk was determined by centrifugation method similar to the determination of water-holding capacity of food fiber.

– To check the purity of the culture, an ultra-small preparation was made.

– β-galactosidase activity of lactic acid bacteria was estimated by their acid-forming rate in milk calculating the amount of lactic acid formed during fermentation.

As the factors of physical impact, EC processing was used when passing through the object of direct current, industrial frequency current and alternating current with a frequency of 50 to 10,000 Hz, as well as acoustic treatment of the object in the same frequency range. Specific frequencies from 50 to 20000 Hz were chosen for the experiment, starting with the power frequency of 50 Hz, and then 100, 200 ... with a step-by-step increase in the frequency of 2–2.5 times while maintaining the power and duration of exposure. In the experiments with alternating current treatment, the duration of exposure was 15 minutes, and in the experiments with acoustic treatment it was 5 minutes. Power impacts did not exceed 10^{-4} W/kg. The EC method and acoustic treatment were used because these methods are simple and convenient for energy generation.

RESULTS AND DISCUSSION

All the experiments were carried out 4–5 times. Processing of experimental data was carried out using the method of mathematical statistics. The standard deviation of the directly measured value was calculated as follows:

$$\Delta x_i = \sqrt{\frac{\sum_{j=1}^{N}\left(x_i^j - \bar{x}_i\right)^2}{N(N-1)} + \sigma_{cons.}^2}, \qquad (1)$$

where x_i is the measured quantity; N – number of direct measurements; j – serial number of direct measuring; $\sigma_{cons.}^2$ the magnitude of the consistent error is the sum of both the instrument error and the round-off errors in the measurements on the instrument scale. The latter can be estimated as $\sigma_{round}^2 = u/\sqrt{12}$, where u is the finest reading. This is justified by the main theorem of calculus of probability.

The average value of the indirectly measured quantity was calculated as

$$\bar{f}(\{x_i\}) = f(\{\bar{x}_i\}), \qquad (2)$$

a mean square deviation of the indirectly measured value was

$$\Delta f(\{x_i\}) = \sqrt{\sum_{i=1}^{M}\left(\frac{\partial f}{\partial x_i}\bigg|_{x_i} \Delta x_i\right)^2}. \qquad (3)$$

The results show that the impact of direct current on the moistened grain in the embryo with "+" polarization causes the concentration of negatively charged ions there, which contributes to the activation of the embryo and favorably affects the $E_{ability}$ of barley of different quality. A typical dependence of $E_{ability}$ value of barley grain from the duration of EC treatment under conditions of strict polarization of the grain embryo is adaptive in nature. At first a sharp change in the indicators happens in the positive or negative way relative to the control value, depending on the sign of polarization of the embryo, and then a gradual change of its value towards the control value takes place, that is there is a primary activation effect ("shock reaction") on the impact of external irritant factor with subsequent adaptation (Fig. 1). The activity of enzymes can increase both at "+" and "–" polarization in the embryo, but at "+" polarization the effects are more significant (Fig. 2). Changing from constant to pulsating mode of passing current through the moistened grain while maintaining the sign of polarization of the embryo leads to a decrease in both positive and negative effects, and as for the $E_{ability}$ value, it is negative on the whole. At the same time, the maximum increase in $E_{ability}$ value relative to control value is 3–10% depending on the quality of barley grain, while the enzyme activity can increase 2–2.5 times (Figs. 1 and 2). It says that the oscillatory nature of the impact has a greater effect on the activity of enzymes than the polarization of the embryo, that is associated with a characteristic feature of biological systems (oscillatory processes).
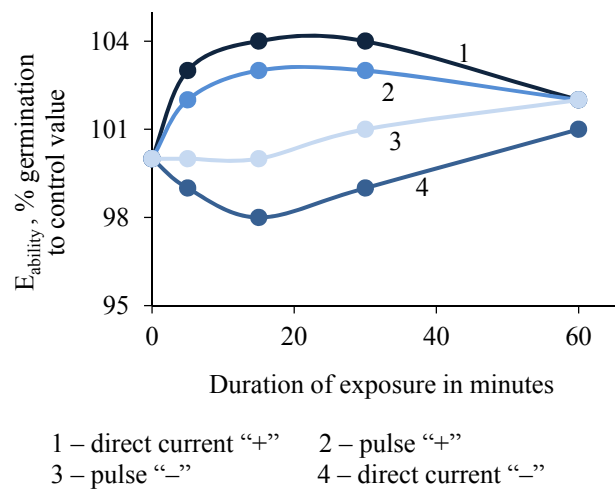

1 – direct current "+"   2 – pulse "+"
3 – pulse "–"   4 – direct current "–"

Fig. 1. Dynamics of changes in the ability of barley germination depending on the parameters of EC treatment with unidirectional current.

1 – direct current "+"   2 – pulse "+"
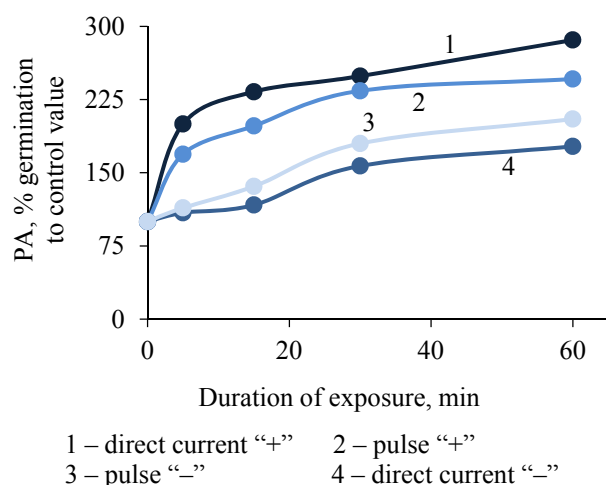3 – pulse "–"   4 – direct current "–"

Fig. 2. Dynamics of changes in the proteolytic activity of barley malt depending on the parameters of EC treatment with unidirectional current.

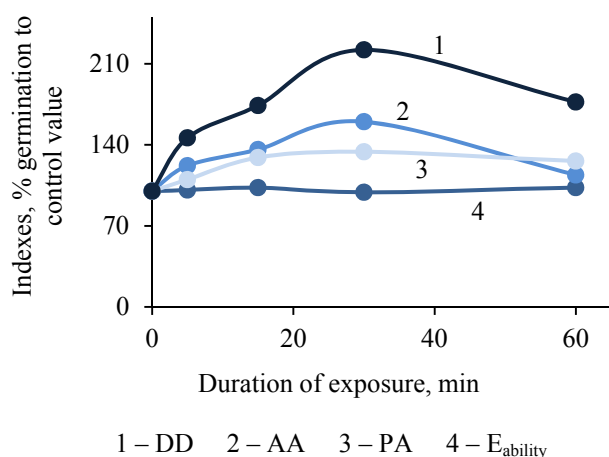1 – DD   2 – AA   3 – PA   4 – $E_{ability}$

Fig. 3. Indexes of sprouted barley after treatment with alternating current of industrial frequency.

EC treatment of moistened barley grains by alternating current of industrial frequency allows realizabion of external steady impact of the given power at fluctuations of values of amplitude from maximum positive to maximum negative-going. The results of the experiments show that while using alternating current, when there is no unidirectional movement of particles inside the biological object, there is no decrease in germination (Fig. 3).

Figs. 1 and 2 use the following symbols: direct current "+" is the impact of unidirectional direct current with positive-going polarization of the grain embryo; direct current "–" is the impact of unidirectional direct current at negative-going polarization of the grain embryo; pulse "+" is the impact of unidirectional pulsating current with positive-going polarization of the grain embryo; pulse "–" is the effect of unidirectional pulsating current at negative-going polarization of the grain embryo.

From the point of view of increasing the number of germinated seeds, EC treatment with current of industrial frequency has no apparent advantage, but in terms of enhancing enzymatic activity is much more effective than EC treatment with direct current. The

favorable influence of the vibration treatment the efficiency of enzyme systems can prove that the germinating grain has self-oscillatory modes of activity. This fact conform sto the literature data [19–24]. In addition, on an industrial scale, it is impossible to implement conditions that ensure strict polarization of the embryo of each grain, while AC processing gives a specific technology solution and array of process vessels.

It is common knowledge that the main purpose of germination of barley to malt is the accumulation of amylolytic enzymes in the grain, that allows the use of barley malt not only for beer, but also in the processes of saccharification of vegetable raw materials. However, when malting, each enzyme plays an important role and good quality of the finished malt can be obtained with their full interaction. To convert as much malt extract as possible into a solution, combined action of hydrolytic enzymes, such as amylases, proteases, and citas, is significant. One of the main results of malting is a change in grain structure because of the combined action of cytolytic and proteolytic enzymes that dissolve the cell walls of the grain endosperm. Cell walls are formed mainly by hemicellulose and gummi substances. Hemicellulose and gum substances consist of 80–90% of β-glucan and 10–20% of pentosans, but they have different solubility in hot water due to the large difference in the molecular weight [25]. The composition of cell walls also includes protein (5%) and insoluble fiber (cellulose) (2%). Enzymes of the cytolytic complex hydrolyze non-starch polysaccharides of the cell walls, resulting in the formation of a framework permeable to amylolytic and proteolytic enzymes.

The role of proteolytic enzymes is not only in the cleavage of high-molecular proteins to amino acids, which are necessary for the development of the embryo and for the synthesis of enzymes, but also in the hydrolysis of protein substances, firmly connected to the walls of endosperm cells. Thus, the process of dissolution of the cell walls of starch grains is facilitated by enzymes cytolytic complex.

The action of amylolytic enzymes appears mainly in mashing, and in the process of germination starch is hydrolyzed by amylases slightly. Germination is stopped when the accumulation of amylolytic enzymes in the grains becomes maximum.

After EC treatment while malting the improvement of dissolution of the grain endosperm makes the quality of malt higher. When mashing such malt, wort viscosity decreases and its filtration facilitates.

Being self-oscillatory in nature, the main biochemical processes in biological objects determine the possibility of regulation of the main indicators of germinating barley and malt obtained from it by varying the frequency of the given power. Thus, the effect of current frequency on barley malting was studied as the next phase of this work.

Figs. 4 and 5 show comparative diagrams of the effect of low-intensity EC treatment on the change of the efficiency of the hydrolytic enzymes main groups at different stages of the malting process. The results are presented for the entire range of investigated current frequencies.
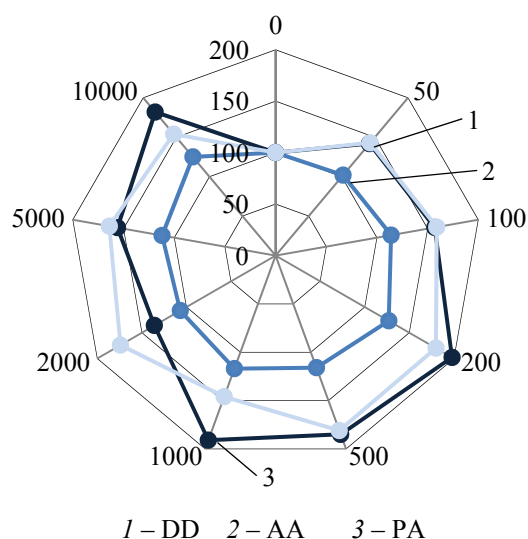
1 – DD   *2* – AA   *3* – PA

Fig. 4. Comparative diagram of the efficiency of hydrolytic enzymes of malt obtained using EC method of barley grain processing after the first soaking.

1 – DD   *2* – PA   *3* – AA
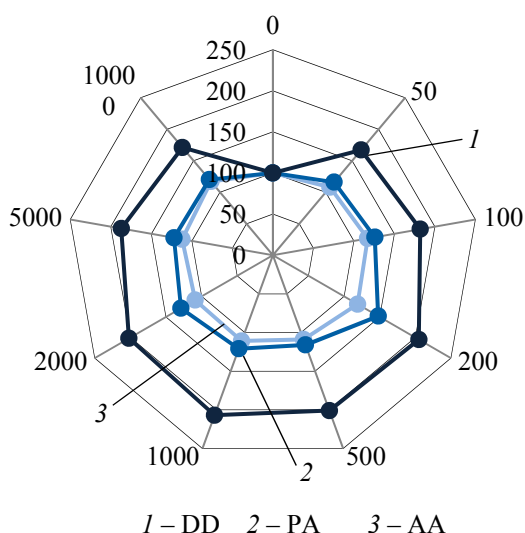
Fig. 5. Comparative diagram of the efficiency of hydrolytic enzymes of malt obtained using EC processing of barley grain after the second soaking.

The analysis of the comparative diagrams shows that as a result of the given power, the activity of proteolytic and cytolytic enzymes increases in an advanced way. Under the same modes of EC treatment, the activity of amylases increases by 1.1–1.2 times, and the activity of the combination of proteolytic and cytolytic enzymes increases by 1.5–20 times relative to the control value. Similar results were obtained in the study of EC exposure using an industrial frequency current in a time interval of 5–60 min (see Fig. 3).

Thus, low-intensity EC treatment of moistened grain mass of barley with alternating current is an effective way to improve the quality of barley malt. The frequency of the current is an important parameter of impact. If one change it, it is bossible to control the growth and biochemical processes in the germinating grain.

To confirm the priority role of the oscillatory nature of the physical effects that increase the growth and activate biochemical processes in biological systems, a physical factor of a different nature such as steady acoustic vibrations was chosen. They being physical entities are mechanical vibrations in elastic medium. Low-intensity acoustic treatment can be implemented using acoustic (sound) waves. This is interesting from both a scientific and practical point of view. In contrast to the EC impact, acoustic treatment of barley for germination to malt can be carried out before soaking (dry-air mode). It is an effective and fairly simple method of processing in production conditions.

After low-intensity sound processing, similar to AC treatment, the change in enzyme activity is much higher than the change in $E_{ability}$. Proteolytic enzymes show the greatest sensitivity to the effects, their activity in some modes of treatment increases by 2–3 times (Fig. 6). Depending on the frequency of exposure and quality of grain AA can be increased relative to the control value to 142 %, PA – up to 299 %, DD – up to 229 %.

Fig. 7 shows the growth and enzyme efficiency of barley after low-intensity acoustic impact on moistened grain mass. A comparison of the parameters is given for the frequency mode in which no negative effects were observed. It can be seen that the change in enzyme activity is much higher than the change in $E_{ability}$, and proteolytic and cytolytic enzymes react to the given power is much stronger than amylolytic ones do. This is in accordance with the results obtained while processing dry grain under the same conditions, as well as the results of low-intensity EC processing of raw grain. The characteristic feature is the bimodal nature of the dependence of different indexes of the obtained malt on the frequency of the used exposure.

On the basis of the obtained results, technological schemes, array of process vessels of continuous processes for producing high quality barley malt using low-intensity, low-energy EC and acoustic treatment were substantiated from the scientific point of view and worked out. Experimentally established dependences can be used not only in making innovative technologies of malting, but also in technologies for producing sprouts of different cultures for use in human nutrition as a source of active enzymes, a complex of mineral and organic substances, various vitamins, including antioxidant vitamins [26].
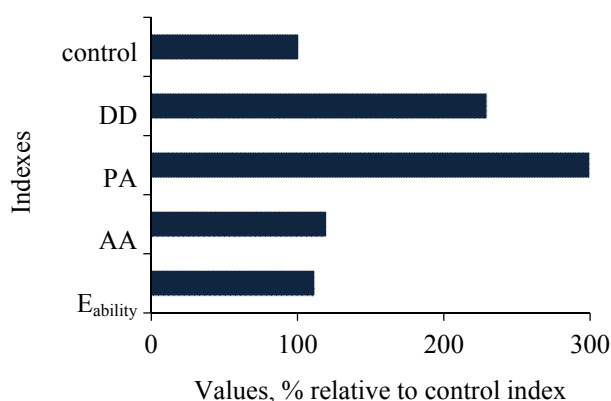
Fig. 6. Comparative characteristics of barley sprouted after acoustic impact on dry grain at f = 5000 Hz.
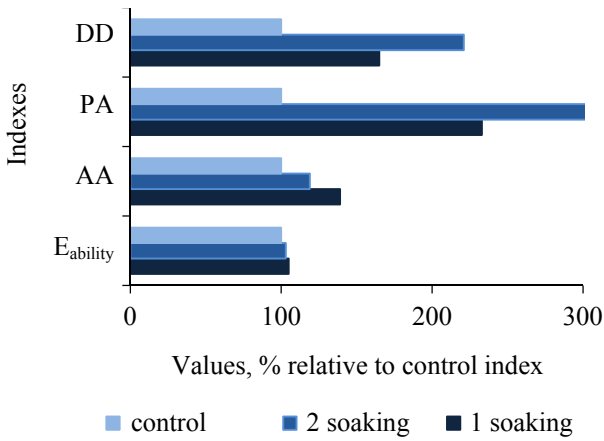
Fig. 7. Comparative characteristics of barley parameters after acoustic impact on raw grain at f = 10000 Hz.

technological properties was obtained, the addition of that in minced meat providesd an increase in the quality of the finished product, and also allowed more efficient use of expensive meat raw materials due to its greater yield [27]. A method of applying the combined action of proteolytic enzymes and low-intensity acoustic treatment to modify the properties of tough meat raw materials and meat raw materials with a high content of connective tissue was proposed. This processing led not only to the improvement of consumer goods properties of the finished product, but also to an increase in its yield. In addition, the new type of impact was easily integrated into standard technologies [19].

To correlate the vibrational nature of biochemical reactions with the frequency of the given power, it was interesting to study the impact of low-intensity physical effects on the efficiency of enzyme preparations in model experiments of hydrolysis of biopolymers. In experiments with model enzyme systems, as well as in experiments with barley grains, the bimodal nature of the dependence of enzyme activity on the frequency of the given power was observed (see Figs. 8 and 9). They are a stable maximum biological effect of physical factors at a frequency of f = 200 Hz and diverse effects in the frequency range of 1000–5000 Hz.

The obtained results became the basis of the scientific concept of a fundamentally new method of modification of biotechnological processes by the combined action of enzymes and extremely low doses of physical factors impact [19, 27]. The combined action of cytolytic enzymes and current modified the properties of wheat fiber "Vitacel WF 200". On the basis of this, a filler with improved functional and

Fig. 8. Impact of current frequency on amylolytic activity: (a) enzyme preparation of Amilosubtilin G10H (substrate is starch), (b) barley malt.
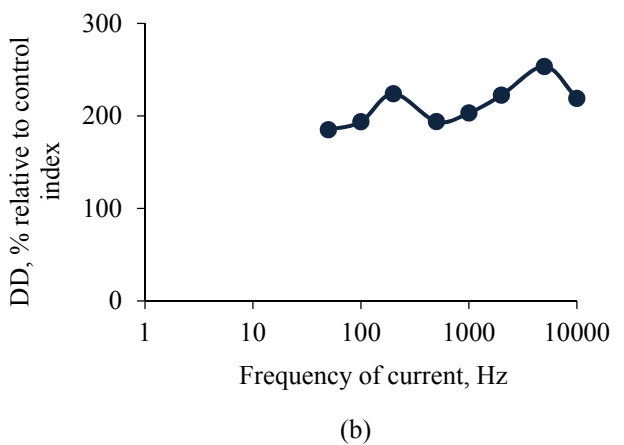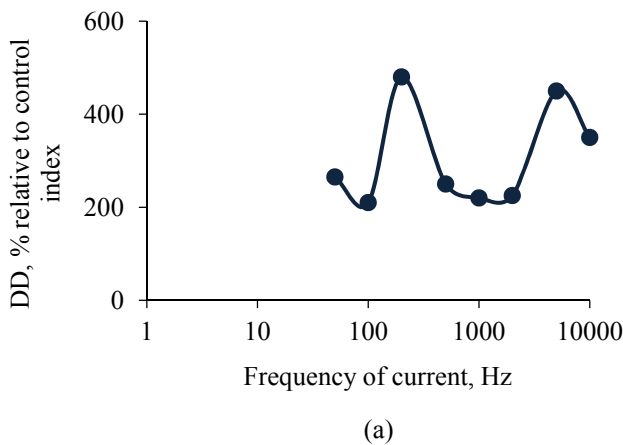
Fig. 9. Impact of current frequency on cytolytic activity: (a) preparation of LAMINEX®BG Glukanase Complex of enzymes (substrate is WF 200 wheat fiber), (b) barley malt.
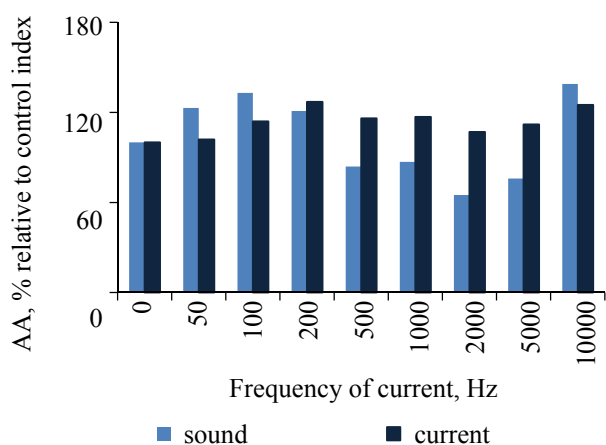
Fig. 10. The effect of frequency of physical forces on efficiency of barley malt amylolytic enzymes.

Fig. 11. The effect of frequency of physical forces on efficiency of barley malt proteolytic enzymes.

In the experiments concerning the current impact on barley grain in the frequency range of 50–10000 Hz, when the energy of the quantum (E_Q) of vibrational and wave effects did not exceed 10^{-10} eV and the thermal effects were minimized, we observed the analogy of the biological action of EC and acoustic treatment such as the maximum activity of hydrolytic enzymes using frequencies of 100–200 Hz and 10000 Hz (see Figs. 10, 11). These results allowus to come to the conclusion that the biological effect of the physical factors of oscillatory nature depends mainly on the variable component of the impact, its frequency. The results presented in this paper conform to the results of other scientists who conducted research on biological systems and confirmed the fundamental role of vibrational processes in the life of biosystems [21, 28–31].

The coincidence of the two frequency ranges of the maximum activity of enzymatic hydrolysis processes in the model reactions and the maximum increase in barley growth under the impact of alternating current and acoustic treatment led to the conclusion about the main role of the structural resonance of the active key hydrolytic enzyme with its two own vibration frequencies and offered a qualitative physico-chemical model of enzymatic processes sensitive to external low-intensity physical forces having vibrational nature.

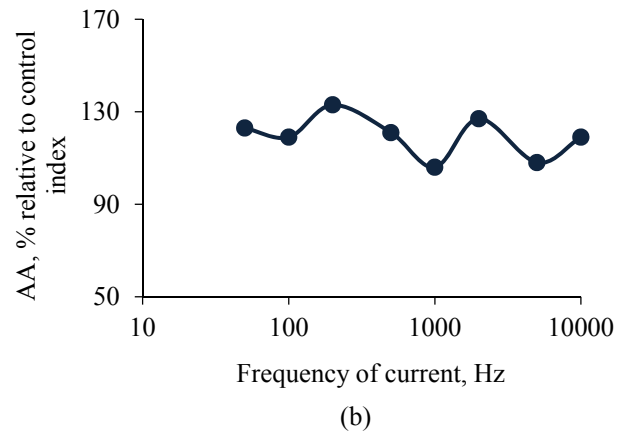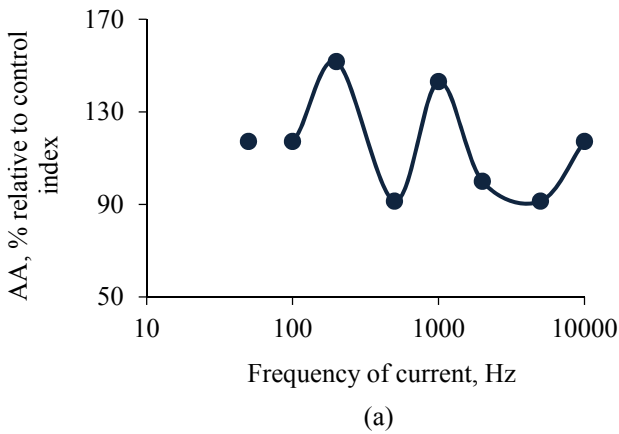The role of the frequency factor was confirmed in the experiments on the modification of the lactose fermentation process when using extremely low doses of acoustic exposure. Two strains were studied such as *ST-13 Str. thermophlus* and *CT-95 Str. thermophlus*, which had the highest and lowest β-galactosidase activity, respectively. Technological properties of strains were determined by experimental and control samples during fermentation of milk. Control samples were not subjected to acoustic treatment.

The results showed that both single and double acoustic treatment of the *CT-13 Str. thermophilus* strain at different exposures in the frequency range 100 Hz – 5 000 Hz had virtually no effect on the acid-forming ability and enzyme efficiency, but the *CT-95 Str. thermophilus* strain showed a significant difference in the change of these parameters in comparison with the control samples (Fig. 12). The strain subjected to low-intensity acoustic treatment at a frequency of 2000 Hz for 5 minutes (selected strain) had the greatest index of lactose-fermenting activity, that was the most active acid and gave the greatest increase in the quantity of cells.
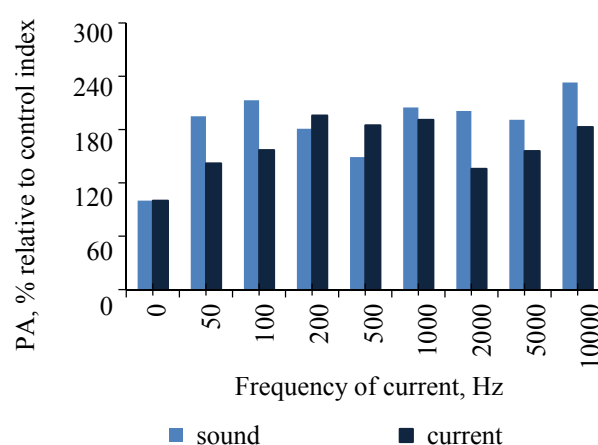
The Selected *ST-95 Str. thermophilus* strain with high β-galactosidase activity was used in the experiments to obtain fermented baked milk with low percentage of lactose. In this case, the control samples were the samples of milk obtained using the model of symbiotic starter consisting of *Str. thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus.* Experimental samples of fermented baked milk were obtained using the selected *ST-95 Str. thermophilus* strain, and *Lactobacillus* delbrueckii subsp. Bulgaricus in the ratio of 4:1.

Fig. 13 shows the dynamics of changes in the active acidity in the process of fermentation of baked milk using a typical and experimental starter. The analysis of the results indicates a more intensive fermentation process in the period of first 4 hours after the introduction of the experimental starter into the baked milk. In addition, in the experimental samples, when producing the fermented baked milk, calcium Caseinate phosphate complex of dairy raw materials reached the isoelectric point (pH 4.58–4.64) faster than in the control samples, that led to its curdling and the formation of a dense clot.
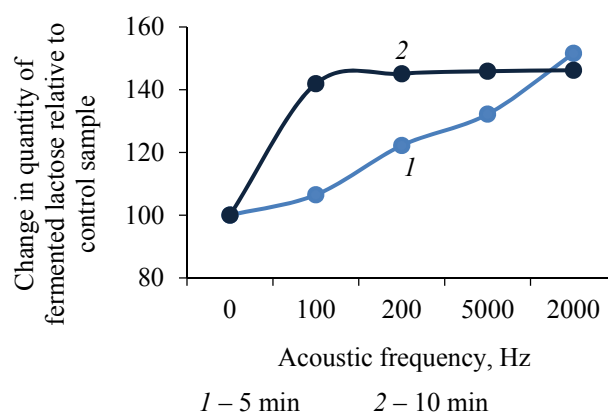
Fig. 12. The impact of acoustic treatment parameters on the amount (in % to control samples) of fermented lactose.
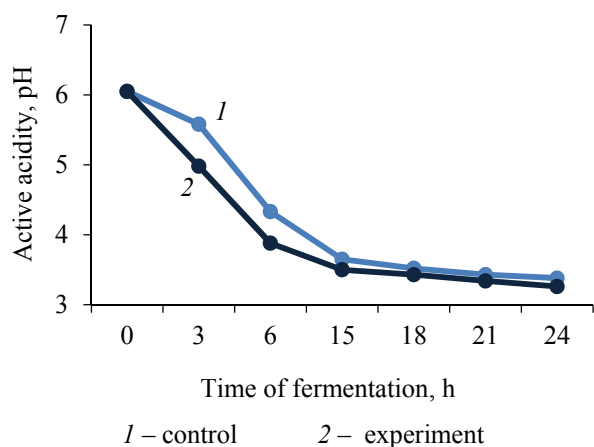
Fig. 13. Dependence of the active acidity of baked milk during its fermentation on the time of fermentation.

Determination of the mass fraction of lactose in the feedstock, as well as in experimental and control samples of fermented baked milk showed that the amount of lactose in the experimental fermented baked milk was less than 30% compared to the feedstock, while the amount of lactose in fermented baked milk produced with the help of a traditional starter (unselected strain), decreased by an average of 7.5%.

The organoleptic characteristics of the test samples were the same as those of the control sample: the clot was dense, smooth, with a cream color, homogeneous throughout the mass, characteristic sour-milk taste and with a pronounced taste of baked milk without off-flavour and smells. That is, in the development of experimental samples of fermented baked milk, the product was obtained to the maximum extent close to the traditional product, but with a low percentage of lactose.

CONCLUSION

Thus, using extremely low doses of physical forces of vibrational nature to regulate the life of biological systems is a promising scientific approach to work out innovative technologies for processing food raw materials. It will accelerate the main technological stages of production, improve product quality at low material and energy costs. The principle of the combined effect of enzyme preparations and low-intensity physical factors can be used in food biotechnology to make new food for different purposes.

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest.

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Genetic identification of bovine leukaemia virus

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Abstract: Molecular genetic research methods make it possible to evaluate the genetic diversity of bovine leukemia virus (BLV) and are the most informative approaches to its genetic identification. Molecular genetic research methods work well for the phylogenetic analysis of sequenced nucleotide DNA sequences of the provirus, as well as for the polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) according to the phylogenetic classification of the pathogen. The purpose of the research was to study the scientific and methodological approaches to the genetic identification of bovine leukemia virus, integrated into the molecular monitoring of infection of cattle with BLV genotypes. The authors used PCR-RFLP-genotyping and comparative phylogenetic analysis of aligned nucleotide sequences of the *env* gene fragment of the BLV provirus isolates to detect the genotypic affiliation of the cattle from twenty-one livestock farms of the Republic of Tatarstan. As a result, isolates of four out of ten BLV genotypes were found in the Tatarstani cattle, namely genotypes 1, 4, 7, and 8. The research involved a comparative analysis of 505 nucleotide sequences of a fragment of the BLV *env* gene, including those deposited in GenBank NCBI. The analysis confirms the inconsistency of several earlier PCR-RFLP typing strategies with the current approach in assessing the genotypic diversity by phylogenetic analysis. The improved strategy of PCR-RFLP genotyping of BLV corresponds with its modern phylogenetic classification. The strategy makes it possible to identify all the known genotypes of the viral pathogen. Its validity has been proved by *in silico* modelling of restrictogrammes and a phylogenetic analysis of the *env* gene fragment of 57 reference isolates of ten BLV genotypes that generate 57 genotype-associated combinations of diagnostically significant PCR-RFLP profiles.

Keywords: Bovine leukaemia virus, BLV, cattle, gene, genotype, genetic identification, PCR, RFLP, sequencing

INTRODUCTION

Enzootic Bovine Leukosis (EBL) is a chronic infectious disease of a tumorous nature. It causes significant economic damage to the dairy and beef cattle industry due to poor production, low quality, cattle mortality, and expensive epidemic prevention measures [1, 2].

Foodstuffs of infected animals can be dangerous to humans due to the harmful metabolites it contains. The causative agent affects all kinds of raw material (milk,

meat, by-products) and products, remaining a potential source of human infection [3–5].

Pasteurization of milk inactivates the virus but does not degrade its genome. The genetic material of the provirus maintains its integrity in canned meat [6]. Moreover, there are dairy products with partial pasteurization regime, e.g. classic cheeses, granulated cottage cheese, powdered milk with a low heating temperature, etc. The temperature processing parameters used in accordance with the regulatory and technical documentation cannot destroy harmful metabolites and, in some cases, do not kill the virus [7].

According to some researches, DNA of BLV provirus was found in epithelial cells of human mammary glands, including those of breast cancer patients. The hypothesis states that BLV may destabilize the host genome, thus leading to cancerous degeneration of cells [8–12].

Obtaining high-quality raw materials of animal origin is the most important challenge for meat and dairy industry. The challenge includes the development of functional and gerodietic foods [13–15].

According to the requirements of the Technical Regulations of the Customs Union "On safety of milk and dairy products" (TR CU 033/2013), BLV preventive measures and eradication activities are extremely important, given the significant prevalence of this incurable disease in the Russian Federation [16, 17].

An early genetic diagnosing of the pathogen is part of the system of anti-epizootic measures, followed by the removal of infected animals from the herd. Molecular genetic research methods make it possible to assess the genetic diversity of BLV [18]. This is the most informative approach to the gene identification of the virus. Molecular genetic research methods work well for the phylogenetic analysis of nucleotide DNA sequences of the provirus, as well as for PCR-RFLP analysis according to the phylogenetic classification of the pathogen [19].

The current phylogenetic classification of BLV includes ten genotypes. The first seven genotypes were described by Argentinean scientists in 2009 [20], while genotype 8 was described by researchers from Russia [21–23], Croatia [24], and a European team of scientists [25] in 2011–2013. Genotype 9 was investigated by a team of Argentinean, Chilean, and Japanese scientists in 2016 [26]. Genotype 10 was described by a team of researchers from Thailand and South Korea [27] in 2016.

The objective of the current research was to study the scientific and methodological approaches to the genetic identification of BLV integrated into the molecular monitoring of infection of cattle herds with BLV genotypes. The following tasks were set:

– to establish the genotypes of BLV isolates in Tearstain cattle;

– to define the types of BLV isolates with deciphered nucleotide sequences of the *env* gene fragment, depending on the chosen gene identification strategy;

– to improve the strategy of PCR-RFLP-genotyping of BLV and make it consistent with the modern phylogenetic BLV classification.

STAGY OBYECTS AND METHODS

The research involved a total of 179 blood samples from AGID-positive cows. The samples were provided by agricultural enterprises from 21 districts of the Republic of Tatarstan. The samples were genetically examined for BLV. The examination included a phylogenetic analysis of sequenced *env* gene fragment of the pathogen and a PCR-RFLP-genotyping consistent with the phylogenetic classification of the infectious agent.

To extract DNA from the whole conserved blood, we used a commercial PCR diagnostic kit, 'DNA-Sorb B', produced by the Central Research Institute of Epidemiology of the Federal Supervisory Service for Consumer Rights and Human Welfare, Ministry of Health of the Russian Federation.

Nested PCR with extracted samples of BLV proviral DNA was performed with external (*env*5032 and *env*5608) and internal (*env*5099 and *env*5521) primers initiating the generation of *env* gene 444 bp fragment of causative agent at the final stage of reaction [28].

Restriction endonucleases used in PCR-RFLP-genotyping of BLV were consistent with its phylogenetic classification: *Bst*YI (isoshizomer *Bst*X2I), *Hph*I (isoshizomer *Asu*HPI), *Hae*III, *Pvu*II, *Ssp*I. The NEBcutter v.2.0 web resource was used for PCR-RFLP modelling.

For the detection of the obtained results of PCR and PCR-RFLP analysis, 2.5% agarose gel horizontal electrophoresis was applied with a TBE buffer (pH 8.0) containing ethidium bromide. The electrophoregrammes were examined in a UV-transilluminator ($\lambda = 310$ nm). The sizes of the generated DNA fragments were compared with standard DNA molecular weight markers (SibEnzyme Ltd, Russia).

Sequencing of the PCR amplification products of the *env* gene fragment of detected BLV provirus isolates was performed on ABI PRISM 3100 Genetic Analyser (Applied Biosystems, USA) in the laboratory of Scientific and Technical Complex Sintol (Russia). Internal oligonucleotide primers *env*5099 and *env*5521 were used as sequencing. The sequenced fragments of the *env* gene of BLV provirus isolates were aligned with the corresponding nucleotide sequences of the reference BLV isolates from GenBank with the help of BLAST and MEGA-4 programmes. The last stage included a phylogenetic analysis.

RESULTS AND DISCUSSION

The study featured a PCR-RFLP-genotyping and a comparative phylogenetic analysis of the aligned sequences of the *env* gene fragment of BLV provirus isolates from 21 districts of the Republic of Tatarstan.

As a result, out of 179 identified isolates, ten isolates belonged to genotype 1; 106 isolates belonged to the cluster of genotype 4; 55 were characterized as genotype 7, and the remaining eight provirus isolates belonged to genotype 8 (Table 1).

According to the results obtained by PCR-RFLP-genotyping and phylogenetic analysis of sequenced *env* gene fragment, there are four out of ten currently known BLV genotypes in Tatarstan: 1, 4, 7, and 8.

Fig. 1 shows the genotypes of BLV isolated with the help of phylogenetic analysis of nucleotide sequences of *env* gene fragment.

An additional assessment of the heterogeneity of the reference BLV representatives for the *env* gene included an analysis of the intra- and intergenotypic heterogeneity of genotypes. The data in Table 2 indicate that it is impossible to use the 'heterogeneous' criterion for assessing the genetic diversity of BLV.

As part of the next task, BLV isolates with the decoded nucleotide sequences of the *env* gene fragment were identified according to the chosen genetic identification strategy. The degree of consistency of genotypic approaches was assessed by comparing the data of the *in silico* PCR-RFLP and the phylogenetic analyses.

A comparative analysis of 505 nucleotide sequences of the BLV *env* gene locus, including those deposited with GenBank NCBI, confirms the inconsistency of a number of earlier PCR-RFLP typing strategies [28–30] with the current approach in assessing the genotypic diversity by means of phylogenetic analysis.

Thus, the BLV isolates that belong to the Belgian subgroup according to D. Beier et al. (2001) [28], belong to genotype 4 according to the phylogenetic classification; Australian subgroup can be referred to genotypes 1, 3, 6, 8, or 9; Japanese subgroup – to genotypes 1, 6, or 7. In addition, the genotyping strategy [28] includes 11 additional unique combinations of PCR-RFLP profiles, conditionally identical to 11 unclassifiable BLV subgroups (Table 3).

Besides, BLV isolates that belong to genotype 1 and 7 according to the phylogenetic analysis, can be referred to Australian and Japanese subgroups, as well as to three unclassifiable subgroups, according to the strategy of D. Beier et al. (2001) [28]; genotypes 2, 5, and 10 belong to two unclassifiable subgroups;

genotypes 3 and 8 – to Australian subgroup; genotype 4 – to the Belgian subgroup and four unclassifiable subgroups; the genotype 5 – to two unclassifiable subgroups; genotype 6 – to the Australian, Japanese and two unclassifiable subgroups; genotype 9 – to the Australian and one unclassifiable subset (Table 3).

BLV isolates that were genotyped according to M. Licursi et al. (2002) [29] as genotype 1 may belong to genotypes 1, 4, 6, or 7 according to the phylogenetic classification; genotype 3 – to genotypes 1, 6, or 7; genotype 5 – to genotypes 1, 3, 6, 7, or 9; genotype 6 – to genotypes 2, 4, 5, or 7 (Table 4).

For the genotyping strategy described in [29], there are 19 unique combinations of PCR-RFLP profiles that are conditionally identical to 19 unclassifiable BLV genotypes (Table 4).

Besides, BLV isolates that are genotyped according to phylogenetic analysis as genotype 1 may refer to 1, 3, 5, and three unclassifiable BLV genotypes according to the strategy of M. Licursi et al. (2002) [29]; genotype 2 belongs to genotype 6 and two unclassifiable genotypes; the genotype 3 – to genotype 5 and one unclassifiable genotype; genotype 4 – to genotypes 1 and 6 and five unclassifiable genotypes; genotype 5 – to genotype 6 and two unclassifiable genotypes; genotype 6 – to genotypes 1, 3, and 5 and three unclassifiable genotypes; genotype 7 – to genotypes 1, 3, and 6 and four unclassifiable genotypes; genotype 8 – to one unclassifiable genotype; genotype 9 – to genotype 5; genotype 10 – to three unclassifiable genotypes (Table 4).

It should be mentioned that, when analyzing *in silico* PCR-RFLP data from 505 BLV representatives, we found not a single nucleotide sequence of the *env* gene fragment that would belong to genotypes 2 and 4 according to M. Licursi et al. (2002) (Table 4). This fact did not make it possible to prove the actual existence of PCR-RFLP profiles indicated for these two BLV genotypes.

Table 1. Distribution of 179 genotyped samples of BLV provirus DNA according to 21 districts of the Republic of Tatarstan, Russian Federation

| Districts		Number of analysed samples	BLV genotypes									
			1	2	3	4	5	6	7	8	9	10
1	Aznakaevsky	10	–	–	–	9	–	–	1	–	–	–
2	Al'keyevsky	13	–	–	–	5	–	–	8	–	–	–
3	Arsky	7	–	–	–	6	–	–	1	–	–	–
4	Buinsky	7	–	–	–	2	–	–	3	2	–	–
5	Vysogorsky	4	–	–	–	4	–	–	–	–	–	–
6	Drozhanovsky	12	–	–	–	4	–	–	7	1	–	–
7	Zainsky	8	–	–	–	7	–	–	–	1	–	–
8	Klaibitsky	7	–	–	–	7	–	–	–	–	–	–
9	Laishevsky	13	–	–	–	12	–	–	1	–	–	–
10	Leninogorsky	19	–	–	–	15	–	–	4	–	–	–
11	Mamadyshksy	10	10	–	–	–	–	–	–	–	–	–
12	Menzelinsky	6	–	–	–	6	–	–	–	–	–	–
13	Musl'umovsky	4	–	–	–	–	–	–	4	–	–	–
14	Nizhnekamensky	14	–	–	–	8	–	–	5	1	–	–
15	Pestrechinsky	1	–	–	–	1	–	–	–	–	–	–
16	Rybnoslobodsky	8	–	–	–	8	–	–	–	–	–	–
17	Sarmanovsky	2	–	–	–	2	–	–	–	–	–	–
18	Spassky	9	–	–	–	3	–	–	6	–	–	–
19	Tukaevsky	9	–	–	–	4	–	–	3	2	–	–
20	T'ulyachinsky	6	–	–	–	–	–	–	6	–	–	–
21	Chistopolsky	10	–	–	–	3	–	–	6	1	–	–
	Total number of samples	179	10	–	–	106	–	–	55	8	–	–
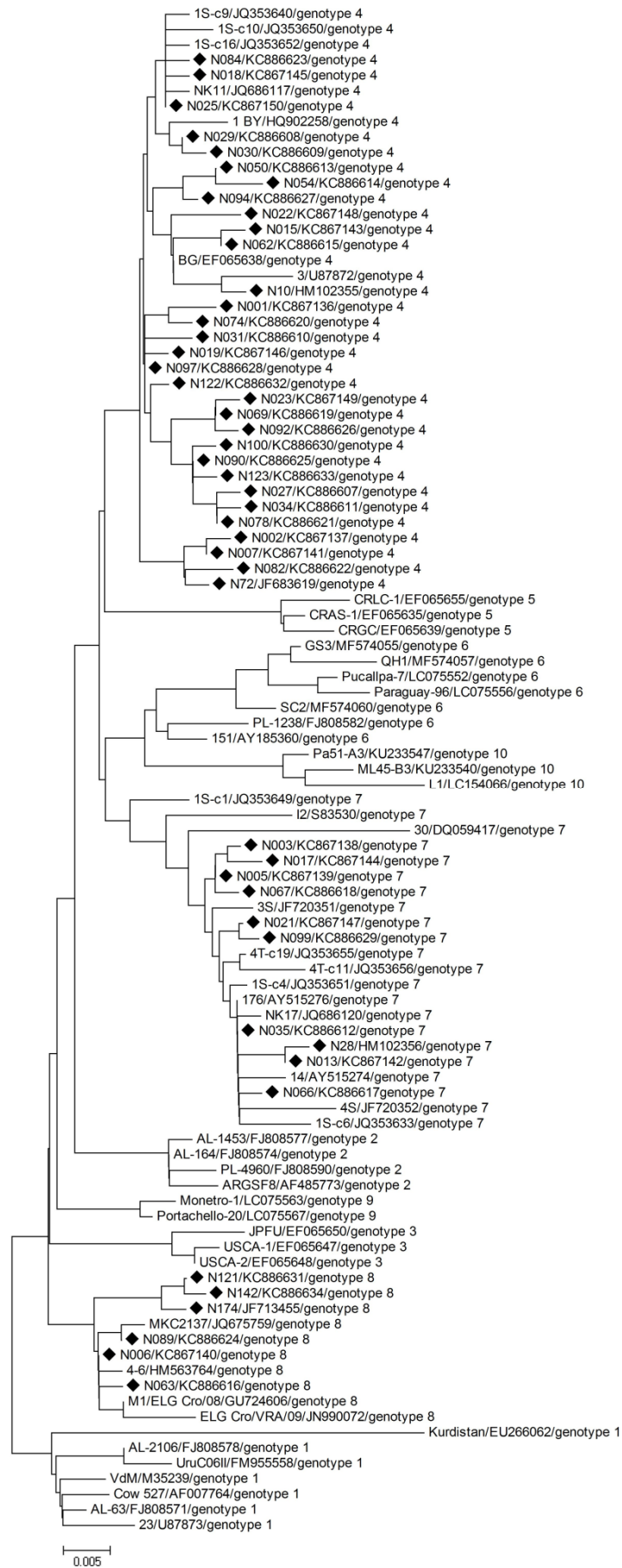
Fig. 1. Dendrogramme of 99 isolates of 10 BLV genotypes, based on a phylogenetic analysis of the *env gene* fragment [MEGA-4: algorithm NJ, 400 nt, 99 seq.] Legend: black diamond marks GenBank NCBI nucleotide sequences of the *env gene* fragment of BLV provirus isolates in the Republic of Tatarstan.

Table 2. Intra- and intergenotypic heterogeneity of reference BLV representatives according to *env* gene (% ratio)

GENOTYPE	PHYLOGENETIC CLASSIFICATION OF BLV									
	1	2	3	4	5	6	7	8	9	10
1	0–5	3–6	3–7	3–7	3–7	3–7	3–8	2–6	3–6	4–7
2	3–6	0–1	3–4	2–4	4–5	3–5	3–5	2–4	3	4–6
3	3–7	3–4	0–2	3–5	4–5	3–5	3–6	3–4	3	4–6
4	3–7	2–4	3–5	0–3	3–5	2–5	2–6	2–4	2–4	3–5
5	3–7	4–5	4–5	3–5	0–2	4–6	3–5	4–5	4–5	5–6
6	3–7	3–5	3–5	2–5	4–6	0–4	3–6	2–5	3–5	3–5
7	3–8	3–5	3–6	2–6	3–5	3–6	0–4	2–5	3–5	4–6
8	2–6	2–4	3–4	2–4	4–5	2–5	2–5	0–2	2–3	3–5
9	3–6	3	3	2–4	4–5	3–5	3–5	2–3	0–1	4–5
10	4–7	4–6	4–6	3–5	5–6	3–5	4–6	3–5	4–5	0–2

Table 3. Comparison of *in silico* data for PCR-RFLP (typification according to D. Beier et al., 2001) and the phylogenetic analysis of the BLV *env* gene fragment

PCR-RFLP genotyping		PCR product (bp)	RFLP fragments (bp)			BLV genotypes										N
			*Pvu*II	*Bam*HI	*Bcl*I	1	2	3	4	5	6	7	8	9	10	
Subgroup	Belgian	444	280/164	444	225/219	–	–	–	142	–	–	–	–	–	–	142
	Australian	444	444	316/128	225/219	57	–	4	–	–	28	70	21	19	–	199
	Japanese	444	444	316/128	219/121/104	8	–	–	–	–	6	1	–	–	–	15
	?	444	444	444	225/219	43	–	–	1	–	–	2	–	3	17	66
	?	444	444	316/128	444	1	–	–	–	–	14	2	–	–	–	17
	?	444	444	316/128	225/191/28	1	–	–	–	–	–	–	–	–	–	1
	?	444	280/164	316/128	225/219	–	36	–	–	10	–	3	–	–	–	49
	?	444	280/164	316/128	444	–	1	–	–	–	–	–	–	–	–	1
	?	444	280/164	316/128	219/189/36	–	–	–	–	1	–	–	–	–	–	1
	?	444	280/164	444	444	–	–	–	1	–	–	–	–	–	–	1
	?	444	208/164	253/191	225/219	–	–	–	1	–	–	–	–	–	–	1
	?	444	280/164	444	219/121/104	–	–	–	4	–	–	–	–	–	–	4
	?	444	444	242/128/74	225/219	–	–	–	–	–	1	–	–	–	–	1
	?	444	444	444	444	–	–	–	–	–	–	–	–	–	7	7

Legend. N is the number of analysed BLV isolates with an established PCR-RFLP profile; "?" – an unclassifiable BLV taxon.

Table 4. Comparison of *in silico* data of PCR-RFLP (typification according to M. Licursi et al., 2002) and phylogenetic analysis of a fragment of the BLV *env* gene

PCR-RFLP genotyping		PCR product (bp)	RFLP fragments (bp)			BLV genotypes										N
			*Bcl*I	*Hae*III	*Pvu*II	1	2	3	4	5	6	7	8	9	10	
Genotype	1	444	225/219	198/94/87/32/27/6	444	98	–	–	1	–	28	68	–	–	–	195
	2	444	219/121/104	312/94/32/6	444	–	–	–	–	–	–	–	–	–	–	–
	3	444	219/121/104	285/94/32/27/6	444	8	–	–	–	–	6	1	–	–	–	15
	4	444	219/121/104	198/94/87/32/27/6	444	–	–	–	–	–	–	–	–	–	–	–
	5	444	225/219	285/94/32/27/6	444	1	–	3	–	–	1	1	–	22	–	28
	6	444	225/219	198/94/87/32/27/6	280/164	–	35	–	139	9	–	3	–	–	–	186
	?	444	444	198/94/87/32/27/6	444	1	–	–	–	–	12	–	–	–	7	20
	?	444	225/191/28	198/119/94/27/6	444	1	–	–	–	–	–	–	–	–	–	1
	?	444	225/219	312/94/32/6	444	1	–	–	–	–	–	–	–	–	–	1
	?	444	444	198/94/87/32/27/6	280.164	–	1	–	1	–	–	–	–	–	–	2
	?	444	219/189/36	198/94/87/32/27/6	280/164	–	–	–	–	1	–	–	–	–	–	1
	?	444	225/219	285/94/32/27/6	280/164	–	–	–	2	1	–	–	–	–	–	3
	?	444	444	198/87/49/45/32/27/6	444	–	–	–	–	–	1	–	–	–	–	1
	?	444	225/219	225/94/87/32/6	444	–	–	–	–	–	–	–	21	–	–	21
	?	444	225/219	285/94/32/21/6/6	444	–	–	1	–	–	–	–	–	–	–	1
	?	444	225/219	198/121/87/32/6	280/164	–	–	–	1	–	–	–	–	–	–	1
	?	444	225/219	198/119/94/27/6	280/164	–	–	–	1	–	–	–	–	–	–	1
	?	444	219/121/104	285/94/32/27/6	280/164	–	–	–	4	–	–	–	–	–	–	4
	?	444	225/219	198/87/49/45/32/27/6	444	–	–	–	–	–	–	2	–	–	–	2
	?	444	225/219	198/119/94/27/6	444	–	–	–	–	–	–	1	–	–	–	1
	?	444	444	198/121/87/32/6	444	–	–	–	–	–	1	1	–	–	–	2
	?	444	225/219	198/87/49/45/32/27/6	280/164	–	1	–	–	–	–	–	–	–	–	1
	?	444	444	198/94/81/32/21/6/6	444	–	–	–	–	–	1	–	–	–	–	1
	?	444	225/219	198/94/81/32/27/6/6	444	–	–	–	–	–	–	–	–	6	–	6
	?	444	225/219	279/94/32/27/6/6	444	–	–	–	–	–	–	–	–	–	11	11

Legend. N is the number of analysed BLV isolates with an established PCR-RFLP profile; "?" – an unclassifiable BLV taxon.

Table 5 compares the *in silico* data of PCR-RFLP according to the strategy by H. Fechner et al. (1997) [30] and the phylogenetic analysis of a fragment of the BLV *env* gene.

Thus, the BLV isolates identified according to H. Fechner et al. (1997) [30] as variant group A belong to genotype 4 according to the phylogenetic classification; variant group B – to genotypes 1, 6, or 7; variant group C – to genotypes 1, 3, 6, 7, or 9; variant group D – to genotypes 1, 4, or 7; variant group F – to genotypes 2, 5, or 7; variant group G – to genotype 1 (Table 5).

For this typing strategy [30], there are 17 unique combinations of PCR-RFLP profiles that are conditionally identical to 17 unclassifiable variant BLV groups (Table 5). Besides, BLV isolates genotyped by phylogenetic analysis as genotype 1 are characterized as variant groups B, C, D, G and three unclassifiable variant groups of BLV according to the strategy of H. Fechner et al. (1997) [30]; the genotype 2 belongs to variant group F and one unclassifiable variant group; genotype 3 – to variant group C; genotype 4 – to

variant groups A, D and three unclassifiable variant groups; genotype 5 – to variant group F and two unclassifiable variant groups; genotype 6 – to variant groups B and C and four unclassifiable variant groups; genotype 7 – to variant groups B, C, D, F and two unclassifiable variant groups; genotype 8 – to variant group E; genotype 9 – to variant group C and one unclassifiable variant group; genotype 10 – to three unclassifiable variant groups of BLV (Table 5).

The priority task of the research was to improve the strategy of PCR-RFLP genotyping of BLV by making it consistent with the phylogenetic classification and taking into account the update information on the genetic diversity of the ten known BLV genotypes.

505 BLV isolates were generated during the analysis of restriction mappings of the *env* gene locus according to 5 restriction enzymes. The interpretation of their *env*-PCR-RFLP profiles actually reflects the strategy of PCR-RFLP genotyping of BLV in accordance with its phylogenetic classification. The data are represented in Table 6.

Table 5. Comparison of *in silico* data of PCR-RFLP (genotyping according to H. Fechner et al., 1997) and the phylogenetic analysis of a fragment of the BLV *env* gene

	PCR–RFLP genotyping	PCR product (bp)	*Bam*HI	*Bcl*I	*Bgl*I	*Hae*III	*Pvu*II	1	2	3	4	5	6	7	8	9	10	N
Variant group	A	444	444	225/219	328/116; 444	198/94/87/32/27/6; 198/121/87/32/6; 198/119/94/27/6; 285/94/32/27/6	280/164	–	–	–	142	–	–	–	–	–	–	142
	B	444	316/128	219/121/104	328/116; 444	285/94/32/27/6	444	8	–	–	–	–	6	1	–	–	–	15
	C	444	316/128	225/219	328/116; 444	198/94/87/32/27/6; 285/94/32/21/6/6; 285/94/32/27/6; 198/87/49/45/32/27/6; 198/119/94/27/6	444	56	–	4	–	–	28	70	–	19	–	177
	D	444	444	225/219	328/116	198/94/87/32/27/6	444	42	–	–	1	–	–	2	–	–	–	45
	E	444	316/128	225/219	328/116	225/94/8732/6	444	–	–	–	–	–	–	–	21	–	–	21
	F	444	316/128	225/219	328/116	198/94/87/32/27/6	280/164	–	36	–	–	9	–	3	–	–	–	48
	G	444	316/128	225/219	444	312/94/32/6	444	1	–	–	–	–	–	–	–	–	–	1
	?	444	316/128	444	328/116	198/94/87/32/27/6	444	1	–	–	–	–	2	–	–	–	–	3
	?	444	316/128	225/191/28	328/116	198/119/94/27/6	444	1	–	–	–	–	–	–	–	–	–	1
	?	444	444	225/219	444	285/94/32/27/6	444	1	–	–	–	–	–	–	–	3	–	4
	?	444	316/128	444	328/116	198/94/87/32/27/6	280/164	–	1	–	–	–	–	–	–	–	–	1
	?	444	316/128	219/189/36	328/116	198/94/87/32/27/6	280/164	–	–	–	1	–	–	–	–	–	–	1
	?	444	316/128	225/219	444	285/94/32/27/6	280/164	–	–	–	1	–	–	–	–	–	–	1
	?	444	316/128	444	328/116	198/87/49/45/32/27/6	444	–	–	–	–	–	–	1	–	–	–	1
	?	444	444	444	328/116	198/94/87/32/27/6	280/164	–	–	–	1	–	–	–	–	–	–	1
	?	444	253/191	225/219	328/116	198/94/87/32/27/6	280/164	–	–	–	1	–	–	–	–	–	–	1
	?	444	444	219/121/104	328/116	285/94/32/27/6	280/164	–	–	–	4	–	–	–	–	–	–	4
	?	444	316/128	444	328/116	198/121/87/32/6	444	–	–	–	–	–	–	–	1	–	–	1
	?	444	316/128	444	444	198/94/87/32/27/6	444	–	–	–	–	–	–	11	–	–	–	11
	?	444	242/128/74	225/219	328/116	198/94/87/32/27/6	444	–	–	–	–	–	–	1	–	–	–	1
	?	444	316/128	444	444	198/121/87/32/6	444	–	–	–	–	–	–	1	–	–	–	1
	?	444	444	225/219	328/116	198/94/81/32/27/6/6	444	–	–	–	–	–	–	–	–	–	6	6
	?	444	444	444	328/116	198/94/81/32/27/6/6	444	–	–	–	–	–	–	–	–	–	7	7
	?	444	444	225/219	444	279/94/32/27/6/6	444	–	–	–	–	–	–	–	–	–	11	11

Legend. N is the number of analysed BLV isolates with an established PCR-RFLP profile; "?" – an unclassifiable BLV taxon.

Table 6. An improved strategy for PCR-RFLP-genotyping of BLV, consistent with the phylogenetic classification

G	BLV isolate	GenBank A/N	PCR product (bp)	RFLP fragments (bp)					C	N
				*Pvu*II	*Ssp*I	*Hph*I	*Hae*III	*Bst*YI		
1	AL–63	FJ808571	444	444	399/45	224/220	198/94/87/32/27/6	198/128/118	1	56
1	Cow 527	AF007764	444	444	399/45	224/220	285/94/32/27/6	198/128/118	2	8
1	23	U87873	444	444	399/45	224/220	312/94/32/6	198/128/118	3	1
1	AL–2106	FJ808578	444	444	399/45	224/220	198/94/87/32/27/6	246/198	4	42
1	UruC06II	FM955558	444	444	399/45	224/220	285/94/32/27/6	246/198	5	1
1	VdM	M35239	444	444	399/45	224/181/39	198/94/87/32/27/6	316/128	6	1
1	Kurdistan	EU266062	444	444	399/45	220/196/28	198/119/94/27/6	198/128/118	7	1
2	AL–164	FJ808574	444	280/164	399/45	224/220	198/94/87/32/27/6	198/128/118	8	34
2	PL–4960	FJ808590	444	280/164	399/45	224/220	198/87/49/45/32/27/6	198/128/118	9	1
2	ARGSF8	AF485773	444	280/164	399/45	444	198/94/87/32/27/6	198/128/118	10	1
2	AL–1453	FJ808577	444	280/164	444	224/220	198/94/87/32/27/6	198/128/118	11	1
3	USCA–1	EF065647	444	444	399/45	444	285/94/32/21/6/6	198/128/96/22	12	1
3	USCA–2	EF065648	444	444	399/45	444	285/94/32/27/6	198/128/96/22	13	2
3	JPFU	EF065650	444	444	399/45	444	285/94/32/27/6	198/128/118	14	1
4	BG	EF065638	444	280/164	399/45	224/220	198/94/87/32/27/6	444	15	115
4	3	U87872	444	444	399/45	224/220	198/94/87/32/27/6	444	16	1
4	1S–c16	JQ353652	444	280/164	399/45	444	198/94/87/32/27/6	444	17	16
4	N023	KC867149	444	280.164	399.45	224.220	198/94/87/32/27/6	253/191	18	1
4	1_BY	HQ902258	444	280/164	444	224/220	198/94/87/32/27/6	444	19	7
4	N034	KC886611	444	280/164	399/45	224/220	198/121/87/32/6	444	20	1
4	1S–c9	JQ353640	444	280/164	399/45	224/220	198/119/94/27/6	444	21	1
4	NK11	JQ686117	444	280/164	399/45	224/220	285/94/32/27/6	444	22	6
4	1S–c10	JQ353650	444	280/164	399/45	220/145/79	198/94/87/32/27/6	444	23	1
5	CRAS–1	EF065635	444	280/164	399/45	224/181/39	198/94/87/32/27/6	316/128	24	8
5	CRGC	EF065639	444	280/164	399/45	224/181/39	285/94/32/27/6	316/128	25	1
5	CRLC–1	EF065655	444	280/164	444	224/181/39	198/94/87/32/27/6	316/128	26	2
6	PL–1238	FJ808582	444	444	399/45	224/220	285/94/32/27/6	316/128	27	7
6	151	AY185360	444	444	399/45	224/220	198/94/87/32/27/6	316/128	28	27
6	GS3	MF574055	444	444	399/45	444	198/94/87/32/27/6	316/128	29	11
6	SC2	MF574060	444	444	399/45	224/220	198/94/87/32/27/6	242/128/74	30	1
6	QH1	MF574057	444	444	213/186/45	444	198/94/81/32/21/6/6	316/128	31	1
6	Pucallpa–7	LC075552	444	444	399/45	444	198/94/87/32/27/6	316/79/49	32	1
6	Paraguay–96	LC075556	444	444	399/45	444	198/121/87/32/6	316/128	33	1
7	N28	HM102356	444	444	444	224/137/83	198/94/87/32/27/6	294/128/22	34	7
7	176	AY515276	444	444	444	224/137/83	198/94/87/32/27/6	316/128	35	53
7	I2	S83530	444	444	444	224/220	285/94/32/27/6	316/128	36	1
7	14	AY515274	444	444	444	145/137/83/79	198/94/87/32/27/6	316/128	37	1
7	30	DQ059417	444	444	444	444	198/87/49/45/32/27/6	316/128	38	1
7	3S	JF720351	444	280/164	444	224/137/83	198/94/87/32/27/6	316/128	39	3
7	4T–c19	JQ353655	444	444	399/45	224/137/83	198/94/87/32/27/6	316/128	40	3
7	1S–c4	JQ353651	444	444	444	224/137/83	198/94/87/32/27/6	316/79/49	41	1
7	NK17	JQ686120	444	444	444	224/137/83	198/87/49/45/32/27/6	316/128	42	2
7	4S	JF720352	444	444	444	224/137/83	198/119/94/27/6	316/128	43	1
7	1S–c6	JQ353633	444	444	444	224/137/83	198/121/87/32/6	316/128	44	1
7	4T–c11	JQ353656	444	444	444	224/137/83	285/94/32/27/6	316/128	45	1
7	N067	KC886618	444	444	444	224/137/44/39	198/94/87/32/27/6	316/128	46	1
7	1S–c1	JQ353649	444	444	444	224/220	198/94/87/32/27/6	444	47	2
8	M1/ELG_Cro/08	GU724606	444	444	399/45	224/220	225/94/87/32/6	198/128/118	48	13
8	N174	JF713455	444	444	399/45	224/220	225/94/87/32/6	316/128	49	4
8	ELG_Cro/VRA/09	JN990072	444	444	444	224/220	225/94/87/32/6	198/128/118	50	2
8	4–6	HM563764	444	444	399/45	224/137/83	225/94/87/32/6	198/128/118	51	1
8	MKC2137	JQ675759	444	444	399/45	444	225/94/87/32/6	198/128/118	52	1
9	Monetro–1	LC075563	444	444	399/45	224/171/49	285/94/32/27/6	198/128/118	53	19
9	Portachello–20	LC075567	444	444	399/45	224/171/49	285/94/32/27/6	246/198	54	3
10	Pa51–A3	KU233547	444	444	399/45	224/220	198/94/81/32/27/6/6	444	55	12
10	ML45–B3	KU233540	444	444	399/45	224/220	279/94/32/27/6/6	444	56	11
10	L1	LC154066	444	444	444	224/220	198/94/81/32/27/6/6	444	57	1

Legend. G – genotype; C – combination; N – the number of analysed BLV isolates with the established combination of PCR-RFLP profiles.
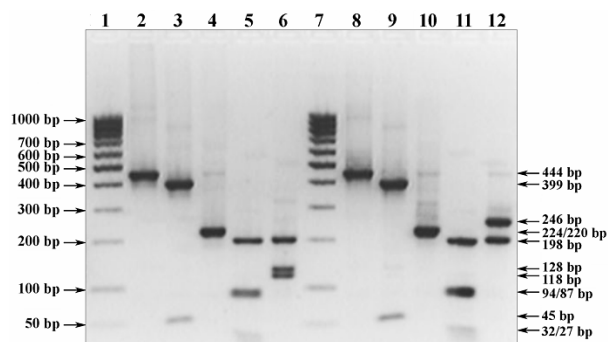
Fig. 2. Electrophoregramme of combinations of PCR-RFLP profiles (C1 and C4) of genotype 1 (an improved BLV genotyping strategy).

Legend. 1, 7) DNA markers 100 bp + 50 bp (SibEnzyme). 2–6) PCR-RFLP profile of BLV N-1 provirus isolate (C1, genotype 1): 2) *Pvu*II-RFLP (444 bp); 3) *Ssp*I-RFLP (399/45 bp); 4) *Hph*I-RFLP (224/220 bp); 5) *Hae*III-RFLP (198/94/87/32/27/6 bp); 6) *Bst*YI-RFLP (198/128/118 bp). 8–12) PCR-RFLP profile of BLV N-4 provirus isolate (C4, genotype 1): 8) *Pvu*II-RFLP (444 bp); 9) *Ssp*I-RFLP (399/45 bp); 10) *Hph*I-RFLP (224/220 bp); 11) *Hae*III-RFLP (198/94/87/32 / 27.6 bp); 12) *Bst*YI-RFLP (246/198 bp).
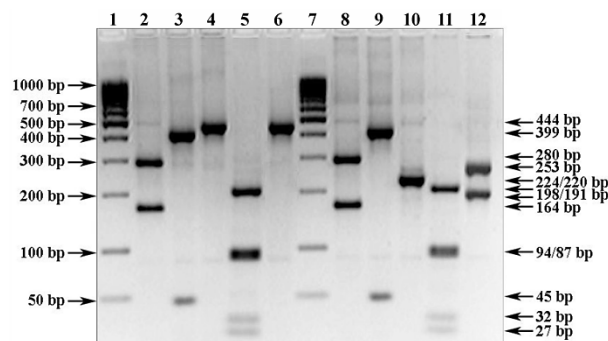

Fig. 3. Electrophoregramme of combinations of PCR-RFLP profiles (C17 and C18) of genotype 4 (improved BLV genotyping strategy).

Legend. 1, 7) DNA markers 100 bp + 50 bp (SibEnzyme). 2–6) PCR-RFLP-profile of the BLV N015 provirus isolate (C17, genotype 4): 2) *Pvu*II-RFLP (280/164 bp); 3) *Ssp*I-RFLP (399/45 bp); 4) *Hph*I-RFLP (444 bp); 5) *Hae*III-RFLP (198/94/87/32/27/6 bp); 6) *Bst*YI-RFLP (444 bp); 8–12) PCR-RFLP-profile of the BLV N023 provirus isolate (C18, genotype 8) *Pvu*II-RFLP (280/164 bp); 9) *Ssp*I-RFLP (399/45 bp); 10) *Hph*I-RFLP (224/220 bp); 11) *Hae*III-RFLP (198/94/87/32 / 27.6 bp); 12) *Bst*YI-RFLP (253/191 bp).

The PCR-RFLP-genotyping strategy of BLV, which we have improved, is consistent with its phylogenetic classification. The new classification makes it possible to identify all the ten currently known genotypes of the viral pathogen (Table 6).

It should be noted that genotype 1 is characterized by seven combinations of *env*-PCR-RFLP profiles (C 1-7), genotype 2 – by four combinations (C 8-11); genotype 3 – by three combinations (C 12-14); genotype 4 – by nine combinations (C15-23); genotype 5 – by three combinations (C 24-26); genotype 6 – by seven combinations (C27-33), genotype 7 – by fourteen combinations (C 34-47); genotype 8 – by five combinations (C 48-52); genotype 9 – by two combinations (C 53-54); genotype 10 – by 3 three combinations (C 55-57) (Table 6).
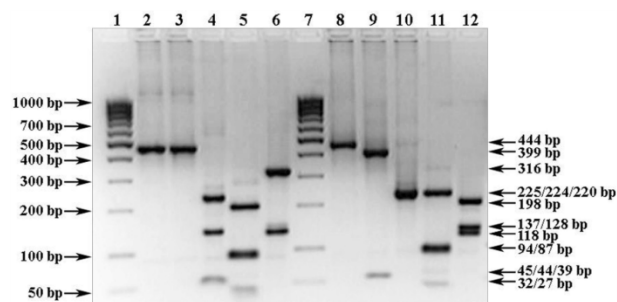

Fig. 4. Electrophoregramme of combinations of PCR-RFLP profiles (C46 and C48) of genotypes 7 and 8 (an improved BLV genotyping strategy).

Legend. 1, 7) DNA markers 100 bp + 50 bp (SibEnzyme). 2–6) PCR-RFLP-profile of the BLV N067 provirus isolate (C46, genotype 7): 2) *Pvu*II-RFLP (444 bp); 3) *Ssp*I-RFLP (444 bp); 4) *Hph*I-RFLP (224/137/44/39 bp); 5) *Hae*III-RFLP (198/94/87/32/27/6 bp); 6) *Bst*YI-RFLP (316/128 bp). 8–12) PCR-RFLP profile of the BLV N006 provirus isolate (C48, eighth genotype 8) *Pvu*II-RFLP (444 bp); 9) *Ssp*I-RFLP (399/45 bp); 10) *Hph*I-RFLP (224/220 bp); 11) *Hae*III-RFLP (225/94/87/32/6 bp); 12) *Bst*YI-RFLP (198/128/118 bp).

It should be emphasized that genotypes 8 and 9 can be easily identified even with the use of one restrictase, *Hae*III, generating RFLP fragments (225/94/87/32/6 bp) that are characteristic of genotype 8; *Hph*I – generating RFLP fragments (224/171/49 bp) that are characteristic of genotype 9. Representatives of genotypes 2 (*Bst*YI and *Pvu*II), 3 (*Hae*III and *Hph*I), and 5 (HphI and PvuII) can be identified with two restriction enzymes (Table 6).

Figs. 2–4 show illustrative examples of the implementation of the strategy of BLV PCR-RFLP-genotyping in accordance with its phylogenetic classification.

As one can see from the electrophoregramme in Fig. 2 (tracks 2–6), the PCR-RFLP profile of the BLV N-1 provirus isolate is identified as combination 1 (C1) of the *env*-PCR-RFLP profile of genotype 1, which includes at least 56 isolates deposited in the GenBank NCBI (Table 6).

The PCR-RFLP profile of the BLV N-4 provirus isolate (Fig. 2, tracks 8-12) characterizes combination 4 (C4) of the *env*-PCR-RFLP profile of genotype 1, with at least 42 identified representatives (Table 6).

The PCR-RFLP profile of the BLV N015 provirus isolate (Fig. 3, tracks 2–6) is identified as combination 17 (C17) of the *env*-PCR-RFLP profile of genotype 4. It includes at least 16 representatives (Table 6), two of which affect cattle in Tatarstan. According to GenBank NCBI, these nucleotide sequences of the *env gene* fragment are isolate N015 (GenBank A/N: KC867143) and isolate N062 (GenBank A/N: KC886615).

The PCR-RFLP profile of the BLV N023 provirus isolate (Fig. 3, tracks 8-12) is identified as combination 18 (C18) of the *env*-PCR-RFLP profile of genotype 4 (Table 6). Its nucleotide sequence of the *env* gene fragment from the GenBank NCBI is the only variant for the given combination (isolate N023, GenBank A/N: KC867149).
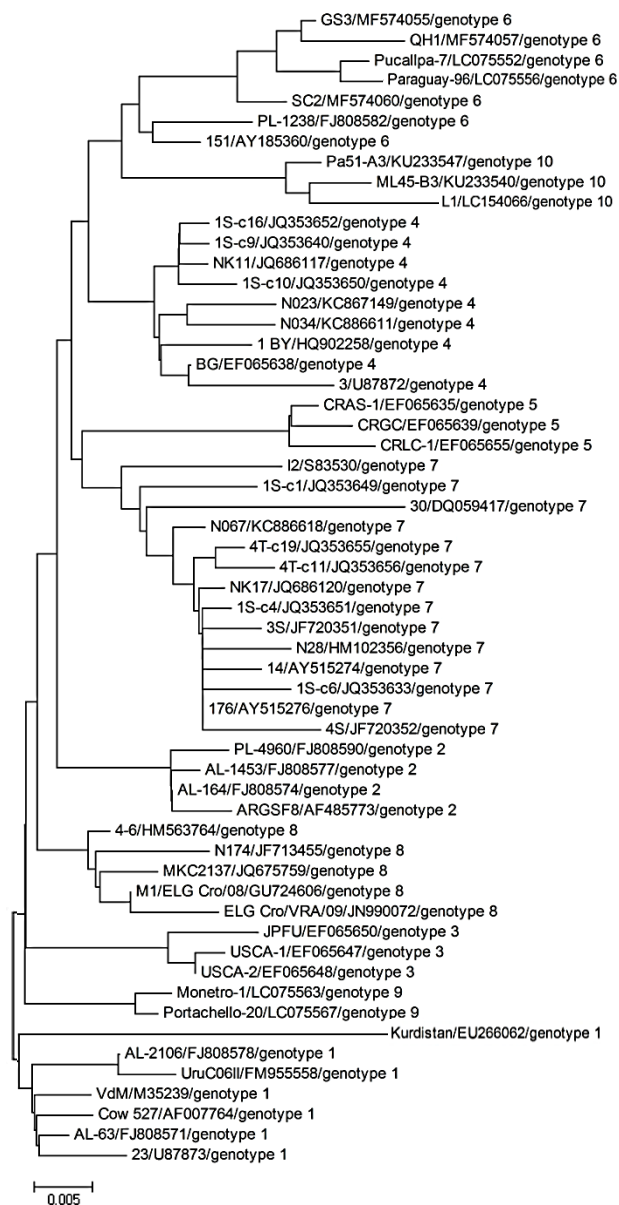
Fig. 5. Phylogramme of 57 reference isolates of the 10 currently known BLV genotypes, built on the basis of a phylogenetic analysis of the *env* gene fragment [MEGA-4: NJ algorithm, 400 nt, 57 seq.].

The PCR-RFLP profile of the BLV N067 provirus isolate (Fig. 4, tracks 2–6) characterizes itself as combination 46 (C46) of the *env*-PCR-RFLP profile of genotype 7 (Table 6). Its nucleotide sequence of the *env* gene fragment from GenBank NCBI is the only one for this combination (isolate N067, GenBank A/N: KC886618).

The PCR-RFLP profile of the BLV N006 provirus isolate (Fig. 4, tracks 8–12) belongs to combination 48 (C48) of the *env*-PCR-RFLP profile of genotype 8. It includes at least 13 representatives (Table 6), three of which affect cattle populations in Tatarstan. According to GenBank NCBI, their nucleotide sequences of the *env* gene fragment are isolate N063 (GenBank A/N: KC886616), isolate N006 (GenBank A/N: KC867140), and isolate N089 (GenBank A/N: KC886624).

The improved strategy of PCR-RFLP-genotyping corresponds with the modern phylogenetic classification of BLV and makes it possible to identify all its known genotypes. Its accuracy is based upon *in silico* modelling of restrictogrammes and the phylogenetic analysis of the *env* gene fragment of 57 reference isolates of the ten known BLV genes (Fig. 5). They produce 57 diagnostically significant genotype-associated combinations of PCR-RFLP profiles.

CONCLUSION

To determine the genotypes of BLV isolates obtained from Tatarstani cattle, we performed a phylogenetic analysis of the *env* gene fragment sequences and a PCR-RFLP analysis that corresponded with the phylogenetic classification of the infectious agent. The genotypic composition of 179 identified BLV isolates detected in cattle from livestock farms of 21 districts of the Republic of Tatarstan was represented by genotypes 1 (10 isolates), 4 (106 isolates), 7 (55 isolates), and 8 (8 isolates). Thus, we state the fact that four out of ten currently known BLV genotypes circulate on the territory of the Republic of Tatarstan, namely genotypes 1, 4, 7, and 8.

After that, we classified the BLV isolates with decoded nucleotide sequences of the *env* gene locus according to the chosen genetic identification strategy. Subsequently, we assessed the degree of consistency of genotypic approaches by comparing *in silico* PCR-RFLP data and the results of the phylogenetic analysis. We used 505 corresponding sequences, including those deposited in GenBank NCBI. As a result, we managed to prove that a number of previously used PCR-RFLP typing strategies were inconsistent with the current approach in assessing the genotypic diversity of BLV with the help of the phylogenetic analysis. The inconsistency of the three PCR-RFLP strategies for BLV typing with the modern phylogenetic classification is associated, among other things, with the on-going knowledge acquisition in the sphere of the genetic diversity of the ten known BLV genotypes.

During the final stage of the research, we improved the strategy of PCR-RFLP-genotyping of BLV to make it consistent with the phylogenetic classification. The new version takes into account the new data about the genetic diversity of BLV. It also includes an interpretation of the PCR-RFLP profiles of 505 BLV isolates. The interpretation resulted from a restriction mapping of the *env* gene fragment according to 5 restriction endonucleases. The improved strategy of PCR-RFLP-genotyping allows one to identify all the currently known BLV genotypes. The improved strategy owes its accuracy to *in silico* modelling of restrictogrammes and the phylogenetic analysis of the *env* gene of 57 reference isolates of ten BLV genes that generate 57 diagnostically significant genotype-associated combinations of PCR-RFLP profiles

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest related to this article.

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Hydrocolloid effect on the stabilization of vegetable purees in the process of freezing, refrigerating and defrosting

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Abstract: The analysis of modern concepts of the use of hydrocolloids for the stabilization of mashed products of plant origin in the freezing – storage – defrosting circle is given. The effect of hydrocolloids on the structure of vegetable purees using the example of xanthan gum, starch and inulin was studied. The most significant characteristics of the structure was identified and their descriptors were considered. Experimental data on the effect of hydrocolloids on the microstructure of vegetable purees was obtained. The expediency of using starch and xanthan gum in combination with inulin was shown. To create a viscous structure, modified starch with small granules that exhibit thermal stability in cold storage and further heat treatment were most suitable. Inulin was also not subject to changes during temperature treatments for the products with a pH higher than 3.5, but probably would not be widely used as a structurant for vegetable purees due to the weak expression of the structure-forming properties. The synergistic combinations of inulin and xanthan gum improved the organoleptic characteristics of the product, especially in cold storage. In addition, the ability of inulin to form a smooth structure in vegetable purée when enveloping the oral cavity was revealed, despite the fact that the initial product had a rather coarse fibrous consistency due to the plant origin of the ingredients. Thus, it is promising to use inulin in combination with thickeners to give the product prebiotic properties with the improved perception in consumers. The results of the studies confirm the possibility of storing vegetable purees at low temperatures for a long time without the deterioration of the structural and organoleptic properties resulting from the slowly occurring degradation processes of the individual components of the product at low temperatures, provided that the hydrocolloids under study are used.

Keywords: Hydrocolloids, vegetable purees, frost, puree texture, inulin

INTRODUCTION

Frozen vegetable purees are a relatively new form factor of the popular type of food products. The increasing popularity of their use as substitutes for fresh puree or crushed vegetable raw materials in producing sauces, dressings, spreads, fillings and soups, multicomponent frozen ready-made products (soup-mashed potatoes) and semi-finished products is related to the convenience to use, and also to a longer shelf life.

The processes of freezing and defrosting have a negative effect on plant tissues causing structural changes and degradation in puree sensory properties.

Structure is understood as the internal structure of the product and the nature of interaction between its individual elements (particles), which cause the reaction of tactile sensations to the physical stimuli that result from the contact with the food product [19]. Structural properties are closely related to rheological properties but the latter does not cover all the factors that determine the structure of food products. Thus, the concept of structure is complex and includes a number of such physical properties of the product as appearance, sensations in the oral cavity, dispersion, and viscosity.

It should be noted that the terms *structure* and *texture* are treated differently in the world literature.

The Russian-language term "texture" in American and English literary sources is treated as "structure". Therefore, to avoid a different interpretation, the authors give the definition of the term "texture" used in this paper. In the Russian-language literature, texture refers to the preferential orientation and relative arrangement of the elements that make up the product.

The desired structure can be obtained by adding supplementary ingredients to the product. Thus, to increase the viscosity and induce gelling, water-soluble biopolymers, hydrocolloids, are used.

Modified starches xanthan gum, galactomannans (guar gum and locust bean gum), and cellulose derivatives have been most used among food hydrocolloids as thickeners in the food industry. As gellants, modified starches, gelatin, agar, gum arabic, carrageenans, pectin, etc. are applied. A lot of hydrocolloids show both of these properties simultaneously [2].

For the products with neutral pH, in most cases, starches which can be combined with the ingredients that reduce the sense of viscosity in the mouth, such as oxidized starches, and also hydrocolloids with a low viscosity are used as thickeners. Opaque thickeners may be used for opaque products, for example, starches from waxy maize varieties.

In the products subjected to heat treatment in the production process, such ingredients as xanthan gum or heat-resistant methylcellulose are used to maintain viscosity. They have the ability to form an extended spatial network [1, 2].

For the products with pH = 3.5–4.0, such hydrocolloids as xanthan and guar gum, locust bean gum and their combinations are used, while starches are of limited use. The reason is that when combined with heat treatment and a low pH, starches and some hydrocolloids undergo partial molecular degradation [16].

Xanthan gum is an exopolysaccharide of microbiological origin obtained by fermentation. In the dissolved form, it has a high viscosity and thixotropy. It forms a spatial network structure [1].

An important property of vegetable hydrocolloids (such dietary fibers as inulin, pectin, etc.) is their prebiotic effect, which increases the nutritional value of the products that contain these components for the consumers that adhere to the principles of healthy nutrition [17, 18].

Different types of dietary fibers play a different role in the formation of the structure and can be conditionally classified into thickening and non-thickening ones. The second group includes short chain molecular oligosaccharides or mono-, di- and trisaccharides that are able to bind water well. Their introduction into the composition of the product increases the sweetness of the product and swelling capacity, improves consistency and sensations in the oral cavity, and changes the boiling point (for liquid products), the freezing point as well as the product density [1].

A significant amount of studies is devoted to the study of the effect of hydrocolloids on the pureed products subjected to freezing, refrigerated storage and subsequent defrosting.

In [3], the effect of cryoprotecting on the taste and structure of frozen/defrosted mashed potatoes was studied. Kappa-carrageenan, xanthan gum, chitosan, inulin, trehalose, oat fiber, starch and hydroxypropylmethylcellulose was used as cryoprotectants either individually or in the form of compositions.

The analogous results of the studies were obtained in [4]. The authors estimated the effect of introduction of inulin in various concentrations both independently and with the addition of cryoprotectants (kappa-carrageenan and xanthan gum) on the visco-elastic properties and microstructure of fresh and frozen/defrosted mashed potatoes. The results of the study showed that if the introduction of inulin resulted in a smoother (soft) and creamy texture of the product, its concentration did not significantly affect the rheological properties of puree, elasticity and the overall sensory evaluation of the samples. At the same time, the addition of kappa-carrageenan and xanthan gum resulted in significant changes. Apart from that, the fresh mashed potatoes had a more rigid structure than the samples of frozen/defrosted purees. This led to the conclusion that inulin can be used as a texturing agent that agrees with [5].

Nevertheless, the final conclusion in [4] stated that inulin as a texturizing agent for mashed potatoes would have limited industrial application in view of its partial hydrolysis (the shortening of the molecular chain) during heat treatment that interferes with gel formation.

It has been established in [6] that dietary fibers (pea fibers, inulin and their compositions) can be applied as functional ingredients for the enrichment of frozen/defrosted mashed potatoes. When introduced into the puree, the fibers exhibited opposite properties (softness – with inulin, hardness – with pea fibers), which is explained by different mechanisms of their interaction with the starch matrix of mashed potatoes. The simultaneous introduction of pea fibers in a low concentration (< 15 g/kg puree) and inulin in large concentrations (> 45 g/kg puree) in recommended to achieve a physiological effect. Herewith there was not an adverse effect on the texture and rheological properties, color and overall sensory perception of the product.

Other pureed objects were analized in [7, 8]. The effect of mixtures of tapioca starch, low-esterified starch and calcium on the stability of fruit pureed fillings in the processes of freezing and baking of bakery products has been studied [9]. It has been shown that a "tapioca starch-pectin" composition can simulate the viscoelastic properties of waxy modified corn starch, which is commonly used in the food industry.

In [10], the effects of introduction of inulin and polydextrose on the physicochemical and sensory properties of banana puree in the cold storage process have been studied.

Thus, at the moment, the range of the studies devoted to the problem of texture stabilization with pure hydrocolloids and their compositions in the process of freezing, refrigerating and defrosting is

extremely limited, and a number of issues remain unresolved.

The paper is aimed at studying the effect of the addition of stabilizers and thickeners on the taste and texture of the vegetable purees subjected to freezing, storage and subsequent intensive thermal effects in microwave treatment. To justify their choice and dosage, a complex analysis texture requirements, the cost of the finished product, and stability during heat treatment (freezing, heating, and cooking) was used.

STUDY OBJECTS AND METHODS

The samples of single-component vegetable purees from carrots, beets and zucchini were considered.

The experimental samples were prepared as follows. The vegetables were hand-washed, cooked until ready in water, the broth was poured. After cooling, the raw material was cleaned, cut into cubes and crushed in a knife mixer (the blade speed was 6.28 r/s). The hydrocolloids were introduced into the products obtained in the form of dry powder. The samples were homogenized (the blade speed was 5.24 r/s) then wiped through a stainless steel sieve ($\varnothing$ 1.5 mm). Some of the samples were analyzed fresh, the others were frozen in plastic containers at –18°C (the control temperatures were measured in the thermal centers of the samples.) The frozen samples were evacuated and placed in cold storage at –18°C. Defrosting was carried out in a microwave oven until the temperature in the thermal center reached 20 ± 5°C, then samples were heated to a consumption temperature (50°C).

One type of hydrocolloids or their compositions were introduced in each test sample. In the study, hydrocolloids were represented by xanthan gum E415, modified starch E1414, and inulin.

The frozen samples were stored for two months. After defrosting, the taste and structure were evaluated.

Table 1. Descriptors of the structure of vegetable purees and their corresponding sensory indices [1]

No.	Structure descriptor	Sensory perception or estimation method
1	Density	The ability of the product to drain from a spoon
2	Water separation	Visual observation of the surface of the puree, as well as after immersion in a spoon
3	Texture	Visual observation of the surface after mixing the product with a spoon
4	Density (sensation in the oral cavity)	Resistance to the fluidity of the product kneaded between the palate and the tongue
5	Viscosity	Perceived cohesiveness of the mixed product when it is drained from a spoon
6	Smoothness	Sensation in the oral cavity determined when the product is compressed between the palate and the tongue

A combination of several methods was used to determine the structure [11–13], including free choice profiling and a procrustean analysis [14]. The organoleptic estimation of the samples was carried out using a quantitative descriptive analysis [15] based on the development of a list of terms and procedures for determining the characteristics of the structure that describe the properties of the product (descriptors). Each descriptor was assigned with an appropriate touch index. The severity of a descriptor for a given product was estimated with a certain scale with the help of a group of tasters as the organoleptic (sensory) perception of the product quality. To eliminate duplication, the multistage estimation of the results was carried out.

During the analysis, some of the most significant characteristics of the structure were identified, and their descriptors were defined (Table 1).

The tasting group included 20 people without special training. For each of the descriptors of the texture, a 7-step scale was used (1-no property is detected, 7-the property is distinct). The tasters also estimated the general perception of the samples on the basis of all the sensory indexes using a similar scale. The results of the sensory analysis were statistically handled.

Scanning electron microscopy (SEM) was used to analyze a change in the texture of purees. The microstructure of fresh, frozen and defrosted purees was examined using a ZEISS EVO 50 microscope (Karl Zeiss, Germany). For the deionization of the material, a layer of aluminum with a thickness of 20 nm was deposited on the pre-dehydrated plates of the puree samples by thermal evaporation. Sputtering was carried out at a VUP-4 (Russia) vacuum universal station. Micrographs were obtained using a SmartSEM digital system.

RESULTS AND DISCUSSION

Sensory studies have been carried out and the severity of descriptors for vegetable purees as well as the overall estimation of the taste and color of the product have been numerically estimated (Table 2).

The results of statistical processing of sensory estimation of experimental samples are graphically presented in the form of histograms (Fig. 1) for each descriptor. The analysis showed the distinct effect of hydrocolloid species on such descriptors as texture and smoothness (Fig. 1a and b) as well as on color and the overall sensory estimation of taste (Fig. 1c and d). The values of the other controlled descriptors were approximately at the same level for one or another type of frozen/defrosted puree regardless of the type of the structurant used (with the exception of fresh mashed potatoes). Therefore, the histograms of these descriptors are not shown here.

The change in the intensity of descriptors of experimental samples is also clearly visible in the case of the graphical interpretation of the results of sensory estimation using the profile method. This method shows in a more comprehensive manner the pattern relating to the sensory comparative estimation of samples in general rather than the image in Cartesian coordinates.

Table 2. Results of the statistical processing of sensory evaluation of experimental samples

Sample/ No. / description / composition				Structure descriptors						Taste	Color
				1	2	3	4	5	6		
Carrot puree	1	fresh	no additives	7.0	1	6.0	6	3.0	4.0	7.0	7
	2	frozen/defrosted	no additives	6.5	1	6.0	6.5	2.0	4.0	4.0	7
	3	frozen/defrosted	xanthan gum 0.5%	6.5	2	6.5	6.5	2.0	5.0	4.0	6
	4	frozen/defrosted	starch 5%	6.0	1	7.0	6.5	3.0	3.0	5.0	5
	5	frozen/defrosted	inulin 2%	6.0	1	6.0	6.0	2.0	5.0	3.0	6
	6	frozen/defrosted	xanthan gum 0.5%, inulin 2%	7.0	1	6.0	6.5	2.0	6.0	5.0	4
	7	frozen/defrosted	starch 5%, inulin 2%	6.5	1	6.0	6.5	2.0	2.0	4.0	4
Beet puree	1	fresh	no additives	7.0	1	6.0	5.0	3.0	4.0	7.0	7
	2	frozen/defrosted	no additives	7.0	1	6.5	5.0	2.0	3.0	5.0	7
	3	frozen/defrosted	xanthan gum 0.5%	7.0	1	5.5	5.0	2.0	4.0	2.5	7
	4	frozen/defrosted	starch 5%	7.0	1	6.5	6.0	2.0	2.0	5.0	5
	5	frozen/defrosted	inulin 2%	7.0	1	7.0	6.0	3.0	5.0	4.0	6
	6	frozen/defrosted	xanthan gum 0.5%, inulin 2%	7.0	1	5.5	6.0	3.0	4.5	3.5	7
	7	frozen/defrosted	starch 5%, inulin 2%	7.0	1	6.5	7.0	2.5	1.0	6.0	7
Zucchini puree	1	fresh	no additives	7.0	1	6.0	5.0	4.0	3.0	7.0	7
	2	frozen/defrosted	no additives	7.0	2	5.0	4.0	3.0	3.0	4.0	7
	3	frozen/defrosted	xanthan gum 0.5%	7.0	1	6.0	6.0	4.0	5.0	5.0	7
	4	frozen/defrosted	starch 5%	7.0	1	6.0	6.0	3.5	1.0	3.0	3
	5	frozen/defrosted	inulin 2%	7.0	1	6.0	5.5	4.0	5.0	4.5	7
	6	frozen/defrosted	xanthan gum 0.5%, inulin 2%	7.0	1	4.0	5.0	4.0	5.0	3.0	6
	7	frozen/defrosted	starch 5%, inulin 2%	7.0	1	6.0	6.0	4.0	1.0	3.0	3

■ carrot,   ■ beetroot,   ■ zucchini
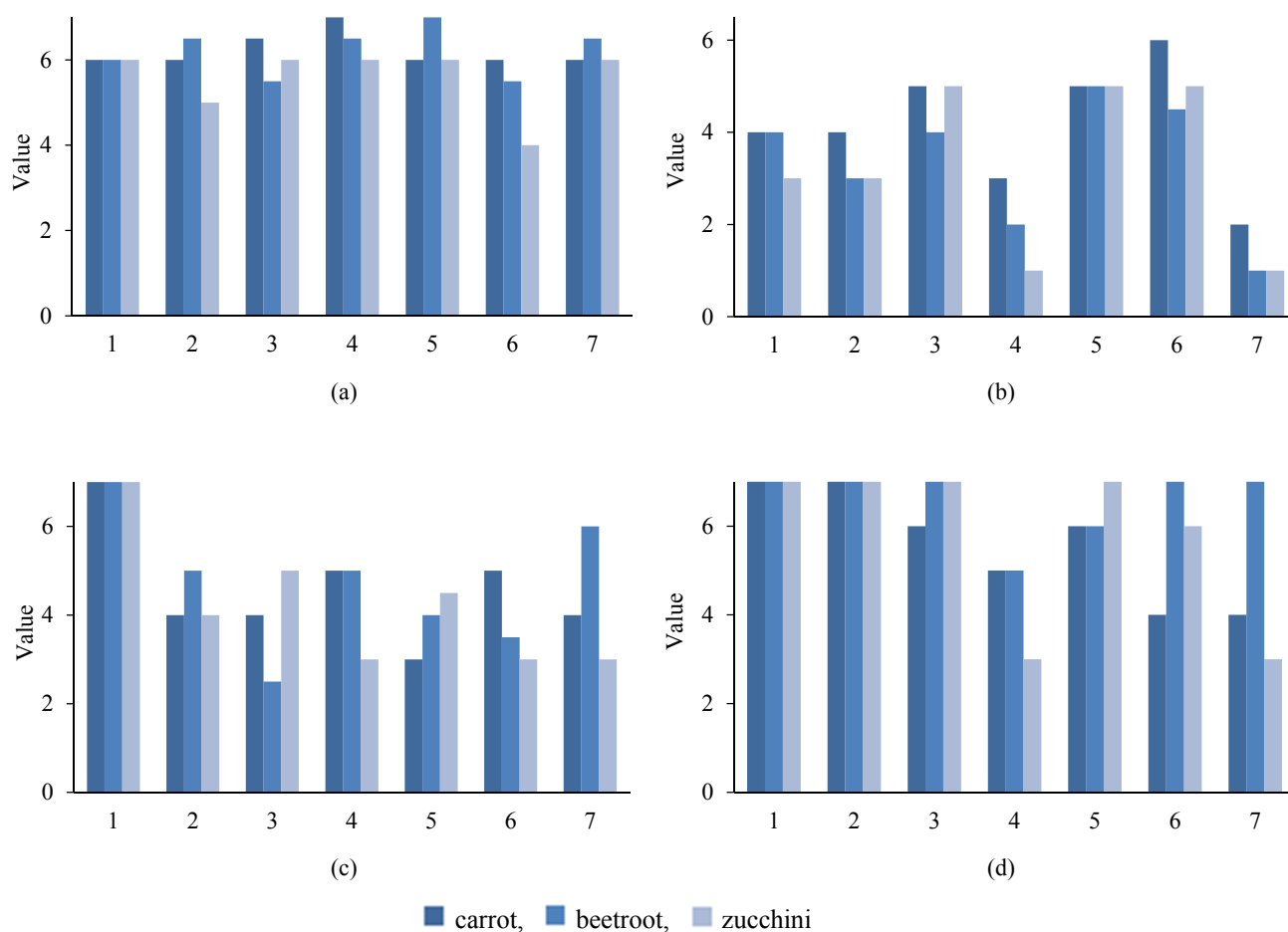
11 – fresh, no additives; 2 – frozen/defrosted, no additives; 3 – xanthan gum (0.5%); 4 – starch (5%); 5 – inulin (2%); 6 – xanthan gum (0.5%) + inulin (2%); 7 – starch (5%) + inulin (2%).

Fig. 1. Results of statistical processing of sensory evaluation of experimental samples: (a) texture, (b) smoothness, (c) taste, and (d) color.

Each specific profilogram shows the values of descriptors of puree samples on the divergent rays with and without the addition of a certain composition that were subjected to have undergone the same refrigeration treatment.

Negative qualities (water separation, texture, and viscosities) are given in advance at the bottom of the profiles below the horizontal one. This is for the convenience of analyzing the results, because slipping down the profilogram sample with the additive indicates a negative effect on the quality of the hydrocolloids introduced into the sample. Profilograms are grouped depending on the composition of hydrocolloid or composition administered.

Xanthan gum exerted its effect on the structure irrespective of the technological process and formed viscosity of the samples regardless of the conditions and types of processing (Figs. 1a, 2, 5). The fresh samples as well as frozen/defrosted ones with the addition of xanthan gum are rated as thick ones. When the surface of the puree was visually observed, and after the spoon was immersed therein, there was no water separation (Fig. 2).

The starch imparted the most pronounced density in the mouth as well as visually (Figs. 3 and 6). There was no water separation in both the fresh and frozen/defrosted samples. The starch also had an effect on taste and color (Figs. 1c, 1d, and 3). It was especially pronounced for the zucchini puree, which

acquired a whitish shade, as well as on the carrot puree (gray tint). In general, the puree with xanthan gum was estimated as more palatable to taste than the puree with starch at the same values of structure descriptors (Fig. 1c).

A significant effect of inulin and xanthan gum on the sense of smoothness of the samples in the oral cavity was noted after squeezing the product between the palate and the tongue (Figs. 4, and 5). At the same time the starch provided a granular structure (Figs. 1a, and 2) especially noticeable for the zucchini puree because of its natural, more tender taste and not such a fibrous structure as in the puree made from carrots and beets.

It was established that in the vegetable purees susceptible to freezing, storage and defrosting, inulin has a low thickening capacity and does not exhibit any synergistic properties with xanthan gum and starch. The weak thickening capacity of inulin can supposedly be associated with the partial molecular degradation of the gel in the freezing-storage-defrosting cycle. However, the frozen/defrosted puree samples containing inulin had more pronounced flavor (Fig. 1c) and smooth texture (creaminess) (Fig. 4) than their analogues without additives.

During the visual observation of the surface after mixing the product with a spoon (descriptor-texture), the samples with inulin had a more glossy, flat surface (Fig. 1a).

Fig. 2. Profilograms for change in the descriptors of the structure of the samples that were subjected to refrigeration: (a) carrot, (b) beetroot, and (c) zucchini.
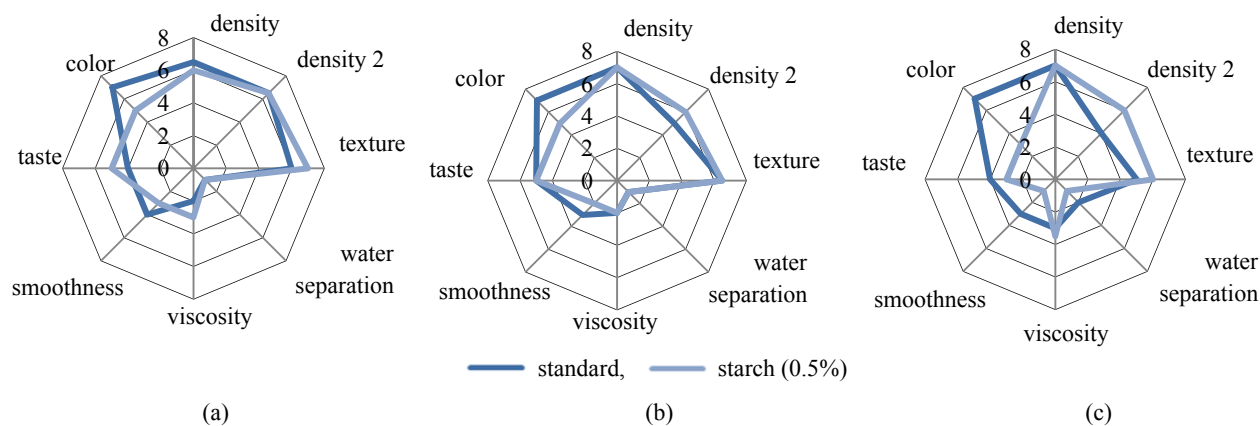
Fig. 3. Profilograms for change in the descriptors of the structure of the samples that were subjected to refrigeration: (a) carrot, (b) beetroot, and (c) zucchini.

Fig. 4. Profilograms for a change in the descriptors of the structure of the samples that were subjected to refrigeration: (a) carrot, (b) beetroot, and (c) zucchini.
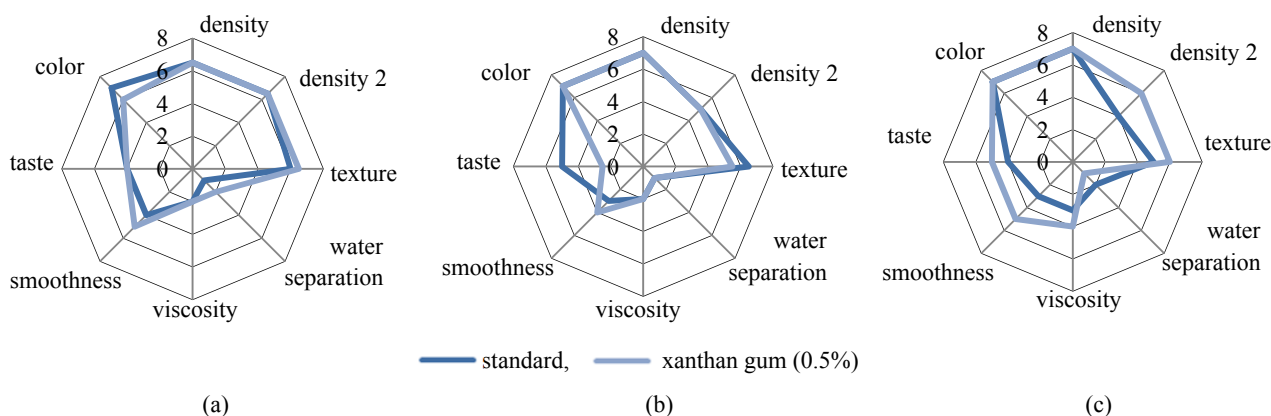

Fig. 5. Profilograms for a change in the descriptors of the structure of the samples that were subjected to refrigeration: (a) carrot, (b) beetroot, and (c) zucchini.
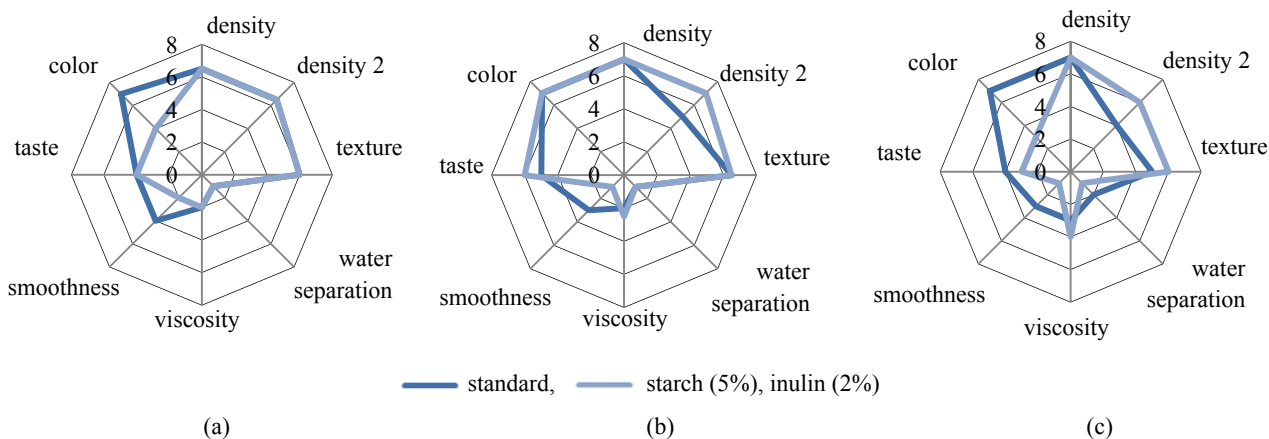
Fig.6. Profilograms for a change in the descriptors of the structure of the samples that were subjected to refrigeration: (a) carrot, (b) beetroot, and (c) zucchini.
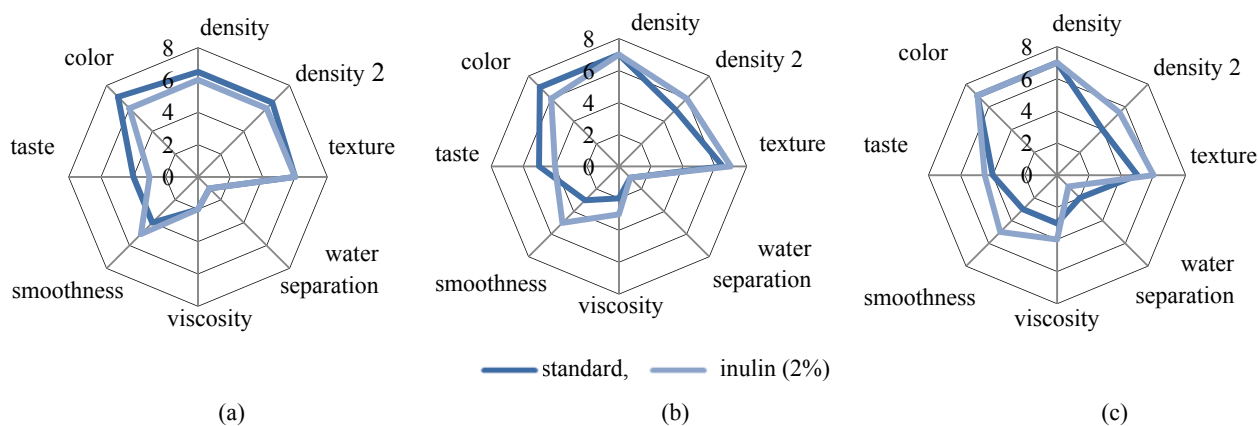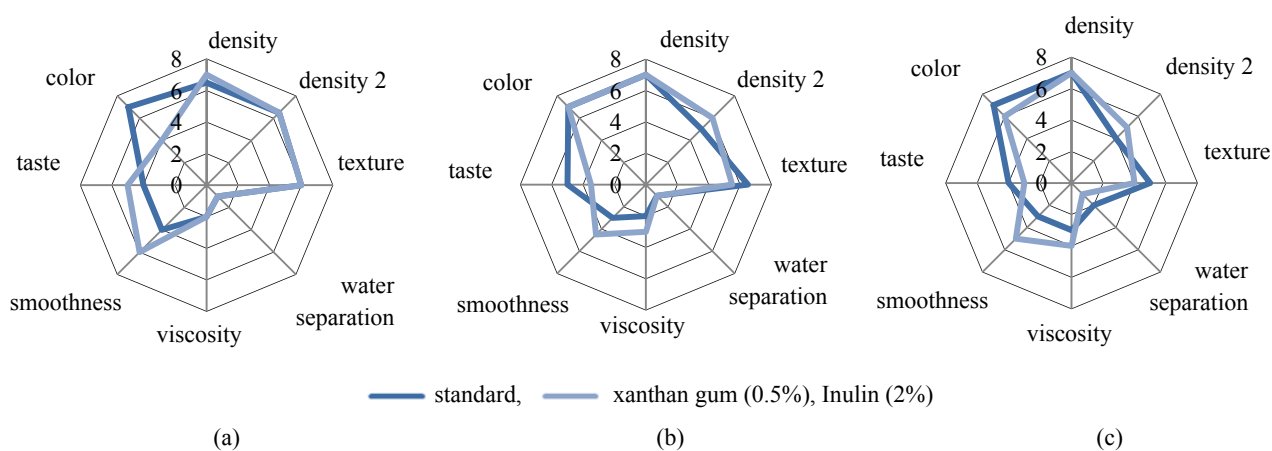
It has been established that the undesirable taste of xanthan gum and starch is covered by a pleasant aftertaste provided by the introduction of inulin into the puree. Thus, the optimal samples of taste estimation were obtained with the addition of inulin and its combinations with starch or xanthan gum (Fig. 1c). Hence, the simultaneous incorporation of prebiotics (inulin) and stabilizers in small amounts into the formula ensures the preservation of high taste qualities of vegetable purees and a stable structure during the storage period and in the heat treatment processes.

In all the samples studied, as a result of introduction of hydrocolloids, the formation of weakly structured gel with the pronounced retention capacity in relation to the components of the products was noted, as confirmed by the microstructural analysis (Figs. 7, and 8).

Fig. 7. Effect of hydrocolloids on the microstructure of zucchini puree: (a) the fresh puree with no addition of hydrocolloids after the freezing-storage-defrosting cycle ; (b) the frozen/defrosted puree with the addition of 5% starch; (c) the frozen/defrosted puree with the addition of 0.5% xanthan gum; (d) the frozen/defrosted puree with the addition of 2% inulin.
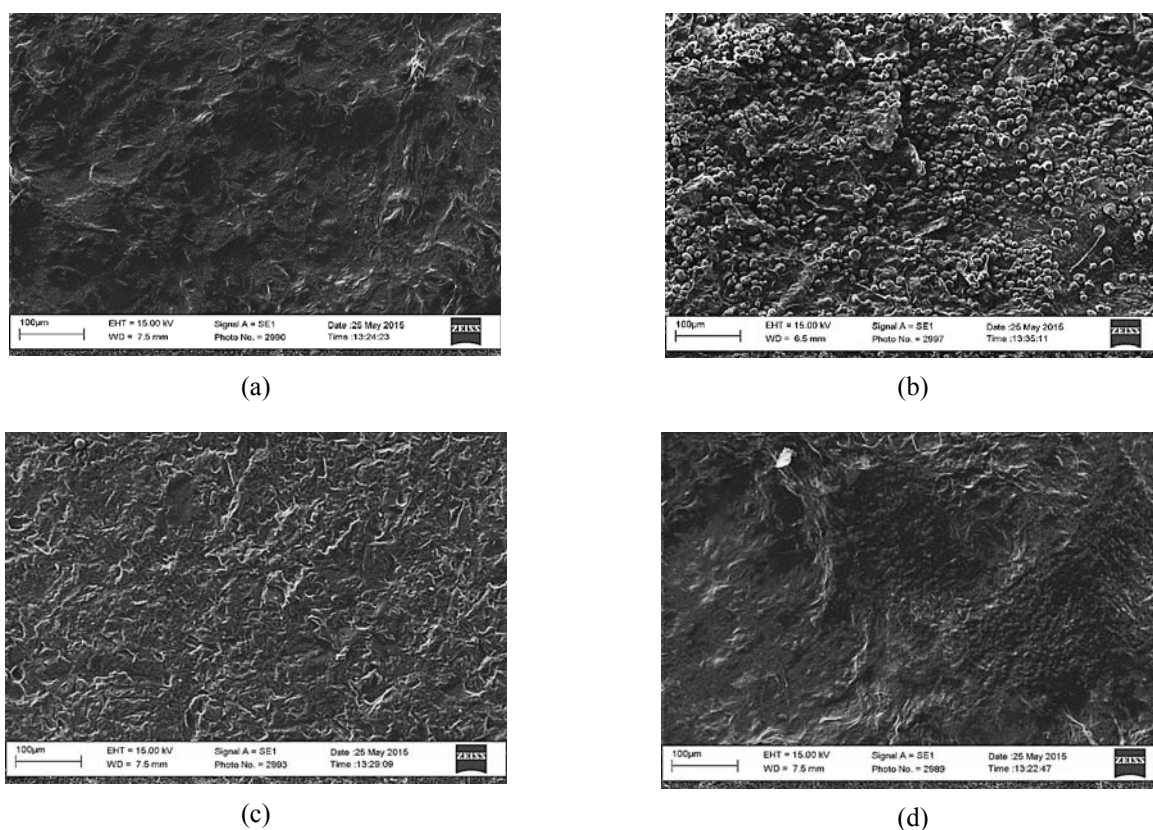

Fig. 8. Effect of hydrocolloids on the microstructure of carrot puree: (a) the fresh puree with no addition of hydrocolloids after the freezing-storage-defrosting cycle ; (b) the frozen/defrosted puree with the addition of 5% starch; (c) the frozen/defrosted puree with the addition of 0.5% xanthan gum; (d) the frozen/defrosted puree with the addition of 2% inulin.
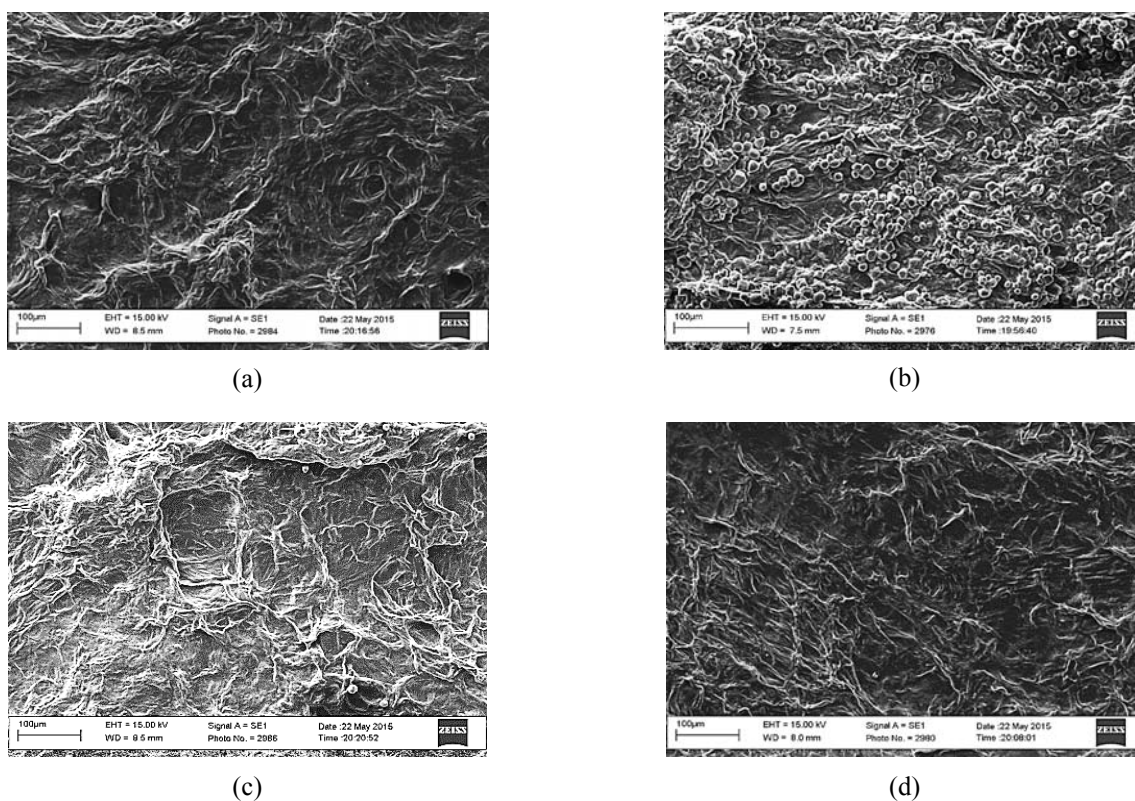
Figs. 7b and 8b show, indicates that the grains of starch do not degrade or degrade insignificantly as during the entire freezing-storage-defrosting treatment cycle, this hydrocolloid. The sensory evaluation of experimental samples of mashed potatoes with starch marked a granular structure.

In the samples with xanthan gum (Figs. 7c and 8c), the presence of a developed spatial structure was noted, which explained high values of descriptors of the structure (density, texture, etc.) for the vegetable purees and a softer structure compared to the puree with the addition of starch (descriptor–smoothness).

Figs. 7d and 8d present the resulting cellular structure of puree with the addition of inulin in the form of weak gel characterized by a low shear modulus, an insignificant shear stress, and weak viscoelastic properties noted in the sensory evaluation.

CONCLUSION

A possibility of using traditional products (xanthan gum and modified starch) and products unused earlier for this group (inulin) of structurizing ingredients due to various manifestations of their basic structure-forming properties in the freezing-refrigerated storage-defrosting cycle has been proved.

In the cold state, the binder acts as a stabilizer to prevent settling and should not lose its binding properties when heating the sample. Due to this, the modified starches with small granules which exhibit a thermal stability in cold storage and further heat treatment are most suitable for creating a viscous structure.

Inulin is also not subject to any changes during temperature treatments for the products with a pH higher than 3.5, which include the study objects, but probably will not be widely used as a structure-forming agent for vegetable purees due to the weak of structure-forming properties. The synergistic combinations of inulin and xanthan gum improve the organoleptic characteristics of the product, including consistency, especially in cold storage. In addition, the ability of inulin to form a smooth structure in vegetable purée when enveloping the oral cavity has been revealed despite the fact that the initial product has a rather coarse fibrous consistency due to the plant origin of the ingredients. Thus, it is promising to use inulin in combination with thickeners to give the product prebiotic properties with the improved perception in consumers.

The results of the studies confirm the possibility of storing vegetable purees at low temperatures for a long time without the deterioration of the structural and organoleptic properties resulting from the slowly occurring degradation processes of the individual components of the product at low temperatures provided that the hydrocolloids under study are used.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Complex of polyphenols sorbed on buckwheat flour as a functional food ingredient

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Abstract: An innovative approach to creating a new generation of specialised foods for dietary therapy of type 2 diabetes can involve planned adding of plant polyphenols to their formulafions. The marked antioxidant properties of polyphenols largely determine their potential antidiabetic effects. However, the use of food polyphenols for prophylactic purposes is limited by their low bioavailability, which makes it expedient to search for technological approaches aimed at obtaining polyphenolic matrices with high biological activity, increased digestibility, and stability. This study objective was to purposely extract and concentrate the polyphenols by sorbing them from an aqueous solution of the bilberry leaf extract (BLE) on buckwheat flour and to assess their storage stability. A number of experiments on optimal parameters selection for sorbing polyphenols from the BLE on buckwheat flour were performed. The parameters included the concentration of the extract solution, the solution/sorbent ratio, the pH of the solution, the temperature and the time of sorption. The sorption on the polyphenol matrix was determined from the difference in their contents in the initial solution of the extract and in the supernatant after centrifugation by the Folin-Ciocalteu method. The effects of exposure to light, temperatures, and humidity on the polyphenol compounds in the dry BLE and in the food matrix contents during storage was analysed by the FTIR spectroscopy. The experiments determined the optimal conditions for the BLE polyphenol sorption on buckwheat flour by incubation of a 2% BLE solution pH = 3.6 with the portion of buckwheat flour at the ratio of 1g/50 cm^3 solution for 45 minutes at 25°C. When storing the food matrix, there was no significant degradation of the polyphenolic compounds in the food matrix, which indicates an increase in the stability of the polyphenols sorbed on buckwheat flour. This paper presents the results that are scientifically and practically relevant for the nutritiology experts who devise promising technological approaches to expanding the range of functional food ingredients of the antidiabetic character.

Keywords: Diabetes, polyphenols, bioavailability, bilberry leaf extract, buckwheat flour, sorption, food matrix, functional food ingredient

INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine diseases, characterised by a steady morbidity increase, a high incidence of vascular complications leading to early disability, high mortality, and significant economic costs of medical and social care [1]. An essential part of preventing DM as an alimentary-dependent disease and its clinical manifestations is optimising and personalising dietary prevention, which involves developing new functional and specialised food products (SFPs) that correct the carbohydrate and lipid metabolism disorders in a specific way [2]. The innovative approach to the development of a new generation of SFPs for diet

therapy of type 2 diabetes can be the usage of plant polyphenols as functional food ingredients (FFI) in their contents, i.e. minor biologically active substances (BAS) with proven in clinical and in vivo studies pronounced hypoglycemic, hypocholesterolemic and antioxidant properties [3]. For many years clinical research and experiments on laboratory animals with induced or genetically determined diabetic manifestations and have been studying the hypolipidemic, and hypoglycemic properties of individual plant polyphenols as such or within the extracts [4]. Using plant polyphenols in metabolic disorders is proved very promising by the study results evidentiating their effectiveness and safety through the

implementation of one of the main principles of phytotherapy, namely: act not only on the affected organ, but also on the body's conjugate systems [5, 6]. According to contemporary theories, the pathogenic basis of type 2 diabetes includes two major disorders: developing the insulin resistance in the peripheral target tissues and reducing insulin secretion. Hyperglycemia and glucose autooxidation cause the oxidative stress, which in turn damages phospholipids in the plasma membranes of the target tissues and beta cells of Langerhans islets. Thusan, inevitable "vicious circle" characterised by a systemic metabolic disorder and concomitant clinical complications arises. The marked antioxidant properties of polyphenols largely determine their potential antidiabetic effects. The polyphenol influence on the cellular level is determined by the interaction with the cell membranes, which modulates the phase state of the membrane lipids and its structural organisation [1, 7]. There is no doubt that polyphenols influence the nuclear and cytoplasmic proteins expression. An important component of the physiological activity of polyphenols is their participation in signaling systems of the cell [1]**.** However, a widespread use of the proven properties of food polyphenols for prophylactic purposes is limited by their very low bioavailability, often not allowing achieving the expected beneficial effects in carbohydrate and lipid metabolism disorders in clinical conditions. So, after a normal meal, the concentration of the metabolites from the phenolic compounds in the blood rarely exceeds 1 nM, although in the gastrointestinal tract it can amount to over 1 mM [8]. Thus, it is obviously expedient to obtain the FFI with the highest possible polyphenol content. The modern priority in increasing the efficiency for the polyphenolic SFPs includes a wide range of technological solutions, such as using microspheres and microcapsules as transport; producing solid dispersions; practicing administration of polyphenols with substances "enhancing" their absorption; generating phospholipid-polyphenol complexes, and polyphenol-protein complexes [8]. Sorbing polyphenols from berry juices and plant extracts on based of soy flour increases their stability and prolongs their shelf life, as shown in [9–11].

Due to the wide range of polyphenols in bilberry leaves, this plant extracts are customarily used in traditional medicine to reduce the symptoms of diabetes mellitus [12]. Previously, we tested hypoglycemic and hypolipidemic properties of an aqueous solution of the BLE when it had been administered intragastrically to the genetic line of obese rats Zucker Rats Crl: ZUC-Leprfa rats in a dose of 2 g/kg of the animal's body weight for 28 days. The extract consumption helped improve the response of the insulin-sensitive tissues to the exogenous administration of glucose and insulin and prevented the increase of blood glucose level [13].

This study objective was to purposely recover and concentrate the polyphenols by sorbing them from an aqueous solution of the BLE on brown buckwheat flour, to assess their storage stability in order to justify the possible use as a FFI in the SFP contents. Accordingly, the research objectives included, first, determining the optimal sorption conditions by varying the concentration ratio of polyphenol extract and flour weights, pH values of the incubation mixture, adsorption time, and parameters of the protein matrix microstructure. Secondly, conducting a comparative analysis of the degradation kinetics for the polyphenols in a solution of the BLE and the polyphenols sorbed on brown buckwheat flour (hereinafter referred to as the food matrix) when stored at the specified temperature and humidity. The choice of the brown buckwheat flour as the absorbing matrix for polyphenols is conditioned by its use in modern medical practice as a diet ingredient for obesity, diabetes, kidney disease, with the view of lowering cholesterol and total lipids, and strengthening the blood vessel walls [14].

STUDY OBJECTS AND METHODS

The dry bilberry leaves extract (OOO "KHARMS", St. Petersburg, Russia) with a moisture and ash contents of 61% and 16.8%, respectively, was the sbudy object. The total contents of flavonoids and hydroxycinnamic acids are 13.67 ± 0.11 mg/g and 11.68 ± 0.22 mg/g, pespecfively. The total content of proanthocyanidins equals 15.5 ± 0.22 mg/g [15]. The content of polyphenol compounds expressed in mg-eq. of gallic acid is 95.5 ± 1.1 mg-eq. g.a./g extract.

An experimental sample of brown buckwheat flour was obtained by grinding an industrial batch of food flour (OOO HLEBZERNOPRODUKT, Taganrog, Russia) by means of a knife grinder GRINDOMIX GM200 (Retsch, Germany) at 8000 rpm for 10 min. Mass content of protein in the sample was 9.7%; carbohydrates, 70.0%; ash, 1.6%; and moisture, 5.5%.

Granulometric characteristic. The granulometric parameters of the brown buckwheat flour sample and the food matrix were determined by electron-scanning microscopy. The samples were applied in a thin layer on a stage with a double-sided conductive tape, followed by ion-plasma spraying of gold to increase the contrast of the image. The micrographs were obtained with a scanning electron microscope AIS 1800C (Seron Technologies Inc) in the secondary electron mode in a high vacuum. By means of the obtained micrographs, the criteria for the size and shape of the particles were calculated: equivalent radius (characterises the average particle size of an irregular shape) and ellipticity (characterises the deviation degree for the shape of a particle from a spherical one), according to formulas

Equivalent radius:

$$r_{eq} = \frac{(l+b)}{2} \qquad (1)$$

Ellipticity:

$$k_э = \frac{l}{b} \qquad (2)$$

where, *l* is the length of the particle, *b* is the width of the particle.

To compare the values obtained between the groups, the Kruskal-Wallis nonparametric test was used.

Determining the optimal sorption conditions. The polyphenols sorption from the aqueous solutions of the BLE on brown buckwheat flour was conducted with a constant stirring of the incubation mixture on a magnetic stirrer, and after the incubation, the centrifugation of the resulting suspension (a centrifuge Beckman J6B (AL-TAR, USA)) at 4000 rpm for 20 min took place. The supernatant was separated from the precipitate by decantation. The precipitate was freeze-dried by means of a LS-500 unit (OOO "PROINTEH", Russia). The dependence of polyphenols sorption on flour the pH value of the incubation mixture was determined with the help of a flour sample of 1g in 50 cm^3 of the incubation mixture of a 0.5% extract solution at a pH range of 2.0–7.0. The sorption took place for 30 minutes at a temperature of 25°C. The sorption dependence on the incubation time of the mixture was determined by means of a 0.5% extract solution in the time interval of 15–90 min at a pH of 3.6, a temperature of 25°C, using a flour sample of 1 g. The sorption dependence on the incubation temperature of the mixture was determined using a flour sample of 1 g in the mixture of 50 cm^3 of a 0.5% extract solution at a pH of 3.6 for 30 min in the temperature range of 25–50°C. The sorption dependence on the extract solution concentration was determined in the range of 0.5–2.5% at a temperature of 25°C, and pH of 3.6 for 30 min using a flour sample of 1 g. The stoichiometric analysis of the polyphenol sorption on brown buckwheat flour was conducted by mixing 100 cm^3 of 0.5% extract with different amounts of flour (1–5 g) for 30 min at a temperature of 25°C and pH of 3.6. The specific content of total polyphenols sorbed on the flour, expressed in mg equivalent of gallic acid (as indicated below), was calculated from the difference in their content in the initial extract solution and in the supernatant after centrifugation.

Evaluating polyphenols stability in the dry bilberry leaves extract and as a part of the food matrix. Half the samples of the dry BLE and the samples of polyphenols in the food matrix were placed in a climate chamber (manufactured by *POL-EKO-APARATURA,* Poland) at 50°C and a relative humidity of 50%. The second half was kept at the same temperature and humidity conditions with an additional exposure to light. The studies were conducted for 7 days. The control points for sampling after the beginning of loading into the climatic chamber were as follows: 16 h, 24 h, 40 h, 48 h, 64 h, 72 h, 88.5 h, 136.5 h, 144 h, 161 h.

Measuring the total polyphenols. The contents of total polyphenols in the analysed solutions of the BLE and supernatants, sampled after the sorption, was determined spectrophotometrically by the Folin-Ciocalteu method [16]. The optical density of the solutions was measured at a wavelength of 765 nm using a spectrophotometer SpectroQuest 2800 (UNICO, USA). In the solutions, the concentration of total polyphenols in mg-eq. g.a. was determined by

means of a calibration curve obtained by using a gallic acid standard (97.5%, Sigma, USA).

Measuring polyphenols desorbed from the food matrix. A 0.5 g portion of the food matrix was placed in a 20 cm^3 vial the adsorbed polyphenols were eluted with 8 cm^3 of a 1% solution of glacial acetic acid in 80% ethanol in an ultrasonic bath "Sapphire 2.8 TTC" (OOO "Sapphire", Russia) for 5 minutes at 55°C. The mixture was centrifuged at 4000 rpm for 10 min, and the supernatant was transferred to a pear-shaped flask. The elution in the same mode was repeated twice more. The eluates were combined, and the alcohol was evaporated on a rotary evaporator IR1M3 (OAO "Khimlaborpribor", Russia) at 60°C to achieve 15% of the initial volume of the combined eluents. The resulting concentrate was transferred to a graduated cylinder, the volume was adjusted to 50 cm^3 with distilled water. In the obtained solution, the content of total polyphenols was determined by the Folin-Ciocalteu method.

Measuring the kinetic parameters of polyphenols degradation. The effects of exposing the polyphenolic compounds in the dry BLE and in the food matrix to light, temperature, and humidity were analysed by the IR Fourier transform spectroscopy using a spectrometer Tensor 27 (Bruker Optik GmbH) and an adapter MIRacleATR (Attenuated total reflection, ATR), and using a software Opus 6.0 [17]. Study parameters: a resolution was 4 cm^{-1}, the number of scans equaled 64, and the scanned area ranged from 4000 cm^{-1} to 600 cm^{-1}. Before measurement, the samples were ground in an agate mortar until a uniform consistency was obtained. Each sample was analysed in 4 replicates. The obtained spectra were processed in an automatic mode, including the following steps: the error correction of the ATR spectrum, the correction of moisture and carbon dioxide contents, reduction to the baseline, and normalisation. The calculations used the average value for the three spectra closest to each sample. To determine the kinetic characteristics during oxidation, the second derivative was calculated at 13 smooth points. The spectra of the second derivatives were analysed in two bands: in the range of 1725–1731 cm^{-1} corresponding to a strong band of stretching vibrations of the carbonyl group of the -CH$_2$-CHO aldehyde group; and also in the range of 2920–2960 cm^{-1} corresponding to the strong band of absorption for valence antisymmetric vibrations of -CH$_2$ bonds [18].

Calculating the kinetic parameters for the accumulation of polyphenol degradation products was carried out by the first-order equation for the reactions [19]:

$$\frac{\ln C_0}{C} = kt, \qquad (3)$$

where k is the reaction rate constant, t is the storage time, C_0 is the initial concentration, and C is the concentration at time t.

The half-transformation period for the functional groups studied, respectively, was determined by the equation:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k}, \qquad (4)$$

Preparing a functional food ingredient (FFI) in experimental quantities. To carry out experiments to assess the stability of the BLE polyphenols sorbed on crushed buckwheat flour, a 200 gr batch of food matrix was obtained. An experimental flour sample of 120 g and the dry BLE of 120 g, which was dissolved in 6 dm³ distilled water (2% solution) were used for this purpose. Then the solution was acidified with 1N HCl to pH of 3.6, centrifuged at 3000 rpm for 10 min. After this, a slight insoluble precipitate was removed, and 120 g of an experimental flour sample was added. The sorption was carried out at 25°C for 45 minutes with a constant stirring, then the mixture was centrifuged at 4000 rpm for 20 min, the precipitate was separated from the supernatant by decantation. Subsequently, 50 cm³ of distilled water was added to the precipitate, the mixture was stirred for 5–7 minutes and centrifuged at 4000 rpm for 10 min. The precipitate (food matrix) was separated from the supernatant by decanting and freeze-drying. The food matrix yield was 100 g. The content of the polyphenols in the food matrix was determined by eluting the polyphenols according to the procedure described above.
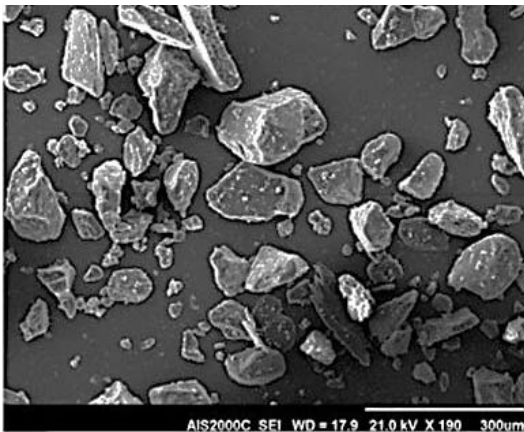
(a)
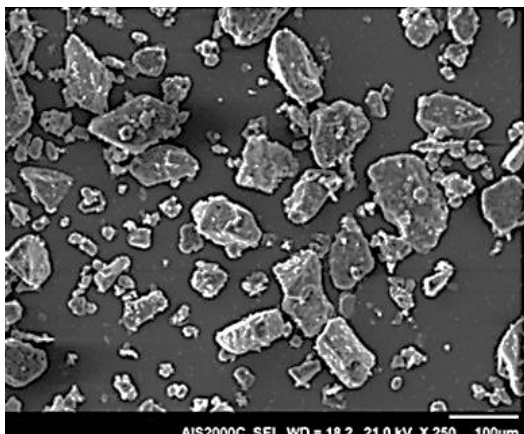
(b)

Fig. 1. Micrographs of the buckwheat flour samples obtained by scanning electron microscopy: (a) flour before grinding (large particles with even edges); (b) flour after grinding (fine particles with even edges).

RESULTS AND DISCUSSION

To carry out experiments to optimise the conditions for sorbing the BLE polyphenols on flour, an experimental batch of buckwheat flour was obtained, ground on a knife mill GRINDOMIX GM200 (Retsch, Germany) at 8000 rpm for 10 min. The average particle size of the initial sample of flour was 181.6 ± 5.1 μm, the average particle size of the ground flour was 134.9 ± 3.9 μm (Fig. 1).

Fig. 2 shows the graph of dependence of the polyphenols sorbing on the ground buckwheat flour on the pH of the incubation mixture. With an increase in pH from 2.0 to 7.0, there was a decrease in the average values of sorption of the BLE polyphenols from 1.7 ± 0.8 to 3.5 ± 0.4 mg-eq. g.a. /g flour.

Changing the incubation temperature in the range of 25–50°C had no significant effect on the sorption efficiency, as follows from the data presented in Fig. 3.

Fig. 4 shows the graph of dependence of the sorption of the BLE polyphenols on their concentration in the incubation mixture.
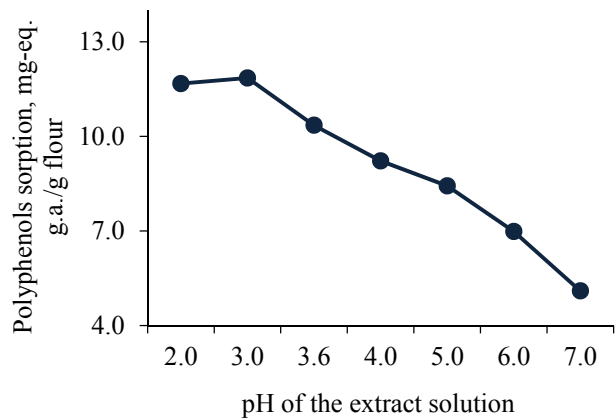

Fig. 2. Dependence of the BLE polyphenols sorbing (mg-eq. g.a./g flour) on buckwheat flour on the pH of the incubation mixture (0.5% the BLE solution, a flour weight of 1 g, incubation for 30 minutes at 25°C).
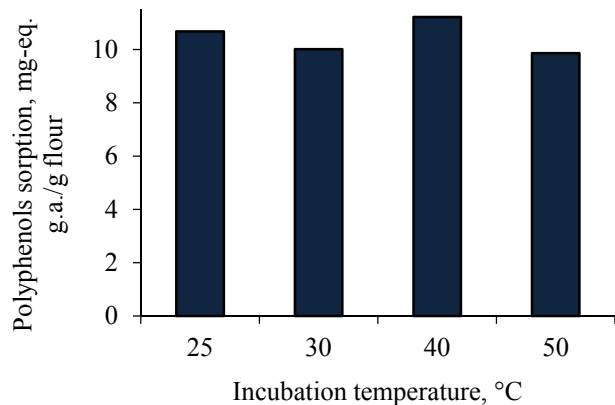

Fig. 3. Sorption of the BLE polyphenols at different incubation temperatures (0.5% BLE solution, a pH of 3.6, a flour weight of 1 g, incubation for 30 min).
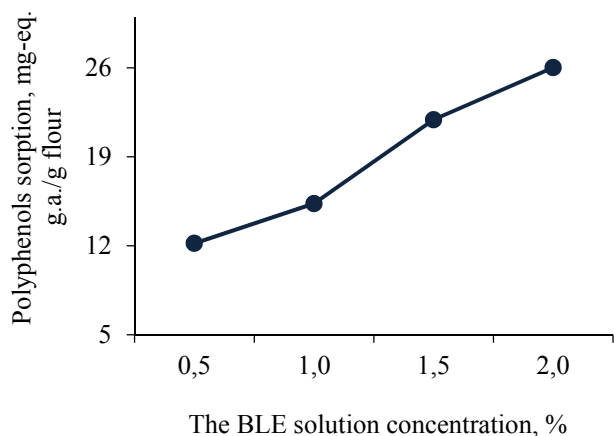
Fig. 4. Dependence of the polyphenols sorption (mg-eq. g.a./g flour) from the BLE solution on buckwheat flour on a pH of the incubation mixture (the BLE solution with a pH of 3.6, flour weight of 1 g, incubation for 30 minutes at 25°C).
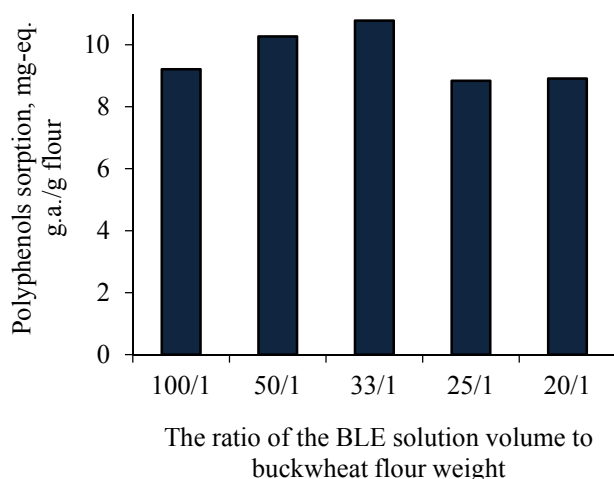

Fig. 5. Sorption of the extract polyphenols on buckwheat flour at various ratios of the BLE solution volume to buckwheat flour weight, ml/g (0.5% BLE solution, pH of 3.6, incubation for 30 min at 25°C).

With an increase in the concentration of the BLE polyphenols in the range of 0.5–2.0%, an increase in sorption is observed ranging from 12.1 ± 0.8 to 26.0 ± 1.6 mg-eq. g.a./g flour. With a further increase in the BLE concentration, gelling occurred, which prevented a correct evaluation of sorption.

Fig. 5 presents the data on the dependence of the BLE polyphenols sorption on the ground buckwheat flour on the ratio of the solution volume and the flour weight.

The change in the ratio of the BLE solution volume/buckwheat flour weight from 100/1 to 20/1 did not have a significant effect on the BLE polyphenols sorption (Fig. 5).

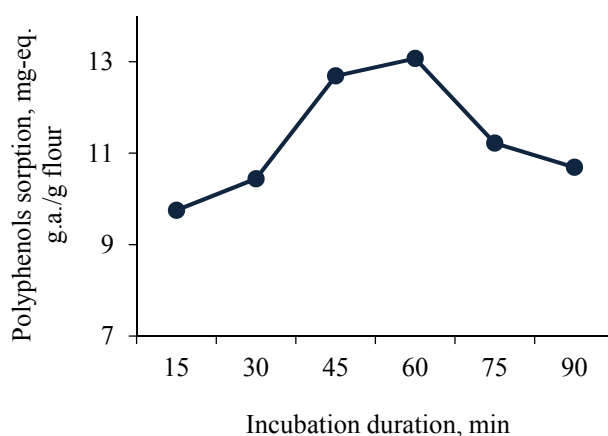Fig. 6 shows the graph of dependence of the polyphenols sorption on the incubation duration.

Fig. 6. Dependence of the BLE polyphenols sorptoion on buckwheat flour on the duration of the mixture incubation, mg-eq. g.a./g flour (0.5% BLE solution, pH of 3.6, flour weight of 1g, incubation at 25°C).

With an increase in the incubation duration from 30 to 60 min, the polyphenols sorption increased from 10.44 ± 0.40 to 13.07 ± 0.26 mg-eq. g.a./g flour. A further increase in the incubation time led to an unreliable decrease in sorption.

To carry out an experiment to assess the degradation degree of the BLE polyphenols in the food matrix during storage, an experimental batch of the product was obtained. The content of polyphenols in it was 23.7 ± 0.5 mg-eq. g.a./g flour.

Upon storage of the initial dry BLE for 72 h, the content of polyphenols decreased from 95.5 ± 1.1 mg-eq. g.a./g extract in the original sample to 86.1 ± 0.7 mg-eq. g.a./g extract when stored in the dark and up to 88.4 ± 1.3 mg-eq. g.a./ g extract when stored in the light. The further storage did not change these values. When storing the food matrix, both in the dark and in the light for the whole period (161 h), there was no reliable decrease in the polyphenols content expressed in gallic acid equivalents (22.9 ± 0.2 mg-eq. g.a./g flour).

The representative curves of the studied samples in the relevant ranges of wavelengths prior to the start of storage and at the end of the experiment are shown in Fig. 7a, b.

When studying the samples of the BLE and the complex during the storage, the influence of light on the degradation of polyphenols in them was not revealed.

The degradation of the polyphenolic compounds was more pronounced for the BLE, yet there were no significant changes revealed in the contents of the -CH_2-CHO aldehyde group (oxidation of -OH polyphenol groups to the corresponding keto- (=O) groups) and -CH_2-bonds (polymerisation accompanied by the formation of longer limiting aliphatic chains) from the complex with the protein matrix during storage.

Fig. 8 shows the accumulation curves for the functional groups under study, as well as the corresponding kinetic curves. The concentration of substances stands for the value of the area under the curve of the corresponding absorption band.

(a)

(b)
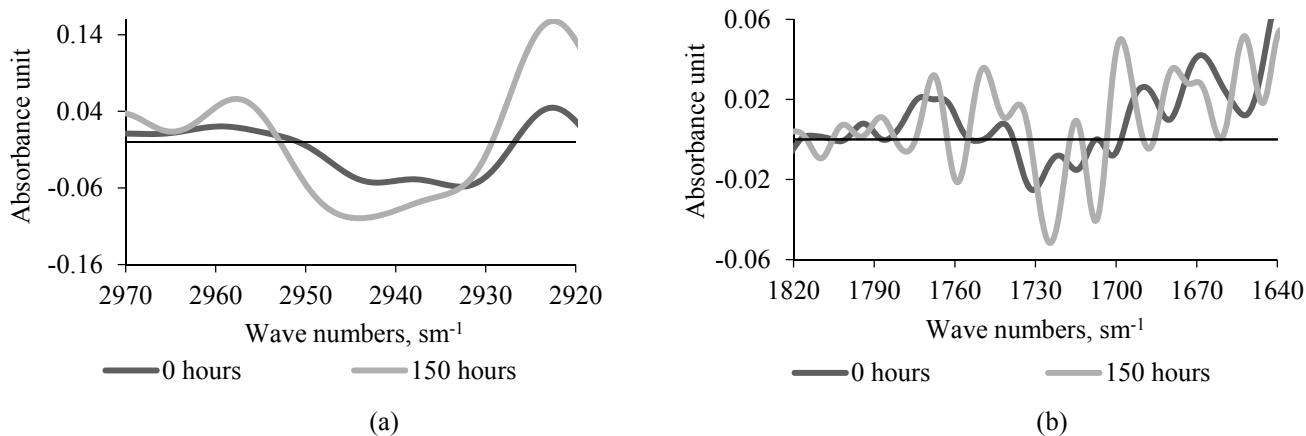
Fig. 7. Representative curves of the second derivatives of the IR spectra for the BLE in the ranges responsible for (a) the valence antisymmetric vibrations of the $-CH_2$ bonds; (b) stretching vibrations of the carbonyl group $-CH_2-CHO$ (each curve is the average value of three spectra of the sample (64 scans each) for the same storage time).

(a)

(b)

(c)

(d)
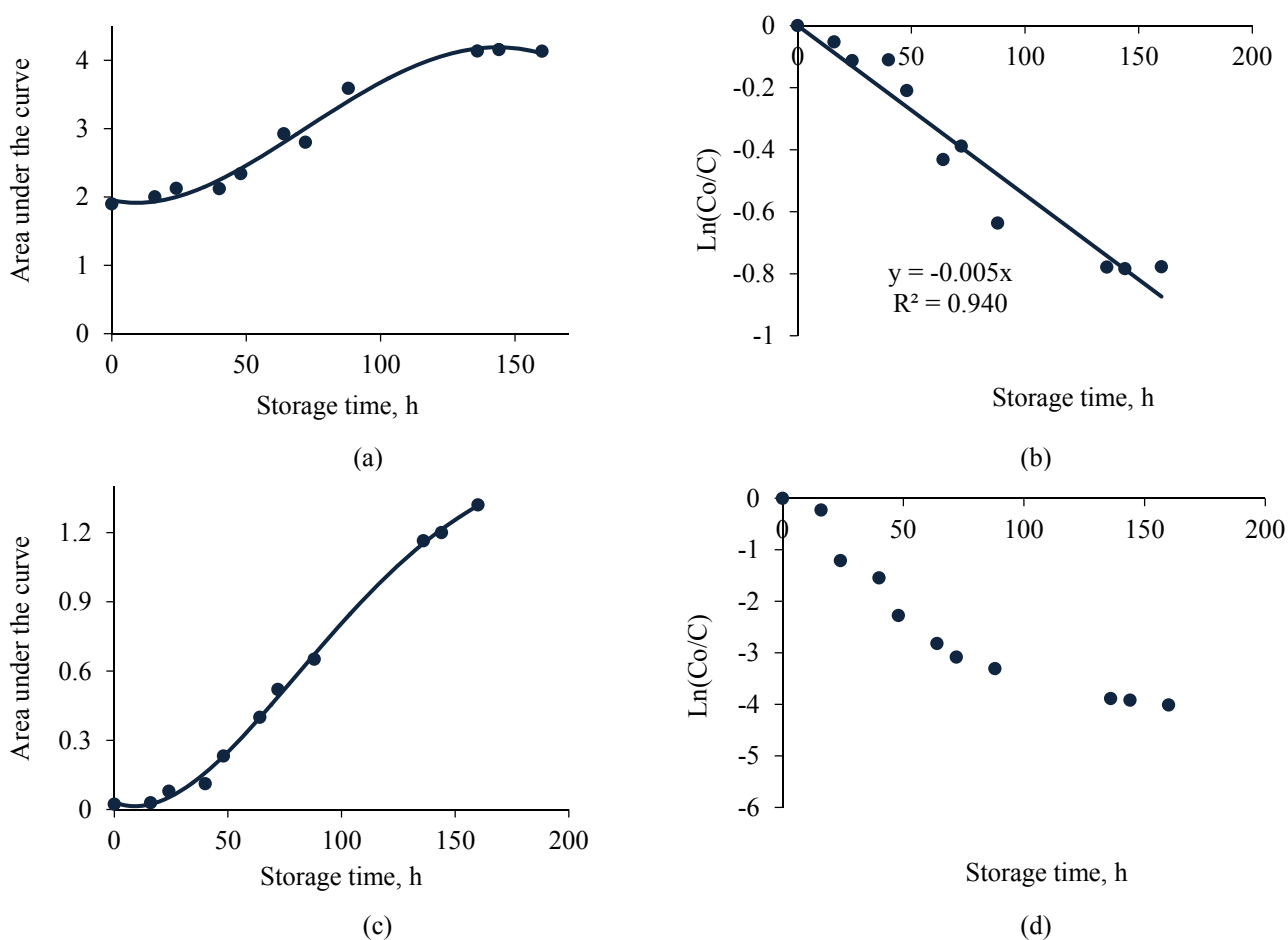
Fig. 8. Accumulation curves for the products of the polyphenols degradation in the BLE samples: (a) CH_2-bonds, (c) CH_2-CHO aldehydes groups) and their corresponding kinetic curves (b, d).

As one can be see from Fig. 8, a linear relationship exists between the logarithms of the functional groups concentrations and the storage time, which leads to the conclusion that the accumulation of these groups proceeds as the first-order reaction.

Thus, the kinetic parameters for the degradation of polyphenolic compounds in the BLE were obtained by calculation (Table 1).

Table 1. The kinetic parameters for the degradation of the BLE polyphenols

Functional group	Reaction	k, s^{-1}	$t_{1/2}, h$
$-CH_2-$	Polymerisation	1.53×10^{-6}	126.0
$-CH_2-CHO$	Oxidation	8.44×10^{-6}	22.8

The data obtained during the storage experiment with the samples evidentiate that the degradation rate for the polyphenols both in the BLE extract and in their complex with buckwheat flour does not depend on the light exposure at a temperature of 50°C and a relative humidity of 50%.

Polymerisation and oxidation of polyphenolic compounds from the BLE are the first-order reactions with the reaction rate constants $k = 1.53 \times 10^{-6}$ s^{-1} and $k = 8.44 \times 10^{-6}$ s^{-1} and the half-reaction period equal to $t_{1/2} = 126$ h and $t_{1/2} = 22.8$ h, respectively, at a temperature of 50°C.

CONCLUSION

In the course of the experiments, the optimal conditions determined for the sorption of polyphenols from the BLE on buckwheat flour were defermined: the concentration of the extract solution was 2%, the solution pH was 3.6, the ratio of the flour weight and the extract solution volume was 1g/50 cm^3, the temperature was 25°C, and the incubation time was 45 min. Under these conditions, the maximum value of the polyphenols sorption, determined by their elution from the food matrix, was 23.7 ± 0.5 mg-eq. g.a./g flour. The experimental batch of the food matrix was prepared by incubating the 2% BLE solution (pH 3.6) with the buckwheat flour (ground with the knife mill) at a ratio of 1 g/50cm^3 solution for 45 min at 25°C. The obtained food matrix can be used as a functional food ingredient in the specialised food products that correct carbohydrate and lipid metabolism disorders.

Considering that the obtained FFI is a powder providing an unstable suspensions in water, it can be recommended to be used in food products having a dense food matrix. Examples of such products are fruit and vegetable purees, viscous dairy products, and flour based products.

When storing the food matrix for 161 hours at a constant temperature (50°C) and humidity (50%) with the exposure to light and without significant reduction in light, the reliable decrease in the polyphenols contents, expressed in equivalents of the gallic acid by the Folin-Ciocalteu technique, was not observed. According to the data obtained by the FTIR spectroscopy, there was no reliable degradation trend for the polyphenolic compounds of the food matrix, which indicated that the sorption of the polyphenols on buckwheat flour increased their storage stability significantly.

This paper presents the new results that are scientifically and practically relevant for the nutritiology experts, who devise promising technological approaches to expanding the range of functional food ingredients of the antidiabetic character.

CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

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Intensification of thermal and rheological processes in a scraped-surface apparatus

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Abstract: The operation parameters of a special heat exchange scraped-surface apparatus ware studied and mathematically described in the article. The feature of the apparatus was the use of perforated cleaning devices in order to increase the turbulence of a product. The developed device can be used in the dairy, meat, and fat and oil industry to cool cream, animal fats, margarine emulsions, cooking fats, and other viscous food products. The increase in the productivity of the apparatus was achieved as a result of the more intensive mixing of the cleaned wall layers with the bulk of the cooled product due to the presence of cylindrical holes in the slats with a diameter of at least 0.05–0.1 of the diameter of the working cylinder. As a result of processing experimental data on heat exchange taking into account energy dissipation, a calculated criterial heat exchange equation for the nonisothermal motion of products and their different flows – laminar and transient – was obtained explicitly. In addition, the article considers the effect of starting modes on the operation of apparatus with mixing devices. These data can make it possible to take into account the possible deviations of parameters caused by nonsteady operating modes. On the basis of the data obtained, we have proposed assumptions about the degree of impact of viscosity and inertia in the considered range of parameters on a starting mode. The results of the study are relevant since they allow us to intensify the thermal processes in this type of common apparatus by 10–12%.

Keywords: Heat exchange, viscosity, rheology, mixing, dairy products

INTRODUCTION

A significant proportion of food products have viscous and quasi-viscous properties.

Viscous media require new engineering knowledge on the development of their production technology and techniques, taking into account the rheological properties of processed raw materials and finished products.

The data on the patterns of changes in rheological characteristics make it possible to affect the quality and structure of products both by adding supplements and by regulating the treating modes of a product.

The method of intensifying heat exchange when treating viscous products by mixing the treated medium in apparatus with special blade-type mechanisms is used widely. It is necessary to study this method deeper, especially when heat-treating viscous media

with non-Newtonian properties. Mixing viscous materials requires a lot of energy. Therefore, to reduce the power used for mixing, it is necessary to minimize the speed of rotation, taking into account the rheological characteristics of the medium treated and ensuring the required treating mode.

In many cases, it may be reasonable to use mixing devices for such apparatus that come into contact with the heat exchange surface in order to clean a product layer. This method was studied by L.K. Nikolaev, B.L. Nikolaev, and a number of other authors [1, 2, 3, 4, 5]. Nevertheless, there are a lot of aspects of operation of apparatus with cleaning devices that require a deeper study, in particular, when heat-treating viscous media with non-Newtonian properties such as cream, animal and cooking fats, margarine emulsions, meat and fish mincemeat, and other viscous food

products. There are also some ideas for changing the design of mixing mechanisms to activate the process that requires development.

The statement of the problem of nonsteady heating or cooling of an infinite cylinder is based on the cases when:
– axial heat fluxes are negligible as compared to radial ones;
– the coefficient of convective transfer of heat from the outer surface α and the ambient temperature t_c are constant;
– there is a symmetry of the initial temperature distribution θ_g (r) round the radius of the cylinder;
– the internal heat sources are assumed to be absent (q = 0).

The assumptions made correspond to the following mathematical model of the process of nonsteady thermal conductivity of cylindrical bodies:

$$\left. \begin{array}{c} \dfrac{\partial \theta}{\partial \tau} = \alpha\left(\dfrac{\partial^2 \theta}{\partial r^2} + \dfrac{1}{r}\dfrac{\partial \theta}{\partial r}\right) \\ \dfrac{\partial \theta}{\partial r}\bigg|_{r=0} = 0;\ -\lambda\cdot\dfrac{\partial \theta}{\partial r}\bigg|_{r=R} = \alpha\cdot\theta\big|_{r=R};\ \ \theta\big|_{\tau=0} = \theta_0(r) \end{array} \right\} . \quad (1)$$

However, this theoretical model does not take into account a number of significant practical circumstances that affect the process, such as the curvature of the surface, the frequency of scraper passages, the non-Newtonian nature of ryazhenka [fermented baked milk], viscosity, and a lot of other food products. The solution of the arising engineering problems can be implemented by experimental methods ensuring the extension of the obtained results using the similarity theory and dimensional analysis.

The time-varying parameters of a number of technological processes are also important parameters of theoretical description of the processes carried out in a great number of food industry equipment. The starting modes of machines and apparatus can be considered a common example of such nonsteady processes. In many cases, it is necessary to take into account the effect of starting mode characteristics since the absence thereof results in inaccurate measurements, as well as in errors in the choice of operating modes of equipment. Thus, the estimation of the features of the starting mode is necessary [12, 13]. The studies of thermal and hydro-dynamic processes in apparatus with cleaning mixing devices are presented in the works by Begachev V.I., Braginskiy L.N., Glukhov V.P., Pavlushenko I.S., and Pavlov M.G. [7]; Konviser I.A. [8]; Konsetov V.V., Kudryavitskiy F.M., and Novichkov A.N. [9], Pavlushenko I.S. and Gluz M.D. [12], Abichandani H. [19]; de Goede R. and de Jong E. J. [20]; Dumont E., Fayolle F., and Legrand J. [21], et al.

The studies of the mentioned above authors were carried out using the equipment that consisted of an apparatus with mixing devices that differed from the experimental plant used in our paper. Part of the considered results of the studies can be regarded only as a first approximation.

STUDY OBJECTS AND METHODS

To successfully design and calculate the considered type of mixer, it is necessary to have the formulae for calculating the energy consumed in the flow apparatus with mixing devices obtained from the experimental data. Some data were obtained using directly an industrial mixer equipped with a set of sensors. To study the process of interaction of a rubber scraper with the cooling surface and the working body deeper, a special experimental laboratory plant based on a modified rotational rheometer was designed and tested [7, 8, 9]. A series of experimental studies were carried out using this modified rheometer. The manufactured scrapers were installed on the rotor cylinder of the rheometer. Alternative stator cylinders were used fo study for various volumes of operating area. When carrying out the studies, a set of methods was used that made it possible to determine the rotation speed of the rotor with a high accuracy, as well as to determine the dynamic pattern of the speed to estimate the starting modes.

Experimental plant no. 1, a scraped-surface heat exchanger, is shown in Fig. 1, 2 and 3 where:
1 – cooling jacket, 2 – working cylinder, 3 – inflator, 4 – motor-reducer, 5 – product supply branch, 6 – coolant outlet, 7 – elastic tape, 8 – metal perforated slat, 9 – hole in the blade, 10 – gap between the blade sections, 11 – crosspiece holder, 12 – shaft, 13 – product outlet, 14 – coolant supply branch, 15 – cleaning blade, 16 – inflator blade, 17 – elastic tape bolt, 18 – crosspiece hub bolt, 19 – crosspiece hub.
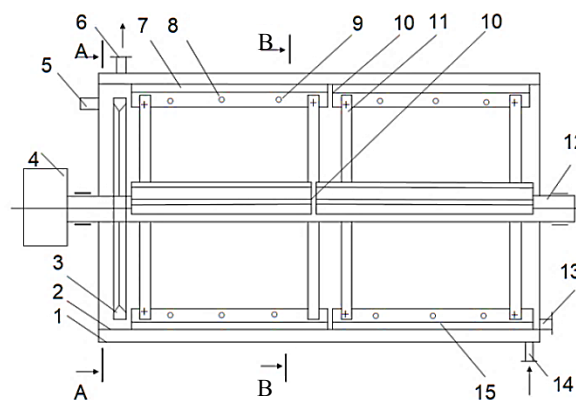

Fig. 1. Experimental plant no. 1 for studying thermal and hydrodynamic processes.
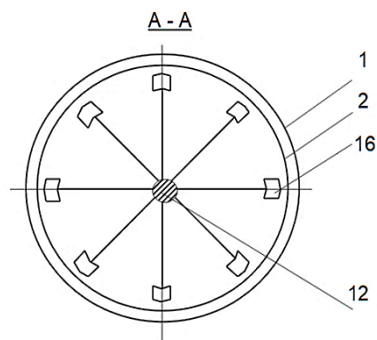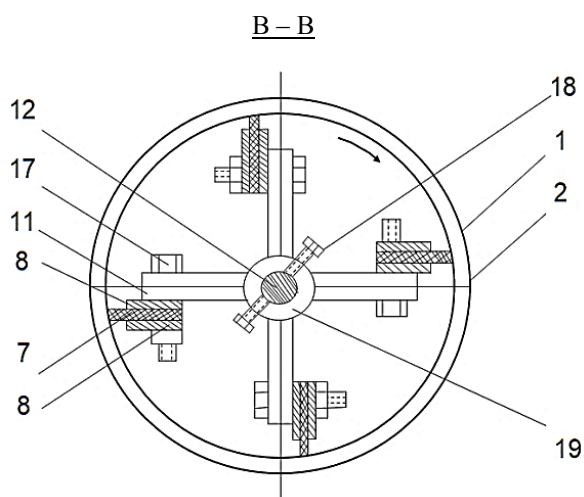

Fig. 2. Inflator.

B – B

Fig. 3. Cleaning devices of the experimental plant.

The experimental plant is similar to a T1-OM-2T heat exchanger in design, but at the same time it is distinguished by the fact that in order to increase the productivity of the apparatus, the mixing device located in the working cylinder has some perforated cleaning-type blades installed at an angle of 90° to the surface of the working cylinder. Each of the blades is made of two metal plates, between which an elastic tape the upper edge of which rises above the plates by no more than 15–10 mm is clamped. The distance between the peripheral edges of diametrically located blades is greater than the internal diameter of the working cylinder D_c by 0.5–0.1 mm. The blades have holes of at least 0.05–0.1 D_c and are divided into sections with a length of no more than 3 D_c, with each successive blade displaced in relation to the previous blade in the longitudinal axial direction by 0.2 D_c, (the blade width is 0.2–0.4 D_c). There is also an inflator in

the apparatus the diameter of which for the peripheral edges of the blades is equal to 0.9 D_c. This combination of features and their distinctive relationship make it possible to increase the productivity of the apparatus.

The new design allowed us to conduct further experimental studies of heat exchange and consumed energy. As a model medium, 2.5% fat ryazhenka was used. The studies were performed within the range of the Reynolds criterion from 90.7 to 6380, while the effective viscosity values were found within the range from 0.03 to 0.14 Pa·s. The studies were carried out in the rheology laboratory of the Mega-department of biotechnologies and low-temperature systems of the ITMO University.

The Table 1 presents the results of the studies of rheological processes.

RESULTS AND DISCUSSION

Studies of the hydrodynamic processes in an apparatus with scraping mixing devices were conducted.

The obtained data, when there is a change in the product temperature and there are different values of a shear rate gradient, show that the effective viscosity of the studied product depends significantly on the value of a product velocity and temperature gradient.

When treating ryazhenka in a heat exchanger with cleaning devices at values of the Reynolds criterion below 2400, there is a laminar motion of the medium in the apparatus. With a further increase in the Reynolds criterion, there is a transitional flow range.

Taking into account the previous large-scale studies carried out by a number of authors [1, 2, 5, 14, 15], to apply the obtained data to a wider range of equipment and treated media, a criterial heat exchange equation for Newtonian and non-Newtonian fluids was proposed taking into account the similarity theory:

Table 1. Change in the rheological characteristics of 2.5% fat ryazhenka during heat treatment

Product temperature t_{pr}, °C	Temperature of the heat exchange surface t_{at}, °C	Product density ρ, kg/m³	Scraper agitator rotation speed n, r/s	Consumed energy N, W	Effective viscosity μ_{ef}, Pa·s	Reynolds criterion Re
			Heating ryazhenka			
18.4	24.8	1031.1	0.783	1.5	0.140	90.7
22.4	27.2	1028.1	1.250	2.9	0.105	192.5
23.2	27.2	1027.9	1.800	4.7	0.086	338.3
24.5	27.8	1027.4	2.617	8.0	0.070	604.0
29.9	33.4	1024.8	3.367	8.8	0.048	1130.0
31.2	34.2	1024.3	4.300	14.0	0.042	1649.0
32.7	35.3	1023.7	5.200	17.1	0.036	2325.0
			Cooling ryazhenka			
31.9	26.6	1023.9	1.367	2.3	0.071	310.0
30.3	26.1	1024.7	1.933	4.1	0.064	486.7
29.6	25.5	1024.8	2.217	5.1	0.062	576.2
28.0	24.8	1025.8	2.567	6.3	0.060	690.1
26.7	23.9	1026.2	3.367	10.8	0.057	953.2
24.5	22.1	1027.3	4.367	14.9	0.049	1440.0
22.0	20.4	1028.4	8.500	41.7	0.039	3158.0
20.5	19.5	1028.9	11.83	160.8	0.030	6380.0

$$Nu = B \cdot \mathrm{Re}^a \cdot \mathrm{Pr}^b \cdot \left(\frac{\mu}{\mu_{\text{ст}}} \right)^{0.14}, \qquad (2)$$

where: $Nu = \dfrac{\alpha \cdot l}{\lambda}$ is the Nusselt criterion;

$\mathrm{Re} = \dfrac{\omega \cdot l \cdot \rho}{\mu}$ is the Reynolds criterion;

$\omega = \pi \cdot D \cdot n$ is the speed of the cutting edge of the scraper, m/s;

$\mathrm{Pr} = \dfrac{c \cdot \mu}{\lambda}$ is the Prandtl criterion;

$l = \pi \cdot \dfrac{D}{z}$ is a characteristic dimension which is a distance between the outer edges of the cleaning devices, m;

D is the internal diameter of the apparatus, m;
z is the number of blades of the mixing device, pcs;
μ and μ_{at} are the effective viscosity of the product at the average product temperature and at the wall temperature, respectively, Pa·s;
B is the empirical coefficient;
a and b are the dimensionless coefficients determined in the course of the experiments.

The use of l in the calculations, which is a distance between the edges of the blades of the mixing device, allows us to take into account the effect of the number of scrapers and the inner diameter of the working cylinder.

When performing the theoretical description of the operation of a great number of apparatus and measuring equipment, an important problem is the technological processes the parameters of which can vary with time. Equipment starting modes are one of the common examples of such nonsteady processes. Variable parameters lead to deviations in the hydrodynamics of a medium, can affect the heat exchange in heat exchange equipment, they also lead to significant changes in a number of other processes carried out in food processing equipment. In many cases, ignoring the effect of starting mode parameters results in measurement errors and the selection of incorrect operating modes for machines and apparatus.

To describe theoretically the operation process of this type of apparatus, it is possible to present the hydrodynamic pattern of a flow, which is a nonsteady flow in a flat gap, in a simplified form:

$$\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x}(\rho v_x) + \frac{\partial}{\partial y}(\rho v_y) + \frac{\partial}{\partial z}(\rho v_z) = 0 \qquad (3)$$

$$\rho \left(\frac{\partial v_x}{\partial t} + v_x \frac{\partial v_x}{\partial x} + v_y \frac{\partial v_x}{\partial y} + v_z \frac{\partial v_x}{\partial z} \right) = \qquad (4)$$

$$= -\frac{\partial p}{\partial x} + \left(\frac{\partial \tau_{xx}}{\partial x} + \frac{\partial \tau_{yx}}{\partial y} + \frac{\partial \tau_{zx}}{\partial z} \right) + \rho g_x$$

where v_x, v_y, v_z are the projections of flow velocities; x, y, z are the coordinates; ρ is density; p is the pressure; g_x is the projection of the acceleration of gravity; τ are the components of the stress tensor.

Since there is a shear flow of a viscous fluid in the experimental apparatus, it is necessary to take into account an equation that relates the rate of shear deformation and stress.

$$\tau_{yx} = \eta \frac{\partial v_x}{\partial y}. \qquad (5)$$

To simulate the motion of an incompressible viscous fluid between the plates of the apparatus, let us take the lower plate as a stable one and the upper plate is the one that constant voltage is applied to. The Eqs. 3–5 take the following form in the partial derivatives:

$$\frac{\partial v_x}{\partial t} = \frac{\eta}{\rho} \frac{\partial^2 v_x}{\partial y^2}. \qquad (6)$$

The boundary and initial conditions are assumed to be equal to:

$$v_x(0,t) = 0; \quad v_x(y,0) = 0;$$
$$\frac{\partial v_x}{\partial y}(H,t) = \frac{\tau_H}{\eta}; \quad \frac{\eta}{\rho} = \mu. \qquad (7)$$

Let us transform the Eq. 6 in the partial derivatives to the equations represented in the ordinary derivatives:

$$v_x(y,t) = f(y)\psi(t). \qquad (8)$$

Then:

$$f(y) \frac{\partial \psi(t)}{\partial t} = v\psi(t) \frac{\partial^2 f(y)}{\partial y^2}. \qquad (9)$$

Therefore:

$$\frac{1}{v\psi(t)} \cdot \frac{\partial \psi(t)}{\partial t} = \frac{1}{\varphi(y)} \cdot \frac{\partial^2 \varphi(y)}{\partial y^2}, \qquad (10)$$

$$\frac{1}{v\psi(t)} \cdot \frac{\partial \psi(t)}{\partial t} = \frac{1}{f(y)} \cdot \frac{\partial^2 f(y)}{\partial y^2}. \qquad (11)$$

Hence:

$$\frac{1}{f(y)} \cdot \frac{\partial^2 f(y)}{\partial y^2} = -k^2, \qquad (12)$$

$$\frac{1}{v\psi(t)} \cdot \frac{\partial \psi(t)}{\partial t} = -k^2. \qquad (13)$$

In the ordinary derivatives, the equations take the form:

$$\frac{\partial^2 f(y)}{\partial y^2} + k^2 \varphi(y) = 0, \qquad (14)$$

$$\frac{\partial \psi(t)}{\psi(t)} = -k^2 v \partial t. \qquad (15)$$

Let us integrate the Eqs. 14 and 15 with allowance for the boundary and initial conditions (7) that determine the constants C_1, C_2 and C_3:

$$f(y) = C_1 \cos ky + C_2 \sin ky, \qquad (16)$$

$$\psi(t) = C_3 e^{-k^2 \nu t}, \tag{17}$$

$$v_x(y,t) = (C_1 \cos ky + C_2 \sin ky)C_3 e^{-k^2 \nu t}. \tag{18}$$

The solution to the Eqs. 16–18 allows us to carry out the mathematical study of the development of the velocity profile of a flow in the space between the cylinders and to calculate the starting mode time of the apparatus:

$$v_x(y,t) = \frac{\tau_H}{\eta}\left[\left(\frac{y}{H}\right) - \frac{8}{\pi^2}\sum_{n=1,3\ldots}^{\infty}\frac{\sin\left(\frac{n\pi}{2}\right)}{n^2}e^{-\frac{\nu n^2 \pi^2 t}{4H^2}}\sin\left(\frac{n\pi y}{2H}\right)\right]. \tag{19}$$

where τ_H is tangential stresses constant voltage, η is the dynamic viscosity coefficient.

The analysis of the data shows that when studying the initial phase of the apparatus with mixing devices, it is necessary to take into account the starting period.

Considering a number of assumptions in deriving the formula (19) for the experimental studies of starting modes, Plant no. 2 shown in Fig. 4 and 5 was used.

It is a modified Volarovich viscometer [10, 11] equipped with devices for measuring the rotor speed. In addition, the modification included the use of alternative external cylinders and the installation of scraping action simulating devices on the rotor cylinder. The experiments consisted in measuring the dependence of rotor speed on the time from the starting moment, while a constant torque was having an impact on the rotor. A series of experiments were carried out with external cylinders with a radius of 19 mm, 34 mm, and 72 mm with a radius of the inner cylinder of 16 mm. As a model medium, 2.5% fat ryazhenka as a sample of a food product with non-Newtonian properties and 99.5% glycerin which is a medium with Newtonian properties were used. The use of glycerin is caused in particular by its well-known physical characteristics.

As a result of processing the experimental data obtained using Experimental plant no. 1, the dimensionless coefficients a, b, c for the criterion equation (2) were obtained.

The exponential factor of the Reynolds criterion was determined as a result of processing the experimental data obtained in the logarithmic coordinates:

$$\lg\left(\frac{Nu}{\left(\frac{\mu}{\mu_{ÑÒ}}\right)^{0.14}}\right) = f(\lg(Re)), \tag{20}$$

The study was carried out for the groups of experiments the Prandtl criterion in which was: $Pr_1 = 388$, $Pr_2 = 2462$.

The graphical-analytical processing of the obtained dependences presented in Fig. 6 showed that the both groups of experiments had the same slope to the abscissa axis. The exponent a of the formula (2) was determined as the slope of the graph to the axis of abscissae and was equal to $a = 0.59$. The value of the exponent indicated the significant effect of the Reynolds criterion on heat exchange.
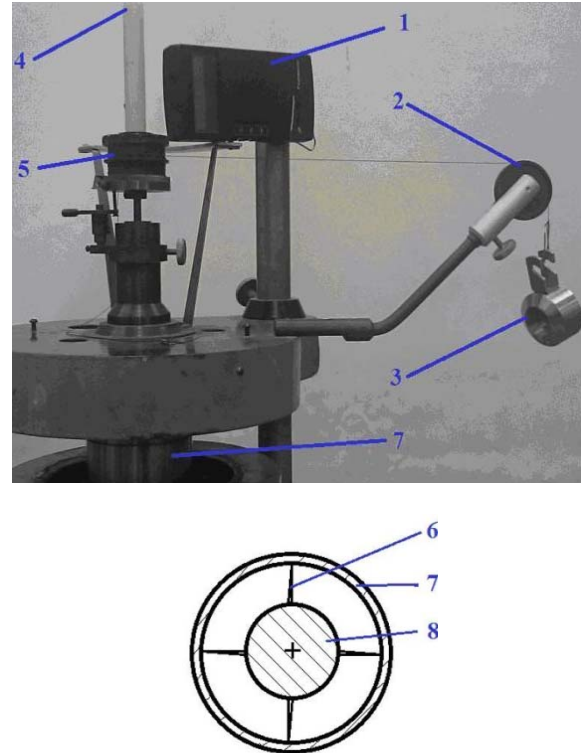
Fig. 4. Experimental plant No. 2 for the study of starting modes in apparatus with cleaning devices:
1 – digital non-contact tachometer; 2 – block;
3 – weight; 4 – tachometer mark; 5 – pulley;
6 – scraper; 7 – stator capacity; 8 – rotor cylinder.
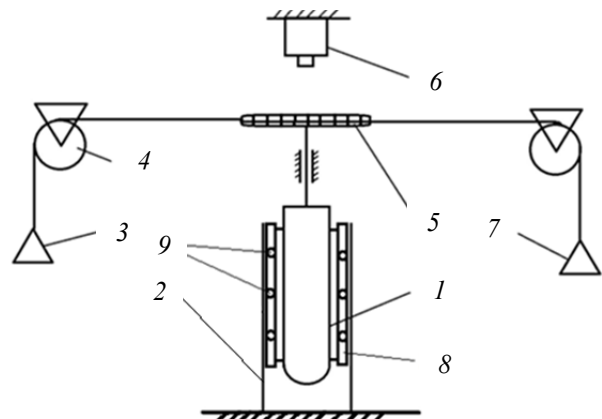
Fig. 5. Diagram of the experimental plant based on a modified rheometer: *1* – rotor cylinder; *2* – stator capacity; *3, 7* – weight; *4* – block; *5* – pulley; *6* – electronic IT 5-ChMTermit contactless tachometer, *8* – scraper, *9* – holes in the blades of the working bodies.
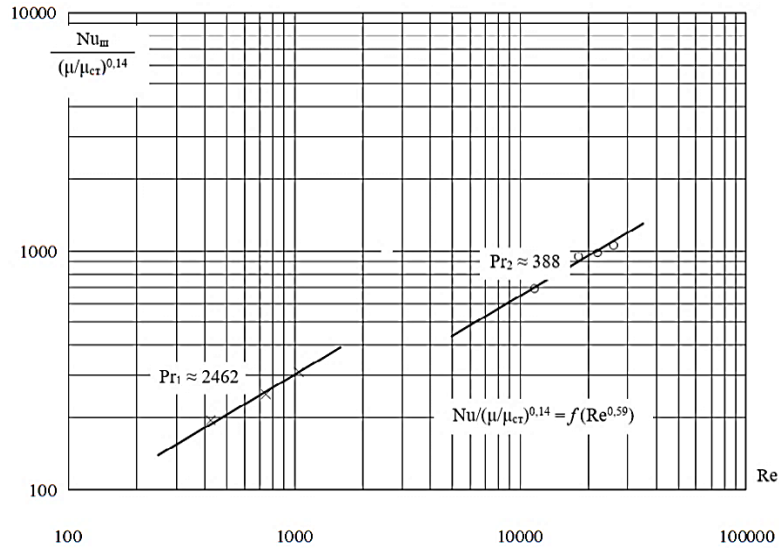
Fig. 6. Dependence of the Nusselt criteria and the Reynolds criterion in an apparatus with mixingdevices – the determination of the exponent of degree of the Reynolds criterion.
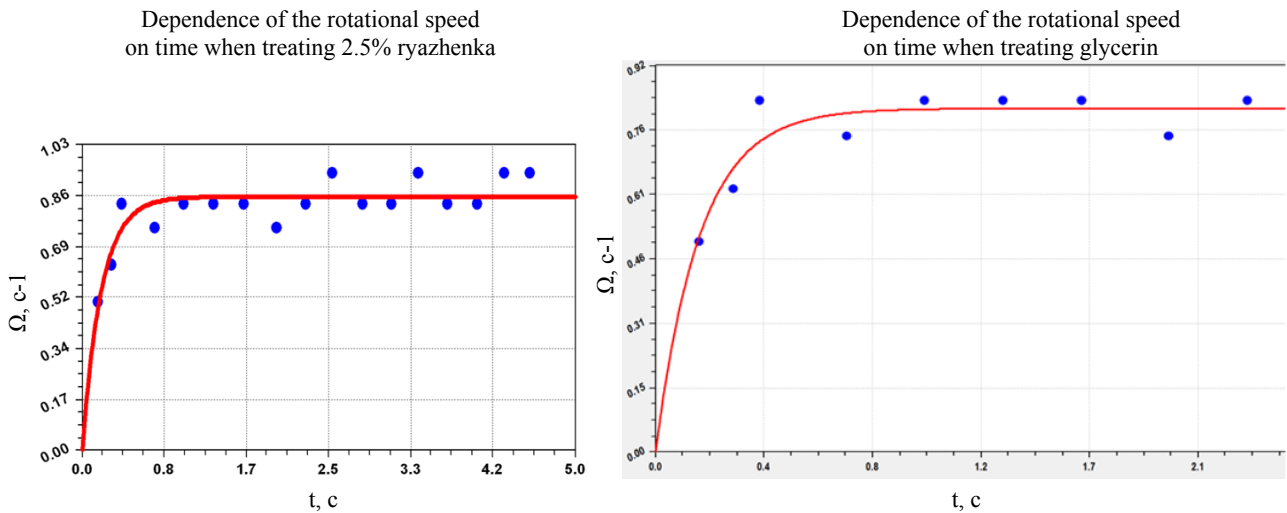
Fig. 7. Dependence of the rotor speed on time from the starting moment for 2.5% fat ryazhenka and for 99.5% glycerin.

Similarly, the effect of the Prandtl criterion on heat exchange was determined. The obtained graphical dependence uses the Reynolds criterion coefficient obtained at the previous stage.

$$\lg\left(\frac{Nu}{\mathrm{Re}^{0.59}\left(\dfrac{\mu}{\mu_{CT}}\right)^{0.14}}\right) = f\left(\lg(\mathrm{Pr})\right). \qquad (21)$$

As a result of graphical-analytical processing of the experimental data, the value *b* of the exponential factor for the Prandtl criterion for the formula (2) was found to be equal to 0.37.

To obtain the coefficient *B* of the formula (5), the data are processed mathematically on the basis of the dependence (2):

$$\lg\left(\frac{Nu}{\mathrm{Pr}^{0.37}\left(\dfrac{\mu}{\mu_{CT}}\right)^{0.14}}\right) = f\left(\lg(\mathrm{Re})\right). \qquad (22)$$

As a result of the made experiments and mathematical processing of the experimental data, the criterial equation (2) takes the form:

$$Nu = 0.923 \cdot \mathrm{Re}^{0.59} \cdot \mathrm{Pr}^{0.37}\left(\frac{\mu}{\mu_{CT}}\right)^{0.14}. \qquad (23)$$

As a result of processing the experimental data obtained using Experimental plant no. 2, the starting mode parameters of the apparatus the character of which is close to an exponential dependence were obtained, they can be described in the form of a

formula (24), the coefficients a and b in this formula have been determined by processing the data using CurveExpert (Fig. 7).

$$\Omega = a(1 - e^{b\tau}) \qquad (24)$$

where Ω is the rotor speed, s^{-1};
a and b are the empirical coefficients:
$a = 0.626 \cdot 10^{-1}$; $b = 5.32$ – the correlation coefficient for 2.5% fat ryazhenka is: $R = 0.964$; the rms deviation $\delta = 0.0607$.

For 99.5% glycerin, $a = 0.814$; $b = 5.89$; the correlation coefficient $R = 0.957$; the rms deviation $\delta = 0.0425$.

CONCLUSION

The effect of the Reynolds and Prandtl criteria on the heat exchange in an apparatus with modified cleaning devices has been determined. For the scraper devices with holes, there was an increase in the Reynolds criterion by 3–5% compared to the measurements made for the devices without holes.

In order to apply the obtained data to a wider range of equipment and treated media, a criterial heat exchange equation for Newtonian and non-Newtonian fluids taking into account similarity simplexes was proposed that takes into consideration the similarity theory. The effect of the Reynolds and Prandtl criteria on the heat exchange in the apparatus were determined.

The lawfulness of describing the characteristics of the starting mode were established using an exponential dependence of the form of $\Omega = a (1 - e^{-bt})$. The experimentally expected theoretical predominance of the effect of medium density on the starting mode compared to the effect of viscosity properties were established. The value of the coefficient of the exponent b differed by 8–15% for the measurements with ryazhenka and glycerin under other equal conditions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this article.

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Hydrodynamics and mass transfer with gel formation in a roll type ultrafiltration membrane

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Abstract: At this point in history, mankind faces a daunting challenge: how are we to produce high-grade foods without damage to the environment? The only possible rational solution lies in the efficient use of natural raw materials. However, the practical side of the matter cannot be resolved without innovative food equipment designed on the basis of the latest scientific achievements. The current research features the theory and practice of curd whey and skimmed milk ultrafiltration. It focuses on the main operating parameters of the equipment involved and the maximum permissible value of the milk solids content. The experiment included whey, obtained as a byproduct of cottage cheese processing, and skimmed milk, obtained by whole milk separation. The membrane method in the processing of secondary dairy raw materials allows for an environmentally-friendly waste-free production. It is a promising trend in the modern food industry: it creates opportunities for a large range of novel dairy products, beverages, and animal feed, as well as for other sustainable technologies. The paper describes how the volume of permeate flux mass transfer and the selectivity of polysulfonamide ultrafiltration membranes (PSA-20 and PSA-50) depend on the volume of operating load and circulation rate during whey and skimmed milk separation. The authors analyzed the mass transfer and the hydrodynamics in the channel of a roll type baromembrane, including the gel formation process. They established the influence of the milk solids weight ratio in the liquid polydisperse system on the permeate flux volume and the selectivity of the polysulfonamide ultrafiltration membrane (PSA-50).

Keywords: Milk whey, membrane technology, ultrafiltration, permeate flux, membrane selectivity

INTRODUCTION

Whey is a byproduct of curds, cheese and casein. It contains up to 50% of whole milk solids [8, 9], which makes it a valuable secondary raw material. However, whey is often treated as production wastes and discharged into the environment, thus causing serious environmental damage [8, 9]. Such type of dairy wastes can be recycled by ultrafiltration. Unfortunately, baromembrane separation of whey is not profitable for medium and small milk processing plants [8, 9]. Nevertheless, it is possible to increase the process efficiency by rationalizing it.

When whey is ultrafiltrated with the maximum permissible value of the concentration factor, the permeate flux through the polymer membrane can be increased while maintaining a specific selectivity index.

The current research provides a theoretical and experimental justification of the main operating parameters and the maximum permissible value of milk solids content in whey and skimmed milk during their separation by ultrafiltration.

STUDY OBJECTS AND METHODS

The experiment involved whey, obtained as a byproduct of cottage cheese processing, and skimmed milk, obtained by whole milk separation. Tables 1 and 2 feature the basic physicochemical characteristics of the dairy raw material and the operational parameters of the polymer membranes. A scientific data analysis [8, 9] and an analysis of the authors' own research [1–4, 8] have made it possible to establish that the best option available for ultrafiltration separation of secondary dairy raw materials are polysulfonamide

ultrafiltration membranes PSA-20 and PSA-50 produced by Vladipor (ZAO STC Vladipor), Russia.

According to the main parameter (extreme delay), these membranes can be used for ultrafiltration of any dairy raw material. Under the same conditions, the difference between polyamide membranes and cellulose acetate ones lies in their extended service life and greater cost. However, PSA membranes allow for a more rigid washing and a higher operating pressure. The process of tangential ultrafiltration was carried out in a special laboratory installation. The surface area of the membrane was < 0.5 m². The operating pressure, the circulation rate of the liquid polydisperse system in the baromembrane channel, and its temperature varied in the following ranges: ΔP = 0.1–0.4 MPa, V = 0.05–0.45 m/sec and t = 8–18°C accordingly. The permeate flux volume Q and the selectivity of the PSA-20 and PSA-50 membranes Ψ were determined experimentally. The Ψ index was calculated by the following formula:

$$\Psi = \frac{V_2 \cdot C_2}{V_1 \cdot C_1} \cdot 100\%, \tag{1}$$

where: V_1 – initial volume of the separated system; C_1 – the mass fraction of dispersed particles; V_2 and C_2 – the volume of the retentate and the mass fraction of the dispersed particles.

The confidence coefficient of the results obtained is 95%.

RESULTS AND DISCUSSION

Theoretical aspects of mass transfer with gel formation in a roll type baromembrane. The process of baromembrane separation provides maximum use of the membrane surface. To ensure the separation in the tangential flux, manufacturers often use the spiral roll filter element. A simultaneous flux in the axial and radial directions makes it possible to reduce the pressure drop in the liquid system and to reduce the size of stagnant zones in the operating channel [1–3].

Many studies focus on a general description of the fluid motion in thin channels with permeable walls [1–3, 12]. However, the flux in the roll type baromembrane channel has a distinct feature: the trajectories of the particles of the fluid are curvilinear, which means that they are influenced by the centrifugal force. This aspect should be taken into account when developing a mathematical description of its hydrodynamics.

A system of equations to describe the steady flow with a constant viscosity coefficient should include the Navier-Stokes equation and the continuity equation in the projections on the axis of the cylindrical coordinate system.

In the following equation, r is the curvature radius of the membrane channel at its inlet; b and h – the half-width of this channel in the directions of the axes r and z respectively; w, u, v are the characteristic values of the corresponding velocities v_r, v_v, v_z. To simplify the mathematical expressions, r was replaced with variable y by using the formula $r = R + y$.

Table 1. Physicochemical characteristics of whey for ultrafiltration

Factor	Cottage cheese whey	Skimmed milk
Milk solids content, %, minimum	6.5	8.0
Including:		
Lactose	5.0	5.0
Protein	1.1	2.8
Fats	0.1	0.1
Mineral substances	0.3	0.8
Acidity, °T	45	21
Index pH	5.0	5.5
Density, kg/m³	1023	1030
Optical density of 10 % solution	0.26	0.32

Table 2. Performance parameters of PSA ultrafiltration membranes (product of ZAO STC Vladipor, Russia)

Performance factors	Membrane type	
	PSA-20	PSA-50
Pressure, MPa	0.1–0.4	0.1–0.5
Extreme delay, kDa	45–50	50–55
Temperature, °C	5–40	10–50
pH of washing environment	2–10	2–12
Useful life, hours	< 3500	< 3000

Taking into consideration that

$$\frac{\partial}{\partial r} = \frac{\partial}{\partial y},$$

we proceed to dimensionless variables:

$$Z = h\bar{z}, \ Y = b\bar{y}, \ v_r = w\bar{v}_r, \ v_v = u\bar{v}_v,$$

$$v_z = v\bar{v}_z, \ P = P_r + \Delta P\bar{P},$$

where P_r – the pressure of the liquid system at the outlet from the membrane channel, ΔP – gradient of the operating pressure in the device.

The dimensionless parameters $wh/(bv)$ and $ur/(Rv)$ can be equated to 1, in accordance with the principle of least action. Assuming that $v = uh/R$, while $w = ub/R$, and taking into consideration that the term that contains the pressure gradient has the same order of magnitude as the terms that include the coefficient of viscosity, it can be written that

$$\Delta P = \rho v\bar{u}R_v/h^2 \tag{2}$$

The equation including $R_v = vh/v$ (small dimensionless parameter) can be solved by power expansion. The first approximation makes it possible to obtain the following system of equations:

$$0 = -\frac{1}{1+\varepsilon\bar{y}}\frac{\partial\bar{P}}{\partial v} + \frac{\partial^2 v_v}{\partial\bar{z}^2}, \tag{3}$$

$$0 = -\frac{\partial\bar{P}}{\partial\bar{y}} + \varepsilon^2\frac{\partial^2\bar{v}_r}{\partial\bar{z}^2}, \tag{4}$$

$$0 = -\frac{\partial\bar{P}}{\partial\bar{z}}, \tag{5}$$

where $\varepsilon = w/u$.

Taking into consideration that the pressure $\bar{P}$ of the liquid system is a parameter independent of the vertical coordinate and based on the small value of the parameter ε, the subsequent simplification of the equation system (3–5) leads to a system of the following differential equations:

$$\frac{\partial \bar{v}_r}{\partial \bar{y}} + \frac{\partial \bar{v}_v}{\partial v} + \frac{\partial \bar{v}_z}{\partial \bar{z}} = 0, \tag{6}$$

$$\frac{\partial \bar{P}}{\partial \bar{v}} + \frac{\partial^2 \bar{v}_v}{\partial \bar{z}^2} = 0, \tag{7}$$

$$\frac{\partial \bar{P}}{\partial \bar{y}} = 0, \tag{8}$$

$$\frac{\partial \bar{P}}{\partial \bar{z}} = 0. \tag{9}$$

We assume that the value of the working pressure $\bar{P}$ of the liquid system changes significantly only along the length of the channel. Then the radial velocity component v_r will be zero, and the dependence of the longitudinal component v_v on the value of the vertical coordinate will be determined by equation (7). After defining v_v, the vertical component v_z can be calculated by equation (6).

To determine the velocity profile of the liquid system for equations (6–9), the following boundary conditions must be introduced:

$$\bar{v}_v = 0 \text{ where } \bar{z} = \pm 1. \tag{10}$$

The upper wall of the membrane channel is impenetrable to permeate

$$\bar{v}_z = 0 \text{ where } \bar{z} = +1 \tag{11}$$

on the bottom wall

$$\bar{v}_z = -v_w(v) \text{ where } \bar{z} = -1. \tag{12}$$

The tangential component: the solution for equation (7) with boundary condition (10) can be written as follows:

$$\bar{v}_v = -\frac{1}{2} \frac{\partial \bar{P}}{\partial v} (1 - \bar{z}^2) \tag{13}$$

By substituting expression (13) into equation (6) with subsequent integration, we determine that

$$\bar{v}_z = \frac{1}{2} \frac{\partial^2 \bar{P}}{\partial \bar{v}^2} \left(z - \frac{\bar{z}^3}{3} \right) + C, \tag{14}$$

where C – an integration constant that does not depend on the values of the variable z; however, it can be determined by the value of the variable v for the general case. The expression for the constant C can be found by using the boundary condition (11):

$$C = -\frac{1}{3} \frac{\partial^2 \bar{P}}{\partial v^2}. $$

In accordance with

$$\bar{v}_z = \frac{1}{2} \frac{\partial^2 \bar{P}}{\partial v^2} \left(\bar{z} - \frac{\bar{z}^3}{3} - \frac{2}{3} \right), \tag{15}$$

we use boundary condition (12); by putting (15) $\bar{z} = -1$ in expression (15) and then integrating it, we describe

the pressure distribution along the length of the membrane channel as

$$\frac{\partial \bar{P}}{\partial v} = \frac{3}{2} \int_0^v v_w(v) dv + C_1. \tag{16}$$

To determine the integration constant C_1, it is necessary to adopt the condition that u is the average flux rate of the liquid system at the entrance to the membrane channel; in this case we obtain the following expression in the dimensionless variables:

$$\int_{-1}^{1} \bar{v}_v |_{v=0} d\bar{z} = 2. \tag{17}$$

Hence, $C_1 = 3$, which makes it possible to rewrite equation (16) as follows:

$$\frac{\partial \bar{P}}{\partial v} = -3 \left(1 - 0.5 \int_0^v v_w(v) dv \right). \tag{18}$$

After integrating expression (18), we obtain the following equation that determines the pressure distribution in the liquid:

$$\bar{P} = \bar{P}(0) - 3 \left(v - 0.5 \int_0^v \int_0^v v_w(v) dv dv \right). \tag{19}$$

The expression can be used to calculate the mass transfer during gel formation on the membrane surface. The expression has a significant effect on the permeate flux and membrane selectivity.

It should be noted that the parameters of the mass transfer process, complicated by gelling, have already been determined. However, the following convective diffusion equation describes a stationary process only for the cases when the coefficient of molecular diffusion does not depend on the concentration of matter in the liquid system, and only for baromembrane separation of liquid in a straight channel [1–3]:

$$Pe \left(\bar{v}_r \frac{\partial \bar{c}}{\partial \bar{y}} + \frac{\bar{v}_v}{1 + \varepsilon \bar{y}} \frac{\partial \bar{c}}{\partial v} + \bar{v}_z \frac{\partial \bar{c}}{\partial \bar{z}} \right) = \frac{R_v^2}{R_w^2} \frac{\partial^2 \bar{c}}{\partial \bar{y}^2} +$$
$$+ \frac{R_v}{R_w Re(1 + \varepsilon \bar{y})} \frac{\partial \bar{c}}{\partial \bar{y}} + \frac{R_v^2}{Re^2(1 + \varepsilon \bar{y})^2} \frac{\partial^2 \bar{c}}{\partial v^2} + \frac{\partial^2 \bar{c}}{\partial \bar{z}^2}, \tag{20}$$

where $\bar{c} = (c - c_в)/(c_д - c_в), c_в$ – the concentration of the released substance at the entrance to the baromembrane channel, $c_д$ – concentration of gel formation; Pe – Peclet number, calculated according to the penetration speed and half-height of the membrane channel.

However, if in equation (20) we assume that $R_v \to 0$ and $\varepsilon \to 0$ (where $\frac{R_v}{R_w} \to 0$ and $R_v/Re \to 0$) while considering the approximation $\bar{v}_r = 0$, then the convective diffusion equation can be simplified and written as follows:

$$Pe \left(\bar{v}_v \frac{\partial \bar{c}}{\partial y} + \bar{v}_z \frac{\partial \bar{c}}{\partial \bar{z}} \right) = \frac{\partial^2 \bar{c}}{\partial \bar{z}^2}. \tag{21}$$

Considering the connection (18), velocity components $\bar{v}_z$ and $\bar{v}_v$ in equations (13) and (15) can be represented as

$$\bar{v}_z = \frac{v_w(v)(3\bar{z} - \bar{z}^3 - 2)}{4}. \tag{22}$$

$$\bar{v}_v = \frac{3}{4}\left(2 - \int_0^v v_w(v)dv\right)(1 - \bar{z}^2). \qquad (23)$$

Since the ultrafiltration process usually involves very small values of the molecular diffusion coefficient D (in comparison with the reverse osmotic membrane separation), then $Pe \gg 1$. And that means that the change in the concentration of the substance released on the membrane occurs only in the thin diffusion perimembrane layer. Proceeding from this, one can further transform equation (21) by introducing a new independent variable ŋ instead of $\bar{z}$:

$$\bar{z} = -1 + \text{ŋ}. \qquad (24)$$

Assuming that for the thin diffusion perimembrane layer ŋ $\ll$ 1, equations (22) and (23) can be simplified and presented as follows:

$$\bar{v}_z = q, \bar{v}_v = \frac{3q\text{ŋ}}{2}, \qquad (25)$$

$$q(v) = 2 - \int_0^v v_w(v)dv. \qquad (26)$$

Hence, the final equation to define the concentration will be

$$Q\frac{\partial\bar{c}}{\partial\text{ŋ}} + \frac{3q\text{ŋ}}{2}\frac{\partial\bar{c}}{\partial v} = a^2\frac{\partial^2\bar{c}}{\partial\text{ŋ}^2} a^2 = Pe^{-1} \qquad (27)$$

The boundary conditions for equation (27) will be

$$c = 0 \text{ where } \eta = \infty, \qquad (28)$$

because outside the zone of the diffusion perimembrane layer (where $\eta = \infty$) concentration c of the substance on the membrane equals its concentration at the entrance to the channel $c_в$:

$$(v_z, c)_{z=-h} = D\frac{\partial c}{\partial\bar{z}}|_{z=-h} \qquad (29)$$

which means that the convective flux of the substance on the membrane to its surface must be compensated by its diffusion flux away from the surface (where $\bar{z} = -h$).

Assuming that the concentration of the substance on the membrane is equal to the concentration of gel formation, equation (29) can be rewritten in dimensionless variables:

$$-\frac{c_д}{c_д - c_в}v_w = a^2\frac{\partial c}{\partial\text{ŋ}}|_{\eta=0} \qquad (30)$$

By introducing the dimensionless variable

$$\xi = \text{ŋ}\left(\frac{q}{2a^2v}\right)^{\frac{1}{3}}, \qquad (31)$$

we solve equation (27) with the given boundary conditions (28) and (30). Thus,

$$\frac{d^2\bar{c}}{d\xi^2} + (\xi^2 + V_w)\frac{d\bar{c}}{d\xi} = 0, \qquad (32)$$

with boundary conditions

$$\bar{c} = 0 \text{ where } \xi = 0, \qquad (33)$$

$$\frac{c_д}{c_д - c_в} = \frac{\partial\bar{c}}{\partial\xi}|_{\xi=0}, \qquad (34)$$

in expression (32) $V_w = V_w\left(\frac{2v}{qa^4}\right)^{\frac{1}{3}}$.

Differential equation (32) with boundary conditions (33) and (34) can be solved as follows:

$$\bar{c} = 1 - \frac{c_дV_w}{c_д - c_в}\int_0^\xi e^{-\left(\xi^{\frac{3}{3}}\right)-V_w\xi}d\xi. \qquad (35)$$

Multiplier V_w is defined from the condition

$$1 - \frac{c_в}{c_д} = V_w\int_0^\infty e^{-\left(\xi^{\frac{3}{3}}\right)-V_w\xi}d\xi. \qquad (36)$$

Thus, the performed analysis of the laws of hydrodynamics and mass transfer established that the calculation of the membrane element of a roll type baromembrane reduced itself to determining the concentration of the substance at the membrane outlet with the help of equations (34), (36) and the equation of material balance.

The modern popular methods of mathematical description of transmembrane mass transfer during membrane filtration have a number of significant limitations, which substantially narrows their practical use since they cannot be applied directly to whey ultrafiltration. Hence, the main operating parameters of the process have to be determined experimentally in order to approbate the results obtained theoretically. The experiment allowed the authors to conclude that PSA-20 and PSA-50 membranes were especially effective in the ultrafiltration separation of skimmed milk and whey.

Dependence of the permeate flux and the selectivity of PSA-20 and PSA-50 membranes during whey and skimmed milk ultrafiltration on the operating pressure and circulation speed of the separated system in the baromembrane channel. The driving force of the ultrafiltration process in polydisperse liquid systems is the transmembrane pressure Δp [1–3, 7, 10, 11]. Therefore, at the first stage of the studies, the authors determined experimentally the dependences of the permeate flux G of the preselected membrane types on the pressure Δp in the circulation loop of the ultrafiltration device at constant values of other process parameters. Fig. 1 and 2 show graphs of functions of G = f(Δp) type for membranes PSA-20 and PSA-50, obtained by whey and skimmed milk ultrafiltration.

The analysis of the experimentally obtained dependences of G = f(Δp) type revealed an identical change in the permeate flux through PSA-20 and PSA-50 membranes: the growth of G in the channel of both membranes was proportional to the increase in operating pressure. However, tgφ$_i$ = dG/dΔp of the graphs for skim milk was lower than that for curd whey. Since the physical significance of tgφ$_i$ is the rate of the increment of the function G = f(Δp), it can be concluded that in whey ultrafiltration the permeate flux through the membrane G and its intensity increase. This phenomenon can be explained by the fact that the physicochemical composition of skimmed milk by mass fraction of milk solids is 2–3% higher than that of whey.
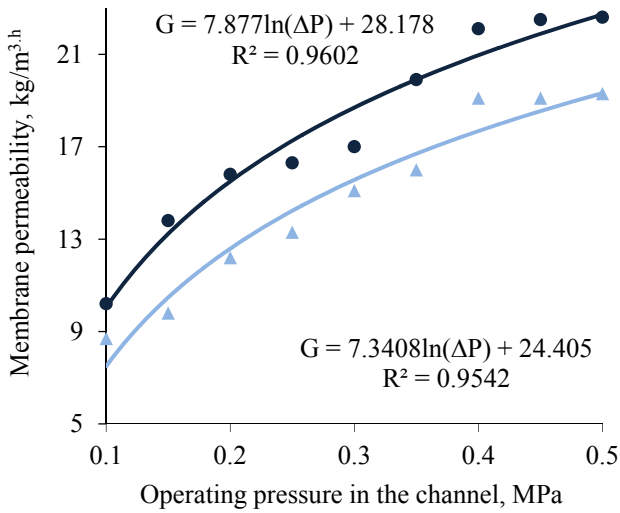
Fig. 1. Dependence of the permeate flux volume *G* in the PSA-50 membrane (● – curd whey, ▲ – skimmed milk) on the operating pressure *Δp* in the channel (V = 0.05–0.45 m/sec and t = 8–18°C, MS = 8–8.2%).
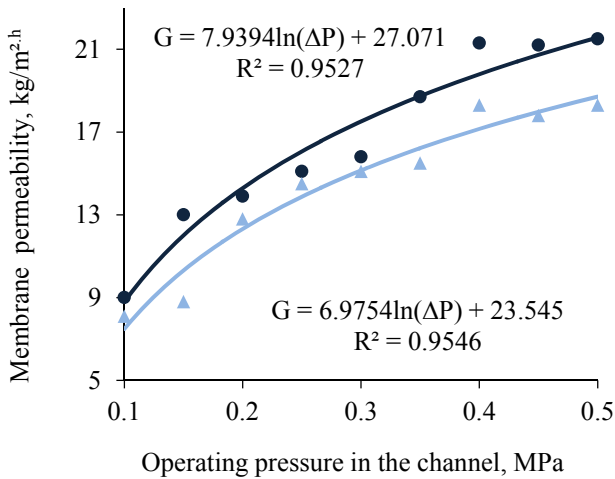
Fig. 2. Dependence of the permeate flux volume *G* in the PSA-20 membrane (● – curd whey, ▲ – skimmed milk) on the operating pressure in the channel ΔpV = 0.05–0.45 m/sec and t = 8–18°C, MS = 8–8.2%).

The analysis of the graphs of G = f (Δp) function revealed that a significant increase in the permeate flux through membranes occurs under the same conditions when the operating pressure rises from Δp = 0.15–0.17 MPa to Δp = 0.32–0.34 MPa. From further on, parameter *G* virtually does not change. When the *Δp* parameter rises up to 0.44–0.46 MPa during skimmed milk ultrafiltration, it produces a slight effect on the permeate flux. In case of whey ultrafiltration, *G* tends to decrease. Based upon the position of the sieve model of the ultrafiltration separation process, a significant increase in membrane selectivity at Δp > 0.44–0.46 MPa is probably associated with the mechanical blocking of pores by protein particles, complicated by the deformation of the membrane structure to some extent, which reduces the initial size of the flow area of the pore space. This suggests that the choice of the optimal region of the operating pressure in the membrane channel should be limited by the range Δp = 0.32–0.42 MPa.

It should be taken into account that the selectivity and the value of permeate flux through the membranes in the tangential flow of the separated liquid medium in the baromembrane contour is influenced by the circulation velocity index V (m/sec), in addition to the operating pressure. Graphical dependences G = f(V) and Ψ = f(V) for PSA-20 and PSA-50 membranes were obtained experimentally by ultrafiltration of curd whey and skimmed milk (Figs. 2 and 3, Figs. 4 and 5 correspondingly).

Fig. 3. Dependence of the permeate flux volume *G* through PSA-50 membrane (● – curd whey, ▲ – skimmed milk) on the velocity circulation of the separated system in the membrane channel (ΔP = 0.1–0.4 MPa, t = 8–18°C, MS = 8–8.2%).
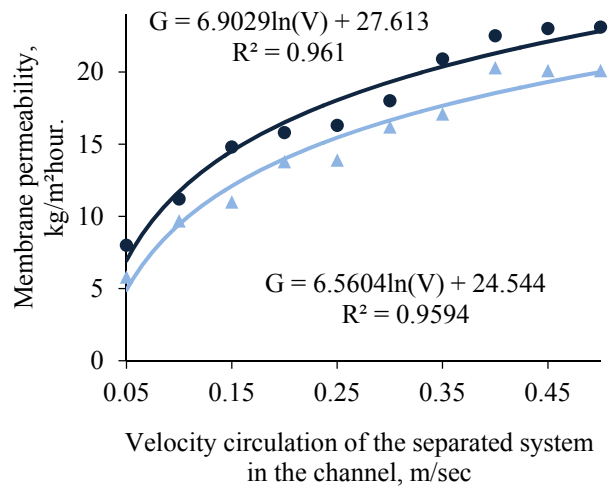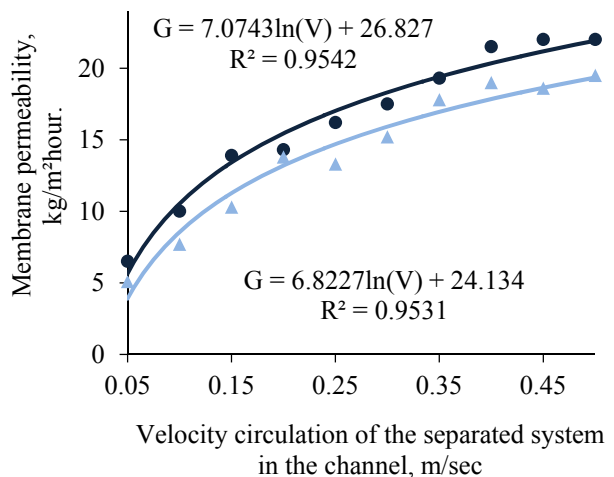

Fig. 4. Dependence of the permeate flux volume *G* through PSA-20 membrane (● – curd whey, ▲ – skimmed milk) on the velocity circulation of the separated system in the membrane channel (ΔP = 0.1–0.4 MPa, t = 8–18°C, MS = 8–8.2%).

$$\Psi = 94.432V^{-0,012}$$
$$R^2 = 0.9544$$

$$G = 93.16V^{-0,016}$$
$$R^2 = 0.9519$$
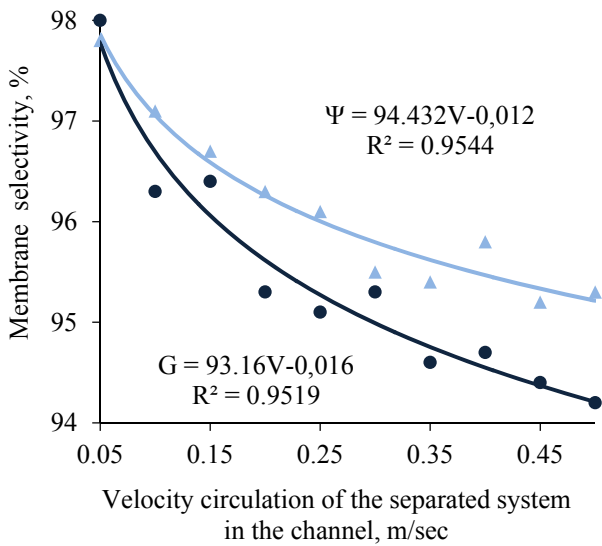
Fig. 5. Dependence of PSA-50 membrane selectivity Ψ (● – curd whey, ▲ – skimmed milk) on the velocity circulation of the separated system in the membrane channel ($\Delta P = 0.1$–0.4 MPa, t = 8–18°C, MS = 8–8.2%).

$$\Psi = 94.848V^{-0.007}$$
$$R^2 = 0.9506$$

$$\Psi = 94.021V^{-0.011}$$
$$R^2 = 0.9514$$

Fig. 6. Dependence of PSA-20 Ψ membrane selectivity (● – curd whey, ▲ – skimmed milk) on the velocity circulation of the separated system in the membrane channel ($\Delta P = 0.1$–0.4 MPa, t = 8–18°C, MS = 8–8.2%).

The analysis of the graphical dependencies $G = f(V)$ and $\Psi = f(V)$ shows that a significant increase in the permeate flux through the membrane is accompanied by an increase in the circulation rate of both curd whey and skimmed milk, within the range V = 0.16–0.36 m/sec If the sieve model of the ultrafiltration process is taken into account, an increase in membrane selectivity Ψ at V > 0.3 m/sec can be explained by the fact that the unstable deposition areas on the membrane surface begin to degrade in the membrane channel at a given circulation speed [5–8]. However, if this parameter grew more than V = 0.3 m/sec in the laboratory device, it was insufficient to remove stable protein fields on the membrane surface [3–7, 10]. This conclusion is supported by the fact that if velocity was increased more than V = 0.3–0.4 m/sec during skimmed milk ultrafiltration, this had almost no effect on the permeate flux through the membrane. And in the case of curd whey, there was a slight tendency to the increase of G, which weakened the intermolecular bonds in the 'membrane – dispersed particles' system. Thus, it can be concluded that the optimal range of the circulation velocity of whey in the baromembrane channel should be limited by the range V = 0.2–0.3 m/sec.

The performance of membrane instalments in industry has demonstrated that the efficiency of the filtration process of secondary dairy raw materials under identical conditions depends on the optimum value of parameter V. This opinion was voiced in a number of papers [1, 9, 10–12] that featured the deposition of dispersed particles on the membrane. It should be noted that cleaning and subsequent regeneration of the membranes can be an important operating parameter of the baromembrane equipment, depending on the contamination of the membrane surface.

The basic requirements usually imposed on baromembrane plants are their high productivity and the minimum membrane volume to membrane surface area ratio. However, it is the end product manufacturing technology that determines both the permissible selectivity index and the parameters of the permeate flux volume and filter elements, which are subject to certain requirements, i.e. the acceptable cost of membranes, their chemical resistance to detergent components, etc. Thus, a high level of permeate flux with a sufficient degree of membrane selectivity should be ensured by the optimal parameters of ultrafiltration of the secondary dairy raw materials. Curd whey is a complex high-molecular polydisperse system. During its baromembrane separation, membrane selectivity and permeate flux are significantly influenced by the level of concentration polarization [1–3, 9–11]. This phenomenon raises the particle concentration in the separated system from the initial value of C_0 to C_{max}. As a result, deposition areas begin to form on the membrane surface, which significantly reduces the permeate flux through the membrane and might block the pores completely. In this case, the concentration polarization level is characterized by the ratio of the dispersed particle concentrations C_0 directly at the surface of the membrane C_1. But in general, C_0 increases with time, and a constant level of concentration polarization can be maintained only by decreasing C_1.

Dependence of the influence of the mass fraction of milk solids on the permeate flux and membrane selectivity. The selectivity and the volume of permeate flux are also affected by the complex state of the dispersed particles. Thus, under the same conditions, the efficiency of membrane filtration of skimmed milk and whey ultimately depends on the milk solids weight ratio in the retentate, since this indicator can accurately estimate the level of concentration of protein particles caught in the membrane. If ultrafiltration is carried out

according to periodic pattern, the milk solids weight ratio will increase with time, together with the level of concentration polarization in the perimembrane zone [5, 6–8]. Many authors [1–5] claim that if the retentatene includes a certain amount of finely dispersed particles that are denser than those of casein dust, the permeate flux may increase, and the membrane selectivity may fall. However, in this case it is necessary to create a high-speed flux therein [1], which is most beneficial in tubular type installations. Therefore, the membrane selectivity and the permeate flux volume, which determine the ultrafiltration efficiency, are likely to depend on some maximum acceptable value in the separated system of milk solids. The analysis of characteristic curves (Figs. 1–6) revealed the similarity in the changes of the main parameters of PSA-50 and PSA-20 membranes. Figs. 7 and 8 show the results of experimental studies in the form of characteristic curves $G = f(DM)$ and $\Psi = f(DM)$. Therefore, at this stage of the study, it is possible to use only PSA-50 membrane, in which G is higher than in PSA-20.
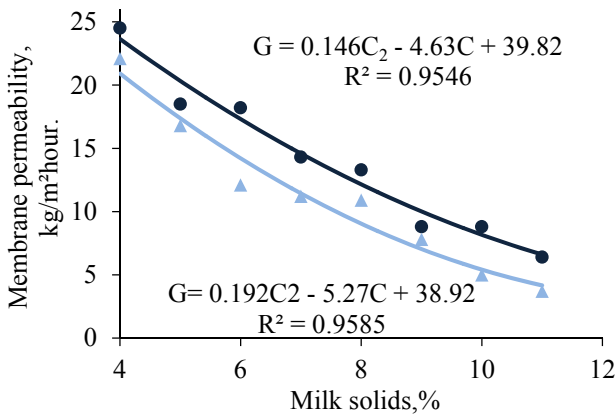
Fig. 7. Dependence of the permeate flux volume G through SPA-50 membrane ($\bullet$ – curd whey, $\blacktriangle$ – skimmed milk) on the milk solids weight ratio ($\Delta P = 0.1–0.4$ MPa, $V = 0.05–0.45$ m/sec, $t = 8–18°C$).
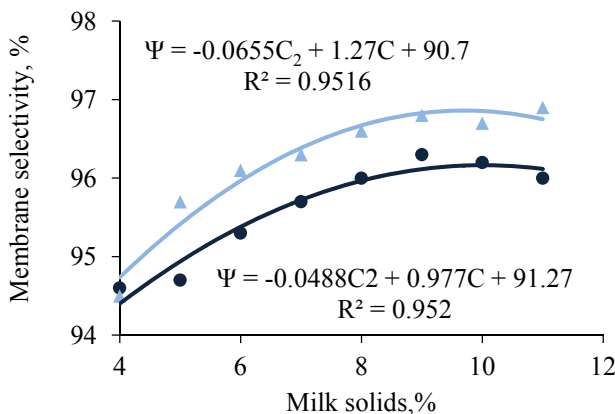
Fig. 8. Dependence of the selectivity of PSA-50 membrane ($\bullet$ – curd whey, $\blacktriangle$ – skimmed milk) on the milk solids weight ratio ($\Delta P = 0.1–0.4$ MPa, $V = 0.05–0.45$ m/sec, $t = 8–18°C$).

As for skimmed milk ultrafication, it was established that the reduction rate value of the permeate flux dG/dMS significantly decreased when the index of milk solids weight ratio reached DM = 8.6–8.9%. However, when DM > 9.2%, this parameter approaches zero. In the case of whey ultrafiltration, dG/dMS tends to zero at DM $\geq$ 10%. The growth rate of the membrane surface selectivity $d\Psi/dMS$ also decreases both during the filtration of skimmed milk and curd whey, assuming its minimum value in the range DM = 8.6–9.9%. If the milk solids weight ratio in the retentate exceeds DM = 10.2–10.4% during whey separation, membrane parameter Ψ reaches 97.5%, and G sinks to 3–5 kg/m²hour.

CONCLUSION

During ultrafiltration of secondary dairy raw materials, a membrane selectivity index higher than 96.0–96.5% is considered economically impractical [1]. Hence, the limiting value of this parameter is MS = 10.0% during curd whey baromembrane separation through a roll type PSA-20 membrane. However, if we take into account the recommendations in [13], then MS should not drop below 20% for milk protein concentrates. This can be achieved by thickening the ultrafiltration retentate in a vacuum evaporator with subsequent semi-finished goods production. At the same time, it is possible to use a less concentrated liquid retentate, e.g. milk drinks [13].

Theoretical calculations of MS (34–36) are similar to those obtained by experimental data. Still, it is necessary to take into account the physicochemical properties of the system, which have a significant effect on the permeate flux and membrane selectivity [1]. It should be noted that very a strong complex deposition appears on the membrane surface when MS = 10.0% during whey ultrafiltration [1]. The stagnant zones in the membrane channels trigger the development of microflora both in the retentate and in the permeate. These factors, together with the physicochemical properties of the system, determine the reduction rate of the permeate flux during baromembrane separation of liquid polydisperse systems. In this regard, it is of great importance to clean membranes from surface contaminants. This ensures the reliability of baromembranes during the ultrafiltration separation of secondary dairy raw materials. However, to determine the optimum parameters of regeneration and washing, a separate experimental research is required.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this article.

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Durability of cutter assemblies and its causative factors

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Abstract: Cutter assemblies operate under stressful conditions: the knives are subjected to high dynamic loads, the cutter shaft rotates at a frequency of $5-100$ s^{-1}, the electric driving motor of the cutter shaft overcomes high starting moments, the bed is subjected to significant static and dynamic loads, the food raw materials and humid atmospheric air in the production room is corrosive to the structural elements, etc. Under the influence of these factors, the cutter assemblies break down, which causes unregulated pauses in food raw materials processing and also requires high expenses for equipment repair. The aim of the paper was to study the durability of the main cutter assemblies and to establish its main determining factors. The presented numerical values of durability of cutter assemblies have been obtained as a result of the planned, warranty and post-warranty practical maintenance of cutters by the engineering team of GEA FOOD SOLUTIONS UKRAINE, LLC, the mechanical supervisor staff of Cherkassk Food Company, LLC and also as a result of scientific research of the processes that provide the operation of these machines and that were carried out at the Cherkassy State Technological University (the Ukraine). The components that operate under the most stressful conditions are knives, a cutter shaft and its bearing assemblies, and the electric driving motor of the cutter shaft. At the same time, the durability of most cutter assemblies is limited by the quality of operation of the cutter head and the durability of the knives. The expenses for the repair or replacement of cutter assemblies can amount to tens of thousands of euros (not including the losses caused by equipment downtime). By applying the appropriate technical solutions and cutter system operating rules, it is possible to significantly improve the durability of a cutter and reduce these expenses.

Keywords: Cutter, durability, repair, knives, fatigue strength, improvement

INTRODUCTION

Cutters are used in the vast majority of processing lines for the production of sausages, canned meat and minced semi-finished products. The design of modern models of cutters makes it possible to implement a number of types and modes of food raw materials processing such as grinding boneless steaks (fresh or frozen) to the state of meal or mincemeat, grinding mincemeat to the state of emulsion, mixing formulation ingredients with simultaneous grinding or without it, vacuumizing raw materials and the heat treatment thereof, which is carried out simultaneously with grinding, the saturation of raw materials with inert gas, and freezing it with liquid nitrogen [1–6].

In order to provide the above processing types, a cutter is equipped with the appropriate assemblies, the main of which are: a cutter head several knife blocks

each of which commonly contains 2 knives; the knives of a special geometric shape made of the appropriate steel grades and heat treated in the appropriate manner; a cutter shaft with bearing assemblies; an electric motor and the belt drive of the cutter shaft drive; a bowl; an electric bowl drive; a bed; an electric cabinet and control panel; a frequency converter, which is part of the cutter shaft drive.

Cutter assemblies usually operate, under stressful conditions: the knives are subjected to high dynamic loads, the cutter shaft rotates at a frequency of $5-100$ s^{-1}, the electric drive motor of the cutter shaft overcomes high starting moments, the bed is subjected to significant static and dynamic loads, the food raw materials and humid atmospheric air in the production room are corrosive to the structural components of the cutter, etc. Under the influence of these factors, cutter

assemblies break down from time to time, which causes unregulated pauses in food raw materials processing and also requires large expenses for equipment repair.

A systematic increase in the durability of cutter assemblies can reduce these negative effects. For this, it is necessary to study the durability of the main cutter assemblies and to establish the main factors that contribute both to a decrease and an increase in this durability.

There are no data on cutter durability in the known literary sources. The only exception is the publications [7–14] that highlight the wear resistance, static and fatigue strength of knives. A study of the durability of the main cutter assemblies is relevant.

The aim of the paper is to study the durability of the main cutter assemblies and to establish its main determining factors.

STUDY OBJECTS AND METHODS

The objects of the studies were GEA Cutmaster V vacuum cutters with 200, 325, 500 and 750-liter bowls; a Laska KR-200-2V vacuum cutter with a 200-liter bowl, a Laska KR-330-2V vacuum cutter with a 330-liter bowl, an atmospheric L5-FKB cutter with a 250-liter bowl, as well as their working bodies (knife heads, knives, bowls), drive components (motors, belt and gear drives, shafts, bearing assemblies and their seals), and electronic control elements.

The studied of GEA Cutmaster V cutters are installed at several meat processing plants in the Ukraine. The studied cutters Laska KR-200-2V, Laska KR-330-2V, and L5-FKB are installed at the meat-packing plant of Cherkassy Food Company, LLC (Cherkassy, the Ukraine).

The durability of the working bodies of cutters and the components of their drive was determined by the mechanical supervisor staff of Cherkassy Food Company, LLC during the planned works on equipment operation, as well as by the engineering team of GEA FOOD SOLUTIONS UKRAINE, LLC during the planned, warranty and post-warranty practical maintenance of cutters. At the same time, the actual operating time of the cutters, the conditions for their operation, the types of failures, their causes and the expenses for their elimination were specified.

The efficiency of cutter knives, their durability and ways to improve it was studied at the Cherkassy State Technological University (the Ukraine) using the following methods. The curvature radius of the cutting edges of the knives was determined using the hard copy proof method with an optical MBS-9 microscope. The pulse-plasma hardening of the cutting edges of the knives was carried out using IMPULS the insfallation of the laboratory of E.O. Paton Electric Welding Institute of the National Academy of Sciences of Ukraine. To determine the effect of pulse-plasma hardening on the microstructure of the surface layers, the corresponding metallographic specimens were made. The metal samples were subjected to grinding in several stages and chemical polishing in a 3% nitric acid solution for 5 s. The microstructure was studied using an optical Neophot-32 microscope. The microhardness of the hardened samples was measured

using metallographic specimens with the help of an M-400 micro-hardness meter from LECO with a load of 50 g. The diffractometric studies of steel samples were carried out in order to identify the factors that provide the hardening of knife material after pulse-plasma treatment. A DRON-UM1 diffractometer, monochromatic Cu-Kα-radiation and the step scan method (35 kV, 25 mA, the exposure time at the point is 10 s, the pitch is 0.05°) were used. A graphite monocrystal set to a diffracted beam was used as a monochromate. The samples were studied in the plane of incidence of the X-ray beam as they were rotated. The hardness of the surface of the knives was measured using a TK-2M hardness tester (by Rockwell) and a Tsh-2 hardness tester (by Brinell). The roughness of the knife surface was measured using a TIME 3221 profilometer.

The strength and vibration resistance of the knives were studied by means of numerical simulation using a T-Flex Analysis software package. The determination of the necessary forces acting on a knife when being in contact with raw materials, as well as the study of hydrodynamics of raw materials during cutting, was carried out with the help of a special FlowVision software package. When studying the hydrodynamics of raw materials, a Sony FS700 digital video camera with high-speed video recording and the corresponding additional equipment (an Odyssey 7Q Convergent Design recorder, Sony SEL-18200 OSS lens and a 9.7" Lilliput 969 A/O/P video monitor) were also used.

The efficiency of raw materials processing using the advanced knife designs was studied by measuring the cutter productivity and energy consumption for the cutter shaft drive. The quality of the finished product was determined by organoleptic analysis in accordance with the current state standards.

RESULTS AND DISCUSSION

The raw material grinding process is carried out with a knife head. High demands are imposed on it as an assembly for imbalance exceeding the permissible value of which can result in a rapid failure of the bearings of the cutter shaft and the shaft itself. After each knife sharpening cycle (in practice-after each working shift), the static balancing of each knife block is performed. In this case, the design of knife blocks must also ensure the dynamic balancing of the knife head in order to eliminate the bending moments that caused by the unbalance of the individual blocks. Poor balancing significantly reduces the life-time of the front (on the knife head side) cutter shaft bearing and also increases the load on the shaft itself.

The knives are subjected to various impacts during operation. The factor the durability of knives is limited by most of all is the wear of their cutting edges. A sharpened knife has a cutting edge with a curvature radius of about 9 microns, which reaches 40–45 microns after one working shift. In the case of the average cutter utilization, the durability of knives is not above 2 months.

This is due to the high wear properties of meat raw materials, including the effect of its surfactants, as well as the sufficiently low allowance of knives for resharpening. The knives are sharpened along their

front edge, which results in a decrease in the width of the knives, and consequently in a decrease in their strength. The knife resharpening allowance, which is regulated by the supporting documents from the manufacturer, is 8–15 mm for the knives of the cutters with a bowl volume of 200–330 liters. Thus, after a rather small number of cycles, a knife becomes unsuitable for further use and is discarded.

The cost of a set of 6 knives ranges for the present time from 200 € (domestic knives made of non-corrosion-resistant steels) to 2000 € (German knives made of special corrosion-resistant steels). Taking this into account, the increase in the wear resistance of cutter knives continues to be a relevant task. One of the ways to increase the wear resistance of knives is their surface hardening, for example, by pulse-plasma treatment, which allows us, according to the authors' studies, to increase the time between failures by 2.2 times. An increase in the microhardness of the surface from $HV_{50} = 3700$ MPa to $HV_{50} = 11,000$ MPa corresponds to this increase in the wear resistance of knives. It has been established that despite a rather high content of austenite, the hardness of the surface layers increases due to the phase hardening as a result of reversal α-γ transformations.

A significant problem for meat processors is the destruction of knives during operation. On average, one or more knives are destroyed at a frequency of up to 3 times a year in the case of two-shift cutter operation for 16 hours a day. At the same time, the knife (or several knives) itself and the raw material being processed (about 200–300 liters) are lost, and the bowl and the cutter head cover are damaged (there are potholes of considerable lengths up to 6 mm in depth). Fig. 1 shows the typical examples of such destructions, which are caused by the high linear velocities of knife parts (100–180 m/sec).

(a)

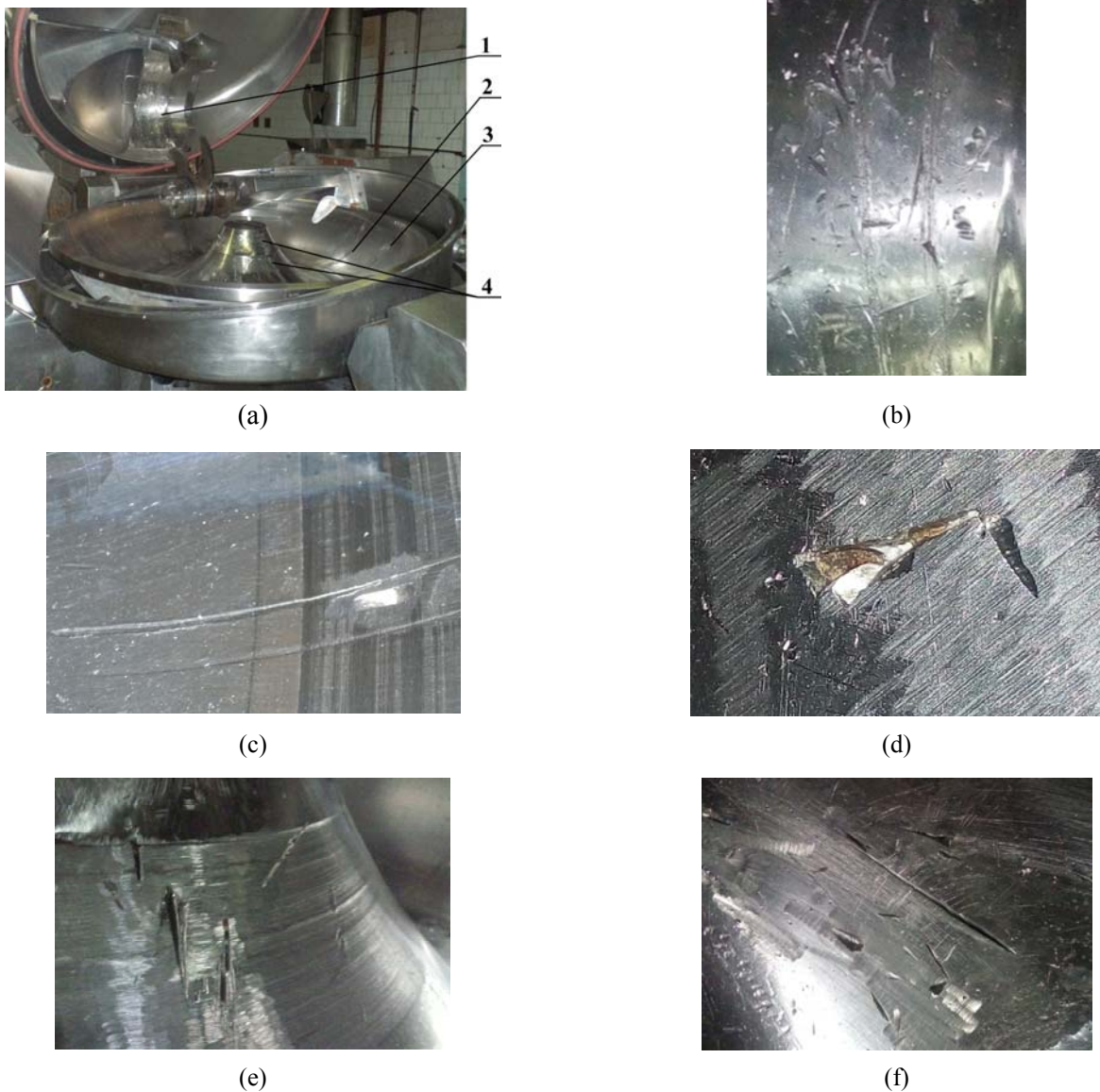
(b)

(c)

(d)

(e)

(f)

Fig. 1. Examples of damage to the construction elements of a Laska KR-330-2V cutter when the knives are destroyed during cutting: (a) general view of the cutter; (b) cover of the knife head (Pos. 1 in Fig. 1a); (c) periphery of the bowl (Pos. 2 in Fig. 1a); (d) periphery of the bowl (Pos. 3 in Fig. 1a); (e), (f) central cone of the bowl (Pos. 4 in Fig. 1a).

Due to the destruction of at least one knife, the balance of the knife head is violated, which results in the development of considerable dynamic forces that affect the cutter shaft and, as a consequence, in the rapid failure of cutter shaft bearings. There can also be the deformation of the knife head cover and the deformation of the support shaft of this cover.

When the knives are destroyed, the front and rear (the side of the knives and the motor side) cutter shaft bearings and the associated wear parts (seals, etc.) are replaced. In some cases, the cutter shaft can be replaced (if there is a further fast bearing failure after the bearings are replaced). The main sign of the need to replace the cutter shaft is an increase in vibration and noise during operation. In addition, the knife cover is levelled, the detected defects are welded (TIG) and polished.

The destruction of knives is caused by a wide range of things. These can be the causes by negligent cutter operation, in particular, by the bones or metal elements that get into the bowl of the cutter together with raw materials. The knife, when running against them, is subjected to great impact loads and is destroyed.

The other causes are due to the design and operation method of knives. During cutting, the cutting forces, the pressure from the raw material supplied by the cutter bowl, and the centrifugal forces act on the knife. They bring the knives to a complex stress-strain state which is followed by the appearance of characteristic stress concentration zones (Fig. 2a) – near the mounting part of the knife and on the back edge of the knife.

The knives make up to 100 revolutions per second. When entering the raw material, they bend under the action of cutting forces, and they unbend on leaving it. In this case, this refers to an oscillatory process which is carried out at a frequency of 100 Hz. The results of authors' studies [15] showed that the knives of modern cutters operate under conditions of the oscillations close to resonance (Fig. 2b). The dynamic factor reaches $\beta = 2.24$ for them, which indicates a proportional increase in the stresses in the knife precisely due to the specific impact of the oscillatory process. Along with insufficient fatigue strength, it is one of the factors of the increased incidence of knife destruction during the operation of modern high-speed cutters.

The authors have developed a new design of high-strength and highly efficient perforated knives. The efficiency of the holes that are located near the mounting part of the knife is insufficient due to the movement of this area with the minimum linear speed V_{min} (the minimum cutting speed) in the upper part of the cutter bowl that is not always filled with raw materials. At the same time, the holes near the mounting part of the knife significantly reduce its strength since they are located in the most stress-bearing part thereof. The improved design of a perforated knife provides the location of holes on the periphery of the body (Fig. 3b). The strength of the knife became higher by 20% in the most stress-bearing part thereof. At the same time, the grinding ability of the knife is improved due to the more advantageous location of the holesthey move in the part of the bowl that is always loaded with raw materials; moreover, they move with the highest linear velocities V_{max}.

As a result of the study of raw materials hydrodynamics during cutting, the authors have developed a technical solution that makes it possible to increase the strength of knives by several times. As is known, the following technical contradiction is fair for cutter knives: "The knife should be as thin as possible to minimize the heating of mincemeat when grinding and the knife should be as thick as possible to ensure its high strength." The heating of mincemeat causes a deterioration in the quality of sausages, and the destruction of the knife during rotation causes significant complex losses for a meat processing plant. As a result of the studies of raw materials hydrodynamics, it has been established that, in contrast to the known concepts (Fig. 4a), when flowing over the upper part of the knife profile, the flow of raw material, after moving along the blade rake, moves around the upper horizontal side of the knife with no contact to it (Fig. 4b). The new established knowledge have made it possible to propose a was differentiated increase in thickness of knives new way to increase their strength (Fig. 4b). At the same time, the above requirements are met at the same time-the low heating of raw materials (due to the small thickness of the blade in the raw material contact area), and the knife strength increased by more than 2.2 times (due to the increased thickness in the rear, the most stress-bearing, part of the knife that does not contact to the raw materials).

In modern cutters, the knives are fully loaded by the cutting power in a very short time interval–1/400 sec. In this case, it refers to shock-type load, and therefore the material of the knife must be adapted to such operating conditions, in particular, have sufficient viscosity.

This problem is solved by the choice of steel grades with a medium (steel 40X13, 50X14MoW) or high (65Mn steel) carbon content and the appropriate heat treatment of the most loaded knife areas (steel normalization or hardening).

One of the leaders in the production of cutting knives, the German company GW Steffens GmbH, produces them from the patented M92 steel. According to the manufacturer, it has the best combination of the following properties: a high wear resistance, high corrosion resistance, a high viscosity and fatigue strength. As shown by the chemical analysis of the material of these knives, the steel contains 0.7–0.9% of carbon, up to 1% of manganese, up to 1% of silicon, 1% of molybdenum, 15% of vanadium, 1% of niobium, and about 13% of chromium. The steel has a higher carbon content compared to 65Mn steel and at the same time the improved viscosity, corrosion resistance, and hardenability.

Different manufacturers of knives provide various thermal treatments thereof. Fig. 5 shows the results of the experimental measurement of hardness of foreign (a) and domestic (b) knife areas. As one can see from Fig. 3a, the knives manufactured by GW Steffens GmbH have a hardened working part (up to 56 HRC units) and a normalized mounting part (up to 20 HRC). Due to this, the improved ability to resist shock loads is provided for the area where the working and mounting parts of the knife are adjacent to each other (1 in Fig. 2).

(a)   (b)
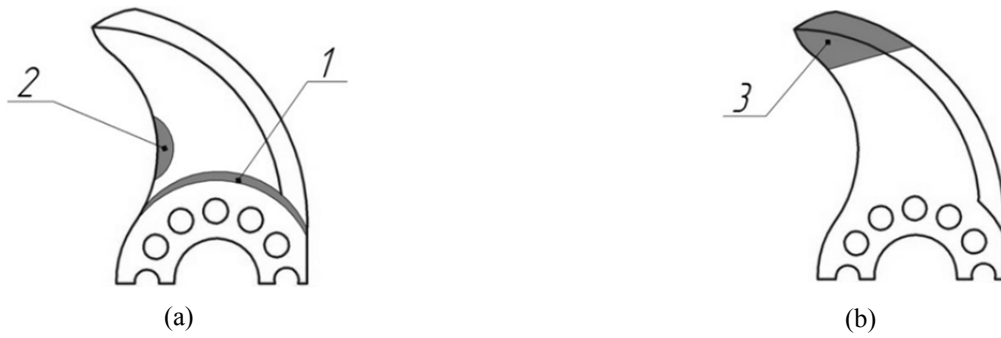
Fig. 2. Stress-strain state of knives: (a) stress concentration zones under static load (*1* – area near the mounting part of the knife, *2* – area on the back edge of the knife); (b) area of the maximum deformations *3* caused by the knife oscillations during cutting at frequencies close to resonant ones.

(a)   (b)
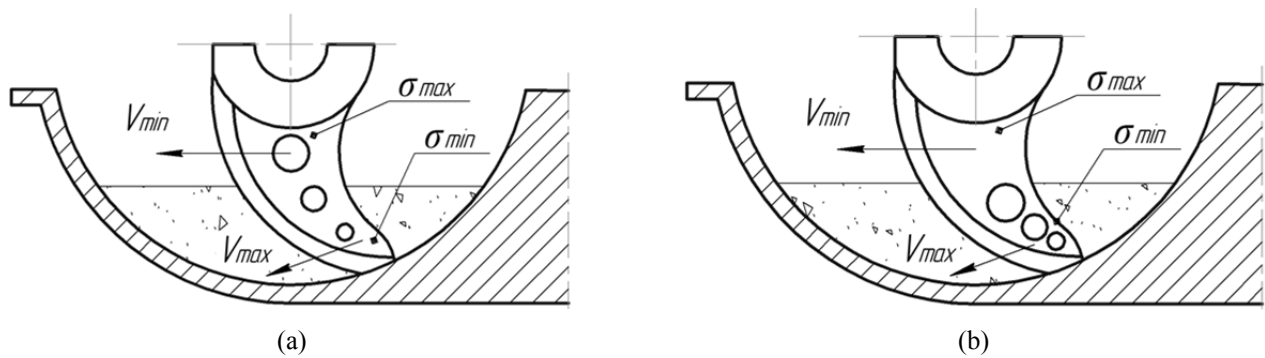
Fig. 3. Operation diagram of perforated cutter knives: (a) usual knife; (b) hardened knife.

(a)   (b)
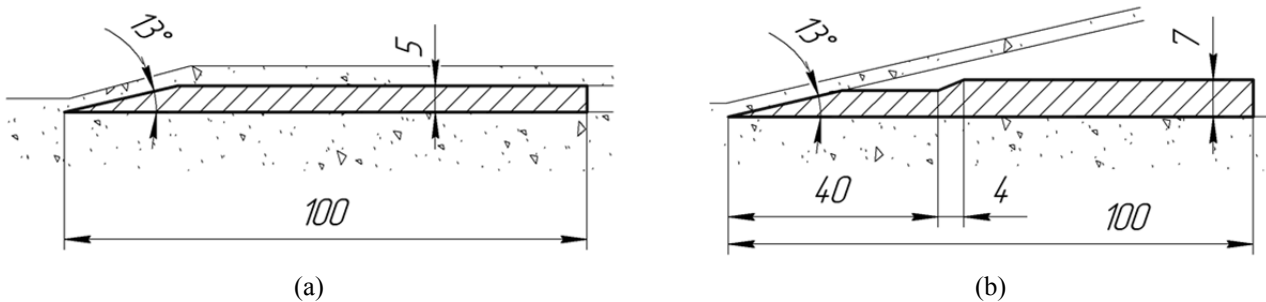
Fig. 4. Flow diagrams for raw materials and the cross-sections of cutter knives: (a) standard knife; (b) hardened knife.

(a)   (b)
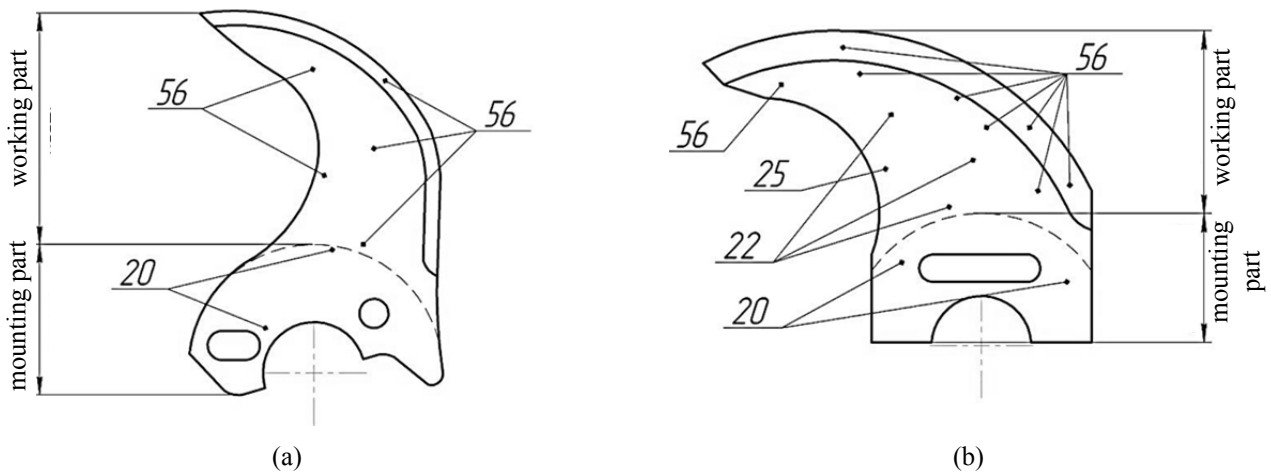
Fig. 5. Results of the experimental measurement of hardness of the knives of various manufacturers by Rockwell: (a) the knife of a Laska KR-330-2V cutter produced by the German company GW Steffens GmbH; (b) the domestic knife of an L5-FKB cutter.

The knives of L5-FKB and L5-FKM cutters manufactured in the Ukraine (Cherkassy) are heat-treated in the other way. The blade is hardened to a hardness of 56 HRC using high frequency currents, and the other parts of the knife remain normalized to a hardness of 20–25 HRC (Fig. 5b). This heat treatment method provides the high viscosity of the knife not only in the area where the working and mounting parts are adjacent to each other, but also at the stress concentration point on the back edge (4 in Fig. 6b).

However, as the results set forth below, show, such heat treatment methods do not provide adequate knife strength. Most of knife failures are caused by a fatigue failure. Fig. 6 shows a knife destroyed by fatigue stresses, as well as characteristic metal fracture zones.

The fatigue crack began to develop in the stress concentration zone 4 on the back edge of the knife at the point of the chamfer intended for mixing raw materials during the reverse rotation of the knife head. The metal has a fine crystalline structure here (zone 3 in Fig. 6b, c, d). The brittle fracture zone (zone 2 in Fig. 6b, c) has a coarse-grained structure. The fact that the fatigued area 3 is larger than the area of the brittle fracture zone 2 shows that the part has been destroyed by flexural strain and tensile stresses with the stresses reaching moderate values. A small amount 1 of the material taken off during resharpening (2.5 mm) should

be noted especially. This indicates that the knife has run out less than half of its rated life before the breakage and its static strength has not reached a significantly reduced value due to the reduction of the width of the body.

Fig. 7 shows a macrocrack that began to develop on the back edge of the knife of the L5-FKB cutter, the deepest part of the crack is in the stress concentration zone 2 (in accordance with Fig. 2a) on the back edge of the knife.

The fatigue failure of knives can be caused by a number of things: insufficient static strength, the insufficiently high permissible fatigue stresses of metal, the insufficient fatigue endurance of metal, the increased roughness of external surfaces, the presence of tensile stresses in the surface layers after grinding and sharpening, as wellas the corrosion and wear of the surface layers.

The measurement of surface roughness of the knives made in Germany and in the Ukraine (Fig. 8) made it possible to establish that the knives of the Laska KR-330-2V cutter produced by GW Steffens GmbH had a mounting surface with a roughness $Ra = 0.708$ μm and a working surface with a roughness $Ra = 0.053$ μm. The knives of the L5-FKB cutter had both a working and mounting part with a roughness $Ra = 0.216$ microns.

(a)   (b)

(c)   (d)
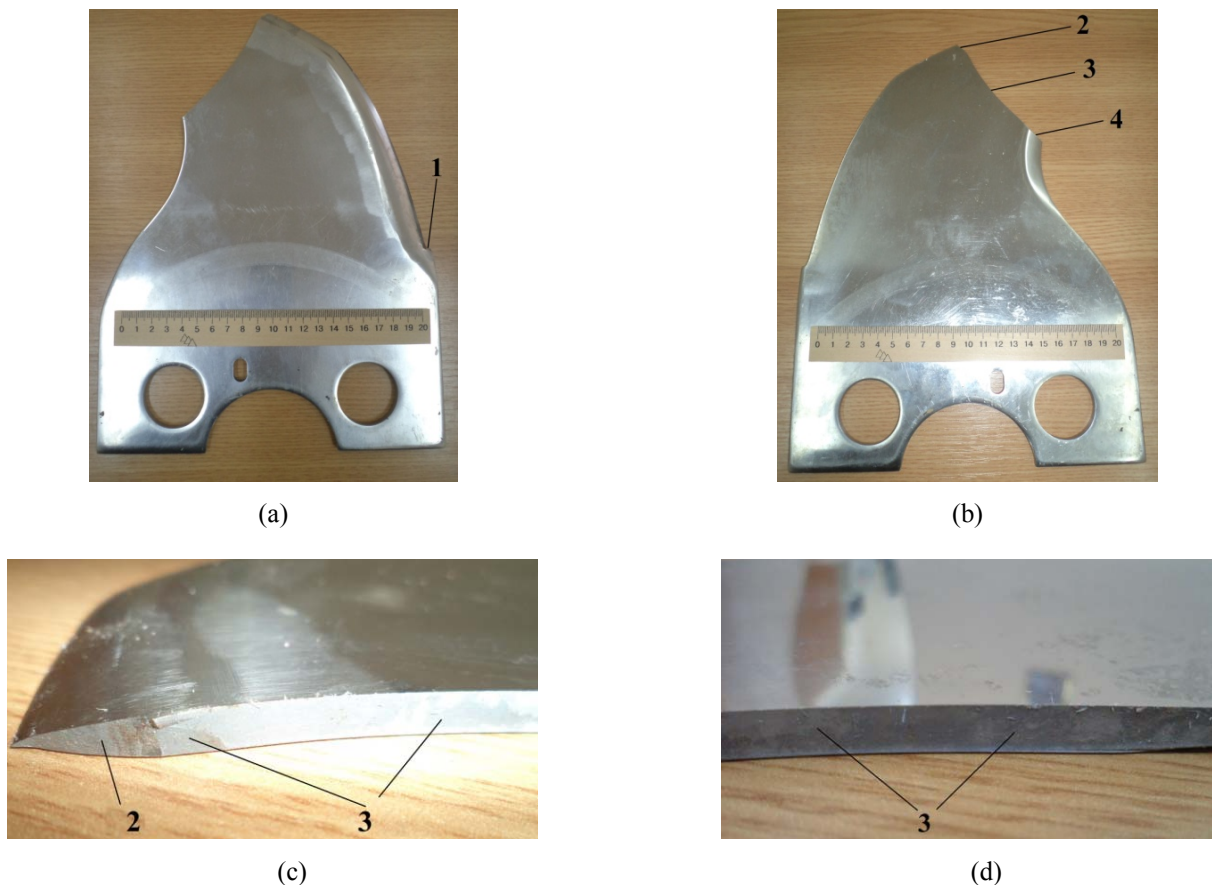
Fig. 6. Example of destruction of a knife of a GEA Cutmaster cutter with a bowl of 500 liters under the influence of fatigue stresses: (a) the general view of the knife from the side of the blade; (b) the general view of the knife from the side of the chamfer on the back edge; (c), (d) the characteristic knife fracture zones; *1* – the value of the metal taken off during resharpening; *2* – the area of coarse metal grains; *3* – the area of fine grains formed under the action of fatigue stresses; *4* – the place where the fatigue crack begins to develop.

(a)

(b)

(c)

(d)
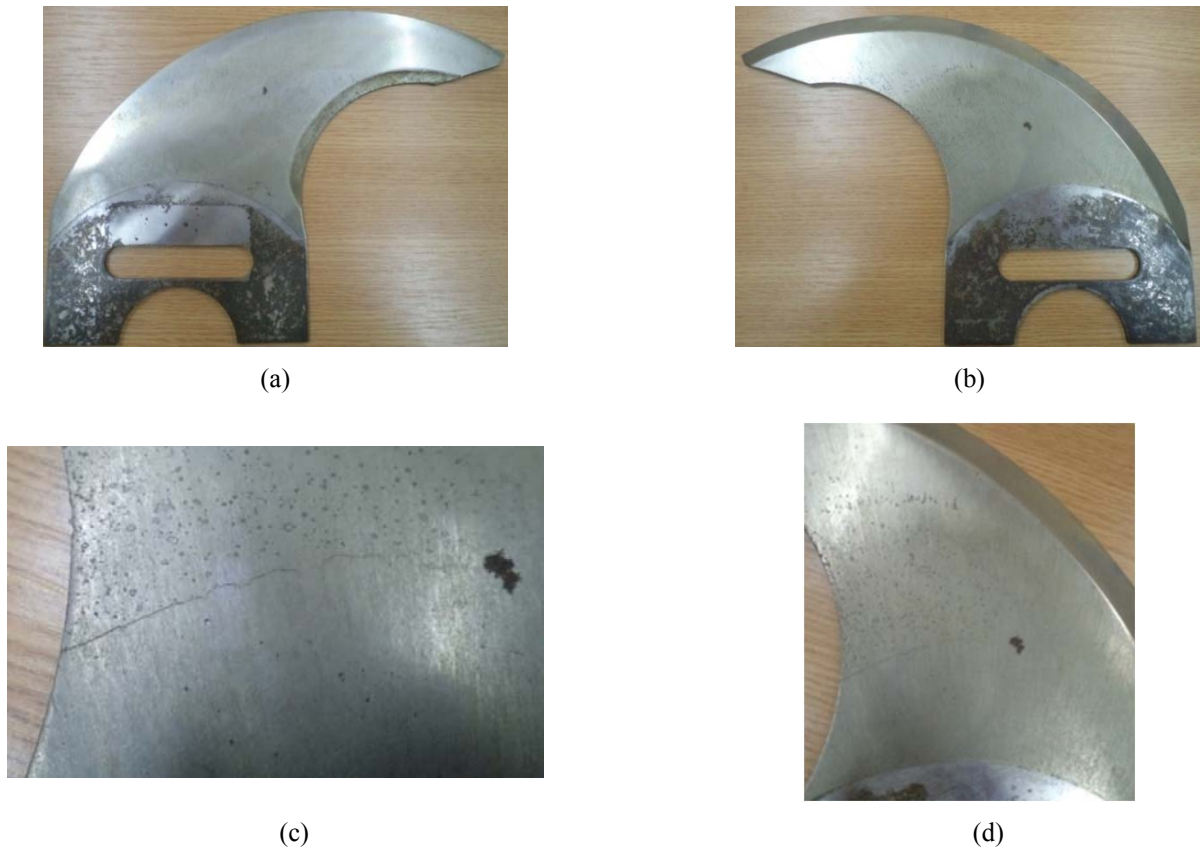
Fig. 7. Fatigue macrocrack on the working surface of the knife body of the L5-FKB cutter: (a) the view of the knife from below, a chamfer on the back edge is shown; (b) the view of the knife from above; (c) the enlarged view of the area the fatigue macrocrack began to develop from; (d) the general view of the macrocrack.

(a)

(b)

Fig. 8. Measurement of the roughness of the knife surface using a TIME 3221 profilometer: (a) the knife of the Laska KR-330-2V cutter; (b) the knife of the L5-FKB cutter.

The data obtained show that the working part of the German-made knife has been polished, while the working part of the Ukrainian-made knife has undergone a grinding operation as finishing. As a result, the L5-FKB knife has a 4-time higher roughness of the working surface, which causes 5–10% of its lower fatigue strength. The back edge of the Laska KR-330-2V knife has a high quality of processing, whereas the back edge of the L5-FKB knife has some significant jags, which is obviously also the source of fatigue cracks in the knife (Fig. 7).

The fatigue strength of the knives is significantly reduced by the fretting corrosion in the area 1 of the mounting part (Fig. 2a) due to the friction along the components of the knife block. In addition, the corrosive effect of the meat medium significantly decreases the fatigue strength due to damage to the surface layer. As known, the corrosive medium reduces the fatigue strength of structural steels by up to 87%, and corrosion resistant by up to 44%. The fatigue strength is also reduced by pre-corrosion (for example, when storing knives) by up to 35%.

The reduction of the risks of knife destruction can be provided by regular flaw detection for the presence of micro-and macrocracks in the repair and mechanical workshops of meat processing plants.

Such defectoscopy should be carried out every 2–3 knife sharpenings, but to date, little attention of production workers is paid to this aspect of cutter operation.

All the above confirms the high relevance of the problem of the insufficient fatigue strength of cutter knives, which requires effective ways to solve it. Given the above factors, it can be argued that none of the heat treatment diagrams shown in Fig. 3 fully meets the requirements for knife cutters. The metal in the areas 1 and 2 (according to Fig. 2a) should be sufficiently viscous to resist shock loads and sufficiently hard to resist fatigue failure and to increase corrosion resistance.

Based on the analysis of the requirements for the construction of cutters and the known methods for their heat treatment, the authors have developed a new way to harden knives. It lies in the fact (Fig. 9) that the whole knife is first annealed to the full depth, then it is normalized or hardened to HB200-350 to the full depth. Then, the blade 1 is hardened to the full depth to a hardness of HRC = 52-66 with the appropriate tempering (for example, by induction hardening with high frequency currents, plasma hardening, etc.). Then, sections 4 and 5 of the knife are face-hardened to a hardness of HRC = 52-66 to a depth of 0.03–2 mm (for example, using surface plastic deformation by means of high-frequency mechanical forging). Further on, all the surfaces of the knife, including the blade (1), the working part (2), the mounting part (3) and the back edge (4) intended for mixing raw materials, are polished. Polishing is carried out to a roughness at which the average height of microroughnesses of the surface is not above 1.0 μm.

At the same time, hardening the blade (1) increases its wear resistance. The normalization or hardening of the core (6) of the mounting part (3) and the working part (2) of the knife increases its viscosity, which is favorable for the operating conditions of the knife (a shock load). Hardening the surface layers (5) of the knife working part (2) increases the fatigue strength and corrosion resistance of these areas. The hardening of the surface layers (5) of the mounting part (3) increases their corrosion and wear resistance under

fretting conditions. Polishing all the surfaces of the knife increases their fatigue strength and corrosion resistance. As a result, the most effective combination of working properties of the cutter knife in comparison with the known analogues becomes possible.

The cutter shaft system is one of the most critical assemblies of the cutter. Bearings is what fails most often (the durability is 12–24 months). They are replaced by two specialists, which causes the corresponding cost of repair work (the bearing replacement lasts for 10–16 hours, the average labor cost per one specialist is 25 euro/hour). The shaft sleeves and cover slides are also replaced (every 24–36 months). The wear parts of the cutter shaft (vacuum seals) are replaced every 6–12 months.

The cutter shaft itself breaks down far less often than the bearings do. This moment is noted for the destruction of the shaft or by the considerably reduced durability of the newly mounted bearings. The shaft is ordered from the manufacturer and is replaced by two specialists.

Both the bearings and the shaft fail for the same reasons, namely because of the high vibration of the knife shaft. Such vibration occurs when processing solid (frozen) raw materials, when using knives of an unregulated design, when one of the knives breaks down and the knife head is poorly balanced.

The decrease in vibration is enabled by a decrease in the outreach of the consoles l1 and l2 (Fig. 10), which is achieved by reducing the thickness and the number of knife blocks (l1), as well as by reducing the length of the pulley (l2) due to the transition from the use of V-belt transmission to the use a toothed gear.

L5-FKB and L5-FKM cutters have paired bearing assemblies (Fig. 10b) in contrast to foreign cutters (Fig. 10a). This results in an increase in the rigidity of the shaft (the load pattern of the shaft as a simple beam is replaced with the load pattern of a beam with two clamped ends) and, as a consequence, in a decrease in its maximum deflection by up to 4 times and an increase in the critical rotation speed by 1.16–1.52 times. As a result of this design of a cutter shaft system, the durability of the shaft and the bearings significantly increases.
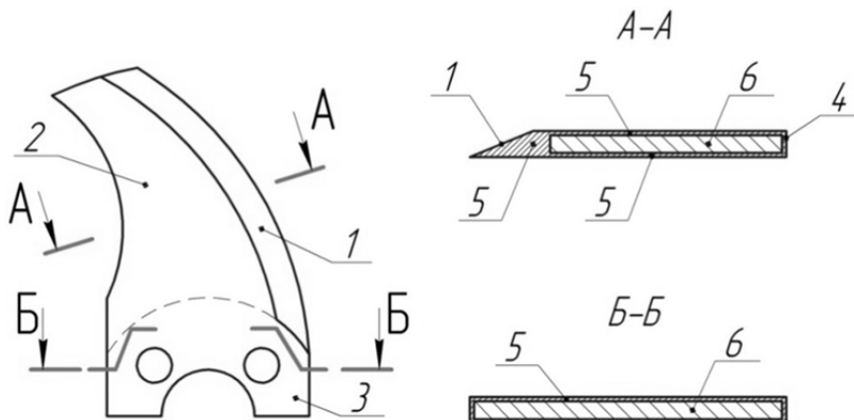
Fig. 9. Hardening pattern of the knife according to the developed method: *1* – knife blade; *2* – working part; *3* – mounting part; *4* – back edge; *5* – face-hardened layer with an increased hardness; *6* – normalized or improved layer with a reduced hardness.

(a)   (b)
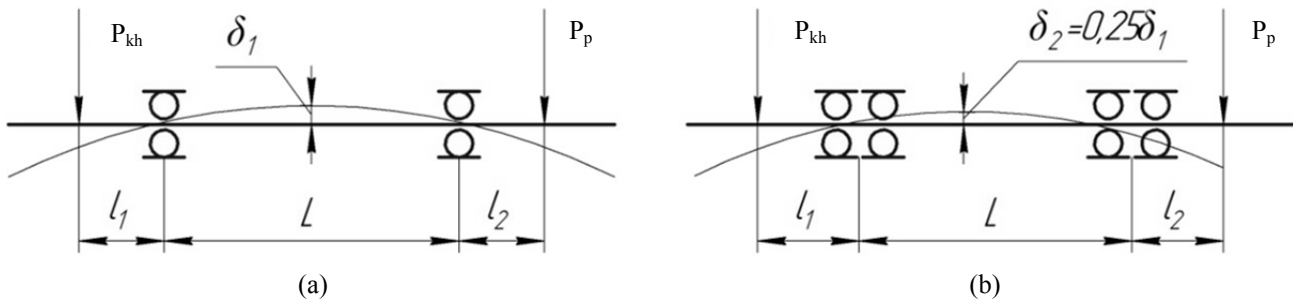
Fig. 10. Installation diagram of the knife shaft in the bearing assemblies: (a) of foreign cutters; (b) of domestic L5-FKB/FKM cutters. P_{kh} is the weight of the knife head; P_p is the gravity of the pulley; L is the distance between the bearing assemblies; l_1, l_2 are the lengths of the consoles; δ_1, δ_2 are the maximum deflections of the cutter shaft.

(a)   (b)
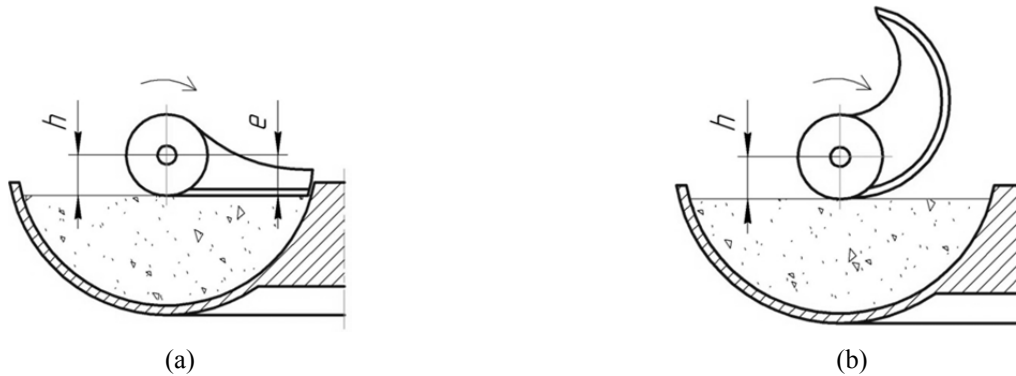
Fig. 11. Diagram for the interaction of the knife with the raw materials: (a) with a straight blade located with the eccentricity e; (b) with a curved blade; H is the distance from the rotation axis of the knives to the raw materials in the cutter bowl.

As mentioned above, the vibration of the shaft is significantly determined by the design of knifes. The more curved the blade, the lower the vibration and vice versa-the more straight the knife blade, the higher the vibration. Fig. 11 shows two corresponding limiting cases. The maximum vibration will be noted when the knife blade is straight and located with the eccentricity e = h where h is the distance from the rotation axis of the knife to the raw materials in the cutter bowl (Fig. 11a). In this case, the load from the cutting forces on the knife will grow from 0 to max instantaneously, which will cause an increased shock load both on the shaft and the knife itself. When using highly curved blades (Fig. 11b), the load on the knife and the cutter shaft will grow gradually, which will reduce the vibration of the shaft.

Since the shape of the knife blades is determined by the type of the product being manufactured (knives with less curved blades are intended for non-structural sausage products, knives with more curved blades-for structural sausage products), then the contradiction between the requirements for the high quality of raw materials processing and low vibration loading of the knife and the cutter shaft is obvious. Relevant is the search for new design solutions for knives that can resolve this contradiction.

In order to reduce vibration during the operation of the cutter knife head, as well as to improve the efficiency of raw materials processing, the authors have developed two knife designs. The knife shown in Fig. 11 is intended for grinding the raw materials of structural sausage products (smoked, etc.). In contrast to standard knives, it has two areas of highly curved cutting edges (7 in Fig. 12). The knife has been designed as mounted on the supporting disc (1), there are grooves (6) for the passage of the raw materials discarded during cutting. Detachable blades (2) with cutting edges (7) are fixed on the body. A lock (5) is also provided for the fastenings (3), (4), (8), (9), (10). All the cutting edges of the presented knife are highly curved, in contrast to standard knives. This increases the smoothness of interaction of the knife with the raw materials and improves the quality of cutting the cooled raw materials used in the production of smoked sausages.

Fig. 13 presents a knife for cooked sausage products developed by the authors. Its design solutions are based on the new results of the studies of hydrodynamics and the quality of raw material processing during cutting. The knife has been made assemblable, the detachable blades (2) have a low metal consumption and prime cost. The design allows the following conflicting requirements to be successfully fulfilled: the increased curvature of the common cutting edge, the small cutting angle for grinding the connective tissue of meat raw materials (the angle α of the blade (2)), an increased cutting angle for the muscle tissue of meat raw materials (the angle β of the dispersing surface (5) of the overlay (4)). The use of such a knife reduces vibration when cutting, improves the processing of raw materials for cooked sausage products, and reduces the operating costs for cutting tools.
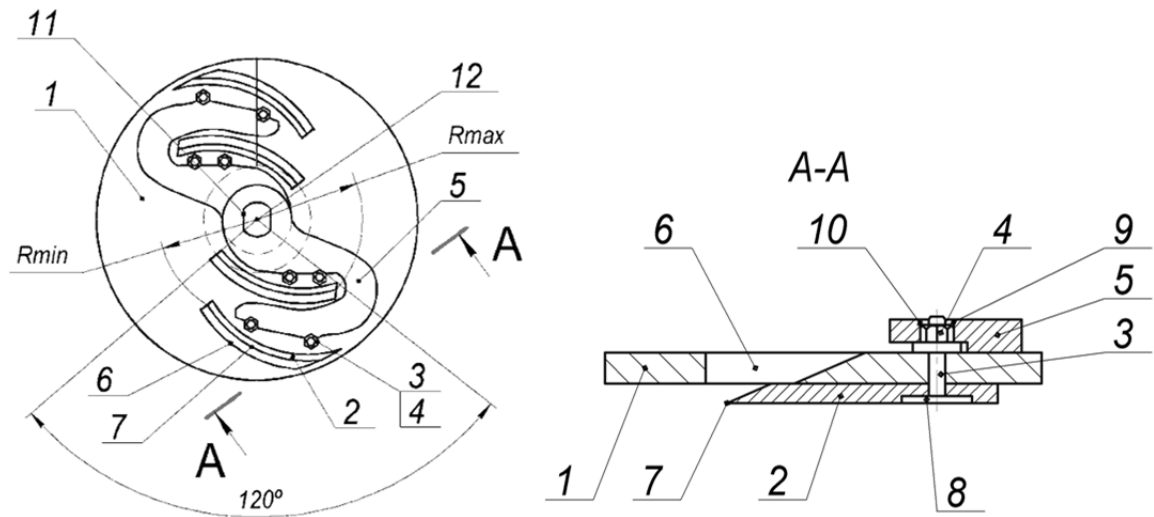
Fig. 12. Knife for structural sausage products: 1 – body; 2 – blade; 3 – bearing assembly; 4 – nut; 5 – lock; 6 – groove; 7 – cutting edge; 8 – bearing plate; 9, 10 – washers.
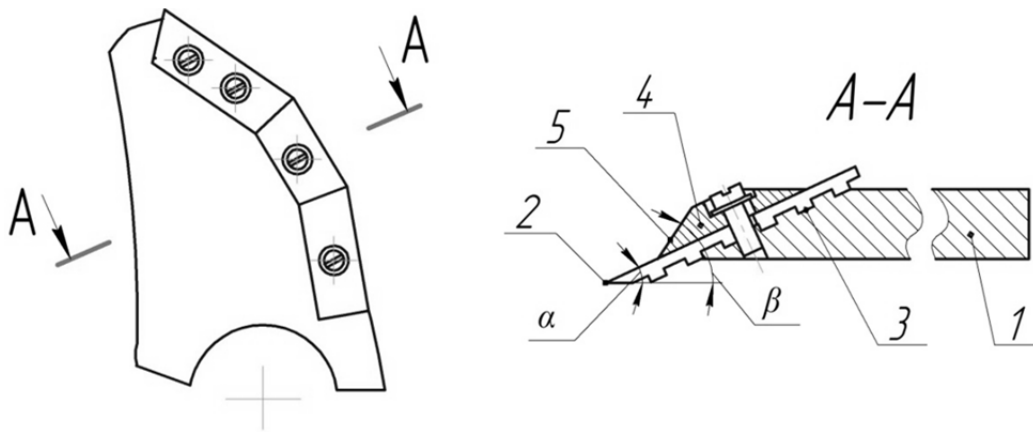
Fig. 13. Knife for cooked sausage products: 1 – body; 2 – blade; 3 – teeth; 4 – overlay; 5 – dispersing surface.

(a)　　　　　　　　　　　　　　　　　　　(б)
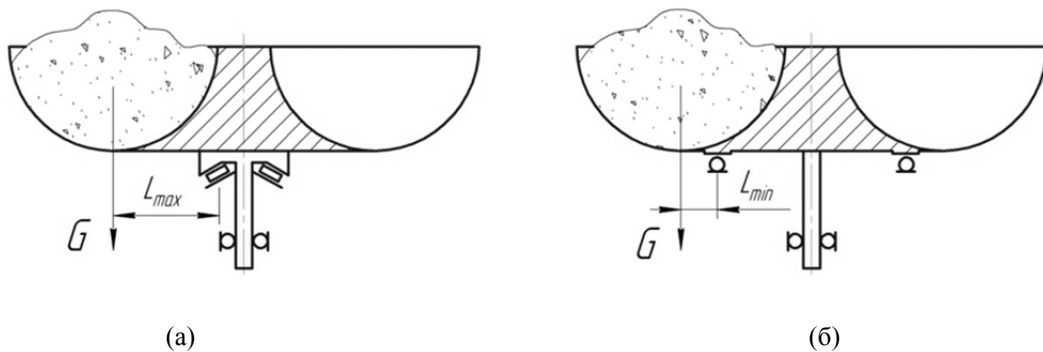
Fig. 14. Origination of a bending moment from the weight of raw material when it is loaded into the bowl: (a) for a bowl with conventional bearing assemblies; (b) for the bowl of the cutters made by LaskaMaschinenfabrikGmbh with a thrust bearing with an increased diameter.

The durability of bearing assemblies of the bowl is quite high (24 months or more). Its reduction is mainly due to the slant of the bowl in the case of the asymmetrical arrangement of raw materials therein. This arrangement of raw material takes place when it is loaded into the bowl (Fig. 14) by means of a mechanical floor trolley lifter – the weight of raw material G acts at a distance l from the bearing

assemblies, which results in the origination of a bending moment. In this case, the shaft bearings of the bowl work under worsened conditions and quickly fail. Some specialists of the Austrian company Laska Maschinenfabrik Gmbh have proposed the use of a thrust bearing of a considerably increased diameter and special design in the bowl supports (Fig. 14b). This makes it possible to significantly reduce the arm l_{min} in

comparison with the bearing assemblies of a standard design (Fig. 14a), which significantly increases the durability of the bowl drive system. The bowl in CutMaster cutters made by GEA is mounted directly on the gear shaft, in this case, the durability of bearing assemblies is more than 9 years.

The cup itself wears out along the surface furthest from the rotation center during operation. Such wear out is caused by the effect of meat raw materials, which, when ground in the cutter head, acquires a high velocity in the direction of about 45° relative to the axis of the cutter shaft (the feed rate is close to the cutting speed, i.e. 70–150 m/s). Such wear out is intensified by the action of raw material surfactants. When the knives are destroyed, the bowl can sustain the most severe mechanical damage. Types of damage and repair are given above (see Fig. 1).

The durability of belt drive belts of the cutter shaft drive and the bowl drive (if a gear-motor is not used for the bowl drive) is 6–12 months. The duration value is determined, mainly, by the culture of operation of the belt drive (the tension of the belts with the appropriate force, preventing the ingress of chemically active substances on the belts, etc.).

Modern cutters use motors with a power from 30 to 320 kW and higher. The electric motor of the cutter shaft drive operates under rather severe conditions: high starting moments (in the absence of a frequency converter in the drive), high air humidity, frequent starts and stops (every 5–7 minutes), the insufficiently effective cooling in high-capacity cutters, etc.

The electric motor bearings are replaced on average every 36 months at a special plant. To this end, the electric motor is dismounted from the machine. The brushes of the electric motor are replaced every 12–24 months. An increase in the durability of the electric motor of the cutter shaft can be provided by the use of a frequency converter in the drive. It will ensure the smooth startings of the engine, by the effective forced cooling of the engine during operation (air or water cooling as in Seydelmann cutters), reduce the moisture condensation in the engine windings (for this purpose, the forced heating of the internal volume of the electric motor during down time is applied for Seydelmann cutters).

The electrical and electronic modules of the cutter, including the control panel, most often fail because of the high humidity in the room, due to the negligent operation of the cutter (the unauthorized actions of personnel during the sanitary treatment of the machine, or mechanical damage) and abnormal situations in the supply network of a meat processing plant. As a repair, the replacement of electrical or electronic components is used.

It should also be noted that the overhaul of a cutter under conditions of its operation is not provided at a meat processing plant. As the parts and assemblies wear out or fail, they are replaced with new ones, the replacement thereof is carried out at different times. If it is necessary to pick up a cutter from a meat processing plant, it reguires four workers (10–16 working hours), one specialist (about three working hours), at the same time, one or two fork lifters, a cargo crane, and a lorry (with a semitrailer) with a capacity of 20 tons should be used. Sometimes picking a cutter up is followed by the dismantling of the ceiling, or walls. The similar forces should also be used when returning the cutter back to the plant.

In general, it can be noted that a cutter remains one of the most important, in the technological sense, machines for making sausage products. The high demand for it in production and its high cost stipulate the increased demand for the durability of its assemblies. The components that operate under the most stressful conditions are knives, a cutter shaft and its bearing assemblies, and the electric driving motor of the cutter shaft. At the same time, the durability of most cutter assemblies is limited by the quality of operation of the cutter head and the durability of the knives.

CONCLUSION

The expenses for the repair or replacement of cutter assemblies can amount to tens of thousands of euros (not including the losses caused by equipment downtime). Applying the appropriate technical solutions and rules of operation of cutter systems can significantly increase the durability of a cutter and reduce these costs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Dynamics of acousto-convective drying of sunflower cake compared with drying by a traditional thermo-convective method

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Abstract: The article is devoted to the dynamics of sunflower cake drying in a fundamentally new acousto-convective way. Unlike the traditional (thermo-convective) method, the method proposed allows extracting moisture from porous materials without supplying heat to the sample. Thermo-vacuum drying helped to determine the absolute and relative initial moisture for the analysed samples of the sunflower cake, which equaled 313.1% and 75.8%, respectively. The kinetic curves for drying by thermo- and acousto-convective methods were obtained and analysed. A study of the acousto-convective drying of sunflower cake showed that the rate of moisture extraction depended on the resonating frequency, while there is an optimal mode in which drying proceeds from two to three times more intensively. In thermo-convective drying of sunflower cake, increasing the temperature of the drying stream twice (from 74.2°C to 127°C) reduces the duration of drying to a final absolute humidity of 40% three times. Comparing the thermo-convective and acousto-convective drying methods showed that twice as much moisture was removed from the samples dried by the (ACDP) with a flow frequency of 790 Hz and at room temperature for a 30-minute interval as with thermal convective drying with a working flow temperature of 127°C. The relaxation mathematical model used to describe the drying phenomenon and the experimental data for sunflower cake drying allows obtaining the quantitative parameters characterizing different modes and methods of drying the samples under study. The article analyses a discrete drying regime that contributes to increasing the efficiency of the acousto-convective mode of moisture extraction.

Keywords: Acousto-convective drying, thermo-convective drying, porous materials drying, moisture extraction

INTRODUCTION

Sunflower is an agricultural oilseed crop, which ranks third in the world in terms of production volumes following peanuts and soybeans [1]. The main product made from sunflower is sunflower oil, which is used in the food industry for cooking and foods. In addition to sunflower oil, sunflower flour is produced from sunflower as well; its nutritional properties are of great significance to humans as both a source of protein [2] and a powerful antioxidant [3]. It is also noteworthy that currently people all over the world are actively discussing ideas on transition from mineral energy sources to renewable ones, including sunflower oil. So, [4–7] deal with the study of diesel fuel production from sunflower oil.

Sunflower is the second in the world in terms of output level in production of vegetable oil from oilseeds. As part of processing sunflower seeds into sunflower oil, the feedstock passes through a number of technological stages, with some of the raw material becomes included into the final product, i.e. oil, and some part turns into waste. The sunflower oil obtained is the main commercial product that makes profit. On the other hand, waste products have to be disposed of, which leads to higher prices for the main products. One of the ways to reduce the cost of the main products is the recycling of production waste and the subsequent use of by-products in livestock breeding and agriculture. One such product is sunflower seed cake, which forms the basis of protein dishes for feeding cattle [8] and small ruminants [9].

A cake appears resultant from the subsequent processing of the sunflower meal. Obtained by processing, the product is a biological material, with relative moisture of about 80%. The high moisture content in the biological material does not allow storing it for a long time with the proper quality, which entails the loss of its useful properties. Therefore, to preserve all useful properties and characteristics of cake, the moisture content in the material must be significantly reduced. In addition, the removal of excess moisture from the product contents will considerably reduce the weight of the final material.

To drain the cake, a traditional thermo-convective approach is used [10, 11], which engages applying the hot, dried air stream to the material. This study proposes an alternative approach for cake drying, based on placing the material to be drained into a high-intensity acousto-convective flow. This technology has proved a noticeable intensification of moisture extraction from various porous biomaterials, such as meat [12], rice [13, 14], and pine nut [15] and inorganic materials, such as granular silica gel [16, 17], cellular aerated concrete [18], wood [19], etc. One of the main advantages of this technology is avoiding heating of the material to be drained, i.e. drying takes place at a room temperature [20]. In addition, the drying process is accelerated.

This work is focused on experimenting with moisture extraction from sunflower cake when drying by the acousto-convective method using a small ACDP developed by Insfitute of Theoveticol and Applied Mechanics, Siberian Branch of Russian Academy of Sciences (ITAM SB RAS) and comparing the results with ones for the thermo-convective drying method.

RESULTS AND DISCUSSION

Thermo-vacuum drying of the sunflower cake. Experiments to analyse the dynamics of heat and mass transfer in a sunflower cake require the data on the initial moisture content. To obtain them, a special study of the dried material in a vacuum oven CHBC-4,3.4,3.4,9/3U24n was carried out. Three control portions with different initial masses were prepared, which differed approximately two and four times from the initial mass of the first sample. Table 1 shows the numerical values of the initial mass of the prepared samples before placing in a vacuum drying oven.

A heating temperature of 50°C, maintained automatically throughout the experiment, was applied with the view to increasing the productivity of the vacuum drying using a temperature controller. After the target temperature was reached within the drying chamber, three prepared control portions were loaded into the vacuum drying cabinet. Then, a vacuum pump was activated; the air pressure in the drying chamber was reduced to 100 Pa.

After a preset time interval, the vacuum pump was deactivated, and the dried samples were briefly removed from the drying chamber for control weighing. The weighing was carried out by the electronic laboratory scales AND EK 610i with a maximum weight of 600 g and 0.01 g readability. The weighing data were processed to evaluate the current moisture contents. The thermo-vacuum drying continued until the current moisture of the samples with a large initial mass was higher than the current moisture contents of the samples with a smaller initial mass. Totally thermo-vacuum drying of the control portions of sunflower cake lasted 24 hours. Table 1 presents the experimental data on weight results after thermo-vacuum drying.

Two expressions are used to evaluate the initial moisture: the first, for an absolute moisture (W); and the second, for relative (w). The absolute moisture is calculated as a ratio of the moisture content to the mass of the absolutely dry material, using the following formula:

$$W = \frac{m - m_0}{m_0} \times 100\% \ , \qquad (1)$$

and the relative moisture is calculated as a ratio of the mass of moisture to the current mass of the material under study:

$$w = \frac{m - m_0}{m} \times 100\% \ , \qquad (2)$$

where m is the current mass of the wet sample, m_0 is the mass of the test sample in an absolutely dry state, i.e. the data obtained after the thermo-vacuum drying experiments. The calculated values of the absolute and relative moisture according to formulae (1) and (2) are presented in Table 1.

Resulting from the analysis of thermo-vacuum drying of the sunflower cake samples, the initial moisture content of the test material was determined. The averaged value of the initial moisture for the three control samples was $W = 313.1\%$ or $w = 75.8\%$. The reported moisture value was taken as the initial W_0 (w_0) in all subsequent experiments with the sunflower cake.

Acousto-convective drying of sunflower cake. The acousto-convective drying of the sunflower cake was carried out on the acousto-convective drying plant (ACDP) of ITAM SB RAS. The ACDP flow-chart is shown in Fig. 1. The ACDP operation is based on a gas-jet radiator of the Hartmann type [21, 22].

The samples of the sunflower cake were placed in a tight gauze sleeve to prevent the loss of fine fractions of the material. The resulting material was placed in a cylindrical container made of a metal stainless mesh with a cell size of 0.7 x 0.7 mm and a wire thickness of 250 μm. The loaded container was closed and fixed to the substrate with screws. The assemblage was placed in the working part of the ACDP and fixed in it with a fastening system.

The experiments were conducted in a heated room with an ambient temperature of 25.1°C, a moisture of 16.7% (3.9 g/m^3), and a dew-point temperature of 1.5°C. The moisture temperature meter IVTM-7 MK-S recorded the temperature and humidity of the air.

The ACDP was first started without the material. After the process stabilisation, parameters of the generated acousto-convective flow were registered in the working part of the ACDP. The working flow

temperature in the ACDP tract equaled 18.8°C. During the experiments, the parameters of the working flow varied depending on the initial data being set, but the pressure in the ACDP prechamber was kept constant by means of a precision airflow adjustment system. The static pressure in the ACDP prechamber for all the experiments was $P_0 = 4.7$ atm.

In this study, three modes of the ACDP operation were chosen by an analogy with [12]. The first operating mode of the ACDP is realised at a resonator depth of 300 mm, with the generated stream frequency of 270 Hz and intensity of 182 dB. The amplitude-frequency characteristics (AFC) of the mode are shown in Fig. 2. The second mode is achieved by decreasing the depth of the resonator to 80 mm, while the frequency of the working flow increases to 790 Hz at an intensity of 175 dB, its AFC is shown in Fig. 3. The third mode corresponds to the zero position of the resonator, that is, the flow goes around the barrier, having no pronounced resonant frequency, and the intensity equals 130 dB.

Figs. 4 and 5 present the processed results on the dynamics of moisture extraction from the sunflower cake samples during the acousto-convective action on them under different operating conditions of the ACDP. These figures show that a 30-minute drying by a flow with operating parameters corresponding to the background mode decreases the absolute moisture content of the material by 94%, and the relative moisture by 7%. For the same time interval, the drying mode with a frequency of 270 Hz decreases the absolute moisture by 117%, and the relative moisture

by 10%. When switching to the next mode of insonation by a working flow with a frequency of 790 Hz, there is a significant intensification of moisture removal, so the absolute moisture for the same half-hour decreases by 185%, and the relative one by 20%.

Table 1. Thermo-vacuum drying of the sunflower cake samples

№	m, g	m_0, g	W, %	w, %
1	19.88	4.86	309.05	75.55
2	40.34	9.78	312.47	75.76
3	81.61	19.54	317.66	76.06
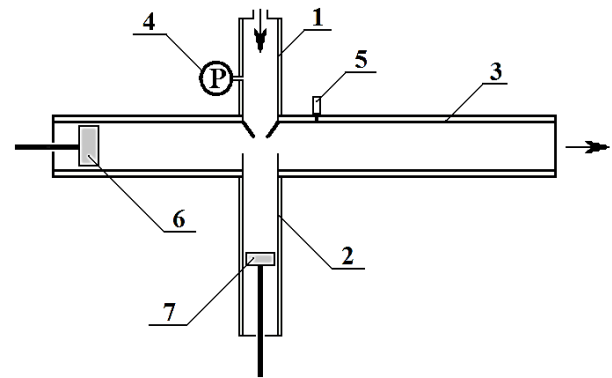
Fig. 1. The ACDP flow-chart: 1 – prechamber, 2 – resonator, 3 – working part, 4 – static pressure gauge, 5 – sensor LH-610, 6 and 7 – adjusting pistons.
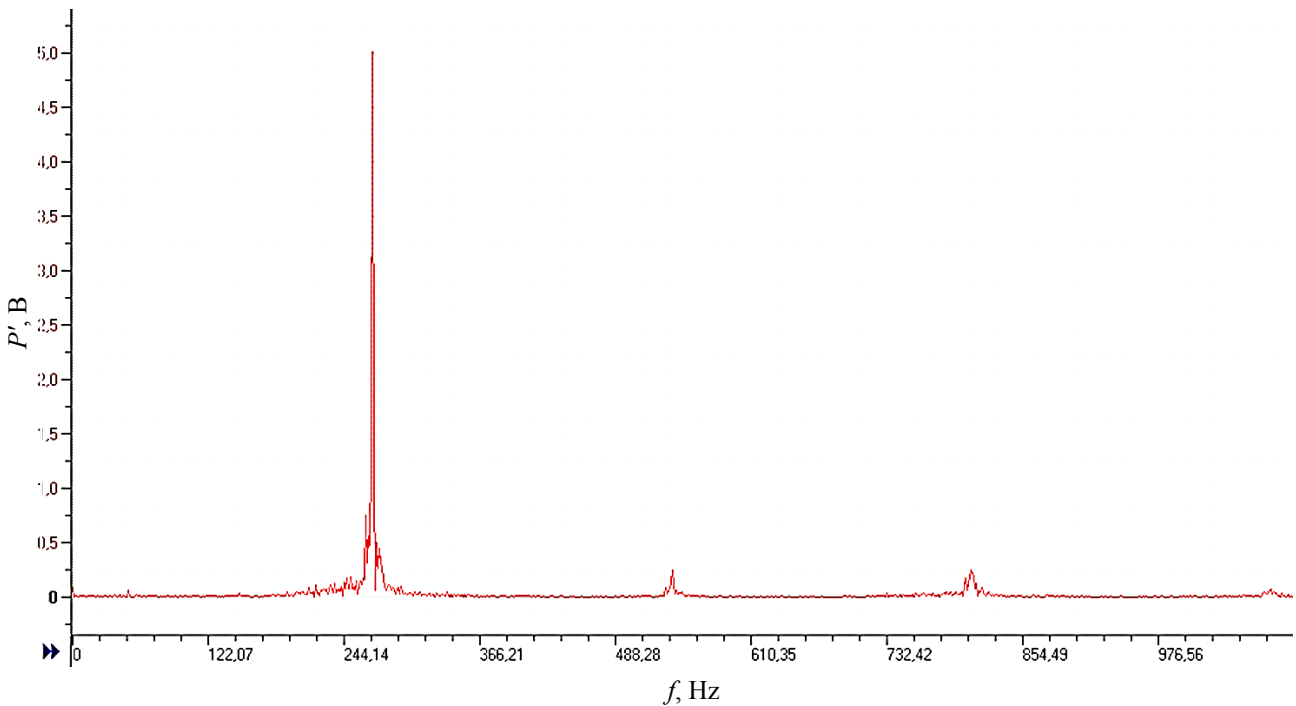
Fig. 2. The AFC of the working flow at a resonator depth of 300 mm.
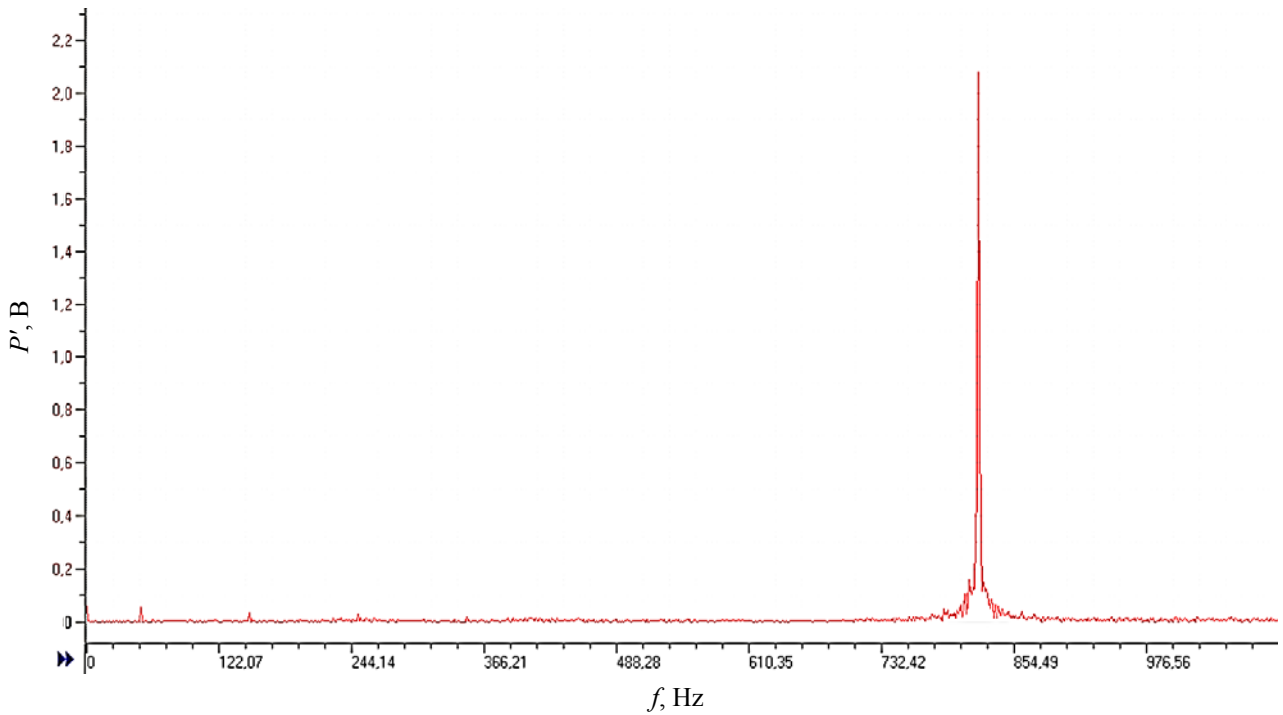
Fig. 3. The AFC of the working flow at a resonator depth of 80 mm.

1 – background   *2* – 270 Hz   *3* – 790 Hz
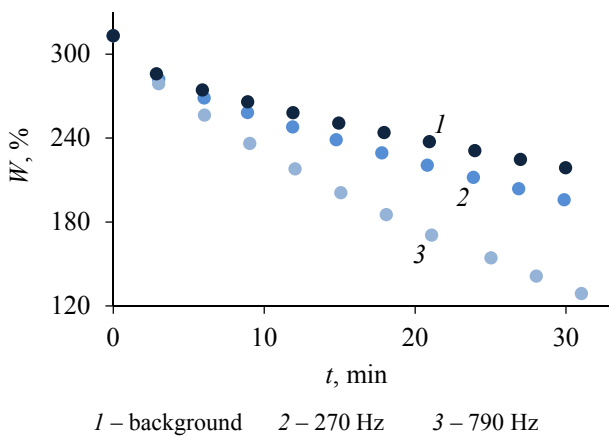
Fig. 4. The absolute moisture change for the sunflower cake during acousto-convective drying by different modes.

1 – background   *2* – 270 Hz   *3* – 790 Hz
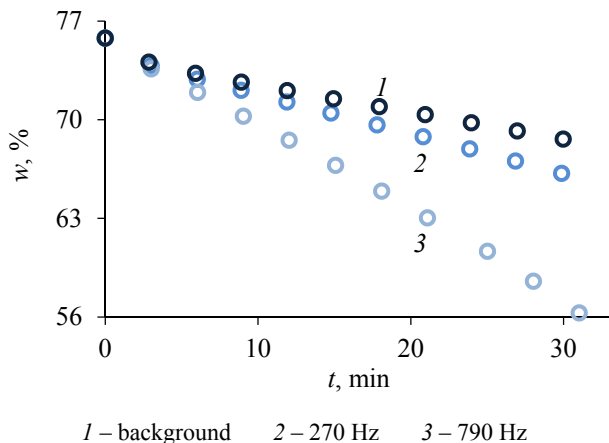
Fig. 5. The relative moisture change dynamics in the sunflower cake during acousto-convective drying by different modes.

Thus, when the material is insonated at a frequency of 790 Hz, the moisture removal is twice as large as when it is insonated at 270 Hz and three times, if there is no resonant frequency in the working flow. Comparing the results of insonation at a frequency of 270 Hz and without a resonant frequency shows that the mode with a resonant frequency of 270 Hz extracts water 1.5 times faster than in the background mode. The obtained result confirms that resonance intensifies moisture extraction from porous materials, in which the resonant frequency value has a significant effect on the rate of the moisture removal.

The understanding of the acousto-convective drying of porous materials is still incomplete today. One of the possible mechanisms describing the physics of extracting moisture from a porous material in a high-intensity acoustic field is presented in [19]. It applies the heterogeneous media mechanics to simulate acousto-convective drying. The mathematical model takes into account the difference of speeds and phase pressures in the porous skeleton and the liquid filling it and satisfactorily describes the initial stage of the drying process (as revealed by the verification of the numerical data obtained in the appropriate experiments). Asaresult, the difference of sound speeds in the skeleton of a porous body and the water caused compression waves, which travel in a solid body and squeeze out liquid onto its surface.

Thermo-convective drying of sunflower cake. Traditionally, the sunflower cake is dried by the thermo-convective method, which is based on heat input to the material, and thus it is necessary to compare the results for the acousto-convective drying dynamics with the ones for the traditional drying. To achieve that, the experiments were conducted with the help of an experimental stand where the sunflower cake was dried with a thermo-convective flow. The heat flow was produced by a thermal gun ETV–4.5/220 T with a

nominal heating capacity of 4.5 kW and a flow rate of 7.6 mps. The heat gun has two modes: the first mode has the minimum temperature of the heat flow; the second one has the maximum temperature of the heat flow. The experimental stand launching and the process stabilisation were carried out without the material. After the process was stabilised, the heat flow temperature at the exit from the heat gun was recorded 74.2°C for the first mode and 127°C for the second mode.

The sunflower cake samples, previously put in the gauze hose, were placed in the center of the heat flow from the heat gun. Every five minutes the samples were removed from the heat flow and weighed. The experiment duration for the thermo-convective drying by the first and second modes was different.

The weight experiment results for the both modes of thermo-convective drying were processed and presented in Figs. 6 and 7. Fig. 6 demonstrates that draining the samples to an absolute moisture of 40% at a heat flow temperature of 127°C took 140 min, and at a temperature of 74.2°C took 460 min. Thus, doubling the temperature of the drying stream led to the acceleration of drying almost by three times, and consequently to cutting down the drying time. This acceleration derived from a change in the dehumidification mechanism, with a temperature below the boiling point, the moisture was extracted in small droplets, and with temperatures above the boiling point, in steam.

Fig. 6. Change in the absolute moisture content of the sunflower cake during thermo-convection drying with a flow at different temperatures.
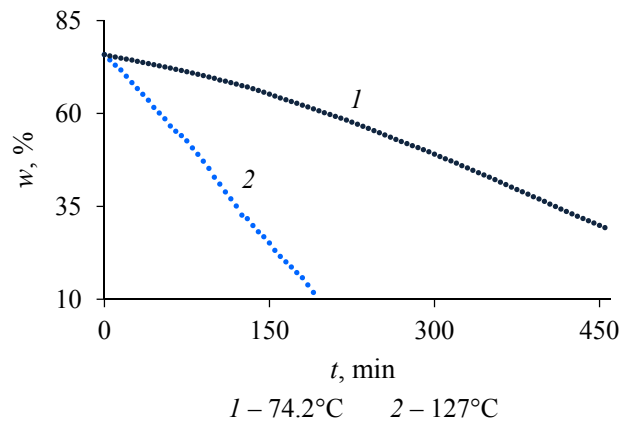
Fig. 7. Change dynamics for the relative moisture content in the sunflower cake when dried by a thermo-convective flow at different temperatures.
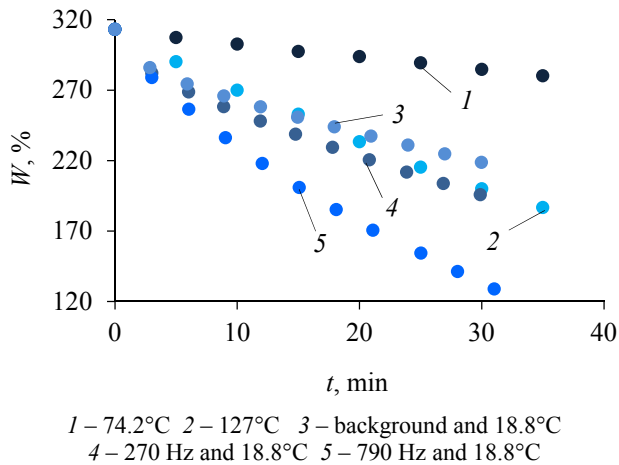
Fig. 8. Modes and methods of drying compared by the change of the absolute moisture in the sunflower cake.
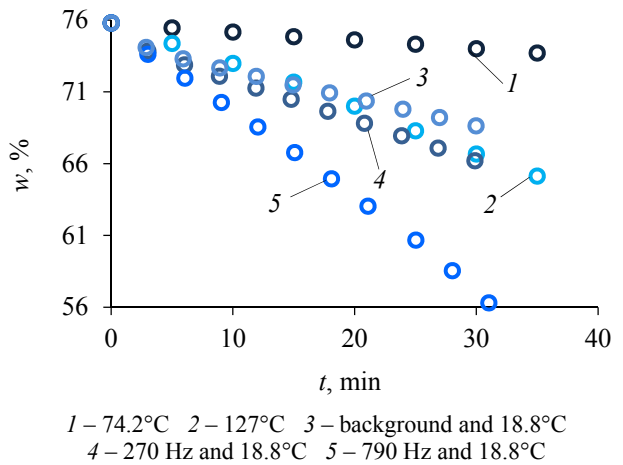
Fig. 9. Modes and methods of drying compared by the change of the moisture content in the sunflower cake.

1 – 270 Hz *2* – 270 Hz *3* – 790 Hz *4* – 790 Hz
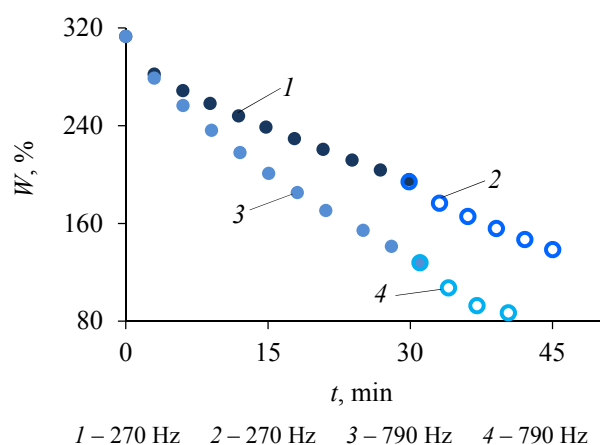
Fig. 10. Moisture extraction dynamics for the sunflower cake samples during acousto-convective drying with an hour-long pause.

In Fig. 7, the characteristic curve of the moisture yield from the sample to be dried shows that the required final relative moisture of 10% can be achieved by thermo-convective drying of the sunflower cake with the second temperature mode of 127°C for 195 min (3.25 h). To assess the two temperature modes, we compared the time when the samples reach relative moisture of 30%. In the first mode, the required moisture was achieved for 450 minutes (7.5 hours), in the second, it took only 135 minutes (2.25 hours). Consequently, the previously determined ratio of one to three holds true.

Comparing dynamics of drying sunflower cake by acousto- and thermo-convective modes. To implement the obtained results in a production facility, it is necessary to compare the dynamics of the sunflower cake drying by acousto- and thermo-convective modes at different temperatures. Figs. 8 and 9 present the curves for moisture extraction from the sunflower cake samples during the first 30 minutes of drying. The slowest is thermo-convective drying at 74.2°C for a specified time interval, the moisture loss is 28.4% (1.8%). A faster moisture yield was recorded for acousto-convective drying by a background mode at 18.8°C, half an hour process resulted in the moisture loss of 94.4% (7.2%). Even faster was thermo-convective drying by the second mode at 127°C, for 30 min the moisture decreased by 113.1% (9.1%). Acousto-convective drying with a resonance frequency of 270 Hz and a flow heat of 18.8°C provided similar parameters, in particular 117.3% (9.6%). The fastest drying of the sunflower cake was realized by the acousto-convective method with a resonance frequency of 790 Hz and a temperature of 18.8°C; the ACDP functioning for half an hour accounted for the moisture loss of 184.3% (19.5%). Thus, the conducted comparison demonstrated that the acousto-convective drying method is twice as efficient as the traditional thermo-convective method. Moreover, [23] shows that power input is reduced by a half. Beyond doubts, these results are indicative of a considerable practical importance for the existing industry.

Therefore, the correct resonant frequency for the working flow can increase the efficiency of acousto-convective drying of sunflower cake, as shown by this case, almost twice, while the power input remains unchanged. If there is no resonance in the working acousto-convective flow or for some reason it disappears, the difference in drying dynamics becomes even more significant; in this study it amounted to almost 2.7 times by the 30th minute of the test.

An interval mode of acousto-convective drying. The experimental results obtained for acousto-convective drying show ed that within the first 15 minutes the moisture extraction dynamics had a significant nonlinearity. So within the first three minutes, the absolute moisture in mode 1 decreased by 31.0%, and in mode 2 – by 34.2%; for the second three minutes in mode 1 – by 13.5%, and in mode 2 – by 22.5%; for the third three-minute interval in mode 1 – by 10.4%, in mode 2 – by 20.3%; the fourth three-minute interval led to a moisture decrease in mode 1 by 10.3%, and in mode 2 – by 18.4%; in the fifth three-minute interval, the moisture reduction in mode 1 was 9.2%, in mode 2 – 17.0%. Thus, within the first 15 minutes the loss of absolute moisture for the sample in mode 1 equaled 74.4%, and in mode 2 – 112.5%.

During the second fifteen-minute interval, the moisture content of the samples decreased linearly, the average value for the five three-minute intervals in mode 1 was 8.6%, in mode 2, 14.4%. For the second fifteen-minute interval, the absolute moisture value in mode 1 decreased by 42.9%, and in mode 2 – by 72.1%, that is, the efficiency of moisture yield decreased for mode 1 by 1.7 times, and for mode 2, by 1.6 times. This trend shows that all subsequent fifteen-minute intervals will result in an even slower drying. To increase the efficiency of acousto-convective drying, it is worthwhile to remove the dried samples from the ACDP after thirty or fifteen minutes and allow those to stand at a room temperature, so that the moisture can redistribute inside the test material, as shown in [12, 13].

This study included experiments on the interval drying of the sunflower cake in which the material to be dried was held outside the acousto-convective flow for an hour. After the withstanding, the samples were placed in the ACDP, operating in the same mode as before the removal of the material from the tract of the working part. The study results, for the ACDP operating in the first and second modes are shown in Fig. 10. Acousto-convective drying in the first mode shows considerable intensification after one-hour withstanding; so within the first three minutes the sample lost 17.6% in absolute moisture, within the second – 10.8%, within the third – 9.8%, and within the fourth – 9.1%. Within the fifth three-minute interval of insonation, the moisture loss equaled 8.3%, which is less than the average value obtained earlier in the drying linear interval for this mode. The total moisture yield after one hour withstanding for the first mode was 55.6%, i. e. the efficiency gain amounted to 12.7%. For the second mode of acousto-convective drying, the efficiency gain held true only within the

first two three-minute intervals; so in the first three minutes, the absolute moisture dropped by 20.5%, and in the second – by 14.4%, which compares well to the average value for the second fifteen-minute area described earlier for the corresponding operating mode of the ACDP.

This resulted in the optimal flow-process chart for acousto-convective drying of sunflower cake, which consists of two technological operations: drying in the ACDP for 15 minutes, and withstanding at the room temperature for an hour. As the flow-process chart demonstrates, no heat is applied to the material to be drained throughout the test drying. The flow temperature is similar to the initial one.

Mathematical description of the experimental data for sunflower cake drying. The mathematical processing of the kinetic curves obtained in the moisture extraction experiments for the sunflower cake was carried out by means of a linear relaxation equation, which had the following form:

$$\frac{dW}{dt} = \frac{W_K - W}{\tau}. \tag{3}$$

The determined value of the initial moisture served the initial condition for Eg. (3)

$$\text{when} \qquad t = 0, \; W = W_0. \tag{4}$$

Here, W_0 is the initial moisture, W_K is the final equilibrium moisture, and τ is the relaxation time of the moisture extraction. The formulated Cauchy problem in Egs. (3) and (4) has an analytic solution in the form:

$$W = W_K + (W_0 - W_K)e^{-t/\tau}. \tag{5}$$

The processing results for the experimental data are shown in Fig. 11. Here, solid and dashed lines represent the results of numerical calculations obtained with the help of Eg. (5) with optimally selected values of τ for the acousto- and thermo-convective drying modes, respectively. The fastest moisture extraction, realized by acousto-convective drying at a frequency of 790 Hz and a temperature of 18.8°C, has a minimum relaxation time of 15 minutes. The next efficient mode of acousto-convective drying at a frequency of 270 Hz and at a temperature of 18.8°C has a relaxation time 30 min. The third place in efficiency is held by the mode of thermo-convective drying at a temperature of 127°C: it has a characteristic relaxation time of 35 min. The fourth in efficiency is the background mode of acousto-convective drying at a temperature of 18.8°C, with $\tau = 40$ min. The slowest moisture extraction from the sunflower cake by thermo-convective drying at 74.2°C has a relaxation time of 180 min, or 3 hours.

To compare quantitatively the determined relaxation intervals for the sunflower cake drying process in the ACDP, there was compiled a summary Table 2, which presents the results of acousto-convective drying for other materials. It is obvious ly that the relaxation times agree well with other materials.
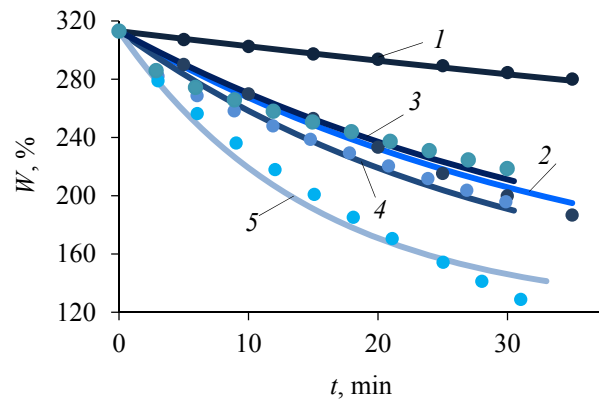
(1) – 74.2°C   (2) – 127°C   (3) – background and 18.8°C
(4) – 270 Hz and 18.8°C   (5) – 790 Hz and 18.8°C

Fig. 11. Mathematical description of the experimental data.

Table 2. Relaxation time for drying various materials in the ACDP

no	Material	Relaxation time, min	Reference
1	pine nuts	20	[15]
2	pine nut shell	7.5	[15]
3	pine nut kernel	13	[15]
4	sorbent	18–50.6	[16]
5	tube assembly along the flow	9	[24]
6	tube assembly across the flow	6	[24]
7	meat fiber	10	[12]
8	cellulous gas-concrete	18	[18]
9	sunflower cake	15–40	this study

CONCLUSION

(1) With the help of thermo-vacuum drying, the initial absolute and relative moisture contents were determined for sunflower cake as 313.1% and 75.8%, respectively.

(2) If the sunflower cake was dried by acousto-convective method at a room temperature (18.8°C), a resonating frequency intensified moisture extraction.

(3) There was determined the quantitative proportion associating the released moisture contents with the sunflower cake acousto-convective drying in different operating modes of the ACDP within 30 minutes:
− at a frequency of 790 Hz and 270 Hz as 2:1;
− at a frequency of 790 Hz and the background as 3:1; and;
− at a frequency of 2 70 Hz and the background as 1.5:1.

(4) As demonstrated, doubling the temperature of the thermo-convective flow drying the sunflower cake (from 74.2°C to 127°C) reduced the duration of drying to a final absolute moisture of 40% by three times.

(5) The comparison of thermo-convective and acousto-convective drying methods showed that within a 30-minute interval the samples dried in the ACDP with a working flow frequency of 790 Hz and at a room temperature yielded moisture twice as much as the samples dried by thermo-convective method with a working flow temperature of 127°C.

(6) The optimal flow-process chart was constructed for interval drying mode of the sunflower cake as consisting of two repetitive technological operations.

(7) The mathematical relaxation model describing the drying process and the experimental data for sunflower cake drying allowed obtaining quantitative parameters that characterised different methods and modes of samples.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Sports and energy drinks

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Abstract: Presently, sports and energy drinks are widely spread not only among athletes, but also among ordinary people of different ages. The purpose of these beverages is to effectively compensate for the loss of water, energy and electrolytes in the human body before or after some exhausting activities. A questionnaire survey on energy drinks conducted in all eight federal districts of the Russian Federation shows that the younger groups of the Russian population (aged 12–17 and 18–30) drink tonic beverages more often than the older groups (aged 31–45 and 45–60). Further, a recent rise in unreasonable consumption of sports and energy drinks among teenagers may lead to various diseases: obesity, type 2 diabetes, heart disease and tooth enamel erosion. Finally, the authors analyse the composition of energy beverages and thoroughly describe each of their main components (L-carnitine, creatine, caffeine, taurine, and juice-containing products). These components are used by athletes due to their effects: L-carnitine helps reduce the signs of physical and mental overstrain, and stimulates working capacity; creatine improves endurance and anaerobic activity; caffeine raises aerobic endurance by increasing the oxidation of fats, thereby helping preserve glycogen in the muscles; taurine plays an important role as an antioxidant protector in the regulation of Ca++ transport, and as a regulator of osmotic pressure in the tissues.

Keywords: Energy drinks, sports nutrition, creatine, caffeine, taurine, L-carnitine

INTRODUCTION

Among specialised food products, sports drinks, as well as energy drinks, have become very popular in the last few decades. Despite an overall decrease in daily consumption of sports drinks, sugar sweetened sports drinks remain popular among the majority of young people, especially high school students. From 2010 to 2015, there was a statistically significant increase in the proportion of teenagers who reported consuming sports drinks in the past 7 days; however, that increase was small (from 56% to 57.6%) [1]. A survey by Cardiff University School of Dentistry [2] showed a large proportion of 12- to 14-year-olds regularly consuming high sugar sports drinks unnecessarily. In particular, it was found that:

− 89% of school children consumed sports drinks, with 68% drinking them regularly (1–7 times per week);

− half of the respondents drank sports drinks for social reasons;

− high sugar content and low pH of sports drinks increased the risk of obesity, type 2 diabetes, heart disease, and tooth enamel erosion; and

− most sports drinks ware purchased by children in local shops at value prices.

Of 160 respondents (87% response rate), 89.4% (143) claimed to drink sports drinks, with 50% drinking them at least twice a week. The main reason for consuming sports drinks was their 'nice taste' (90%, 129/143). Most respondents purchased drinks from local shops (80.4%, 115) or supermarkets (54.5%, 78). More boys claimed to drink sports drinks during physical activity (77.9% *versus* 48.6% girls, P < 0.001), whereas more girls claimed to drink them socially (51.4% *versus* 48.5% boys, NS).

Water plays a vital role in the diet of athletes. It contributes to the accumulation of glycogen in the muscles (composed of 3–4 parts of water per part of glycogen); in addition, water is involved in the regulation of body temperature. Optimal hydration of the body is of vital importance to human health. Depending on age, temperature, climate, health status and physical activity, the athlete's daily need for free fluid can vary from 1.5–2 to 5–6 litres per day (in some cases). Drinking regimen in a balanced, healthy diet of athletes cannot be organic, since its deficiency not only

contributes to a decrease in the performance, but it can also lead to serious violations of the urinary system [3].

To avoid the risk of dehydration and reduce physical disturbances, athletes are advised to use sports drinks that provide compensation for fluid, electrolyte and carbohydrate losses. In some cases, thirst does not always appear in the early stages of dehydration. At the same time, there are data on dehydration developing in the first 10–15 minutes during, for example, a marathon race.

To quench thirst, it is permissible to use mineral water, fruit and vegetable juices and drinks, fruit drinks, tea, tonic drinks, or fresh fruit. The most widely used are specialised sports drinks.

There are three main types of sports drinks that contain different amounts of water, electrolytes, and carbohydrates:
(1) isotonic drinks, containing water, carbohydrates, and 4–8% electrolytes;
(2) hypotonics, containing 6% electrolytes, 2% carbohydrates, and 92% water; and
(3) hypertensive drinks, containing 32% carbohydrates, 4% electrolytes (not all drinks), and water.

For intensive physical training, it is recommended to take isotonic, and for extremely intensive training, hypertensive drinks that contain a high amount of easily digestible carbohydrates and are designed to quickly restore energy reserves.

Rehydration after physical activity is an important part of the recovery process. Biochemical and physiological recovery of the body begins in the first minutes after the end of endurance exercises. To compensate for losses, it is recommended to use a volume of fluid that is at least 50% greater than its amount lost with sweat [4]. In order to quickly restore the resources, dilute solutions of glucose with the addition of sodium chloride are used, since these hypotonic solutions are most effective in reducing the delay in the stomach and absorption in the intestine.

Replenishment of fluid losses in the body of athletes requires regular compliance with the drinking regime. It is shown that the loss of 9–12% of water is an emergency situation for the body which can lead to death. Losing 2% of weight due to water reduces the work ability by 3–7%, while with the loss of 40% of protein, fat and carbohydrates, a person can stay alive for a long time. In severe physical activity, it is necessary to monitor the state of the water balance and continuously replenish the fluid loss. Water comes in when consuming liquids, with food, and as a result of metabolic processes. The first way accounts for about 60% of total water consumption, the second one – 30%, and the third – about 10%. There are also different ways of water loss from the body: 50–60% of water is daily discharged with urine, about 20% with exhaled air, 15–20% with sweat (depending on the load intensity), and less than 5% with faeces. The average person needs about two litres of water per day to make up for the losses. With intensive loads, water intake reaches 3–4 litres per day. It is proved that when the volume of fluid in the body decreases by 2%, the athlete's result may deteriorate by 15%.

It should be noted that the most effective way to compensate for the loss of large amounts of water and salts is to consume weakly acidic and slightly sweet mineralized drinks, of which the hypo- and isotonic solutions of carbohydrate-mineral complexes are the most physiological.

At present, however, there are no sufficient studies to reveal the psycho-physiological effects of various combinations of tonic beverage ingredients on the organism. Therefore, the state needs to control the release, sales and consumption of tonic beverages. According to the Russian Federation Law 'On Protection of Consumer Rights', the product must be safe for the consumer's life and health.

It is now established that inadequate water intake or moderate dehydration may be associated with a risk of developing chronic diseases [5–7]. Hypohydration is a common condition for 16–28% of the population, depending on the age [8], and dehydration among the elderly is often associated with the presence of chronic diseases [9,10].

Sources of water are liquids or drinks (including drinking water, tea, wine, soft drinks) and water contained in food. All products contain water, although the amount of water in food will vary between their individual species. The European Food Safety Authority (EFSA) found that 20–30% of total daily water consumption in Europe came from food [11]. However, the total percentage of water in food varies vary between countries and depends on the types of foods and diets. For example, in Ireland, the amount of water in foods is on average 33% [12], and in China, where people consume more liquid products, such as soups and broths, it is 40% [13].

The human body has a system for monitoring the volume of fluid by hormonal regulation, carried out through osmoreceptors controlling the osmolarity of blood serum and volume receptors that are responsible for the volume of extracellular fluid. The concentration of sodium in serum is the main parameter of osmolarity. Since perspiration is hypotonic, prolonged exercise increases the osmolality of blood serum.

Active athletes can lose up to 2.5 litres of sweat per hour. Sweat contains electrolytes (mainly sodium chloride, but also potassium, calcium, and magnesium) with a sodium concentration of 20–80 mMol/l. Dehydration occurs when more fluid is lost than consumed. It disrupts the fluid and electrolyte balance in muscle cells, the activity of the cardiovascular system and temperature regulation, reducing the athlete's performance.

The most physiological are drinks containing carbohydrates and minerals. They not only rehydrate the body, but also retain the electrolyte balance and energy status (glycogen stores).

Tonic and energy drinks in sports. In recent years, there has been an increase in the consumption of tonic beverages in the Russian Federation [14]. More than 40% of the population periodically use toning beverages, and 30–50% of them are young people aged 12–24, for whom these drinks are contraindicated because of their negative impact on health [15–17]. Today, there is an increased number of tonic drinks in

the market of the Russian Federation. Nevertheless, our survey of 11,850 people of different ages (12–60) and sex in all eight federal districts of Russia showed a fairly low frequency of consumption and no significant differences in the consumption of sweet carbonated drinks between various districts. The survey revealed that the carbohydrate component of sweet carbonated beverages in various districts of Russia, even when consumed frequently (5–6 times a week), did not exceed 3.71% of the total calorie content of the diet or 7.1% of the caloric value of carbohydrates in the diet energy supply [18].

The same survey demonstrated a low frequency of energy drinks consumption. The study showed that individuals aged 46–60 hardly ever used these drinks. Only a small number of individuals aged 31–45 (from 1.25% to 0.32%) consumed energy drinks, but not more than 2–4 times a week. In the 12–17 and 18–30 age groups, there was a slight increase in the number of people consuming energy drinks (Table 1).

Table 1. Energy drinks consumption in various federal districts of the Russian Federation

Federal District	Age groups							
	12–17		18–30		31–45		46–60	
	2–4/week	1/day	2–4/week	1/day	2–4/week	1/day	2–4/week	1/day
Central	1.96	0	2.71	0.19	1.25	0	0	0
North-western	0.99	0	0.90	0.41	0	0	0	0
Ural	0	0	0.30	0	0	0	0	0
North Caucasian	0.33	0	0.21	0.30	0.32	0	0	0
Volga	1.01	0	0.61	0	0	0	0	0
Southern	0	0	5.10	0.60	0	0	0	0
Siberian	0.66	0	2.37	0	0.32	0	0	0
Far Eastern	0	0	1.82	0	0	0	0	0
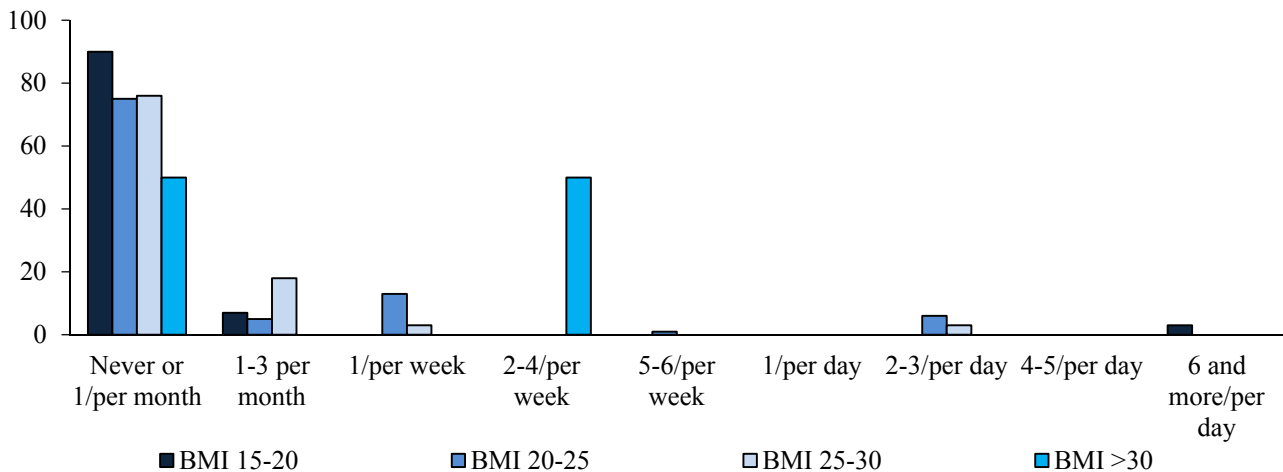
Fig. 1. Non-alcoholic energy drinks (Burn, Red Bull, Adrenaline Rush) consumption by males aged 12–17.
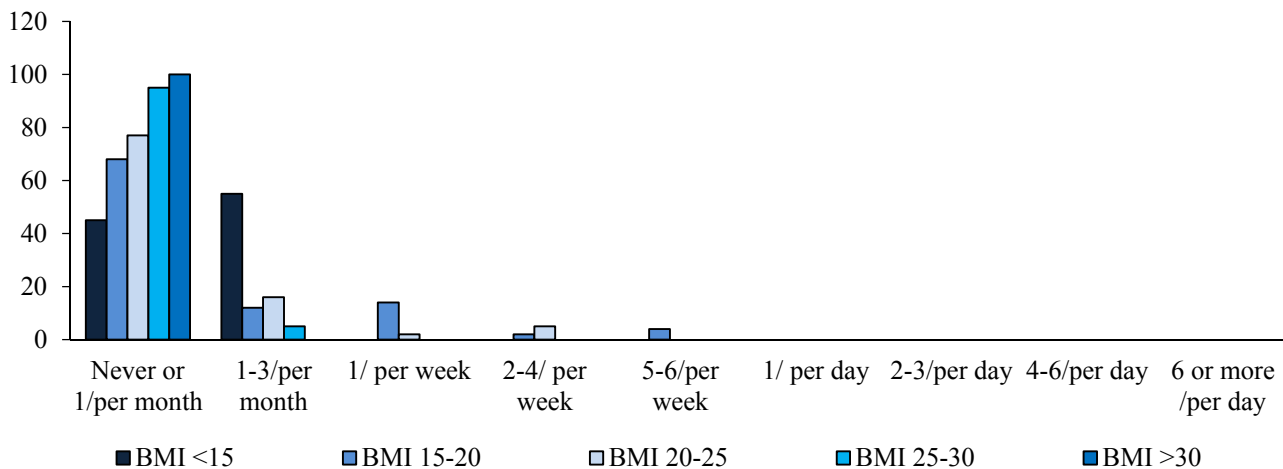
Fig. 2. Non-alcoholic energy drinks (Burn, Red Bull, Adrenaline Rush) consumption by females aged 12–17.
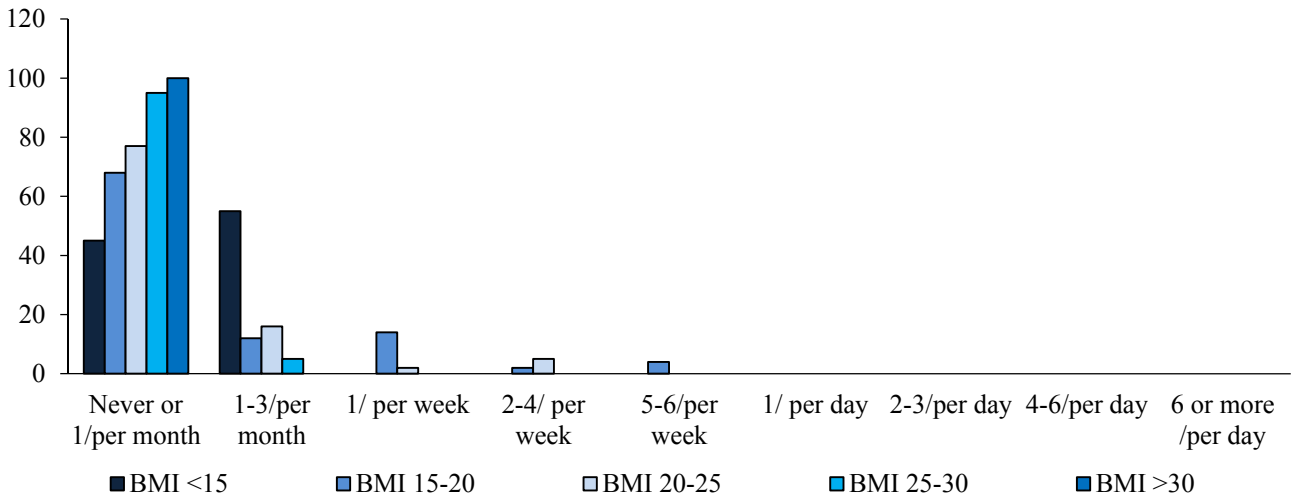
Fig. 3. Non-alcoholic energy drinks (Burn, Red Bull, Adrenaline Rush) consumption by males aged 18–30.
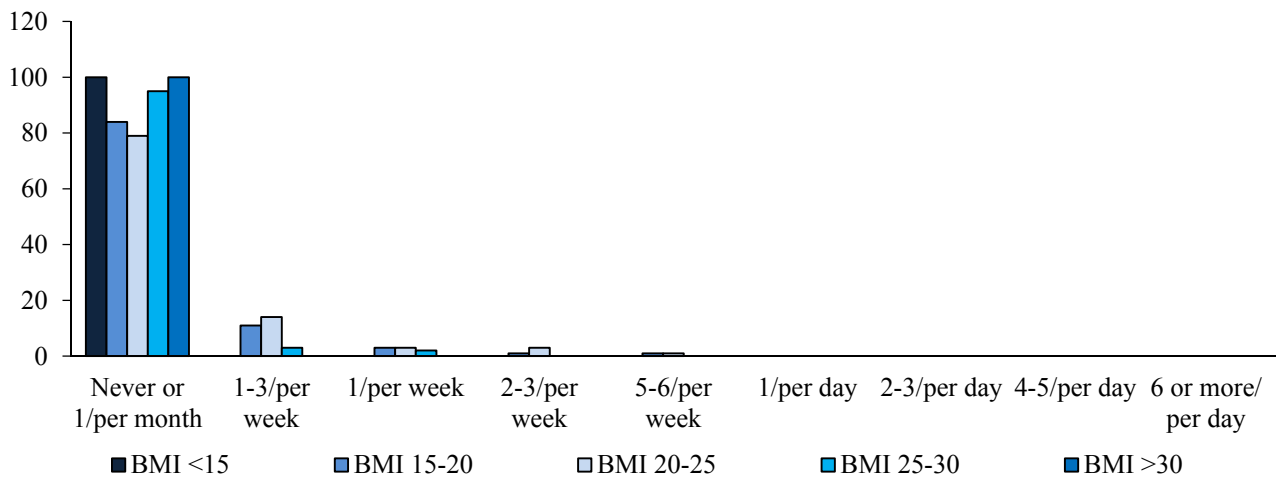
Fig. 4. Non-alcoholic energy drinks (Burn, Red Bull, Adrenaline Rush) consumption by females aged 18–30.

We demonstrated that there was no correlation between the body mass index (BMI) and the frequency of energy drinks consumption (Figs. 1–4) in the main consumer groups (aged 18–30 and 12–17).

The discovery of tonic (energy) drinks in Europe is associated with the Austrian entrepreneur Dieter Mateschitz. In 1984, after studying Asian energy drinks, he modified them to suit European tastes. In 1987, the European market saw the first non-alcoholic energy drink named 'Red Bull Energy Drink,' carbonated and with a lower sugar content than its Asian prototype. Currently, there are over 500 energy drinks consumed in different parts of the world [20]. Energy drinks most often contain a combination of caffeine, taurine, and D-glucurono-lactone among other ingredients, such as synephrine, for example [21].

Synephrine is a biogenic amine of the phenylethanolamine/phenylpropanolamine group. The protoalkaloid p-synephrine is present in the bitter orange (*Citrus aurantium L.*) and other fruits of citrus cultures. The presence of p-synephrine or m-synephrine in food additives containing extracts of *C. aurantium*, indicates adulteration. Only p-synephrine, a natural compound from *C. aurantium* extracts present in food additives, is considered in [21]. The

formulation of drinks, however, also includes with the main components such as tonic compounds, amino acids, B group vitamins, and carbohydrates. Among the most important components of energy drinks are also the methyl-xanthine alkaloid Caffeine and the sulphur-containing amino acid Taurine. Energy drinks are becoming more popular among athletes and individuals engaged in fitness and physical exercise. Unfortunately, scientific literature rarely provides evidence of the ergogenic role of energy drinks in sports. Existing evidence suggests that caffeine in doses contained in energy drinks is probably an insufficient factor for increasing muscle activity.

Along with these components (caffeine, taurine, etc.), tonic and energy drinks contain complex carbohydrates. Complex carbohydrates and water, of course, are significant nutrients for athletes, playing an important role in preventing and inhibiting fatigue during long, intensive training. Nevertheless, to analyse the effectiveness and safety of tonic (energy) beverages in sports, it is necessary to analyse the properties of not only carbohydrates, but also all the biologically active ingredients that make up this type of beverage. This is significant, given the multi-component nature of energy drinks. For example, one of the most popular

drinks (Red Bull) contains 4 g of taurine, 2.4 g of glucuronolactone, 320 mg of caffeine, 108 g of carbohydrates, and 140 mg of B group vitamins in one litre (4 cans). The concentration of carbohydrates in this drink is 11%, with an osmolality of 601 mOsmol/kg H_2O. By contrast, a well-known sports drink (for example, Gatorade Orange) has a lower concentration of carbohydrates (6%) and an osmolality of 297 mOsmol/kg H_2O. Carbohydrates are basic macronutrients in drinks that determine their caloric value. The calorie content of the drink affects the rate of gastric emptying at rest [22] and its intake in the body during exercise [23]. Emptying the stomach is important for ensuring the bioavailability of the drink. For example, ingesting a drink with a carbohydrate concentration of 8% or higher may result in delays in the blood flow and decrease the availability of ingredients to the muscles. In addition, an increase in osmolality of up to 414 mOsmol/kg H_2O reduces fluid absorption from 82% to 68%, compared to water [24]. The combination of high osmolality and carbohydrate concentration (601 mOsmol/kg H_2O and 11%, respectively) probably reduces its absorption compared to a commercial sports drink. It should be noted that the available data allow us to speak not only of the effectiveness of specialised sports drinks components, but also of their safety. The existing fears associated with the risk of undesirable health effects of caffeine intake from all sources, including tonic beverages, are not justified. A thorough analysis conducted by the European Food Safety Agency (EFSA), and, in particular, the EFSA Scientific Committee's report on food allow us to conclude that an intake of up to 300 mg of caffeine per day is a safe amount [25]. This report is based on the study of caffeine content in the diet of pregnant women. Pure caffeine, as well as caffeine consumed as an extract of green tea, or a combination of the main antioxidant components of tea, epigallocatechin gallate, quercetin and caffeine in amounts equal to their content in the tea extract, is not only safe, but it also improves the antioxidant activity of blood plasma and liver and increases the stability of microsome and lysosome membranes [26]. However, in the context of conflicting opinions among experts on the effectiveness of tonic drinks for athletes and concerns about their safety, it seems worthwhile to mention one of the most comprehensive studies into the safety of energy drinks commissioned by the Ministry of Health of the Russian Federation and conducted by the National Medical Research Centre for Psychiatry and Narcology [27]. It was found that giving rats 30–60 mg of energy beverage per day did not affect the dynamics of their body weight growth; nor did it lead to increased alcohol consumption in adulthood, compared to the control animals. The average daily motor activity of those animals which consumed the drink increased, compared to the control animals, and correlated with the volume of consumed beverage, while remaining at a constant level throughout the experiment. Thus, the analysis of research data and the properties of the main biologically active components of energy drinks suggests that both caffeine and taurine, as well as their

combinations in concentrations contained in energy drinks and taken in recommended doses, do not have an adverse effect on the body [28]. Today, there is little information confirming the extensive use of tonic (energy) drinks in sports. There are arguments 'for' and 'against' such practice, so there is a clear need for further experimental and clinical studies into the issue. The safety of energy drinks in the Russian Federation, including in sports, is regulated by the legislative documents. For example, the technical regulations TR CU 021/2011 of the Customs Union stipulate that the amount of caffeine in soft drinks should not exceed 150 mg/l, and in specialised tonic drinks – 400 mg/l. According to Article 4 of the technical regulations TR CU 022/2011 of the Customs Union, soft drinks containing more than 150 mg of caffeine per litre and/or medicinal plants and their extracts in an amount sufficient to provide a tonic effect on the human body should have an inscription 'not recommended for children under the age of 18, pregnant and lactating women, as well as people suffering from increased nervous excitability, insomnia, and hypertension.'

The main biologically active components of specialised sports and energy/tonic drinks. L-Carnitine. L-carnitine is a choline-like quaternary amine discovered by two Russian researchers, V.S. Gulevich and R. Krinberg in 1905. L-carnitine plays an important role in suppressing inflammatory reactions, oxidative stress and apoptosis [29], and ischemic heart disease [30, 31]. Deficiency of acetyl-L-carnitine (ALC) seems to contribute to the risk of developing depression, indicating a dysregulation of fatty acid transport across the inner membrane of mitochondria [32]. L-carnitine is an amino acid derivative that plays an essential role in the cellular metabolism by acylation of its β-hydroxyl group. Carnitine (b-hydroxy-c-trimethylaminobutyrate), a high polar zwitterionic compound, facilitates the transport of long-chain fatty acid across the inner mitochondrial membrane for subsequent b-oxidation [33]. L-carnitine enters the body with food, especially of animal origin, and accumulates mainly in muscle tissue (up to 95% of all carnitine consumed); therefore, its deficiency primarily affects muscular activity [34]. Biologically active is the natural L-stereoisomer of carnitine; thus, only L-carnitine should be used as a food supplement or drug [35, 36]. It should be noted that the antioxidant activity of L-carnitine helps to prevent muscle disorders caused primarily by high physical loads [37]. However, the role of L-carnitine is not limited to participation in the energy metabolism of muscles. In fact, it produces a high pharmacotherapeutic effect on damaged muscle tissue [38, 39]. In humans and animals, L-carnitine is synthesised primarily in the liver and kidneys by the transformation of lysine and methionine with the participation of vitamins C, B3 and B6, folic acid, iron, and a number of amino acids and enzymes [31]. Its main function is to transfer long chain fatty acids from the cytosol into the mitochondrial matrix, where their beta-oxidation occurs, to acetyl-CoA, which is a substrate for the formation of ATP in the Krebs cycle. [40–43]. Fatty acids with a short and medium chain

length (from 4 to 12 carbon atoms) can penetrate into the mitochondrial matrix by diffusion. Fatty acids with a long chain that predominate in the human body (12 to 20 carbon atoms) are activated by acyl-CoA synthetase located on the outer membrane of mitochondria, with the participation of magnesium ions and ATP [44].

These enzymes catalyse a reaction during which a thioether bond occurs between the carboxyl group of the fatty acid and the thiol group of coenzyme A, i.e. CoA, and a fatty acid derivative is formed. At the same time, ATP is split into AMP and inorganic phosphate. The long-chain acyl-CoA is catalysed by the enzyme carnitine acyltransferase I on the outer surface of the inner membrane with a formation of acylcarnitine, which is transported through the internal mitochondrial membrane with the transport protein-carnitine-acyl-translocase [44]. On the inner surface of the mitochondrial membrane, the enzyme carnitine acyltransferase II catalyses the transfer of acyl to intramitochondrial CoA with a formation of acyl CoA, which during the β-oxidation is converted to acetyl-CoA, participating in the Krebs cycle [44]. Free carnitine returns to the outside of the inner membrane of the mitochondria with the same translocase. Fatty acids with a long hydrocarbon radical are transferred through mitochondrial membranes [44]. It is believed that this pathway receives predominantly long-chain fatty acids in the mitochondria. In addition to being a carrier of fatty acids, carnitine also modulates the ratio of acyl-CoA / CoAsSH and supports the free pool (CoA), which is necessary for the functioning of pyruvate dehydrogenase and ketoglutarate dehydrogenase [45] and, therefore, for the operation of the Krebs cycle. A decrease in the intake of carnitine causes a decrease in the CoA content in the matrix and a concomitant increase in the ratio of acyl-CoA/CoAsSH, which inhibits the enzymatic activity of the aforementioned dehydrogenases. Consequently, not only the oxidation of fatty acids is weakened, but also the utilisation of carbohydrates, the catabolism of some amino acids, the detoxification of cytotoxic organic acids and xenobiotics [45]. Also, L-carnitine improves the integrity of membranes, stabilises the immune system, promotes a more economical use of glycogen and glucose stores during prolonged intensive training [29], participates in the exchange of ketones and cholines, and suppresses the formation of lactate and processes of apoptosis [45]. The main source of L-carnitine is meat and dairy products. It should be noted that endogenous synthesis accounts for only 10–25% of the human body's need for carnitine. Therefore, it is extremely important for athletes to take L-carnitine exogenously. Excess accumulation of acyl-CoAs in the mitochondria during increased physical activity inhibits the rate of enzyme-dependent oxidative metabolic processes in various tissues of the body. Carnitine is able to take acyl groups for conversion to acetyl-carnitine, effectively reducing the level of acyl-CoA and increasing the possibility of continuing high intensity exercise [45]. This process is limited by the level of L-carnitine in the muscles, which gradually decreases with the continuation of intensive exercise. Thus, L-carnitine levels in the muscles are associated with the ability to maintain a high level of aerobic oxidation at low levels of lactic acid production [29, 40, 43, 45]. Therefore, the use of L-carnitine in sports can reduce anaerobic lactic energy production and increase more effective aerobic energy production, improving the activity of the respiratory chain in muscles and working capacity during intense physical exertion. It helps athletes reduce the signs of physical and mental overstrain, stimulates their working capacity, increases their appetite, produces cardio-, hepato- and neuroprotective effects, and, at the same time, possesses immunostimulating properties.

Creatine. Creatine monohydrate is one of the most effective products of sports nutrition that increases physical activity and muscle mass during training. Numerous studies show that creatine increases body weight and/or muscle mass during exercise [46]. The increase in muscle mass during 4–12 weeks of training with the use of creatine, compared to the control group, is usually around 900 to 2,250 grams [47], which appears to be the result of an improved ability to perform high intensity exercises allowing the athlete to faster adapt to the load and muscle hypertrophy [48, 49]. The only clinically significant side effect of creatine monohydrate is its ability to increase athletes' body weight [47, 50, 51]. Although there are some studies on possible side effects of creatine, the recent long-term safety studies suggest no obvious side effects [51, 52]. In addition, creatine monohydrate helps reduce the incidence of injury during training [53–55]. Thus, creatine monohydrate and creatine in various formulations are a safe and effective product for increasing muscle mass. The analysis carried out by the International Society of Sports Nutrition (ISSN) allowed the following conclusions to be drawn [56]: 'Creatine monohydrate is the most effective ergogenic food additive currently available to athletes, in terms of increased physical activity of high intensity and muscle mass during training.' There is no conclusive scientific evidence that the short-term or long-term use of creatine monohydrate has a side effect for healthy individuals. It is not only safe, but possibly effective in preventing injuries. With proper control and precautions taken, the use of creatine by young athletes is acceptable and can provide an alternative to potentially dangerous anabolic drugs. Monohydrate is the most widely studied and clinically effective form of creatine to be used in nutritional supplements in terms of its accumulation in muscles and the ability to increase tolerance to high-intensity exercise. Although the combined use of carbohydrates or carbohydrates and proteins in sports creatine-containing supplements also contributes to the accumulation of creatine in the muscles, the effect of such a combined use is not higher than that of using monocomponent creatine monohydrate. The fastest method for increasing muscle creatine is its consumption in the amount of 0.3~g/kg/day for at least 3 days, followed by 3–5 g/day as a maintenance dose. Consuming smaller amounts of creatine monohydrate (2–3 g/day) leads to an increase in muscle creatine content for 3–4 weeks; however, the effectiveness of this supplementation method is little proven. Along with increasing muscle

mass and muscle strength, creatine improves tolerability of physical activity in different conditions [47]. This is especially true when performing loads of high intensity, intermittent exercises, such as several approaches to the bar, repeated sprints and/or physical exercises, including long-distance running and jogging (for example, football) [47]. Creatine is also effective in intermittent high intensity training. It is stated that in addition to these effects, creatine improves the 'critical power' of the athlete [57]. Endurance athletes can also theoretically improve their sports power in various ways. For example, increasing creatine supplies before applying carbohydrate loading (i.e. increasing the dietary intake of carbohydrates before competition in an attempt to maximise carbohydrate stores) improves carbohydrate safety [58–59]. A study [60] showed that taking 20 grams of creatine for 5 days improved endurance and anaerobic activity in elite rowers. Most highly skilled athletes also performed intermittent drills (sprint or speed activity) in an attempt to improve their anaerobic threshold. Since creatine has been reported to increase sprint performance, its use during the training process can improve adaptation to exercise in endurance athletes [61].

Caffeine. Caffeine is the most commonly used pharmacologically active purine compound. In natural sources, caffeine is mainly found in coffee (*Coffea arabica*) and tea (*Camellia cinensis*). The content of caffeine in raw materials and different products varies within fairly wide limits. Coffee beans contain up to 1.5% of caffeine. Even higher is its content in tea leaves – up to 5%. However, caffeine is found not only in coffee and tea, but also in guarana berries and cola nuts. Along with this, caffeine is an ingredient added to a variety of foods, such as baked goods, ice cream, soft caramel, cola drinks and others. Caffeine is also an ingredient in so-called 'energy drinks' and it is present, in combination with synephrine, in certain dietary supplements designed to reduce body weight (increase weight loss) and improve athletic performance. Energy drinks most often contain a combination of caffeine, taurine D-glucurono-lactone and other ingredients. It should be noted that the data obtained in a Russian study [62] on the magnitude of caffeine intake differ from those of foreign researchers [63]. The level of caffeine consumption in Russia significantly exceeds the maximum level of safe daily intake of caffeine (150 mg) established in Russia, although it is lower than the safe level (400 mg/day) established abroad. The content of caffeine in one average consumer package of coffee or tea exceeds its content in one package of energy drinks (12 brands studied). A study of the actual level of caffeine consumption in Russia, which involved 3,600 people (1,600 adults and 2,000 adolescents), showed the following average daily levels of caffeine intake: 344.9 mg by adults (aged 18–44), 225.8 mg by younger adolescents (aged 12–14), and 256.6 mg by older adolescents (aged 15–17) [63]. The FDA (USA) recognises caffeine, the most physiologically active component of energy drinks, as a safe ingredient to use in beverages. Guarana containing caffeine, along with a small amount of theophylline, theobromine and tannins, is also safe,

according to the FDA. The content of ginseng root extract in energy drinks is lower than in dietary supplements, which ensures their safety [62]. The pharmacokinetics of pure caffeine and caffeine in various 'dietary' compositions (drinks, dietary supplements, food products, etc.) may vary. The time of caffeine absorption and elimination, as well as peak plasma concentration rates, differ significantly between dietary and pure caffeine. The slower absorption of dietary caffeine can prolong lipolytic, metabolic, psycho-stimulating, and other effects of caffeine [21]. Recent studies show that caffeine taken as part of dietary supplements before exercise improves aerobic endurance by increasing the oxidation of fats, thereby helping preserve glycogen in the muscles. They also demonstrate that caffeine contained in food additives and beverages contributes to ergogenic effects in anaerobic conditions, and when taken in combination with various other ingredients (e.g. taurine), it produces an enhanced effect [64].

Taurine. Taurine is one of the main components of tonics and sports food products. It is a sulphur-containing amino acid which is not important for protein synthesis, but is the most common amino acid found free in many mammalian tissues. Taurine, first discovered in 1827, has multiple properties that can be effectively used in sports nutrition. There is a considerable number of publications about its important physiological functions that manifest themselves in different tissues of the body, ranging from its classical role as a conjugating agent for bile acids to its functions as an important regulator of osmotic pressure, a modulator of calcium homeostasis and its signalling pathways, and a recently found role as an endogenous antioxidant and anti-inflammatory compound. The classic studies of Huxtable R. J. [65] postulated that taurine was humans is involved in many metabolic functions; in particular, it played an important role as an antioxidant protector in the regulation of Ca++ transport and as a regulator of osmotic pressure in the tissues. Along with this, taurine has an anti-inflammatory effect [66]. Taurine is widely used not only as a medicinal product, but also is a part of many specialised products for sports nutrition and energy drinks. The average dose of taurine in energy drinks is 200–400 mg 100 g.

It should be noted that taurine is a synergist of caffeine. A study published in 2008 [cit. 67] showed that the amount of taurine used in various beverages did not cause any side effects. The interest in taurine significantly increased after it was found that exercise reduced its content in skeletal muscles [67]. Subsequent studies showed that taking 6 g of taurine per day for 7 days could increase exercise tolerance, possibly due to its antioxidant effects [68]. However, there are also conflicting opinions on the effectiveness of taurine in sports practice. For example, it is shown that taking 5 g of taurine per day for 7 days as a dietary supplement does not change its content in the muscle tissue and has no influence on the metabolism in the muscles during exercise [68]. However, there is evidence that the normal level of taurine is important for skeletal muscle function. Taurine appears to play an important role in regulating

the release of Ca+ from the sarcoplasmic reticulum and helps maintain the sensitivity of the contractile elements to Ca+ [69], which is extremely important in sports. Studies offer conflicting data on the effect of taurine on muscle strength, the time of muscle fatigue development and the rate of its recovery. For example, experimental studies in mice demonstrate the absence of such effects in taurine [70]. At the same time, other studies suggest that supplementing the diet with taurine, or adding it to drinking water, leads to the accumulation of taurine in muscle tissue [71]. It was shown that the concentration of taurine introduced in dietary supplements during two weeks increased the content of taurine in rat muscles by almost 40%, which improved muscle contraction [71]. Intensive physical exercises decrease the concentration of taurine in the muscles and have a negative effect on contractility [72]. Another study showed that simultaneous administration of taurine and caffeine during a two-week period increased the running time of the mice and led to a decrease in lactate accumulation in the muscles [73].

Juices and juice-containing products. There is an annual increase in the consumption of juices and juice-containing products in the world. Juices are good for human health, because vegetables and fruits, and, consequently, juices obtained from them, have protective functions [74]. Fruit and vegetable juices supply the body with food ingredients that reduce the risk of cardiovascular and oncological diseases. Flavonoids contained in citrus juices have an anticarcinogenic, antioxidant, and antimutagenic effect [75, 76]. Consumption of juice drinks in Europe and Russia is continuously increasing due to both the marketing activities of producers and a high utility of juices. Blending juices with plant extracts of medicinal, spicy and aromatic raw materials enriches the drinks with vitamins, minerals and other biologically active substances. Such beverages are classified as functional.

Recommendations for health promotion and disease prevention include the need for a person to consume a variety of fruits and vegetables every day [77, 78]. The available data indicate a direct relationship between a diet rich in fruits and vegetables and an improvement in people's health, as well as a reduction in the risk of developing major chronic diseases [80]. In addition, ongoing research shows that biologically active substances contained in plant food sources have a significant effect on metabolism [81]. Despite the fact that consumption of fruits and vegetables is an essential component of a healthy diet, there is no consensus on the effects of freshly squeezed juices on the body [80, 82]. Freshly squeezed juices have a lower fibre content and a higher calorie content per serving.

It is well-known that fruit and vegetable juices contain biologically active substances that have an antioxidant effect. The imbalance between the generation of active oxygen species and the body's antioxidant defence system is an important factor in the development of chronic diseases, such as cardiovascular and oncological diseases, diabetes, and

a number of others [82]. The effects of consuming 100% fruit juices on the mechanisms of antioxidant protection have been thoroughly studied [83–89]. The consumption of natural juices increases the antioxidant activity of blood serum and the effect can persist for several hours depending on the volume and type of juice, and on the individual's subjective characteristics. Apple, orange and grape juice made from homogenised fruit 150 ml had the maximum antioxidant effect within 30 minutes after administration in 10 healthy men (aged 24 ± 1 year) [83]. A significant decrease in the indices of oxidative stress in plasma persisted for 90 minutes (apple and orange juice) and 2 hours (grape juice) after intake [83]. A number of studies reveal a positive effect of fruit and vegetable juices on the state of the endothelium, the inflammatory reaction, and the proliferation of cells involved in the pathogenesis of atherosclerosis and cardiovascular diseases [83]. As is known, a change in lipid metabolism is a risk factor for the development of cardiovascular diseases [87]. A number of studies confirm the positive effect of 100% juices on blood lipid markers [87–92]. There is evidence of a positive effect of diets with a high content of fruits and vegetables, including juices, in the treatment of certain types of cancer [94]. However, it should be noted that fruit juices, as well as drinks with sugar and sugar substitutes, can be risk factors in the development of obesity and type 2 diabetes [95–102].

CONCLUSION

Specialised food products, sports drinks, as well as energy drinks, have become very popular in the last few decades. It is now established that inadequate water intake or moderate dehydration may be associated with a risk of developing chronic diseases. Hypohydration is a common condition in different types of sport activity. Despite an overall decrease in daily consumption of sports drinks, sugar sweetened sports drinks remain popular among the majority of young people, especially high school students. Among the main compounds in sports and energy drinks, Caffeine, Taurine and L-Carnitine are the most commonly used pharmacologically active agents inducing multiple activities. L-carnitine plays an important role in suppressing inflammatory reactions, oxidative stress and apoptosis, and ischemic heart disease. The antioxidant activity of L-carnitine helps to prevent muscle disorders caused primarily by high physical loads. Taurine is one of the main components of tonics and sports food products that is important for skeletal muscle function. The studies published during the last two decades demonstrate the usefulness of sports drinks. Furthermore, multiple international studies also show the safety of sports and energy drinks containing well-known biologically active substances, when taken in recommended doses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Natural sweeteners: health benefits of stevia

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Abstract: *Stevia rebaudiana* (Bertoni), a perennial shrub, is the sweetest plant belonging to the *Asteraceae* family. Stevia leaves are an excellent source of diterpene glycosides stevioside, rebaudioside A-F, dulcoside, and steviolbioside, which are responsible for sweetness and have been utilized commercially for sugar substitution in foods, beverages, and medicines. To the best of our knowledge, a large number of studies have been carried out on composition, health implications, and safety of steviol glycosides. However, commercial production of stevia-incorporated food products needs further research in order to meet the huge global demand. Stevia-incorporated products possess better sweetening potency and maximum consumer acceptability, when compared with other sugar substitutes. Hence, the current research attempts to review the health promoting effects of stevia with special emphasis on its application in the food system. The paper majorly features 1) the anti-hyperglycemic, anti-hypertensive, anti-caries, anti-inflammatory, and anti-cancer benefits of stevia, 2) value-added stevia-incorporated products, e.g. bakery, dairy, and beverages, 3) the effect of incorporation of stevia on physicochemical, rheological, and nutritional food properties, 4) the current status and regulatory perspective of utilizing stevia at national and international level. Due to legislative actions and growing consumer awareness, public interest in natural sweeteners has significantly increased. Since the use of artificial sweetener has recently been questioned, the data the present article provides will be useful for consumers and manufacturers that seek an alternative.

Keywords: *Stevia rebaudiana*; sugar substitutes, health benefits, value addition, product characteristics, consumer acceptability

INTRODUCTION

One out of the six basic taste sensations in humans is sweetness. Honey, coconut sugar, blackstrap molasses, table sugar, agave, high fructose, corn syrup, maple syrup and other natural sweeteners contain glucose, fructose, and sucrose as their primary constituents. However, sweeteners obtained from natural sources possess a high caloric value, which may lead to obesity, diabetes, and cardiovascular diseases. There has been a gradual rise in the number of diabetic patients all over the world. India has become the diabetic capital of the world with about 72 million cases of diabetes in 2017 (International Diabetes Federation, Diabetic Atlas, 2017). Due to the growing health awareness, there has been a huge demand for sugar substitutes that would provide lesser or no calories and possess better sweetening potency. There is a variety of artificial zero-calorie sweeteners on the market, e.g. saccharin, aspartame, acesulfame potassium, cyclamates, etc. However, artificial sugar substitutes became associated with health complications, and the use of these artificial sugar substitutes has subsequently been restricted. Thus, there is a continuous search for high intensity low-calorie or non-caloric sweeteners of natural origin that are safe for consumption. Stevia, which plays an important role as a non-nutritive natural sweetener, emerged as a safe sugar substitute that does not pose any threat to human health [1].

RESULTS AND DISCUSSION

Stevia rebaudiana (Bertoni) is a perennial shrub belonging to *Asteraceae* family, native to Paraguay. Out of 230, only two species – *rebaudiana* and *phlebophylla* – produce sweet steviol glycosides [2]. Stevia leaves contain eleven diterpene glycosides, such as stevioside, rebaudioside A-F, dulcoside, etc. Stevioside and rebaudioside are the sweetest glycosides

present in stevia leaves, which are 250–300 times sweeter than sucrose, and are chemically and thermally stable. Stevioside and rebaudioside obtained from stevia leaves have been utilized commercially in Japan, South America, China, and Korea to sweeten various foods. Dietary supplements containing stevia extracts have been utilized in USA extensively [3]. Market stevia products contain such steviol glycosides as stevioside or rebaudioside A [4].

Health benefits. Stevia is known to provide a wide variety of health benefits (Fig. 1). The leaves possess functional properties superior to those of many other high potency sweeteners. Hence, stevia is likely to become the ultimate natural sweetener in the food industry.

Anti-hyperglycemic. According to WHO global report on diabetes (2016), as many as 422 million adults were suffering from diabetes all over the world, and diabetes-related deaths accounted for 1.5 million. Diabetes mellitus is one of the major metabolic diseases characterized by hyperglycemia. It is a chronic disease resulting either from defects in insulin secretion of β-cells of pancreatic islets (islets of Langerhans) or from the response to insulin, or combination of both. Type 1 diabetes mellitus is juvenile-onset, or insulin independent diabetes mellitus, in which the exact etiology of the disease is unknown, while in type 2 diabetes the risk to predisposal depends upon metabolic and the genetic factors.

Traditionally, the extract obtained from stevia leaves proved its effectiveness in treatment of diabetes [5, 6]. There is a stimulation of insulin secretion from β cells of islets of Langerhans and INS-1 cells by direct action of stevioside and steviol [7, 8]. The antihyperglycemic effect of stevioside was investigated in type 2 diabetic Goto-Kakizaki rats, and it was concluded that the hypoglycemic action of stevioside was due to increased secretion of insulin and induction of genes of glycolytic pathway [9].

A limited number of human studies have been reported to depict the mechanism of stevioside (Fig. 2). A study of acute effects of stevioside was conducted on twelve type 2 diabetic patients. It was observed that there was an average 18% decline in the post-prandial blood glucose levels of the diabetic patients after supplementing the standard test meal with 1 g stevioside. However, the slight increase in the insulin levels was not statistically significant [10]. The effect of steviol glycosides on insulin sensitivity and glucose metabolism have been elucidated [11]. In diabetic rats, intake of steviol glycosides resulted in a decrease in blood glucose levels, along with a decrease in the rate of gluconeogenesis and reduction in insulin resistance. Similar findings suggest that oral administration of medium polar extract of stevia leaves at 200 and 400 mg/kg body weight basis for 10 days in alloxan-induced diabetic rats resulted in delayed but significant antihyperglycemic effect without producing hypoglycemia, along with lesser body weight loss in contrast to standard positive control drug glibenclamide [12].

There was a research on the blood glucose lowering effects of rebaudioside A on the activity of carbohydrate metabolizing enzymes in induced diabetic rats [13]. An increase in the rate of glycolysis and reduction in gluconeogenesis produced a significant antihyperglycemic effect. Another study featured the antihyperglycemic mechanism of stevia. It was found that stevia (400 mg/kg) reduced blood glucose levels better than pioglitazone (10 mg/kg), which can be used to control blood sugar levels in diabetics. There was an elevation in insulin levels due to the impact of stevia on the pancreatic tissue, and the valuable anti-hyperglycemic action was exerted via PPARϒ dependent mechanism and its antioxidant property [14].

Anti-hypertensive. Persistent elevation of systolic blood pressure ($\geq$ 140 mm Hg), or diastolic blood pressure ($\geq$ 90 mm Hg), or both is the principal cause for developing cardiovascular diseases, which are associated with high mortality rates globally. In primary hypertension, etiology is unknown and accounts for 90% of the hypertension cases. By contrast, secondary hypertension is known, and it affects less than 10% of the hypertensive population. It is precipitated by another medical condition affecting kidneys, arteries, heart, or endocrine system.

An early investigation conducted on rats showed that stevia extract at doses greater than those used for the purpose of sweetening resulted in vasodilation and a lower mean arterial pressure in hypertensive rats [15]. A reduction in blood pressure was elucidated upon studying the effectiveness of stevioside (250 mg three times a day for a period of 3 months) in human subjects suffering from mild or moderate primary hypertension [16]. The mechanism by which stevioside produces vasodilatory effect is analogous to that of verapamil, an antihypertensive drug which acts by blocking calcium channels in myocardial and arterial smooth muscle cells (Fig. 3). The antihypertensive effect of stevioside was antagonized by administration of indomethacin (parostaglandin synthesis inhibitor), suggesting stevioside produces reduction in mean arterial pressure via prostaglandin activity [17]. In the study, to evaluate the effect of stevia on renal function, it was reported that steviol increased the excretion of sodium and potassium along with glomerular filtration rate in a dose dependent manner. It acted as a diuretic agent by preferably affecting the Na$^+$-glucose coupled cotransporter in proximal convulated tubule of kidney [18]. The hemodynamic effects of rebaudioside A were investigated in human trial. No significant changes in mean arterial pressure or heart rate were observed after daily intake of 1000 mg/kg body weight Rebaudioside A in healthy individuals who were normotensive or having low normal mean arterial pressure [19].

Anti-caries. Dental caries is a widespread chronic disease in humans and affects oral health. Dental caries may lead to the development of painful sensation in tooth, infections, oral and pharyngeal cancers, oral tissue lesions, and the outcome may be life-threatening if untreated, as in case of cavernous sinus thrombosis and Ludwig's angina.
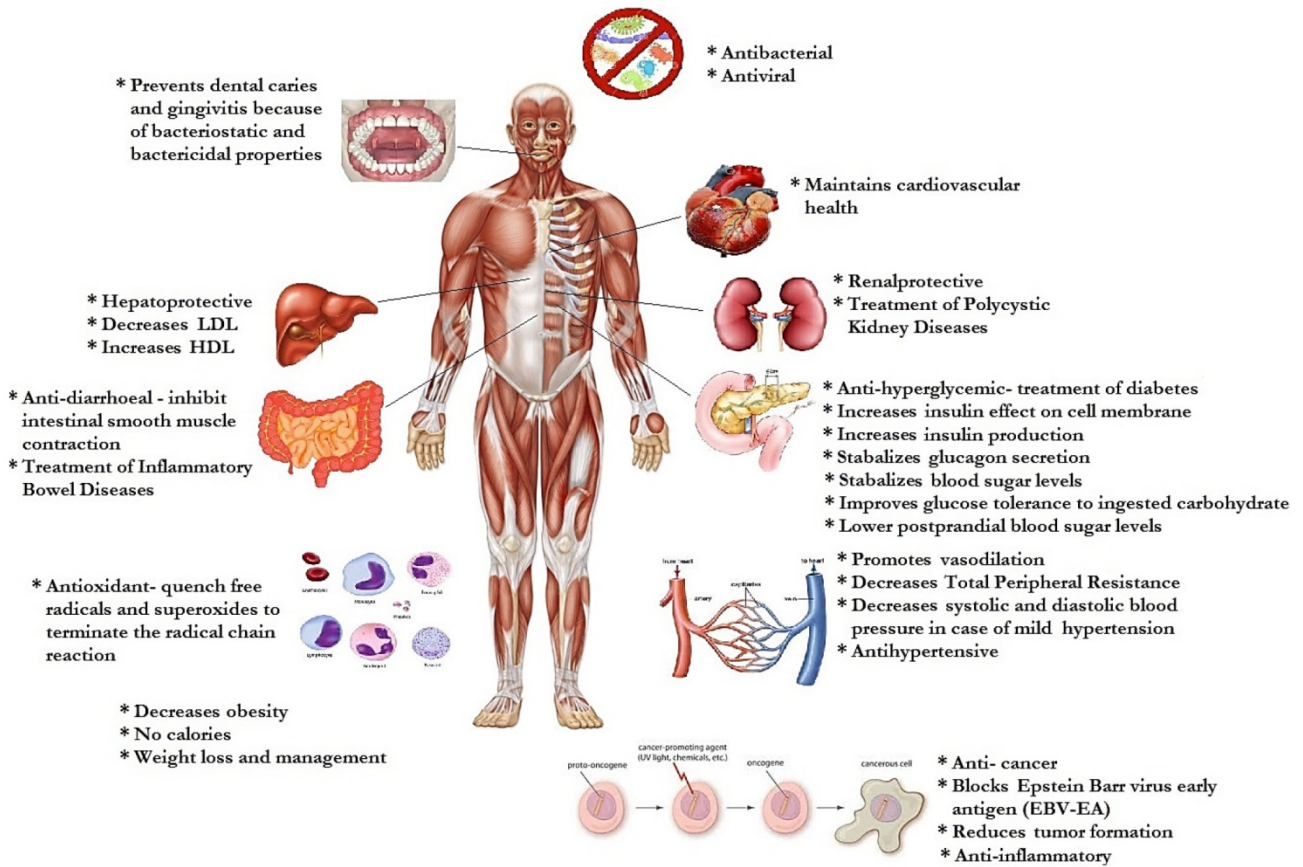
Fig. 1. Health benefits of stevia on various organs.
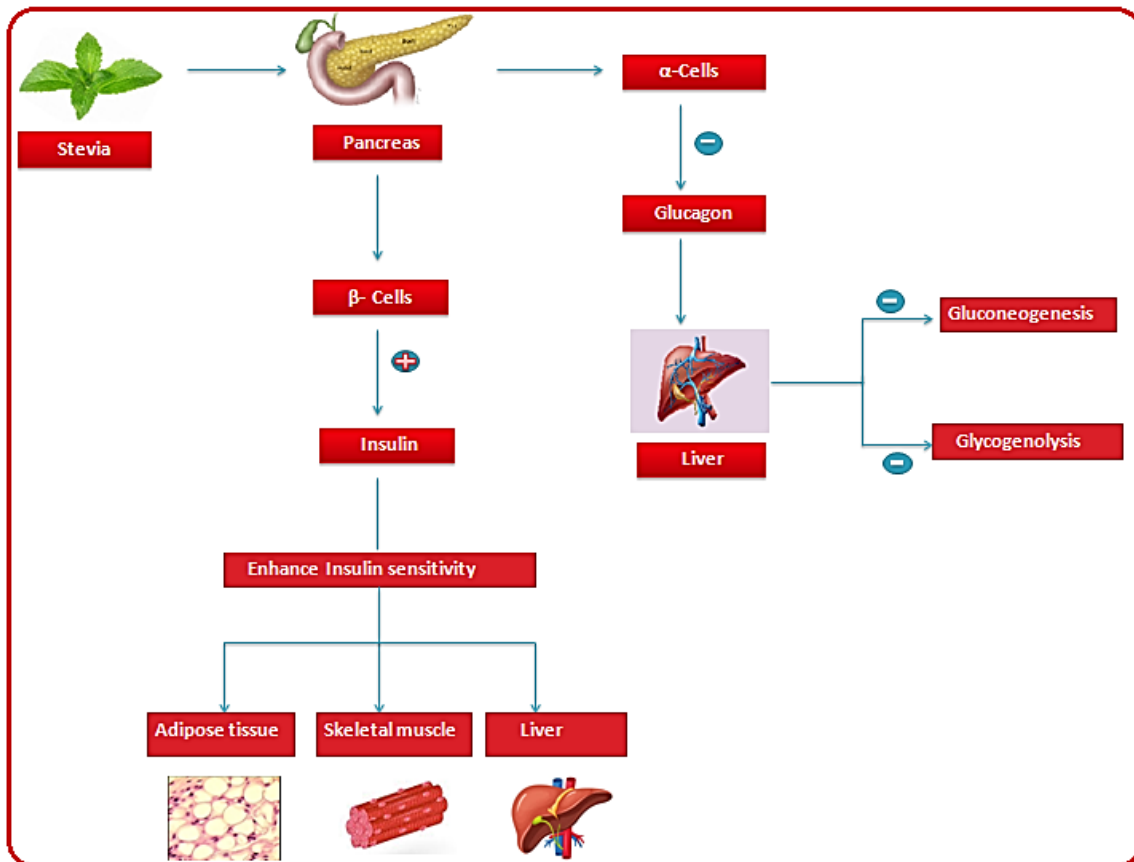
Fig. 2. The possible mechanism of anti-hyperglycemic action of stevia.
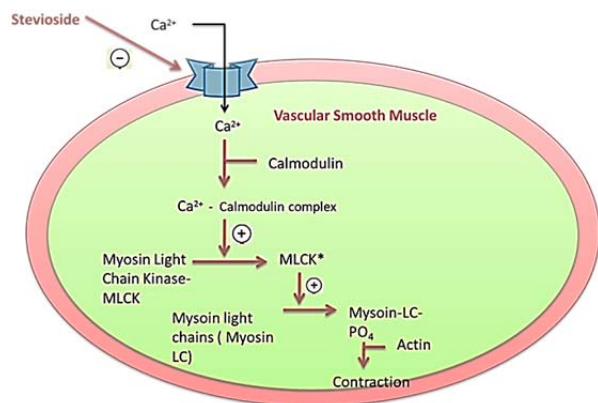
Fig. 3. Antihypertensive action of stevioside. Inhibition of Ca^{2+} influx in vascular smooth muscle resulting in vasodilation and hence reduction in systemic vascular resistance.

Routine consumption of caloric sweeteners, such as sucrose, results in ingestion of carbohydrates, which boosts the amount of harmful microbes in the oral cavity. That may ultimately result in plaque and gingivitis [20]. Stevia is a non-caloric sweetener that possesses antimicrobial properties benefiting oral health via prevention of dental caries. There are 57.82% less chances of developing a plaque with consumption of stevia, compared to sucrose recorded by aid of Silness-Loe plaque index [21]. A study on the cariogenic potential of commercial sweeteners conducted on artificial enamel proved that stevia-incorporated products exhibited antimicrobial activity and showed the least potential to act as cariogenic in contrast to sucrose and other commercial artificial sweeteners [22].

Anti-inflammatory. Inflammatory bowel disease, or IBD, is a group of chronic diseases that involve the inflammation of the alimentary tract. It includes Crohn's disease (inflammation of the entire lining of alimentary canal) and ulcerative colitis (inflammation of the innermost lining of intestine). Ample evidences are available showing that stevioside acts as an anti-inflammatory *in vivo*, as well as *in vitro*. Stevia stem extract was suggested to act as a gastroprotective since it reduced histamine-induced gastric abnormality in rainbow trout [23]. The suggested mechanism of action was inhibition of smooth muscle contraction by blocking the calcium channel. The active substance in the stem extract was stevioside, which was potentially responsible for decreasing the acid secretion caused by histamine and inhibiting the action of pepsin [24]. Similar findings were reported when the anti-ulcerogenic activity of stevia was examined by oral administration of stevia extracts leading to a significant reduction in the free acidity, which resulted in inhibition of gastric lesions [25].

Anti-cancer. Cancer can be defined as a group of diseases involving the abnormal proliferation of cells, which is associated with high mortality rate. The anticancer effect of stevioside, isosteviol, and the derivatives obtained from isosteviol upon microbial transformation was evaluated. All the components

were found to be potent in inhibiting Epstein-Barr virus early antigen (EBV-EA) while the highest potency was exhibited by the derivatives obtained by microbial transformation [26].

The ent-kaurene diterpene glycosides isolated from *Stevia rebaudiana* were investigated for anti-inflammatory activity against inflammation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), and it was found to suppress inflammation along with a significant inhibitory effect on the tumour formation [27, 28]. The methanolic and ethanolic extract of stevioside exhibited anti-cancer potential against the Caco cell line [29]. Stevioside exhibited *in vitro* anticancer activity against MCF-7 cells, which are most commonly used for breast cancer studies in humans. The suggested mechanism of antibreast cancer activity was enhanced expression of proteins participating in apoptotic pathway [30].

Value-added products prepared with incorporation of stevia. Value-added products are prepared for enhancing the value of food items through the addition of ingredients, processing or packaging. Value-added food products are more attractive and usable by the consumer than the original commodity. Commercially, leaves of stevia are exploited directly or after processing in preparation of various value-added products. As a functional food ingredient, stevia has been partially or fully incorporated into baked, dairy, confectionery products, etc. The present review features the commercial use of stevia in various food products and its effect on various properties.

Bakery products. Sweetened food items, such as cakes, cookies, muffins, and biscuits, are the major contributors to sugar intake globally [31]. Sucrose is a crucial ingredient in baked goods. It contributes not only to the taste but also to the characteristic texture and structure [32]. However, excessive consumption of sugar leads to acute elevation in postprandial glucose levels [33]. There is an increased risk of obesity, diabetes, dental caries, and coronary heart disease associated with regular consumption of high amounts of sugar-containing foods.

The use of stevia in baked products is suitable since it maintains its stability throughout the baking process and can be heated up to 200°C [34]. The non-nutritive high intensity sweeteners contribute to the sweet taste of the product, but the maintenance of texture, colour, and flavour is also crucial. Hence, whenever stevia is used as a partial replacement of sugar, other additives, such as bulking agents, hydrocolloids, proteins, etc., should be added to compensate for the loss of texture.

To evaluate the physical properties of muffins sweetened with stevia suggested a study that muffins with 25% of the sucrose replaced by steviol glycosides were ranked best in terms of browning index, texture, cooking yield, and sensory acceptance. Sucrose replacement greater than 50% had a negative impact on the quality characteristics of muffins [35]. Low glycemic index muffins were made by adding stevia and cocoa dietary fibre. The replacement of 20% sucrose with stevia along with substitution of cocoa powder with cocoa fibre did not produce any negative

impact on the quality attributes and consumer acceptability [36]. Functional cookies prepared by 20% replacement with defatted soy flour and powder obtained from stevia leaves were ranked best for all the sensory characteristics [37].

Dairy products. Milk and milk-based products are a vital component of functional foods. Dairy processors are investigating novel technologies to sweeten their products without adding more calories, since people are averted from the consumption of intensely sweetened dairy-based products. There has been a continual search for natural low-calorie alternatives to sweeten dairy products that would reduce the sugar content while maintaining the texture, body and mouthfeel. Stevia has emerged as a suitable choice for dairy products since it preserves its stability, when subjected to heat treatment.

One of the most popular frozen dairy products is ice cream. Sugar influences its texture, viscosity, and freezing point and, hence, plays a significant role in determining the consumer acceptability. The most popular ice cream sweetener is sucrose because of its cost effectiveness and consumer acceptability. However, stevia and other non-nutritive sweeteners are gaining popularity due to health hazards associated with sucrose. The replacement of sugar with stevia in ice cream and kulfi can lead to a significant decrease in the caloric value**.** Studies revealed that ice cream mixtures in which sucrose had been partially replaced with stevia had better sensory scores than those with stevia only [38, 39].

Yogurt is one of the best-known foods that contain probiotics. Sensory analysis of strawberry flavoured yogurt with stevia elucidated that yogurt with a mixture of stevia and sucrose had the best sensory profile [40]. Stevia exhibited a synergistic sweetening effect when used in combination with other sweeteners to sweeten strawberry flavoured yogurt [41].

Flavoured milk supplies vital nutrients equivalent to plain milk. Studies have revealed that flavoured milk is preferred for consumption by children and adults [42]. However, flavoured milk contains a high quantity of sugar, which has been linked to promotion of obesity amongst children and adults [43, 44]. The use of stevia is an appealing alternative in order to reduce the caloric value of flavoured milk. It is of primary importance to maintain the acceptance of the product by the consumer while decreasing the sugar content. In the study [45], the perception of sweetness intensity in skim chocolate milk prepared by addition of extracts of stevia and monk fruit was defermind. It was established that flavoured milk containing stevia had a maximum consumer acceptability.

Beverages. The primary function of stevia is sweetening, but it may modify the flavour in certain cases.

Peach juice was formulated with a blend of stevia (160 mg/L) and sucrose (56 g/L), which led to 25% reduction in calories without affecting the sensory attributes of the product compared to the control sample containing 9% sucrose [46]. Orange juice is one of the most popular non-carbonated beverages globally since it contains high amounts of vitamin C, carotenoids, folic acid, flavonoids, etc. It acts as an antioxidant preventing the damage caused by free radicals to tissues and also decreases the chances of heart disease and cancer [47, 48]. Low calorie orange nectar and orange juice were formulated with the addition of stevia [49, 50]. Other beverages, such as mango nectar and passion fruit juice, were developed with partial replacement of sucrose with stevia and evaluated for their sensory as well as physicochemical parameters [51, 52].

Effect of incorporation of stevia on different properties. *Physicochemical properties.* Physical properties of food material are its measurable and quantifiable characteristics. They are used to describe matter without altering its composition. They elucidate the unique way a food material will react to physical treatments – thermal, optical, electromagnetic, mechanical, etc. Knowing these properties helps design optimum operation parameters and equipment to ensure the quality and safety of foods.

Some important physicochemical parameters for evaluation of ice cream are overrun ratio, first melting point, last melting point, and fat destabilization. The addition of stevia in ice cream resulted in a higher first melting time being the longest for the sample containing stevia and cocoa. Table 1 shows that the last melting time for ice cream containing cocoa with sucrose and cocoa with stevia was lower as compared to plain ice cream with sucrose and plain ice cream with stevia [38]. The overrun ratio for ice cream with stevia and cocoa was found to be the highest, while the overrun ratio was the lowest for plain ice cream with stevia [38]. Similarly, the overrun ratio of soft ice cream increased when stevia was added [39]. Fat destabilization was reported to be slightly higher in ice cream containing stevia [38]. Increasing levels of sugar replacement with stevia in kulfi resulted in a decrease in melting rate [53]. In case of juices, the important physicochemical properties to be evaluated are °Brix and titratable acidity.

Rheological and textural properties. Texture may be referred to as a collection of physical attributes that emerge from structural makeup of food. They are perceptible via sense of touch and associated with disintegration, distortion, and flowability under the influence of force. Some important textural properties in foods are hardness, cohesiveness, springiness (elasticity), adhesiveness, chewiness, gumminess, resilience fracturability, stringiness, and initial modulus.

Viscosity (the resistance to flow) is an important evaluation parameter for ice cream mix. As given in Table 2, a decrease in viscosity of ice cream was observed when nothing but stevia was added. However, the ice cream sample containing both stevia and cocoa possessed the highest viscosity amongst all other ice cream samples. With a gradual increase in stevia concentration in ice cream, the viscosity was relatively lowered, compared to the sample containing sucrose only [39]. A disaccharide produces the solution with high osmolality due to its hydrophilicity and high solubility, as well as its ability to form hydrogen bonds [54].

Table 1. Effect of stevia on physicochemical properties of various food products

Product	Stevia form	Amount of stevia, %	Description	Reference
Ice cream	Stevia leaf powder	0.862	↑ in last melting time ↓ in over run ratio	[38]
Ice cream with cocoa	Stevia leaf powder	0.786	↑ in first melting time ↑ in fat destabilization ↑ in over run ratio	[38]
Ice cream	Stevia powder (> 90% steviol glycosides)	0.02–0.11	↑ in over run ↓ in melting resistance compared to ice cream sweetened with sucrose	[39]
Kulfi	Stevia extract powder with 91.1% stevioside	0.05–0.07	↓ in specific gravity ↑ in the freezing point compared to kulfi sweetened with sucrose ↓ in melting rate ↓ in penetration	[53]
Herbal Kulfi	Stevia powder	0.05–0.07	↓ in melting rate	[58]
Passion fruit juice	Stevia extract	0.09924	↓ in ᵒBrix ↑ in titrable acidity	[52]
Mango nectar	Stevia with 97% Rebaudioside	0.052	↓ in ᵒBrix value was lower as compared to nectar sweetened with sucrose ↑ in ᵒBrix valueas compared to nectars with other sweeteners ↔ in ᵒBrix during storage	[51]
Orange nectar	Stevioside powder (85–95% purity)	0.02–0.06	↓ in titrable acidity during storage ↓ in ᵒBrix with addition of stevioside ↔ in ᵒBrix during storage	[49]

Note. ↑ – increase, ↓ – decrease, ↔ – non-significant

Table 2. Effect of stevia on rheological and textural properties of various food products

Product	Form of stevia	Amount of stevia, %	Description	Reference
Plain ice cream	Stevia leaf powder	0.862	↓ viscosity	[38]
Ice cream with cocoa	Stevia leaf powder	0.786	↑viscosity amongst all samples of stevia	[38]
Ice cream	Stevia powder (> 90% steviol glycosides)	0.02–0.11	↓ viscosity	[39]
Orange nectar	Stevioside powder (85–95% purity)	0.02–0.06	↑ in viscosity	[49]
Mango nectar	Stevia with 97% Rebaudioside	0.052	↑ in viscosity	[51]
Muffin	Stevianna with 98% Rebaudioside A	9.97 and 19.76	↑ in firmness value in contrast to muffin with sucrose ↓ in firmness value in contrast to muffin with inulin ↑ in springiness value in contrast to muffins with inulin and sucrose	[32]
Muffin	Stevia powder with 95% steviol glycosides	0.075–0.300	↑ in hardness and springiness as more sugar was replaced with stevia ↔ in cohesiveness with addition of stevioside	[35]
Muffin	Stevia powder with 95% steviol glycosides	0.09	↑ in firmness with addition of stevia ↓ in porosity when stevia and sucrose were used in combination	[36]
Functional yoghurt cake	Stevia leaves liquid extract	3.33	↑ in firmness Similar deformation to regular yoghurt cake	[56]
Bittersweet chocolate	Stevia extract	0.16	↑ in hardness values in low fat samples sweetened with stevia	[57]

Note. ↑ – increase, ↓ – decrease, ↔ – non-significant

Viscosity of the low calorie nectar (sugar content reduced to 70% compared to the control sample) was found to be slightly higher than that in the blank sample, which was possibly due to incorporation of 0.03% pectin in the sample [49]. Similar results were seen in the case of mango nectar, in which the sample sweetened with stevia exhibited a higher viscosity compared to nectar sweetened with other sweeteners [51]. The critical textural variables for muffins are firmness, springiness, and cohesiveness. The texture profile analysis (TPA) of

muffins revealed that with the increase in replacement of sucrose with stevia, a significant increase in hardness and springiness was observed [36]. The possible reason for this was the decrease in the amount of sucrose in the muffins since sucrose played a significant role in governing the texture [55]. Reduction in the amount of sucrose led to an increase in porosity. However, when sucrose was used in combination with stevia, the value of porosity was slightly lower [36]. In a functional yogurt cake, an increase in firmness and hardness after adding ground stevia leaves has been reported [56]. When bittersweet chocolate was combined with rebaudioside A, which is the sweetest component present in stevia leaves, it resulted in an increase in hardness of low fat chocolate [57].

Nutritional properties. Excessive consumption of food containing high content of sugar and saturated fats is one of the most critical nutritional issues mankind have to face in the current scenario. It has been linked to serious health problems. Health awareness is growing nowadays, and this has led to a significant rise in demand for low-calorie/fat products. Many low-calorie food products have been developed with the addition of dietary fibre or low calorie sweeteners as the consumers are striving to choose healthier food alternatives (Table 3).

Significant reduction of glycemic index and caloric value was reported in ice cream prepared with addition of stevia, which propounds the utilization of stevia as a substitute for sugar and a healthy alternative for people who are predisposed to diabetes [39]. Kulfi samples prepared with incorporation of stevia showed a decrease in calorific value but a significant increase in fat, ash and protein content, due to a decrease in total solids, which resulted from partial replacement of sugar with stevia [53]. Similar results were reported for herbal kulfi that contained both stevia and a mixture of herbs including *Foeniculum vulgare, Ocimum sanctum,* and *Piper betle* [58]. A hot water extract of stevia leaves was used to produce a functional yoghurt cake.

There was a reduction in calories by 35.72% as compared to a regular yoghurt cake. The biological evaluation of yoghurt cake in diabetic rats revealed that there was no significant change in blood levels of cholesterol, alkaline phosphatase, bilirubin, glucose, creatinine, and triglycerides. However, the level of urea and Aspartate transaminase (AST), as well as HDL cholesterol, decreased slightly. Lower doses did not produce any effect on serum glucose, AST, ALT, total cholesterol, protein, and triglycerides, but higher doses produced a slight elevation in these parameters [56]. Similarly, muffins prepared by partial replacement of sugar with steviol glycosides showed a decrease in caloric value [32, 36]. The incorporation of stevia into muffins led to a prolonged release of reducing sugars during the digestion of starch *in vitro*, thus, reducing the predicted glycaemic response [32]. A decrease in energy value was noted when sucrose free chocolates were prepared with the addition of stevia [59].

Sensory properties. Sensory properties of a food product are regarded as one of the most crucial attributes since they are most noticeable by the consumer [60]. Sensory evaluation refers to the scientific technique of invoking, computing, analysing, and interpreting the responses by the perception of senses [61]. The palatability of the product has become the primary criteria for consumers nowadays, whereas such parameters as nutritional value and the wholesomeness of the product are secondary. Therefore, to ensure market success, a product must have the desired sensorial characteristics, i.e. taste, flavour, aroma, mouthfeel, aftertaste, and textural parameters.

As described in Table 4, kulfi prepared with addition of stevia indicated maximum overall acceptability, whereas no significant effect was observed on the body, texture, and flavour of the kulfi if the level of replacement of sugar with stevia was less than 50%. However, a negative impact on the textural properties, as well as flavour, was observed when the level of sugar replacement with stevia exceeded 50% [53].

Table 3. Effect of stevia on nutritional properties of various food products

Product	Stevia form	Amount of stevia, %	Description	Reference
Ice cream	Stevia powder (> 90% steviol glycosides)	0.02–0.11	↓ in calorific value and glycemic index	[39]
Kulfi	Stevia extract powder with 91.1% stevioside	0.05–0.07	↓ in calorific value	[53]
Herbal Kulfi	Stevia powder	0.05–0.07	↑ in fat and protein content ↓ in carbohydrate content	[58]
Muffin	Stevianna with 98% Rebaudioside A	9.97 and 19.76	↓ in calories and post prandial insulin levels	[32]
Muffin	Stevia powder with 95% steviol glycosides	0.09	↓ in caloric value	[36]
Functional yoghurt cake	Stevia leaves liquid extract	3.33	↓ caloric and energy values	[56]
Chocolate	Stevia extract with Glucosyl stevioside	0.5	↓ in energy value	[59]
Orange nectar	Stevioside powder (85–95% purity)	0.02–0.06	↓ in phenolic, ascorbic acid and stevioside content after 2 month storage at room temperature and 4°C	[49]

Note. ↑ – increase, ↓ – decrease, ↔ – non-significant

Table 4. Effect of stevia on sensory properties of various food products

Product	Stevia form	Amount of stevia, %	Description	Reference
Ice cream	Stevia leaf powder	0.862	↑in overall acceptance than sucrose sweetened ice-cream	[38]
Ice cream with cocoa	Stevia leaf powder	0.786	More preferred in terms of taste, color , flavor, melting in mouth and texture	[38]
Ice cream	Stevia powder (> 90% steviol glycosides)	0.04	Higher suitability in terms of taste, texture and mean liking	[39]
Herbal Kulfi	Stevia powder	0.05–0.07	↓ in overall acceptability	[58]
Passion fruit juice	Stevia extract	0.09924	↓ in aroma, flavor and overall impression	[52]
Muffin	0.075	0.075–0.300	↑ in scores for color, porosity and overall quality	[35]
Muffin	Stevia powder with 95% steviol glycosides	0.09	↑ in scores for overall quality, softness and taste	[36]
Bittersweet chocolate	Stevia extract with different Rebaudioside A contents (60%, 80% and 90%)	0.16	Stevia with 60% rebaudioside was more preferred in terms of sweetness, bitterness and melting rate	[57]

Note. ↑ – increase, ↓ – decrease, ↔ – non-significant

Current status and future perspective of utilizing sweeteners at national and international front. The US Food and Drug Administration regulate sweeteners as food additives. Food additives must be approved by the FDA, which publishes a Generally Recognized as Safe (GRAS) list of additives. To date, the FDA has not been presented with scientific information that would support a change in conclusions about the safety of the five approved artificial sweeteners (saccharin, aspartame, sucralose, neotame, and acesulfame potassium). The safe conclusions are based on a detailed review of a large body of information, including hundreds of toxicological and clinical studies. Steviol glycoside has been used as a non-nutritive low calorie sweetener in beverages, teas, and medicines in Japan, China, South America, and Korea. Many international beverage industries use stevia as a sweetener in different fruit juice drinks. The incorporation of stevia into such bakery products as puddings and cakes is highly suitable since stevioside is required in minute quantities to sweeten the product. It neither ferments nor exhibits any browning reaction during cooking, which broadens the field of application in baking and makes it possible to enhance the quality, decrease the calories, and increase the shelf-life. Stevia can be used as a sugar substitute in confectionery to sweeten chewing gums, candies, mints, chocolates, etc. Besides, stevia can be extensively used to sweeten various products, e.g. ice-cream, chocolates, fruit drinks, biscuits, soft drinks, yoghurt, biscuits, beverages like tea, and coffee.

The joint FAO/WHO expert committee on food additives (JECFA, 2008) stated that steviol glycosides were safe for use in foodstuffs and beverages. JECFA also suggested the acceptable daily intake (ADI) of 0–4 mg/kg body weight of steviol glycosides, which is equivalent to 0–10 mg/kg stevioside [62]. Currently, the largest market for production and application of stevia and its glycosides is in China and Japan. In 2009, the use of rebaudioside A was permitted in France. In European Union, the approval for use of steviol glycosides as food additive was granted by European Commission in 2011.

In India, the use of steviol glycosides was permitted by FSSAI in a notification issued in 2015 for a variety of products, including yoghurts, carbonated beverages, jams, fruit nectars, dairy based desserts, ready-to-eat cereals, etc.

CONCLUSION

Diterpene glycosides obtained from *Stevia rebaudiana* can be used to sweeten various foods and beverages without increment in calories. Apart from sweet contents, the other constituents of *Stevia rebaudiana* exert various health benefits, such as anti-hyperglycemic, anti-cancer, hepatoprotective, anti-hypertensive, anti-caries, antioxidant, and antimicrobial. Low-calorie stevia-incorporated products are rich in antioxidants, amino acids, and certain vitamins. They possess many other therapeutic properties in that they are anti-diabetic, anti-hypertensive, antitumor, antiulcer, etc.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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Sport nutrition: the role of macronutrients and minerals in endurance exercises

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Abstract: Athletes' nutrition optimization is very important for the nutritional support in all sport specializations. Macronutrients, as well as minerals and vitamins, are functionally active components that play an important role in nutrition of athletes especially in endurance sport. Optimal use of diets, including specialized sport nutrition, normalizes biochemical, immune, endocrine functions and restores athletes' energy balance at different stages of sport exercises. Non-optimal athletes' nutrition of different age groups, inadequate to their physiological needs, and no personalized approach to athletes' diets, violate their right to adequate safe nutrition, according to international standards and criteria. Nutritional factors are one of the most important key factors in the risk prevention measures for a large number of diet-dependent diseases (e.g. digestive, liver, pancreas, cardiovascular system, endocrine system, and kidney diseases). The review presents the information on energy requirements, balance and availability, types and content of functional products for athletes. It also gives an overview of the specialized food market in Russia.

Keywords: Sport nutrition, minerals, proteins, energy, carbohydrates, specialized products

INTRODUCTION

Optimization of athletes' nutrition, which takes into account the phase state of the organism, athletes' individual, age-sex and other features, is very important for the nutritional support in all sport specializations. It is of a great importance for athletes involved in endurance sport, e.g. sportsmen of the world national teams. Particular attention should be paid to young athletes. A full-value optimal nutrition for all these categories of athletes creates conditions for maximum physical performance, increases a body's resistance to stress and the effects of any unfavorable factors. Control over the adequacy of nutrition, as well as its optimization, is included in the structure of the mandatory athletes' check-ups to ensure timely detection of health and fitness dynamics. Nutritional disorders significantly reduce the effectiveness of training activities, especially in trauma and stress, and increase the risk of pathology development. Along with other factors, they may adversely affect the effectiveness and duration of athletes' professional activity.

The introduction of specialized sport nutrition into the diet is crucially important in the medico-biological support of highly skilled athletes. It contains additional macronutrients as well as essential micronutrients, such as vitamins, minerals and other biologically active substances. There are different types of sport nutrition:

– foods and beverages containing high concentrations of different types of carbohydrates for creating and maintaining the glycogen in muscles for providing energy;
– protein enriched products for enhancing protein synthesis in muscles and adapting to exercises;
– multiple micronutrients (vitamins, minerals, biologically active substances) in different forms; and
– isotonic solutions for rehydration, additional energy supply and etc.

RESULTS AND DISCUSSION

Energy requirements, energy balance, and energy availability. An athlete's energy requirements depend on sport, the training period, competition cycle, and recovery period. It varies from day to day depending on changes in training volume and intensity level. It is well-known that energy consumption is directly proportional to the athletes' physical activity. Therefore, people involved in general fitness programs (for example, exercises for 30 to 40 minutes a day, 3 times a week) can usually meet energy needs, using regular foods in accordance with a normal diet. Their energy consumption can be in the range 1,800–2,400 kcal/day or about 25–35 kcal/kg per day [1, 2]. Athletes who have a moderate level of training (for example, 2–3 hours a day of training, 5–6 times a week) or a high intensity of

training (3–6 hours per day of intensive training for 5–6 days a week) may additionally have energy consumption of 600 to 1,200 kcal or more per hour during a workout [1, 2]. For elite athletes energy consumption during endurance training or a competition can be huge, e.g. the estimated energy expenditure for cyclists participating in the Tour de France was 12,000 kcal per day [3, 4]. In addition, the requirement for calories for athletes with significant body weight (e.g., 100–150 kg) can range from 6,000 to 12,000 kcal per day, depending on the volume and intensity of various training skills [3]. That is why the only way to optimize the athletes' nutrition is the mandatory use of specialized products and dietary supplements. The violation of nutritional status, especially in highly qualified athletes with extreme physical activity, has a significant negative impact on health indicators. It is a serious risk factor for the development of many diet-dependent diseases which may be prevented by diet regulation.

Energy consumption in sports can be calculated in accordance with the recommendations, e.g., of the American College of Sports Medicine [5]. Energy balance occurs when total energy intake (EI) equals total energy expenditure (TEE), which in turn consists of the summation of basal metabolic rate (BMR), the thermic effect of food (TEF), and the thermic effect of activity (TEA).

$$TEE = BMR + TEF + TEA$$

$$TEA = Planned\ Exercise\ Expenditure + \\ + Spontaneous\ Physical\ Activity + Nonexercise \\ Activity\ Thermogenesis$$

Below is the example of calculating of the energy availability (EA) for a sportsman with body weight of 60 kg, body fat 20%, FFM 80% (= 48.0 kg FFM), EI 2,400 kcal/day, and additional energy expenditure from exercise – 500 kcal/day:

$$EA = (EI - EEE)\ /\ FFM = (2,400 - 500)\ kcal\ / \\ /48.0\ kg = 39.6\ kcal/kg\ FFM$$

One should also take into account the peculiarities of the three types of energy production, which differ drastically:
– aerobic energy production, which is typical of sports that require endurance exercises (marathon, skiing, road racing, etc.);
– anaerobic energy production, i.e. the ability to perform muscular work in conditions of oxygen deficiency, which is realized mainly in sports that require short-term energy release (weightlifting, sprinting, etc.); and
– mixed anaerobic-aerobic energy production, which is typical of sports with different alternating exercises (combat sports, game sports, etc.)

Specialized foods that include easily recyclable energy sources, micronutrients and biologically active substances allow regulating and activating the main functional processes (biochemical, immune, cardiac, endocrine etc.) at various stages of the training process.

At the same time, the unbalanced athletes' nutrition, inadequate to their physiological needs, the lack of a personalized approach to athletes' diets violate their right to adequate safe food, according to international standards and criteria (Resolution number 2001/25 of April 20, 2001, the human rights mission of the United Nations).

History of sports nutrition. Sports nutrition is the application of basic nutritional principles to improve the training process, athletic performance and recovery of athletes in post-training periods.

It is believed that the first evidence-based research on athletic nutrition is closely related to the studies of carbohydrate and fat metabolism, conducted in Sweden in the late 1930s. In the late 1960s, Scandinavian scientists began to study the processes of storing, consuming and re-synthesizing glycogen in muscles during long-term sports training. Technologies for assessing the response of human tissues to physical activity were also developed. Later, in 1965, the scientific advances gave an opportunity to a group of researchers at the University of Florida, led by Dr. Robert Cade, to develop and scientifically prove the possibility of using a carbohydrate-containing beverage in sports. Thus, in 1965 *the Gatorade* appeared – one of the first well-known sport drinks.

In the 1970s, physiologists from all over the world, including the leading scientists of the Soviet Union, began to develop a new direction – the physiology of sports. These studies were carried out mainly in highly skilled athletes, especially in long-distance runners as these athletes developed the fastest and most life-threatening depletion of glycogen stores in the muscles. In addition, this sport was easily modeled in laboratories with the use of treadmills and exercise bikes. In this regard, much of the initial research of sports nutrition has been associated with the study of carbohydrate-containing foods.

Many studies in this area were empirical and largely subjective, primarily related to the use of protein products by body builders. Despite a large number of studies on the use of protein products in sports, many fundamental questions about the quantity, quality and timing of protein intake remained unrevealed. Despite the recommendations for optimal protein intake in different sports and for athletes of different ages, many aspects of this problem remained controversial.

In the 1980s, physiological research ultimately contributed to the research conducted by sport physiologists and nutritionists. This was a necessary step, since many aspects of nutrition in sports laid in the plane of dietetics. Physiologists, on the basis of studies by marathon runners and cyclists for long distances, determined the need for consumption of about 8 g of carbohydrates per 1 kg of body weight. But it was nutritionists' domain of competence to determine which drinks, foods, and carbohydrates athletes should to use in their diet to maintain the balance.

Thus, in the 1980s sports nutrition appeared as a new direction in the nutrition science. Taking into account the importance of sports nutrition products in maintaining high sports results, a significant part of the

research was aimed at increasing the endurance of athletes when using sports nutrition products.

By the 1990s, educational programs in many countries appeared not only in sports medicine but also in sports nutrition. They were developed not only for dietitians, but for athletes and coaches.

Winter Olympics 'Sochi-2014' played a special catalytic role in the development of research and educational programs, expanding the range of sports and technology of food production. The event revealed the problem and forced further studies.

Types of specialized products for athletes. The need to use specialized sport nutrition is due to the fact that during training a large volume and high intensity recovery efficiency and basic metabolic functions cannot be accomplished with traditional foods and diets.

In this regard, in the athletes' diets, especially those with high physical activity, various 'specialized foods for athletes', or 'sports nutrition', are introduced.

In accordance with the official regulatory acts No. 1414 of the Ministry of Sports of the Russian Federation, there are following types of sports nutrition:
– carbohydrate (energy) drinks with high concentration of carbohydrates;
– sport/rehydration drinks (isotonic solutions);
– non-liquid carbohydrate nutrition;
– natural proteins of animal and plant origin (animal meat, fish, dairy – casein and whey proteins, egg white, soy protein);
– hydrolyzed proteins with different degree of the hydrolysis (mixture of peptides of different structure and amino acids);
– individual amino acids or mixtures of 2 to 3 amino acids;
– product for body weight control; complexes of vitamins and mineral supplements;
– sports dietary supplements – individual compositions of protein and non-protein nature that activate biochemical processes (carnitine, creatine, succinate, ribose, etc.); and
– additives for recovery after intensive workloads and injuries.

By the effect on metabolism, special nutrients in sports nutrition products are divided into the following groups:
– with metabolic action, i.e. aimed at stimulating the processes of anaerobic and aerobic metabolism;
– with anabolic action, i.e. enhancing the processes of synthesis of substances in the body;
– used to maintain the biochemical homeostasis of the body; and
– aimed at accelerating recovery processes after physical training with antioxidant and antihypoxic effect.

Market of specialized products for athletes in the Russian Federation. At present, in the Russian market there is a large number of specialized food products for athletes (SFPA) with different ingredients that can be characterized both by their 'basic' component and by the intended purpose.

The greatest demand on the sports nutrition market is for protein (59%), followed by vitamins and minerals (50%), amino acids (48%), creatine (38%), energy (30%), and gainers (18%).

The sports nutrition market is rapidly growing. In 2013 it exceeded 1.3 billion rbls. It is more than 70% higher than in 2012. According to IndexBox, the supply in the Russian market of sports nutrition in 2012 was 40% higher than in 2011. The high growth rate of the sports nutrition market was also observed in 2010–2011 (142% and 148% respectively) and extremely high during 2014 Winter Olympics in Sochi.

According to the Discovery Research Group agency, more than 90% of goods at the present sports nutrition market in Russia is occupied by foreign products. The volume of imported goods in value terms following the results of 2012 amounted to more than 1.7 billion rbls., by the end of 2013 – more than 2.0 billion rbls. The protein compositions were more than 40% of the total SFPA and about 17% is creatine-containing SFPA. The analysis shows that most Russian consumers prefer foreign-made goods. Only 13% of consumers choose sports foods of domestic production.

In the end of 2015, the United States became the undisputed leader in the supply of sports nutrition, accounting for 70% of the total import volume. The production of Germany accounted for about 18% of the sports nutrition supplied to the Russian market. Products from Canada, which accounted for about 7% of all supplies, took the third place.

The analysis of specialized products for athlete nutrition held at the Federal Center of Nutrition and Biotechnology (Moscow) showed that over the period 2011–2016, more than 1000 SFPA of various composition and different food and energy values were submitted for examination. Protein and protein-carbohydrate products were most popular, followed by carbohydrate enriched with biologically active substances, crystalline amino acids and their mixtures, isotonic drinks, carbohydrate-mineral complexes with or without vitamins, as well as vitamin and mineral complexes and their combinations. For the last years, the number of biologically active substances of plant origin, mostly from Asian countries, as well as supplements that are used mainly for the nutrition of athletes (creatine and L-carnitine in the form of various compounds, carnosine, lipoic acid, hydroxymethylbutyrate, etc.) has increased dramatically.

About 60% of products are based on concentrates and isolates of whey proteins and about 15–20% –on amino acids. L-carnitine, creatine, glutamine, HMB (hydroxymethylbutyrate), glucosamine, chondroitin, leucine, and arginine are used more often.

Recently, the products of sports nutrition (mainly of foreign origin) contain such new ingredients as hydroxyisocaproic acid (HICA), agmatin, β-alanine, and norvaline.

As for protein-containing products, two types are represented in the market:
– concentrated protein, which consists of 70–90% protein in the form of a monocomponent without or with additives in different compositions with vitamins, minerals, creatine, individual amino acids, digestive enzymes, plant extracts, etc.;

– carbohydrate-protein containing 18–35% protein with or without similar additives.

The most popular ones and often found in the SFPA are:
– milk proteins;
– combination of whey proteins with egg albumin;
– combination of whey and soy proteins;
– meat proteins;
– soy proteins per se;
– proteins of peas;
– collagen hydrolyzates; and
– proteins of plant origin.

Whey proteins are the most commonly used source of protein. They are used as whole and hydrolyzed proteins. Caseins and their salts in the form of caseinates, both as mono-components and as mixtures of these whey protein fractions, are also popular among athletes.

A protein component in the products imported into the Russian Federation and recently undergoing research is almost always represented by whey proteins or their mixture with casein or chicken egg protein. Attention is drawn to the fact that there is practically no soy both in foreign products and those manufactured in the Russian Federation. This fact is apparently explained by the active anti-advertising in Russia on soy due to its genetically modified forms.

In a number of products, hydrolyzed collagen (usually with milk and egg protein) is present as an integral part of the protein base. At the same time, a limited amount of protein products is made on the basis of pure collagen (hydrolyzate), usually with the addition of vitamin and mineral complexes, and is advertised as a source of individual components to maintain the functions of the skeletal muscles.

A sufficiently large segment in the total amount of sports nutrition products is taken by mixtures of crystalline amino acids, which come in the form of capsules, tablets, and in liquid form. A pure BCAA (a mixture of branched amino acids) is the most popular, while BCAA with other components, including various amino acids and vitamins, takes a second place, and a complex of essential and non-essential amino acids comes next. In some cases, as a source of amino acids, products are declared in the form of hydrolysates of milk, whey proteins or collagen.

The carbohydrate component in the products is usually represented by corn maltodextrin and/or simple carbohydrates (sucrose, fructose). The latter are often the basis of liquid forms of products. Carbohydrate products with vitamins and/or mineral components make up the hypo- and isotonic group and are more often present as ready-made beverages or liquid concentrates that require additional dilution.

In addition to protein products, carbohydrate or mixed basis with a high food and energy value, a sufficient amount of products is made up of specialized products that can equally be attributed to biologically active additives to food, but designed to feed athletes: creatine, glutamine, caffeine, taurine; carnitine, glucosamine and chondroitin, omega-3 fatty acids, vitamins and/or vitamins-mineral complexes, herbal compositions and their extracts (guarana, ginseng, ginkgo biloba, green tea, bearberry, garcinia etc.). The listed components, with the exception of plants, are usually offered both as mono-components (in the form of powders, tablets, capsules), and in the form of various combinations.

One of the trends observed in the last few years was the presence of specialized products from abroad, mainly from the USA, except for long-used creatine monohydrate, amino acids in L-form, carnitine and other components that are products of intermediate metabolism in energy cycles or their substrates: lipoic acid, alpha-ketogluthorate, ketogluthorates of amino acids and esters of ketoforms of amino acids, acetylated forms of amino acids, creatine compounds in the form of taurine or ethyl ether yl or hydroxymethylbutyrate, or other compounds (beta-alanine, norvaline, agmatine, etc.). These components are also produced as individual additives and are often present in the composition of sports products in the form of complexes not only in carbohydrate, but also in carbohydrate-protein compositions.

Carbohydrates in the diet of athletes. It is known that the consumption of carbohydrates is extremely important for optimal adaptation to frequent stress signals, which is typical of sports. Adequate and timely intake of carbohydrates is one of the key factors for the recovery of glycogen, the work of muscles and liver [6].

With increasing of physical exercise activity, the total demand for carbohydrates increases significantly. At endurance sports, the daily requirement for carbohydrates is 5–8 g per 1 kg of body weight [7].

Carbohydrates are the key energy factor for both aerobic and anaerobic pathways of metabolism, the main nutrients for muscle contraction during physical exercises of varying intensity. The degree of use and depletion of carbohydrates accumulated in muscles is different for different sports and largely depends on the duration and intensity of the training process, as well as the degree of hydration of the organism and, the athletes' level of training [8]. Along with this, the lack of carbohydrates becomes a limiting factor for the cognitive functions of athletes [9, 10].

The carbohydrate component in the products is usually represented by corn maltodextrin and/or simple carbohydrates (sucrose, fructose). The latter are often the basis of liquid products.

One of the products of European origin is amylopectin barley starch 'Vitargo®' (Sweden). The chain length of its carbohydrates is 500,000–700,000 (carbohydrate chain length of starch-like foods is more than 2.5×10^8 D, maltodextrin – 1,000–10,000 D, dextrose – 180 D). Besides, it is characterized by low osmolality in comparison with other carbohydrates. The molecular structure of the carbohydrate resembles glycogen, which ensures its rapid entry into the blood. Clinical trials have shown it to be significantly more effective than dextrose and maltodextrin. Carbohydrate products with vitamins and/or mineral components constitute a group of hypo- and isotonic agents.

It should be noted that different carbohydrates differ in glycemic index (food rating depending on the response of blood glucose to reference food)

and are accordingly applied for different phases of training, competitive, and recovery processes. Glycemic index of sucrose is 65, fructose – 23, glucose – 100, and maltodextrin – 96.

Given the significant differences in the properties and types of carbohydrates used in sports practice, glycemic index (GI) is extremely important criteria for their usage in different periods of the sports process. It is used to characterize the rate of carbohydrate to glucose conversion in blood using the concept of glycemic index. GI ranks all products in relation to glucose, less often – white bread. The glycemic index is determined by the rate of a given carbohydrate (or product) causing an increase in blood sugar levels. Glucose has a high glycemic index, sucrose – moderate, fructose – low.

Foods with a high GI provide a rapid increase in blood sugar levels. Carbohydrates contained in the relevant products are easily digested and absorbed by the body; they are quickly used to produce energy and glycogen. Foods with a high glycemic index are best used immediately before or immediately after training. When using products with a low glycemic index, the blood sugar level increases more slowly. Carbohydrates from such foods are not acquired immediately, but provide a more lasting effect, so it is more appropriate to use it at least 1.5–2.0 hours before training.

There are special features when using the form of carbohydrates, e.g., combined carbonated drinks used in sports. One of the criteria for assessing the tolerability, and absorption of specialized foods is osmolality, which characterizes the osmotic pressure of liquids and is the sum of cations, anions, and non-electrolytes, i.e. of all kinetically active particles in 1 l of water (or 1 kg of water) and is expressed in MMol per liter (mOsm/l) or MMol per kg (mOsm/kg).

In accordance with the medical and biological requirements for carbohydrate-mineral drinks intended to overcome the effects of dehydration and loss of electrolytes during training and competitions, their osmolality should be in the range of 200–330 mOsm/kg, preferably 270–330 mOsm/kg.

Carbohydrates make a significant contribution to the osmolarity of ready-made beverages. The degree of degradation of complex carbohydrates affects this index of the product, while mono- and disaccharides increase it. Mineral salts being used for replenishment of electrolyte losses also contribute to the osmolarity of beverages.

In this regard, to optimize the carbohydrate-mineral composition of the products being developed, studies have been carried out to determine the osmolarity of solutions of carbohydrates and mineral salts, which, as a rule, form part of hypo- and isotonic drinks. Glucose and fructose, related to monosaccharides, have a high osmolarity, and its values increase directly in proportion to the concentration of solutions. Thus, 6% solutions of glucose and fructose have osmolarity of 309 and 341 mOsm/l, and the sugar solution of this concentration has an osmolality of 180 mOsm/l. The smallest osmolality is represented by solutions of

maltodextrins (dextrose equivalent value of 18.9%): a 20% solution has an osmolality of 200 mOsm/l. In this regard, for the preparation of an isotonic beverage, it is necessary to use several carbohydrate components in ratios, which would provide both the optimal osmolarity and the content of carbohydrates required for the restoration of the organism.

To prepare beverages for the replenishment of the body with carbohydrates and salts, various concentrations of salt-electrolyte solutions are introduced into their formulation: calcium lactate, magnesium citrate, potassium citrate, and citrate and sodium chloride, which affect the osmolarity of drinks. Osmolarity of solutions of all salts should be in a direct proportion to their concentration [11].

The principles and strategies for carbohydrates intake in different phases of the training process are presented in Table 1. The data are from [12].

Proteins in the diet of athletes. Proteins are the main 'building' material of the body. They are part of the muscles, ligaments, skin, and internal organs, used as an energy source (1 g of protein ideally gives 4.46 kcal, however, given the cost of digestion, this figure decreases to about 3 kcal).

The protein of the food hydrolyses into the amino acids, which are then used as a 'building material' for body proteins. Therefore, the amino acid composition of the protein is of great importance, especially leucine, isoleucine, and valine. They are a kind of basis around which the entire metabolism of proteins is built. The proteins of milk, meat, and eggs are optimal in nutrition. Meat is rich in glutamine, eggs in methionine. The most balanced composition of the whey protein is cow milk protein (lactoalbumin) and protein contained in egg yolk. Besides, milk contains casein, which is less valuable as a food protein, but not much. Egg protein (albumin) is also a very valuable component of food. Protein-rich foods are eggs, chicken, turkey, cottage cheese, cheese, yoghurt, kefir, milk, lean beef, fish, beans (peas, beans, lentils), and nuts. The assimilation of proteins is essentially related to its structure. Milk and egg proteins, which are in solution in the form of separate molecules 'rolled up into tangles', are absorbed quite well. However, when we get cottage cheese from milk or cook eggs, a process of protein denaturation takes place, in which some of the bonds in the protein molecules are broken, especially the sulfide bridges and weak bonds between some amino acid residues. At the same time, their assimilation becomes more complicated. On the contrary, proteins contained in meat foods, when heat-treated, become more easily assimilated, although their nutritional value decreases. Soy proteins, which have high biological value and good digestibility, are optimal. Proteins of leguminous plants are better absorbed after a long treatment. Plant proteins are mostly obtained from seeds, where the protein is stored as a 'building material' for the future plant. The proteins contained in mushrooms are undesirable, because they are poorly absorbed by the body (because of their fibrous structure, the presence of carbohydrate residues, etc.).

Table 1. Summary of guidelines for carbohydrate intake by athletes

Situation		Carbohydrate targets	Comments on type and timing of carbohydrate intake
Daily needs for fuel and recovery:			
(1) The following targets are intended to provide high carbohydrate availability (i.e, to meet the carbohydrate needs of the muscle and central nervous system) for different exercise loads for scenarios where it is important to exercise with high quality and/or at high intensity. These general recommendations should be fine-tuned with individual consideration of total energy needs, specific training needs, and feedback from training performance.			
(2) On other occasions, when exercise quality or intensity is less important, it may be less important to achieve these carbohydrate targets or to arrange carbohydrate intake over the day to optimize availability for specific sessions. In these cases, carbohydrate intake may be chosen to suit energy goals, food preferences, or food availability.			
(3) In some scenarios, when the focus is on enhancing the training stimulus or adaptive response, low carbohydrate availability may be deliberately achieved by reducing total carbohydrate intake, or by manipulating carbohydrate intake related to training sessions (e.g,, training in a fasted state or undertaking a second session of exercise without adequate opportunity for refuelling after the first session).			
Light	– Low intensity or skill-based activities	3–5 g/kg of athlete's body weight/d	– Timing of intake of carbohydrate over the day may be manipulated to promote high carbohydrate availability for a specific session by consuming carbohydrate before or during the session, or during recovery from a previous session
Moderate	– Moderate exercise program (e.g., ~1 h/d)	5–7 g/kg/d	
High	– Endurance program (e.g., 1–3 h/d moderate to high-intensity exercise)	6–10 g/kg/d	– Otherwise, as long as total fuel needs are provided, the pattern of intake may simply be guided by convenience and individual choice
Very high	– Extreme commitment (e.g., > 4–5 h/d moderate to high-intensity exercise)	8–12 g/kg/d	– Athletes should choose nutrient-rich carbohydrate sources to allow overall nutrient needs to be met
Acute fueling strategies – These guidelines promote high carbohydrate availability to promote optimal performance during competition or key training sessions			
General fueling up	– Preparation for events < 90 min exercise	7–12 g/kg/24 h as for daily fuel needs	– Athletes may choose carbohydrate-rich sources that are low in fiber/residue and easily consumed to ensure that fuel targets are met, and to meet goals for gut comfort or lighter "racing weight"
Carbo-hydrate loading	– Preparation for events > 90 min of sustained/intermittent exercise	36–48 h of 10–12 g/kg body weight/24 h	
Speedy refueling	– < 8 h recovery between 2 fuel-demanding sessions	1–1.2 g/kg/h for first 4 h then resume daily fuel needs	– There may be benefits in consuming small, regular snacks – Carbohydrate-rich foods and drink may help to ensure that fuel targets are met
Pre-event fueling	– Before exercise > 60 min	1–4 g/kg consumed 1–4 h before exercise	– Timing, amount, and type of carbohydrate foods and drinks should be chosen to suit the practical needs of the event and individual preferences/experiences – Choices high in fat/protein/fiber may need to be avoided to reduce risk of gastrointestinal issues during the event – Low glycemic index choices may provide a more sustained source of fuel for situations where carbohydrate cannot be consumed during exercise
During brief exercise	– < 45 min	Not needed	
During sustained high intensity exercise	– 45–75 min	Small amounts, including mouth rinse	– A range of drinks and sports products can provide easily consumed carbohydrate – The frequent contact of carbohydrate with the mouth and oral cavity can stimulate parts of the brain and central nervous system to enhance perceptions of well-being and increase self-chosen work outputs
During endurance exercise, including "stop and start" sports	– 1–2.5 h	30–60 g/h	– Carbohydrate intake provides a source of fuel for the muscles to supplement endogenous stores – Opportunities to consume foods and drinks vary according to the rules and nature of each sport – A range of everyday dietary choices and specialized sports products ranging in form from liquid to solid may be useful – The athlete should practice to find a refuelling plan that suits his or her individual goals, including hydration needs and gut comfort
During ultra-endurance exercise	– > 2.5–3 h	Up to 90 g/h	– As above – Higher intakes of carbohydrate are associated with better performance – Products providing multiple transportable carbohydrates (Glucose:fructose mixtures) achieve high rates of oxidation of carbohydrate consumed during exercise

The 'ideal' protein contains 40 mg of isoleucine, 70 mg of leucine, 55 mg of lysine, 35 mg of methionine and cystine (in total), 60 mg of phenylalanine and tyrosine (in total), 10 mg of tryptophane, 40 mg of threonine, and 50 mg of valine (in 1 g).

Using the composition of the 'ideal' protein, one can calculate the content of essential amino acids in a given protein relative to the ideal one. This criterion is then used to assess the balance of the diet. Analysis of this indicator immediately reveals what amino acids will be missing in nutrition. For example, if the food lacks sulfur-containing amino acids, you can supplement the diet with egg whites. It should be noted that exercises impose requirements on the quality of the protein, and even interchangeable amino acids must come from food in sufficient quantities.

The indicator of the biological value of the protein (BV) is 'the amount of protein stored by the body when eating 100 grams of this protein food'. For the whey protein of cow milk (lactoalbumin, albumin) BV is almost equal to 100, for casein and soy proteins – 75, and for proteins of meat and fish – 80. For most vegetable protein BV is approaching 50. The exception is the protein contained in potato and nuts. The thermal processing of food leads to a drop in the biological value of the protein, but it is necessary, and not only because of the organoleptic properties of food: eating cheese, eggs, for example, can lead to salmonellosis, and raw milk – to intestinal disorders.

Another widely used criterion is the protein efficiency index (PEI). It is determined by the effect of this protein on muscle growth. Performance indicators for different proteins are also different, but here again whey protein remains the leader. The balance of amino acids and the optimal chemical structure are the most important characteristics of the protein.

The newest criterion for the quality of the consumed protein is the amino acid-adjusted digestibility index (PDCAAS). However, it does not take into account the significant difference in the nutritional value of proteins from different sources. Soy protein, caseinate, and egg white are the leaders – 1 (compare: beef – 0.92, peas – 0.69, canned beans – 0.68, oats – 0.8, canned lentils – 0.2, peanuts – 0.52, wheat –0.40, whole wheat gluten – 0.25).

Dietary protein during a workout acts as a trigger and substrate for the synthesis of contractile muscle fibers and metabolic proteins, and also contributes to structural changes in the ligament apparatus and bone tissue of athletes [13, 14]. Studies show that stimulation of the synthesis of muscle proteins in response to even a single sports load occurs for at least 24 hours, with an increase in sensitivity to the inclusion of dietary proteins in muscle tissue [15].

It is widely believed that the requirements for high physical loads in the protein are increased. It is believed that to increase endurance, it is necessary to compensate for the consumption of muscle protein consumed by oxidative processes. To increase strength, it is useful to give extra protein in order to build muscle mass (the so-called anabolic effect). At the same time,

convincing scientific findings confirming these provisions have not been obtained at present (unlike the additional intake of carbohydrates, for which the effect of increasing stamina is a strictly proven fact). Moreover, giving hypothetical assumptions about the benefits of additional amounts of protein, one cannot ignore the obvious negative effects of its overdose, which can begin with a dose of 2–4 g of protein per 1 kg of body weight.

It is now established that, at high physical exercises, despite the increase in energy consumption, the need for protein does not increase very much. An adult who leads an average lifestyle should receive 11–12 % of the daily calorie intake from proteins (both animal and plant ones, approximately in equal proportions). In intensively trained athletes under certain conditions, the quota of protein intake can be slightly increased in comparison with these indicators.

A special role in the diet of athletes of any age and sports qualifications is given to protein products. Maintaining a balance between the synthesis, breakdown and re-synthesis of protein is the basis of physiological adaptation of athlete's muscles to stresses. The recommended protein intake for athletes varies from 1.2 to 2.0 g/kg per day. It is important to control not only the lack of protein in the diet, but also its excess, which affects not only the athletic performance, but also the safety for the body of athletes, especially young ones. Excessive consumption of protein can lead to osteoporosis, impaired renal function, and other pathologies.

The problem of protein dosing in athletes is still the subject of debates. One thing is clear that protein intake beyond these standards does not increase adaptation to the load [16].

It is now generally accepted that protein intake in the amount of 1.2–1.6 g/kg body weight per day provides the optimal amount of amino acids for growth, maintenance, and recovery of all tissues provided adequate calorie intake. In particular, it was shown that in actively trained cyclists (with more than 5900 kcal diurnal energy consumption), a positive balance of nitrogen is observed when the protein is consumed at 1.4 g/kg body weight, which is only 20–40% higher than the protein requirement among people who lead an average lifestyle [17, 18].

Studies have shown that protein synthesis in muscles is gradually optimized depending on physical loads by assimilating proteins with high biological value. Approximately 10 g of essential amino acids are included in the re-synthesis in the muscle tissue already in the early recovery period [14, 18], which is transformed into a recommended protein intake equal to 0.25–0.3 g/kg body weight or 15–25 grams per average weight of the athlete (60–80 kg). Higher doses of protein (e.g., > 40 g of dietary protein) do not have much effect and can be significant only for athletes with a large body weight or when weight loss is required [19].

The proportion of proteins of animal origin should be at least 60%, which provides the desired optimum

for the amino acid composition. The remaining 40% should account for proteins of plant origin. In special cases, the proportion of animal proteins can be 80%: for example, during training, aimed at the development of speed-strength qualities, as well as increasing muscle mass, or performing long and intense training loads.

Minerals in sport. Mineral substances contained in food are extremely important for body's life. Thus, sodium is the main extracellular ion taking part in water transfer, blood glucose, generation and transmission of electrical nerve signals, and muscle contraction. Potassium is the main intracellular ion that takes part in the regulation of water, and acid and electrolyte balance. Chlorine is necessary element for the regulation of osmotic pressure, the formation of gastric acid (chloride ions concentrate mainly in the extracellular fluid).

With intensive training and increased sweating, additional sodium intake (in the form of salt) is recommended to prevent seizures. Excess sodium negatively correlates with the calcium content. The constancy of osmotic pressure and the constancy of the volume of fluid are important interrelated processes of the body. The change in the amount of salts in the body (their retention or loss) is associated with the corresponding compensatory changes in the volume of the liquid. Sodium regulates fluid balance in the intercellular space. Sodium ions are largely responsible for the distribution of water in the body. Consequently, the increase or decrease in sodium ions leads to a proportional fluid retention or loss. The constancy of the osmotic pressure is maintained by changing the volume of the liquid. Sodium also participates in the transport of amino acids, sugars, and potassium.

Most products, such as cheese, bread, meat products, fish, vegetables, and canned foods contain sodium chloride.

The main part of sodium and chlorine ions is excreted from the body with urine, and with intensive work, physical exertion, especially in conditions of elevated ambient temperatures, with sweat. The demand for sodium sharply increases during physical exertion (long-distance running, marathon, etc.). In this case, it is necessary to increase the amount of salt consumed, taking into account the food content of up to 20–25 g per day [20]. Sodium chloride is widely used as an additive to food. The intake of sodium chloride with food can fluctuate significantly. With excessive consumption of sodium chloride in the body, the liquid is retained. Increased salt intake is one of the main risk factors for the development of arterial hypertension [21], including in athletes.

At the same time, it should be noted, that to avoid the risk of dehydration and reduce performance people, who are engaged in heavy work or exertion, one should use drinks containing carbohydrates and electrolytes during and after physical activity. The use of dilute solutions of carbohydrates and electrolytes (including sodium chloride because of its loss with sweat) has a more favorable effect on the recovery of the organism under severe working conditions than the use of water only [22, 23].

With short physical exertion, there is no need to consume additional amounts of sodium. Evidently, the compensation of the losses of this electrolyte acquires in the course of prolonged heavy physical exertion, to maintain its concentration in the blood plasma and the osmotic pressure. Specialized food products, including beverages, can be used.

The sodium concentration in such beverages varies generally between 20 and 40 Mmol/l. The purpose of adding this electrolyte is not only to recover its reserves. It pursues the goal of maintaining the volume of extracellular fluid, increasing the rate of absorption of water and glucose in the small intestine. Moreover, the addition of sodium to the drink contributes to the desire to drink, and this can increase the amount of fluid consumed, which is favorable for maintaining the volume of extracellular fluid [24, 25].

The inclusion of various carbohydrates in beverages, including glucose, sucrose, and maltodextrin, has certain advantages both in terms of the rate of absorption of water and sugars, as well as in improving the taste of the drink [26]. Taste sensations play an important role, since they can affect the amount of drink consumed.

Thus, with heavy physical exertion and unfavorable environmental factors (high temperature), dilute solutions remain the advantage. In most situations, a carbohydrate concentration of 2–8% is recommended. As noted above, in practice, mixtures of various carbohydrates are often used, including free glucose, sucrose, maltose, and maltodextrin.

The addition of fructose is permissible, but it is worthwhile to avoid the use of high concentrations or fructose alone, since fructose absorption is worse than glucose, and, ultimately, high doses can lead to a risk of diarrhea. Rehydration after physical exertion is an important part of the recovery process. To compensate for losses, it is recommended to use a volume of liquid that exceeds by at least 50% its amount lost with sweat [27]. In order to quickly restore the body's resources, dilute solutions of glucose with the addition of sodium chloride are used, since these hypotonic solutions are most effective in reducing the retention in the stomach and absorption in the intestine.

Replenishment of fluid loss in the body of athletes should occur due to regular compliance with the drinking regime. It is shown that the loss of 9–12% of water is an emergency situation for the body and can lead to death. Loss of 2% of weight due to water reduces the workability by 3–7%, while with the loss of 40% of protein, fat and carbohydrates, a person can stay alive for a long time. In severe physical exertion it is necessary to monitor the state of the water balance and continuously replenish the fluid loss. Water comes in with liquids and foods and as a result of metabolic processes. The first way gives about 60% of the total water consumption, the second one – 30% and the third – about 10%. There are also different ways of removing water from the body. 50–60% of water per day is discharged with urine, about 20% - with exhaled air, 15–20% with sweat (depending on the intensity of exercise), and less than 5% – with feces. The average person needs about two liters of water a day to make up for the losses. With intensive loads, expenditure

increases, reaching 3–4 l per day. It is proved that when the volume of fluid in the body decreases by 2%, the athlete's result may deteriorate by 15%.

Biochemical and physiological recovery of the body begins immediately after physical activity. The most efficient way to compensate for the loss of a large amount of water and salts can be with the help of weakly acidic and slightly sweet mineralized drinks, and the hypo-and isotonic solutions of carbohydrate-mineral complexes which are the most physiological.

CONCLUSION

Optimization of nutrition of athletes is very important for the nutritional support in all sport specializations. Macronutrients as well as minerals and vitamins are functionally active components, especially in endurance sport. Optimal uses of diets, including specialized sport nutrition, improves biochemical, immune, and endocrine body's functions and restores energy balance at different stages of sport exercises. Nutritional factors are one of the most important key factors in the risk prevention measures for a large number of diet-dependent diseases (e.g. digestive, liver, pancreas, cardiovascular system, endocrine system, kidney diseases).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Oxidative stability of edible oils via addition of pomegranate and orange peel extracts

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Abstract: The main objective of the present study was to improve the oxidative stability of sunflower oil (SFO) and soybean oil (SBO). The aqueous ethanol extracts (80% ethanol) of pomegranate and Baladi orange peels were used as natural antioxidants at concentrations of 800 and 1,200 ppm in SFO and SBO in comparison to butylated hydroxytoluene (BHT). Their antioxidant activities were estimated via the Rancimat method and over 24 days of storage at 65°C. The effect of extracts on the stability of sunflower and soybean oils during the storage period was studied by measuring the peroxide value (PV), conjugated dienes (CD) at 232 nm, conjugated trienes (CT) at 270 nm, free fatty acids (FFAs), iodine value (IV), and the refractive index (RI). A great difference in PVs was observed between the control sample and the oil samples containing natural extracts which slowed the rate of peroxide formation. Generally, the results showed that during the storage period at 65°C, the conjugated diene formation followed a similar pattern relative to PV accumulation. The PV, CD, CT, FFA, and RI values of SFO and SBO containing a pomegranate peel extract (PPE) and Baladi orange peel extract (BOPE) at concentrations of 800 and 1,200 ppm were lower than those of SFO and SBO containing 200 ppm BHT, and this trend became apparent during the storage period. The rate of reduction of IV in the control was higher than that in SFO and SBO containing both synthetic and natural antioxidants. These findings confirmed that the natural antioxidants under investigation could be used as alternatives to synthetic antioxidants to improve the oxidative stability of edible oils in the food industry.

Keywords: Pomegranate peel extract, baladi orange peel extract, sunflower oil, soybean oil, oxidative stability

INTRODUCTION

Lipid peroxidation that results from the reaction between unsaturated fatty acids and molecular oxygen is a severe problem for the oil and fat industry [1]. This process not only deteriorates the quality of fats and fatty foods and results in chemical spoilage, but also produces free radicals, such as peroxyl and hydroxyl radicals, and reactive oxygen species (ROS) that are reportedly related to carcinogenesis, mutagenesis, inflammation, ageing, and cardiovascular diseases [2].

Antioxidants are the substances that can prevent or inhibit oxidation processes in the human body and deterioration in food products [3]. Synthetic antioxidants are widely used as food additives to prevent rancidification due to their high performance, low cost, and wide availability [4]. Hence, synthetic antioxidants, such as butylated hydroxytoluene (BHT),

tertiary butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA), are used in edible vegetable oils. These antioxidants used as preservatives in the food industry may be responsible for liver damage and carcinogenesis; for this reason, an interest in the use of natural antioxidants has increased [5].

Plant extracts offer a unique range of applications for holistic health and wellness [6]. Secondary plant metabolites, such as phenolic compounds from plant sources, are highly valuable for their therapeutic attributes as antioxidants [2]. The sources of these metabolites include different plant organs, such as seeds, leaves, and peels.

Pomegranate (*Punica granatum* L.) peels and Baladi orange (*Citrus sinensis*) peels have been studied as potential antioxidant sources due to their high antioxidant activities. The processing of pomegranate

fruits produces 46% peels, 14% seeds, and 40% juice, with by-products representing more than 50% of the total weight [7]. Pomegranate peels contain 249.4 mg/g of phenolic compounds in comparison to only 24.4 mg/g of phenolic compounds in the pulp of pomegranates [8]. Citrus peel that represents approximately half of the fruit mass contains the highest concentrations of flavonoids [9].

These by-products have high antioxidant activities, few studies have dealt with the use of food wastes to stabilize edible oils. Thus, the aim of the present study was to evaluate the antioxidant efficiency of PPE and BOPE as natural antioxidants for improving the oxidative stability of refined bleached deodorized (RBD) sunflower and soybean oils because these peels are the richest sources of phenolic compounds with antioxidant activities.

STUDY OBJECTS AND METHODS

Materials. Freshly refined, bleached and deodorized sunflower and soybean oil samples without any added antioxidants were obtained from Arma Company for Food Industries in the 10th of Ramadan City, Sharkia Governorate, Egypt. The oil samples were immediately stored at -18°C until further analyses. Pomegranate fruits (*Punica granatum* L.) and Baladi orange fruits (*Citrus sinensis*) were obtained from the local markets in Zagazig City, Sharkia Governorate, Egypt. All the chemicals and reagents used in the analytical methods (analytical grade) were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.) and El-Gamhouria Trading Chemicals and Drugs Company, Zagazig, Egypt.

Methods. Preparation of extracts. The plant materials were washed with distilled water and then peeled. The fresh peels were cut into small pieces with a sharp knife for easy drying and directly dried in a hot-air oven at 40°C until completely dried. The dried samples were ground to fine powder using a laboratory blender. The extracts were prepared according to [1] with slight modifications. The material passed through an 80-mesh sieve was used for extraction purposes. The powdered material (10 g) from each sample was extracted with 100 mL of aqueous (80%) ethanol using a magnetic stirrer at room temperature overnight. The extracts were separated from the residues by filtering through a Whatman No. 1 filter paper. The residues were re-extracted twice with the same solvent under the same conditions, and the extracts were pooled. All the combined extracts were concentrated by a rotary evaporator under a vacuum (BÜCHI Rotavapor, Model R-124, Germany) at 45°C to obtain crude extracts.

Incorporation of natural extracts with the oil samples. Fresh RBD sunflower and soybean oil samples without any additives and those containing 1,200 and 800 ppm PPE and BOPE were prepared separately. The crude concentrated extracts of pomegranate and Baladi orange peels were individually added to preheated (50°C) RBD sunflower and soybean oils at concentrations of 800 and 1,200 ppm (w/w). The extracts were weighed and dissolved in 5 mL of 2-propanol to facilitate their dispersion in sunflower and soybean oils [2]. The mixtures were stirred for 30 min at 50°C for uniform dispersion [10]. A synthetic

antioxidant, BHT, was used at its legal limit of 200 ppm to compare the efficiency of the natural antioxidants. A control sample (without any added antioxidant extract) and a sample containing BHT were also prepared for both sunflower and soybean oils under the same conditions. Each 120 g of the prepared sample was placed in an airtight dark brown glass bottle. Subsequently, the prepared oil samples were stored in an oven at a temperature of 65°C for 24 days, whereby each day of storage was equivalent to one month of storage at ambient temperature [11]. The oil samples were periodically analyzed at different intervals.

Determination of oxidative stability by Rancimat. The oxidative stability of the oil samples was estimated as an induction period (hr) according to the method described by [12] using a Rancimat Metrohm Instrument (Ud.CH-9100 Herisau, Switzerland, Model 679) at 100°C with the air pumped at a flow rate of 20 L/h. This assay was conducted at the Oils & Fats Research Dept., Food Technology Research Institute, ARC, Egypt.

Measurement of chemical and physical properties of oils. The peroxide value (PV), free fatty acids, iodine value (measured according to the Hannus method), and the refractive index using a refractometer (INYRL-3-Poland) at 20°C were determined in different oil samples according to the method described by [13].

The UV absorbencies of the oil samples at 232 and 270 nm (K232 and K270) were determined according to the European Official Methods of Analysis [14]. Each oil sample (0.1 g) was dissolved in 10 mL of cyclohexane. The absorption was then read at 232 nm (conjugated dienes) and at 270 nm (conjugated trienes) in a quartz cell with a 1 cm long path using a 1% solution of oil against cyclohexane as a blank in a Pye-Unicam double-beam recording spectrophotometer, model L.

Statistical analyses. The experiments were conducted in triplicate, and the resulting data were expressed as the mean ± SD. The statistical analyses of the data were performed using SPSS software (17.0 for Windows) and analyzed by one-way ANOVA. Significant differences between the means were determined using Duncan's multiple range tests (Duncan, 1955). The significance level was $P \leq 0.05$.

RESULTS AND DISCUSSION

Oxidative stability. The Rancimat method is commonly used to estimate the antioxidative potency of various antioxidants [15]. As shown in Table 1, the addition of the natural antioxidants extracted from pomegranate and Baladi orange peels to sunflower and soybean oil samples resulted in an increase in the oxidative stability of the oils. This increase depended on the concentration of antioxidants as well as the type of oil. The stability of sunflower oil increased from 9.32 hours in the control oil sample (without any additives) to 12.68, 12.88, 14.20, 16.12 and 18.33 hours in the sunflower oil treated with BHT, BOPE (800 ppm), BOPE (1,200 ppm), PPE (800 ppm) and PPE (1,200 ppm), respectively. In addition, the stability of soybean oil increased from 11.70 hours in the control oil sample (without any additives) to 15.00, 15.26, 16.30, 17.74 and 19.60 hours in the soybean oil treated with BHT, BOPE (800 ppm), BOPE (1,200 ppm), PPE

(800 ppm) and PPE (1,200 ppm), respectively. In addition, the results revealed that the PPE showed the highest value for the induction period followed by BOPE. These natural antioxidants are considered beneficial to human health because they inhibit lipid peroxidation and scavenge free radicals. Generally, PPEs exhibit good antioxidant capacities and act as the effective scavengers of several ROS due to their high levels of polyphenolic compounds [16]. Various phenolic compounds present in orange peel extracts prolonged the oxidative stability of the oil samples according to the Rancimat method [17]. The obtained results are close to those reported by [18] and [19].

Peroxide value. PV measures the concentration of peroxides and hydroperoxides formed during the initial stages of lipid oxidation. PV is a good indicator of the primary oxidation products of oils [20]. A saturated iodine solution added to the oil sample reacts with the resulting hydroperoxide from the lipid oxidation and releases free iodine as a product. The liberated iodine is then titrated against sodium thiosulphate. The titration value can be calculated and reported as PV in milliequivalents of oxygen per 1kg of oil [21]. A continuous increase in PV with the increased storage period was observed in all the samples. This increase in the PV is attributed to the formation of hydroperoxides. The results in Table 2 show the extent of the changes in the PV of the sunflower and soybean oils supplemented with PPE and BOPEs at different concentrations compared to the control and sunflower and soybean oil samples containing synthetic antioxidant over the storage period. The PV ranged from 0.96 to 71.87 $meqO_2$/kg oil for sunflower oil supplemented with PPE and from 0.96 to 95.55 $meqO_2$/kg oil for sunflower oil supplemented with BOPE. The sunflower oil sample without any antioxidants (control) reached the maximum PV of 167.36 $meqO_2$/kg oil after 24 days of storage. A great difference in PV was observed between the control and sunflower oil samples containing natural extracts, which slowed the rate of peroxide formation. The PV of sunflower oil containing 1,200 ppm of PPE and BOPE as well as BHT at a concentration of 200 ppm was found to be 62.69, 86.39 and 96.37 $meqO_2$/kg oil after 24 days of storage, respectively. Regarding the effect of the natural antioxidants extracted from pomegranate and Baladi orange peels on the PV of soybean oil during the storage period, the obtained results showed that PV ranged from 0.87 to 57.17 $meqO_2$/kg oil for the soybean oil supplemented with PPE, and from 0.87 to 76.76 $meqO_2$/kg oil for the soybean oil supplemented with BOPE. The soybean oil without any antioxidants (control) reached the maximum PV of 139.19 $meqO_2$/kg oil after 24 days of storage. There was also a great difference in PV between the control and soybean oil samples containing natural extracts. The PV of soybean oil containing 1,200 ppm of PPE and BOPE as well as BHT at a concentration of 200 ppm was observed to be 46.71, 69.09, and 78.99 $meqO_2$/kg oil after 24 days of storage, respectively. Based on PV, the studied antioxidants can be arranged in the following descending order according to their efficiency in maintaining the quality of the studied oils: PPE (1,200 ppm), PPE (800 ppm), BOPE (1,200 ppm),

BOPE (800 ppm), and BHT (200 ppm). The inhibition of oil oxidation was highly dependent on concentration; therefore, all the extracts were effective at 800 ppm as compared to BHT (200 ppm). These data suggest the superior antioxidant activity of pomegranate and orange peel extracts compared to a synthetic antioxidant. These results confirm the findings of [22], [23], and [6].

Conjugated dienes. The estimation of the conjugated dienes (CD) at 232 nm is a good measure of the oxidation state of oils [20]. CDs are hydroperoxides formed with the shift in the position of the double bond(s) during the oxidation of polyunsaturated fatty acids [24]. The results in Table 3 show that the UV absorbance at 232 nm for the sunflower and soybean oils without any additives at the beginning of the storage period were 1.405 and 1.289, respectively. Subsequently, they increased at the end of the storage period to 25.32 and 22.59 for the sunflower and soybean oils, respectively. The content of CDs (in the case of the natural antioxidants extracted from pomegranate peel and Baladi orange peel) was increased at the end of the storage period to reach 10.53 and 9.66 for PPE at a concentration of 1,200 ppm and 15.73 and 13.32 for BOPE at a concentration of 1,200 ppm for the sunflower and soybean oils, respectively. The UV absorbance values at 232 nm for the sunflower and soybean oils treated with BHT at a concentration of 200 ppm at the end of the storage period were 17.47 and 15.73, respectively. Generally, the results showed that during the storage period at 65°C, the conjugated diene formation followed a similar pattern relative to PV accumulation. Both of these indices measure the primary products of lipid oxidation. It was found in [6] that the conjugated diene content of sunflower oil without any additives (control) was 25.55 ± 0.38, whereas the contents in the sunflower oil containing BHA at a concentration of 200 ppm, kenaf seed extract (KSE), roselle seed extract (RSE), and roselle extract (RE) at a concentration of 1,500 ppm were 18.26±0.59, 17.53 ± 0.41, 13.55 ± 0.33 and 15.13 ± 0.46, respectively, after 24 days of storage at 65°C. The obtained results are consistent with the findings of [10] who reported that the conjugated diene and triene values of sunflower oil with and/or without the different extracts were higher than those of soybean oil with and/or without different extracts after 15 days of storage at 65°C.

Table 1. Effect of natural and synthetic antioxidants on the oxidative stability of sunflower and soybean oils as determined by the Rancimat apparatus at 100°C

Oil treatment	Induction period, hours	
	Sunflower oil	Soybean oil
Ctrl	9.32	11.70
BHT-200 ppm	12.68	15.00
BOPE-800 ppm	12.88	15.26
BOPE-1,200 ppm	14.20	16.30
PPE-800 ppm	16.12	17.74
PPE-1,200ppm	18.33	19.60

Note. Ctrl – oil samples without any additives; BHT – butylated hydroxytoluene; BOPE – baladi orange peel extract; PPE – pomegranate peel extract.

Table 2. Changes in the peroxide values (meqO$_2$/kg oil) for the sunflower and soybean oils supplemented with natural and synthetic antioxidants during storage at 65 ± 2°C

Storage time, days	Control	BHT 200 ppm	PPE		BOPE	
			800 ppm	1,200 ppm	800 ppm	1,200 ppm
			Sunflower oil			
0	0.96 ± 0.01	0.96 ± 0.01	0.96 ± 0.01	0.96 ± 0.01	0.96 ± 0.01	0.96 ± 0.01
4	8.77 ± 0.04	4.90 ± 0.03	3.43 ± 0.04	2.86 ± 0.03	4.63 ± 0.08	4.15 ± 0.06
8	24.51 ± 0.16	10.48 ± 0.35	7.03 ± 0.10	5.71 ± 0.04	9.72 ± 0.09	8.63 ± 0.08
12	58.18 ± 0.42	25.82 ± 0.31	16.21 ± 0.18	13.71 ± 0.14	24.27 ± 0.85	20.53 ± 0.66
16	120.38 ± 0.64	53.13 ± 0.49	34.44 ± 0.15	29.44 ± 0.43	50.15 ± 0.65	43.89 ± 0.11
20	149.56 ± 0.20	75.09 ± 1.12	53.63 ± 0.08	46.76 ± 0.62	72.22 ± 0.59	63.95 ± 0.16
24	167.36 ± 0.30	96.37 ± 0.24	71.87 ± 0.63	62.69 ± 0.73	95.55 ± 0.09	86.39 ± 0.18
			Soybean oil			
0	0.87 ± 0.04	0.87 ± 0.04	0.87 ± 0.04	0.87 ± 0.04	0.87 ± 0.04	0.87 ± 0.04
4	6.48 ± 0.02	3.34 ± 0.03	2.58 ± 0.01	2.25 ± 0.02	3.19 ± 0.02	2.93 ± 0.02
8	19.07 ± 0.08	7.82 ± 0.09	5.20 ± 0.04	4.61 ± 0.04	7.42 ± 0.03	6.36 ± 0.05
12	43.24 ± 0.04	20.86 ± 0.30	13.79 ± 0.16	11.82 ± 0.12	18.01 ± 0.14	16.40 ± 0.18
16	96.65 ± 0.05	43.16 ± 0.18	29.58 ± 0.36	25.31 ± 0.12	39.88 ± 0.35	35.66 ± 0.23
20	123.71 ± 0.28	60.81 ± 0.15	44.14 ± 0.29	36.60 ± 0.41	58.25 ± 0.05	52.32 ± 0.33
24	139.19 ± 0.11	78.99 ± 0.50	57.17 ± 0.65	46.71 ± 0.42	76.76 ± 0.23	69.09 ± 0.17

Note. The values are expressed as mean ± SD. BHT – butylated hydroxytoluene; PPE – pomegranate peel extract; BOPE – baladi orange peel extract.

Conjugated trienes. The estimation of conjugated trienes (CT) is a good parameter for measuring the oxidative deterioration of oils: it indicates the effectiveness of antioxidants in oils [21]. CT may be produced by the dehydration of conjugated diene hydroperoxides [25]. The effects of BHT, PPE, and BOPE additions to sunflower oil on ultraviolet absorbance at 270 nm are also shown in Table 3. There were regular increases in CT for all the samples over the storage period. The increase in CT can be arranged in descending order as follows: SFO-Ctrl > SFO-BHT > SFO-BOPE (800 ppm) > SFO-BOPE (1,200 ppm) > SFO-PPE (800 ppm) > SFO-PPE (1,200 ppm). The results showed that the PPE and BOPE controlled the CT values appreciably, and their CT values were lower than those of BHT at every interval revealing the antioxidants efficiency in improving the stability of sunflower oil by delaying the formation of secondary oxidation products. [26] described the antioxidant activity of garlic extracts in sunflower oil as assessed under accelerated conditions when using CT as an indicator of oxidative degradation. With regard to the effect of the natural antioxidants extracted from pomegranate and Baladi orange peels on ultraviolet absorbance at 270 nm in the soybean oil, Table 3 shows a relative increase in the CT contents of soybean oil under accelerated storage. The CT values continued to increase with the increased storage time. The highest CT content was observed for SBO-Ctrl showing a higher intensity of oxidation, followed by SBO-BHT, SBO-BOPE (800 ppm), SBO-BOPE (1,200 ppm), SBO-PPE (800 ppm) and SBO-PPE (1,200 ppm). The CT contents of PPE and BOPE also increased but at a slower rate than that in the control. This finding demonstrated the antioxidant potential of PPE and BOPE in the stabilizing oils. Notably, BHT showed relatively low antioxidant properties in preventing lipid oxidation compared to PPE and BOPE. The similar results were found by [22] who reported that the control exhibited the highest CD and CT contents followed by BHT and PPE. [1] reported that the corn oil without any additives exhibited the highest CD and CT contents followed by BHT, citrus peel extract, and pomegranate peel extract.

Free fatty acids. The free fatty acid content (FFA % as oleic acid) is used as an indicator of oil hydrolysis. FFA are formed due to the hydrolysis of triglycerides in oil and are considered one of the important indicators of oil rancidity [27]. Table 4 depicts the extent of changes in the FFAs in the sunflower and soybean oils stored with PPE, BOPE, and BHT. The SFO samples containing PPE and BOPE at concentrations of 800 and 1,200 ppm had a lower FFA content than the SFO sample containing 200 ppm BHT over the storage period. The FFA values of SFO containing PPE and BOPE were lower than those of SFO without any additives (control). Table 4 also shows the effect of the storage period on FFA content in the soybean oil treated with different antioxidants. The FFA content of the SBO sample without any additives was higher than that of the SBO samples that contained PPE and BOPE. The FFA values of SBO containing PPE and BOPE at concentrations of 800 and 1,200 ppm were lower than those of SBO containing 200 ppm BHT, and this trend became apparent during the storage period. These results confirmed the findings of [23] who reported that the soybean oil treated with an ethanolic extract of orange peel at concentrations of 800, 1,200 and 1,600 ppm showed lower FFA contents than the soybean oil treated with synthetic antioxidants (BHT and BHA, 200 ppm) at the end of the storage period. [28] reported that after 7 days of storage at 80°C, the FFA contents of the sunflower oil supplemented with pomegranate peel extract and sour orange peel extract at a concentration of 800 μg/mL were lower than those of the sunflower oil supplemented with BHT (200 μg/mL).

Table 3. Changes in the conjugated diene and triene values for the sunflower and soybean oils supplemented with natural and synthetic antioxidants during storage at $65 \pm 2°C$

Storage time, days	Control	BHT 200 ppm	PPE		BOPE	
			800 ppm	1,200 ppm	800 ppm	1,200 ppm
DIENE						
Sunflower oil						
0	1.405 ± 0.01	1.405 ± 0.01	1.405 ± 0.01	1.405 ± 0.01	1.405 ± 0.01	1.405 ± 0.01
4	4.94 ± 0.04	2.95 ± 0.02	2.44 ± 0.06	2.26 ± 0.05	2.91 ± 0.06	2.73 ± 0.03
8	8.72 ± 0.03	6.42 ± 0.04	4.15 ± 0.06	3.03 ± 0.06	5.82 ± 0.04	5.21 ± 0.04
12	11.66 ± 0.17	8.82 ± 0.08	5.91 ± 0.04	4.30 ± 0.06	8.15 ± 0.05	7.74 ± 0.07
16	17.79 ± 0.03	13.07 ± 0.13	8.61 ± 0.05	6.38 ± 0.04	11.45 ± 0.06	10.34 ± 0.08
20	21.17 ± 0.06	15.22 ± 0.08	10.92 ± 0.06	8.68 ± 0.10	14.29 ± 0.04	12.76 ± 0.10
24	25.32 ± 0.07	17.47 ± 0.06	13.12 ± 0.04	10.53 ± 0.11	16.88 ± 0.06	15.73 ± 0.11
Soybean oil						
0	1.289 ± 0.01	1.289 ± 0.01	1.289 ± 0.01	1.289 ± 0.01	1.289 ± 0.01	1.289 ± 0.01
4	4.21 ± 0.05	2.61 ± 0.05	2.26 ± 0.09	2.09 ± 0.08	2.52 ± 0.04	2.45 ± 0.05
8	7.57 ± 0.11	5.78 ± 0.06	3.72 ± 0.07	2.67 ± 0.06	5.25 ± 0.05	4.67 ± 0.06
12	10.80 ± 0.23	8.09 ± 0.08	5.27 ± 0.08	3.80 ± 0.06	7.58 ± 0.09	6.93 ± 0.05
16	16.42 ± 0.16	11.95 ± 0.09	7.52 ± 0.10	6.05 ± 0.05	10.78 ± 0.06	9.30 ± 0.11
20	18.87 ± 0.10	14.02 ± 0.08	9.63 ± 0.06	7.83 ± 0.08	13.12 ± 0.07	11.77 ± 0.10
24	22.59 ± 0.18	15.73 ± 0.09	11.85 ± 0.09	9.66 ± 0.08	14.54 ± 0.08	13.32 ± 0.10
TRIENE						
Sunflower oil						
0	0.644 ± 0.03	0.644 ± 0.03	0.644 ± 0.03	0.644 ± 0.03	0.644 ± 0.03	0.644 ± 0.03
4	1.83 ± 0.03	1.19 ± 0.05	0.85 ± 0.05	0.76 ± 0.04	1.05 ± 0.06	0.94 ± 0.02
8	4.06 ± 0.05	2.04 ± 0.06	1.40 ± 0.03	1.14 ± 0.04	2.01 ± 0.08	1.76 ± 0.04
12	6.89 ± 0.09	3.71 ± 0.09	2.49 ± 0.03	2.06 ± 0.06	3.56 ± 0.05	3.15 ± 0.04
16	9.82 ± 0.09	5.86 ± 0.07	3.80 ± 0.02	2.72 ± 0.08	5.37 ± 0.08	4.84 ± 0.06
20	12.84 ± 0.11	8.39 ± 0.10	5.59 ± 0.08	4.23 ± 0.04	7.95 ± 0.12	7.11 ± 0.03
24	15.59 ± 0.10	9.60 ± 0.16	6.99 ± 0.17	5.65 ± 0.10	9.40 ± 0.08	8.66 ± 0.06
Soybean oil						
0	0.461 ± 0.01	0.461 ± 0.01	0.461 ± 0.01	0.461 ± 0.01	0.461 ± 0.01	0.461 ± 0.01
4	1.49 ± 0.03	0.96 ± 0.04	0.71 ± 0.04	0.64 ± 0.03	0.88 ± 0.04	0.82 ± 0.04
8	3.55 ± 0.13	1.88 ± 0.08	1.20 ± 0.04	0.99 ± 0.06	1.76 ± 0.04	1.62 ± 0.08
12	5.36 ± 0.09	3.15 ± 0.06	2.41 ± 0.05	1.87 ± 0.03	3.05 ± 0.06	2.84 ± 0.05
16	7.92 ± 0.12	4.82 ± .04	3.37 ± 0.06	2.57 ± 0.06	4.75 ± 0.08	4.27 ± 0.04
20	10.94 ± 0.07	6.89 ± 0.10	4.91 ± 0.11	3.80 ± 0.09	6.66 ± 0.08	6.25 ± 0.06
24	13.27 ± 0.08	8.36 ± 0.09	6.23 ± 0.08	5.27 ± 0.07	7.98 ± 0.07	7.32 ± 0.03

Note. The values are expressed as mean ± SD. BHT – butylated hydroxytoluene; PPE – pomegranate peel extract; BOPE – baladi orange peel extract.

Table 4. Changes in the free fatty acids content (% as oleic acid) for the sunflower and soybean oils supplemented with natural and synthetic antioxidants during storage at $65 \pm 2°C$

Storage time, days	Control	BHT 200 ppm	PPE		BOPE	
			800 ppm	1,200 ppm	800 ppm	1,200 ppm
Sunflower oil						
0	0.067 ± 0.002	0.067 ± 0.002	0.067 ± 0.002	0.067 ± 0.002	0.067 ± 0.002	0.067 ± 0.002
4	0.129 ± 0.001	0.104 ± 0.001	0.096 ± 0.001	0.091 ± 0.001	0.102 ± 0.001	0.099 ± 0.001
8	0.208 ± 0.001	0.152 ± 0.001	0.130 ± 0.001	0.122 ± 0.001	0.142 ± 0.001	0.137 ± 0.002
12	0.300 ± 0.002	0.199 ± 0.001	0.171 ± 0.001	0.166 ± 0.002	0.196 ± 0.001	0.180 ± 0.002
16	0.413 ± 0.002	0.267 ± 0.002	0.218 ± 0.001	0.212 ± 0.001	0.255 ± 0.003	0.239 ± 0.001
20	0.493 ± 0.002	0.313 ± 0.001	0.253 ± 0.001	0.240 ± 0.001	0.294 ± 0.001	0.278 ± 0.002
24	0.545 ± 0.003	0.346 ± 0.001	0.287 ± 0.002	0.267 ± 0.002	0.331 ± 0.002	0.317 ± 0.002
Soybean oil						
0	0.046 ± 0.001	0.046 ± 0.001	0.046 ± 0.001	0.046 ± 0.001	0.046 ± 0.001	0.046 ± 0.001
4	0.113 ± 0.001	0.088 ± 0.001	0.074 ± 0.001	0.070 ± 0.001	0.080 ± 0.001	0.078 ± 0.001
8	0.191 ± 0.001	0.139 ± 0.001	0.117 ± 0.001	0.108 ± 0.001	0.136 ± 0.001	0.125 ± 0.001
12	0.284 ± 0.003	0.194 ± 0.002	0.158 ± 0.001	0.150 ± 0.001	0.186 ± 0.001	0.171 ± 0.001
16	0.385 ± 0.003	0.255 ± 0.001	0.204 ± 0.002	0.196 ± 0.002	0.247 ± 0.001	0.231 ± 0.001
20	0.460 ± 0.001	0.292 ± 0.001	0.238 ± 0.001	0.223 ± 0.001	0.278 ± 0.002	0.264 ± 0.001
24	0.499 ± 0.002	0.323 ± 0.002	0.267 ± 0.001	0.248 ± 0.001	0.309 ± 0.001	0.296 ± 0.002

Note. The values are expressed as mean ± SD. BHT – butylated hydroxytoluene; PPE – pomegranate peel extract; BOPE – baladi orange peel extract.

Table 5. Changes in the iodine value (gI$_2$/100g oil) for the sunflower and soybean oils supplemented with natural and synthetic antioxidants after storage at 65 ± 2°C for 24 days

Oil type	Storage time, days	Control	BHT (200 ppm)	PPE		BOPE	
				800 ppm	1200 ppm	800 ppm	1200 ppm
SFO	0	135.52 ± 0.91	135.52 ± 0.91	135.52 ± 0.91	135.52 ± 0.91	135.52 ± 0.91	135.52 ± 0.91
	24	100.57 ± 1.23	113.34 ± 1.51	118.17 ± 0.68	119.99 ± 0.76	113.69 ± 0.83	115.72 ± 0.34
SBO	0	138.73 ± 0.72	138.73 ± 0.72	138.73 ± 0.72	138.73 ± 0.72	138.73 ± 0.72	138.73 ± 0.72
	24	109.13 ± 1.48	118.64 ± 0.94	123.99 ± 0.21	125.34 ± 0.40	119.30 ± 1.13	121.27 ± 0.50

Note. The values are expressed as mean ± SD. SFO – sunflower oil; SBO – soybean oil; BHT – butylated hydroxytoluene; PPE – pomegranate peel extract; BOPE – baladi orange peel extract.

Table 6. Changes in the refractive index values (at 20°C) for the sunflower and soybean oils supplemented with natural and synthetic antioxidants during storage at 65 ± 2°C

Oil type	Storage time (days)	Control	BHT (200 ppm)	PPE		BOPE	
				800 ppm	1,200 ppm	800 ppm	1,200 ppm
SFO	0	1.4752	1.4752	1.4752	1.4752	1.4752	1.4752
	12	1.4780	1.4769	1.4765	1.4762	1.4769	1.4767
	24	1.4816	1.4794	1.4784	1.4780	1.4793	1.4789
SBO	0	1.4754	1.4754	1.4754	1.4754	1.4754	1.4754
	12	1.4773	1.4765	1.4761	1.4760	1.4764	1.4763
	24	1.4805	1.4787	1.4777	1.4774	1.4785	1.4781

Note. The obtained results represented the mean of triplicate determinations results. The standard deviation for all the tested samples in this table = 0.000; SFO – sunflower oil; SBO – soybean oil; BHT – butylated hydroxytoluene; PPE – pomegranate peel extract; BOPE – baladi orange peel extract.

Iodine value. Sunflower and soybean oils contain a great deal of polyunsaturated fatty acids (PUFAs). These PUFAs are highly prone to lipid oxidation [29]. During storage, the double bonds of these PUFAs are attacked by free radicals, which results in the formation of conjugated bonds [30]. Hence, the measurement of the amount of the unsaturated fatty acids present in sunflower and soybean oils can be used as a reference to determine the freshness of oil [31]. The freshness of sunflower and soybean oils can be determined quantitatively by measuring the iodine value (IV) [27]. Unsaturated fatty acids react with iodine monobromide and release free iodine. Free iodine can then react with sodium thiosulphate. The results are then calculated and expressed in g I$_2$/100 g. [32] demonstrated that IV highly diminished with increasing the storage time. The double bonds of unsaturated fatty acids in the oil are broken during storage, and IV decreased [33]. The variations in IV for the supplemented oil samples and the control after an accelerated storage period at 65°C for 24 days are shown in Table 5. IV was 135.52 g I$_2$/100 g for all the sunflower oil samples before storage. After 24 days of storage, IV were 100.57, 113.34, 119.99, 118.17, 115.72, and 113.69 g I$_2$/100 g for SFO-Ctrl, SFO-BHT, SFO-PPE (1,200 ppm), SFO-PPE (800 ppm), SFO-BOPE (1,200 ppm) and SFO-BOPE (800 ppm), respectively. However, IV was 138.73 g I$_2$/100 g for all the soybean oil samples before storage. After 24 days of storage, IV were 109.13, 118.64, 125.34, 123.99, 121.27 and 119.30 g I$_2$/100 g for SBO-Ctrl, SBO-BHT, SBO-PPE (1,200 ppm), SBO-PPE (800 ppm), SBO-BOPE (1,200 ppm) and SBO-BOPE (800 ppm), respectively. IV was shown to decrease with the increased storage time for all the samples. The rate of reduction of IV in the control was higher than that in the sunflower and soybean oils containing both synthetic and natural antioxidants. A decreasing in IV in oils is generally attributed to a breakdown in the double bonds of the fatty acids caused by the oxidation process. IV at the beginning and end of the storage period was determined according to [34]. These results are in accordance with those reported by [6] and [35] who reported that after 24 days of storage at 65°C, the rate of reduction in IV of sunflower oil without any extracts was higher than that in the sunflower oil supplemented with natural extracts.

Refractive index (RI). The data in Table 6 show the effect of PPE and BOPE on the refractive index (RI) of sunflower and soybean oils during the accelerated storage at 65°C for 24 days. The RI values of the supplemented sunflower and soybean oil samples and the control demonstrated a gradual increase with the duration of the storage period. After 24 days of storage, the untreated sunflower and soybean oil samples (control) demonstrated higher RT values than the sunflower and soybean oil samples containing natural extracts and BHT. The RI values of the supplemented sunflower oil samples and control were 1.4752 before storage, and this value became 1.4816, 1.4794, 1.4780, 1.4784, 1.4789, and 1.4793 at the end of the storage period for SFO-Ctrl, SFO-BHT, SFO-PPE (1,200 ppm), SFO-PPE (800 ppm), SFO-BOPE (1,200 ppm) and SFO-BOPE (800 ppm), respectively. With regard to the extent of the changes in RI of the soybean oil supplemented with PPE and BOPEs when compared to the control and the soybean oil samples containing synthetic antioxidant after 24 days of storage, the results showed that the RI values of the supplemented soybean oil samples and control were 1.4754 before storage, and this value reaching 1.4805, 1.4787, 1.4774, 1.4777, 1.4781, and 1.4785 at the end of the storage period for SBO-Ctrl, SBO-BHT, SBO-PPE (1,200 ppm), SBO-PPE (800 ppm), SBO-BOPE (1,200 ppm) and SBO-

BOPE (800 ppm), respectively. These results are consistent with [20] who reported that oxidation may result in an increase in RI of edible oils due to oxidative deterioration and increased conjugation.

CONCLUSION

The present study demonstrates that pomegranate and orange peel extracts have strong protective effects against the oxidation of sunflower and soybean oils. In addition, the study showed a positive correlation between the results obtained using the Rancimat method and the oven test (during storage at 65°C for 24 days) in the sunflower and soybean oils treated with the natural antioxidants extracted from pomegranate and orange peels at concentrations of 800 and 1,200 ppm. These findings have confirmed that the natural antioxidants under investigation could be used as alternatives to synthetic antioxidants to improve the oxidative stability of edible oils in the food industry.

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Research Article
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Analysis of myofibrillar and sarcoplasmic proteins in pork meat by capillary gel electrophoresis

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Abstract: Myofibrillar and sarcoplasmic proteins were extracted from pork meat (*M. Longissimus dorsi*) and then separated by capillary gel electrophoresis (CGE). Migration time and peak areas of individual protein molecules in the electropherogram were analysed. The electropherograms obtained after the separation of myofibrillar proteins contained 53 well-separated peaks, of which the following were identified: thymosin, myosin light chain-3 (MLC-3), myosin light chain-2 (MLC-2), troponin C, troponin I, myosin light chain-1 (MLC-1), tropomyosin 1, tropomyosin 2, troponin T, actin, desmin, troponin, C protein, and myosin heavy chain (MHC). The relative concentration of the identified myofibrillar proteins was 74.5%. Of the 56 separated sarcoplasmic proteins the following were identified: myoglobin, myokinase, triosephosphate isomerase, phosphoglycerate mutase, lactate dehydrogenase, glyceraldehyde phosphate dehydrogenase, aldolase, creatine kinase, enolase, phosphoglucose isomerase, pyruvate kinase, phosphoglucomutase, and phosphorylase b. The relative concentration of the identified sarcoplasmic proteins was 83.6% of all sarcoplasmic proteins extracted from the pork meat.

Keywords: Myofibrillar proteins, sarcoplasmic proteins, capillary gel electrophoresis

INTRODUCTION

The quality of meat and meat products depends on the amount and state of proteins present there. Biochemical reactions occurring during processing and storage of meat affect the structure and functional properties of meat protein [1, 2]. These changes lead to two directions: splitting of large protein molecules and aggregating of small protein molecules. The result is protein denaturation that affects the technological properties of meat. Changes in myofibrillar proteins have a great influence on the texture and other properties of meat and meat products [2]. In order to properly manage the processing and storage of pork meat, it is necessary to determine the state of the present proteins. Several techniques are used to analyse myofibrillar and sarcoplasmic proteins in meat.

Polyacrylamide gel electrophoresis (PAGE) is often used for proteins separation [3]. PAGE can be used to determine native and denatured proteins. There are several techniques for carrying out polyacrylamide gel electrophoresis: disc gel electrophoresis [4, 5], one-dimensional SDS-polyacrylamide gel electrophoresis

(1D SDS-PAGE) [6–9], and two-dimensional SDS-polyacrylamide gel electrophoresis (2D SDS-PAGE) [10]. A capillary gel electrophoresis (CGE) was developed as an alternative to the traditional slab-gel PAGE [1, 11, 12]. The separation of proteins by CGE is based on the difference in the electrophoretic mobility of ions in the medium within small capillaries. The size of a molecule has a significant influence on the mobility of the ions [13]. New technical solutions, transferred from high-performance liquid chromatography (HPLC) instruments, enabled the development of high-performance instruments, such as high-performance capillary electrophoresis (HPCE). HPCE is a modern analytical technique based on the principles of classical electrophoresis and chromatographic performance [14]. Different HPCE techniques allow using of empty or filled capillaries. In many cases, a capillary is filled with polyacrylamide gel. When a sample is treated in an SDS-containing buffer, the technique is known as capillary SDS-CGE. It has a similar protein separation possibility as a slab-gel SDS-PAGE. SDS-CGE has several advantages over the traditional slab gel

electrophoresis, including on-capillary detection, great resolving power, a capability of accurate protein quantification and molecular weight determination, and instrumental automation [15]. SDS-CGE provides rapid separation of peptides and proteins from complex mixtures such as meat, milk, grains and other food products [1, 16, 17].

To determine the influence of soluble and insoluble proteins on meat quality, precise methods for quantifying each protein need to be developed. Quantification of isolated proteins is a complex problem. The process of densitometric scanning of coloured SDS-PAGE gels in the traditional slab PAGE is complicated and insufficiently precise. For this reason, several authors recommend the application of new techniques. The aim of this paper is to investigate application possibilities of high performance SDS-CGE for the identification and quantification of myofibrillar and sarcoplasmic proteins in pork meat.

STUDY OBJECTS AND METHODS

Preparation of the capillary electrophoresis (CE) instrument. Electropherograms were obtained using a capillary electrophoresis CE 7100 instrument (Agilent Technologies, PaloAlto, CA) equipped with a non-column DAD for UV detection and spectra collection. Migration time and peak areas of individual protein molecules in the electropherogram were analysed using ChemStation Software (Agilent Technologies, PaloAlto, CA). The preparation of the CE instrument was carried out according to certain recommendations [18].

For CGE analysis using the SDS Gel Buffer from Beckman Coulter, 50 μm ID. Bar-fused silica capillaries were used, with a total length of 33 cm and an effective length of 24.5 cm. One time per day, the capillaries were conditioned as follows: rinsed at high pressure (2 bar) with 0.1 N NaOH during 10 min, with 0.1 N HCl during 5 min and with deionized water during 2 min; then rinsed at high pressure (4 bar) with the SDS Gel Buffer during 10 min. After that, both electrodes were dipped in water. Voltage equilibration was conducted at 16.5 kV during 10 min with 5 min ramping. Prior to each sample application, the capillaries were conditioned as follows: rinsed at high pressure (4 bar) with 0.1 N NaOH during 3 min, with 0.1 N HCl during 1 min, with deionized water during 1 min, and with the SDS Gel Buffer during 10 min. After that, both electrodes were dipped in water.

The injection of the samples was performed electronically using -5 kV for 20 seconds. After dipping the inlet electrode in water, the proteins were separated by using -16.5 kV (-500 V/m^{-2}) during 30 minutes. A pressure of two bars was used to both inlet and outlet vials during the run. After the run was completed, the capillaries were conditioned as follows: rinsed at high pressure (4 bar) with 0.1 N NaOH during 15 min, rinsed at high pressure (3.5 bar) with 0.1 N HCl during 5 min, and with water during 10 min. All the rinses were carried out in the forward direction, that is pressure was used to the inlet vial. The capillary temperature was maintained at 25ºC. The detection wavelength was 220 nm with a bandwidth of 20 nm (no reference wavelength) and a response time of 1 second. The glass vials of 2 mL were used for all utilized reagents. The filling volume was 1.2 mL, except the vials for water dipping, which contained 1.6 mL water, and the vials for waste, which contained 0.6 mL water. Three separate waste vials were used to collect 0.1 N NaOH, 0.1 N HCl/water and the SDS Gel Buffer. The SDS gel buffer was replaced after every sequence of eight runs.

CGE with Beckman Coulter gel. An SDS-MW Analysis Kit was purchased from Beckman Coulter, Fullerton, CA. The Kit contained the following components: 50 μm I.D. bare-fused silica capillaries of 57 cm; SDS-MW Gel buffer – proprietary formulation; SDS; sample buffer – 100 mM Tris-HCl with pH 9.0 and 1% SDS; SDS protein size standard (10 to 225 kDa) 16 mg/mL; internal standard 10 kDa protein 5mg/mL, acidic wash solution 0.1 N HCl; and basic wash solution 0.1 N NaOH.

Preparation of SDS-MW Size Standard. SDS-MW size standard was removed from arefrigerator and left at room temperature for 15 min before starting the sample preparation. The standard was thoroughly mixed and briefly centrifuged in a standard microfuge. 10 μL of the size standard was pipetted into a micro vial, and then 85 μL of the sample buffer, 2 μL of the internal standard, and 5 μL of 2-mercaptoethanol were added, respectively. The vial was tightly capped and thoroughly mixed. The mixture was heated in a water bath at 100°C for 3 min in a closed micro vial. The vial was then placed in a room temperature water bath to cool for 5 min before injection.

Protein extraction from pork meat. For this study, pork meat samples were obtained from *M. Longissimus dorsi* muscle of pigs that lived on a farm near Banja Luka, Bosnia and Herzegovina. The animals were farmed under the same conditions. Before slaughter, they were 6 months old, and their average weight was 110–120 kg. The slaughter was done in an abattoir, under controlled conditions. After slaughtering, the carcasses were cooled to 4°C. The samples for analysis were taken 24 h post mortem and transported to the laboratory under refrigeration. The analysis was carried out immediately after the samples were brought to the laboratory.

The water-soluble proteins were extracted from pork muscles (4 g diluted 1:10 with 0.03 M potassium phosphate buffer, pH = 7.4) at 2°C for 2 min in a homogeniser (Ultraturax, IKA) followed by centrifugation at 5000 rpm for 20 min [19]. The supernatant (sarcoplasmic proteins) was collected for qualitative and quantitative assays. The pellet was washed again by the same procedure. The resulting pellet was then dispersed in 10 volumes of 8 M urea containing 10 ml β-mercaptoethanol per litre, and extracted for 2 min at 2°C in a homogeniser (Ultraturax, IKA) [19]. The homogenate was centrifuged under refrigeration at 5000 rpm for 20 min and the supernatant, containing the myofibrillar proteins, collected for further qualitative and quantitative assays. The sample solutions were filtered using 0.2 μm syringe filters prior to analysis.

Preparation of protein sample. The sample was diluted with the SDS sample buffer for a total 95 μL

volume to obtain a final protein concentration range of 0.2 mg/mL to 2 mg/mL. the amount of protein in the sample was estimated by Lowry's method, using bovine serum albumin (BSA) as a standard. Then 2 µL of internal standard and 5 µL of 2-mercaptoethanol were added. The vial was tightly capped and then thoroughly mixed. The mixture was heated in a water bath at 100°C for 3 min in a closed micro vial. After that, the vial was placed in a room temperature water bath to cool for 5 min before injection.

Protein determination. In order to determine the molecular weights corresponding to the electrophoretic peaks, a calibration curve was obtained using seven proteins in SDS-MW size standard (Beckman Coulter, Inc., Fullerton, CA): uith protein molecular weights of: 10, 20, 35, 50, 100, 150, and 225 kDa. All the proteins were completely separated within 30 min (Fig. 1). The first peak (MT = 12.64 min), corresponding to a 10 kDa protein, was used as an internal standard during the analysis of myofibrillar and sarcoplasmic proteins in pork meat. Within this range, the logarithm of the protein molecular weight is linear with its reciprocal electrophoretic mobility. A standard protein mixture was used to construct a calibration curve (Fig. 2). A regression line was obtained: y (logMw) = 0.088X – 0.0286, R^2 = 0.9874. Protein identification was performed by comparing the obtained molecular weights with the relevant data published in the literature [4, 8, 20, 21].
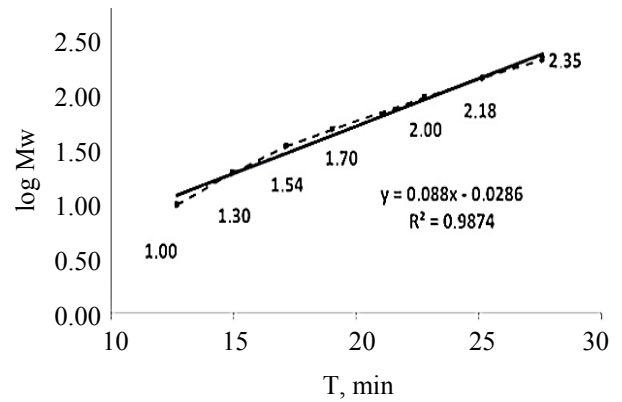

Fig. 2. The calibration curve to assess protein molecular weight.
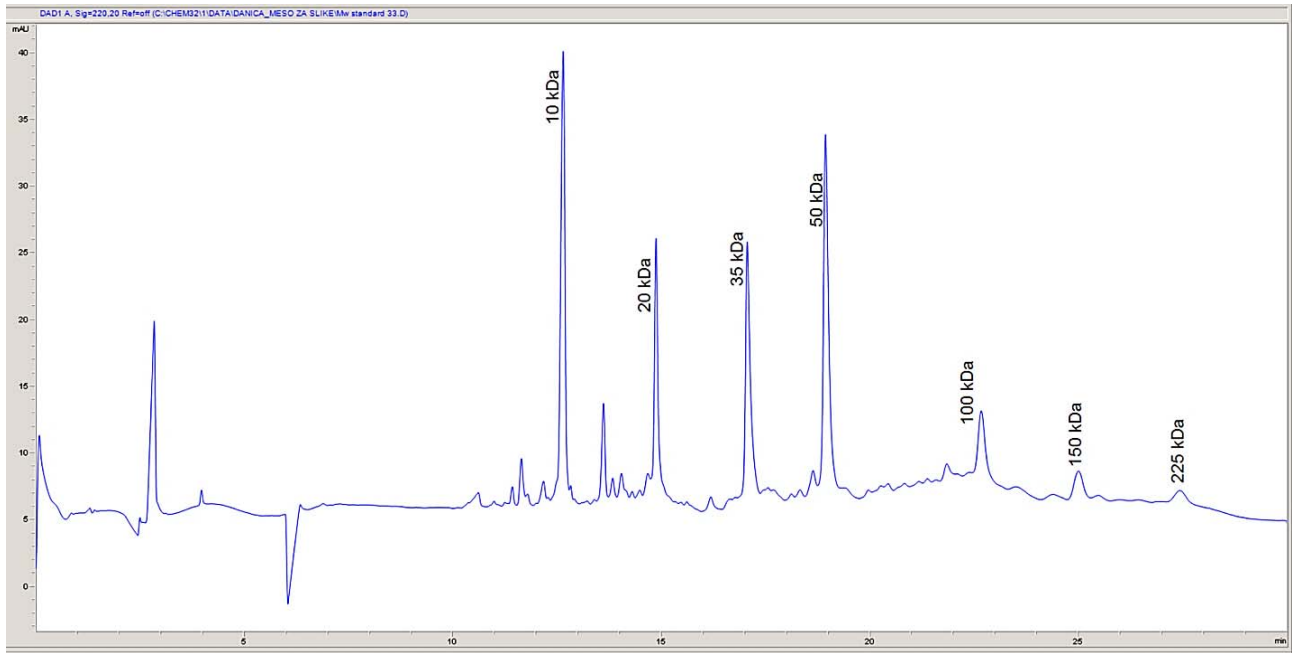
Fig. 1. Capillary SDS gel electrophoresis separation of a standard protein test mixture with a molecular weight range of 10 kDa to 225 kDa, on bare-fused silica capillaries with a total length of 33 cm and an effective length of 24.5 cm, filled with SDS-MW gel buffer, SDS sample buffer – 100 mM Tris-HCl with pH 9.0 and 1% SDS.

Table 1. The migration time and relative concentrations of seven standard proteins

Protein weight, kDa	Migration time			Relative concentration		
	Average, min[1]	Standard deviation (SD)[1]	Coefficient of variation Cv, %[1]	Average, %[1]	Standard deviation (SD)[1]	Coefficient of variation Cv, %[1]
10	12.84	0.17	1.35	25.14	0.49	1.96
20	15.10	0.20	1.33	13.73	0.64	4.46
35	17.32	0.23	1.23	17.34	0.82	4.71
50	19.22	0.25	1.31	25.96	0.49	1.88
100	23.00	0.30	1.31	10.82	0.84	7.73
150	25.34	0.30	1.19	4.13	0.42	10.28
225	27.79	0.32	1.15	2.10	0.24	11.43

Note. [1](n=6)

Fig. 3. The electropherogram obtained after separating myofibrillar proteins from pork meat (*M. longissimus dorsi*).
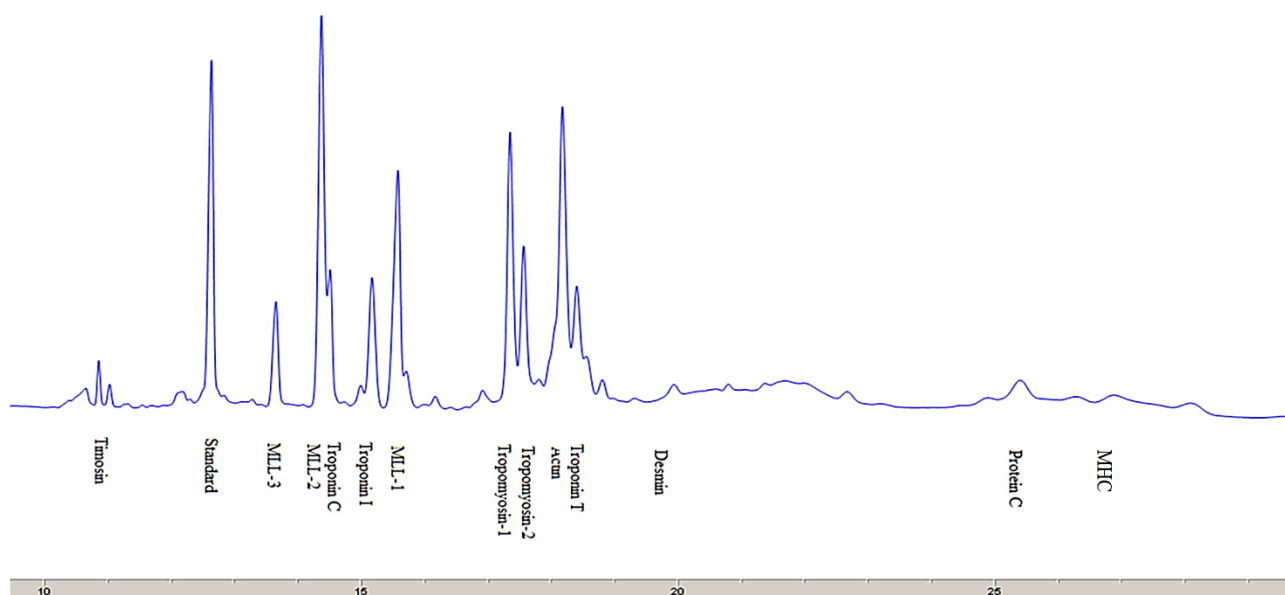
Determination of relative protein concentration. The peak areas and relative protein concentrations were determined by ChemStation Software (Agilent). The areas of peaks in the electropherograms, determined by indirect detection, allowed us to automatically quantify the relative concentration of each protein in the mixture using the capillary instrument software.

Validation method. The repeatability of the protein separation process from a mixture of known molecular weight proteins was evaluated in seven repetitions. The migration time (MT) and relative concentrations of seven proteins (10, 20, 35, 50, 100, 150 and 225 kDa) are shown together with the standard deviation and coefficient variation in Table 1. The results with good repeatability confirm the coefficient of variation in all the measurements of migration time and relative protein concentration being less than 1.35% and 11.43%, respectively. The obtained data were used to identify the separated myofibrillar and sarcoplasmic proteins. It should be noted that all the measurements during the test of repeatability were performed by an analyst using the same measurement procedure and the same measuring instrument in the same working conditions.

RESULTS AND DISCUSSION

There are many proteins in the meat that differ in their chemical and physical characteristics and functions performed in the muscles of living animals and in the meat *post mortem* [22]. The electropherograms of myofibrillar and sarcoplasmic proteins separated by SDS-capillary gel electrophoresis (SDS-CGE) are shown in Fig. 3.

Myofibrillar proteins. Fig. 3 presents an electropherogram obtained after separating myofibrillar proteins from pork meat (*M. longissimus dorsi*). The proteins were separated and detected at a certain time, which was specific and constant for each of the myofibrillar proteins. The total number of peaks obtained after the CGE separation of myofibrillar proteins from the pork meat (*M. longissimus dorsi*) was

52 or 53 (Table 2). The molecular weight of each identified protein was determined on the basis of the migration time and calibration curve obtained on the electropherogram database for the mixture of known molecular weight proteins (Mw standard) (Fig. 2). This technique allowed the separation and identification of proteins to be ranged from 10 kDa to 225 kDa. To separate proteins of very high molecular weight, electrophoretic methods are not sufficient and they need to be combined with other separation techniques. By partial fragmentation of large protein molecules in smaller segments and their analysis, it is possible to obtain qualitative and quantitative data for proteins of high molecular weights [23].

The relative concentration of the separated proteins was determined as a ratio between the corresponding peak area and the total area of all the peaks in the electropherogram (Table 2). Proteins whose migration time was shorter than 10 min (concentration of 0.1%) were not considered. Myofibrillar proteins are characterized by high heterogeneity and a wide range of molecular weights. In order to facilitate electrophoretic studies, it is useful to divide them into several groups that differ by molecular weight: group I (Mw < 20 kDa), group II (Mw = 20–50 kDa), group III (Mw = 50–100 kDa), group IV (Mw = 100–150 kDa). and group V (Mw > 150 kDa). The numbers of peaks separated were as follows: 24–27 peaks in group I (total relative concentration of 26.75–27.87%), 15–17 peaks in group II (total relative concentration of 50.98–53.34%), 6 peaks in group III (total relative concentration of 9.73–10.96%), 1 peak in group IV (total relative concentration of 1.11%), and 4 peaks in group V (total relative concentration 9.08%) (Table 2).

Based on the obtained molecular weights of myofibrillar proteins, the following proteins were identified (Table 3): thymosin, myosin light chain-3 (MLC-3), myosin light chain-2 (MLC-2), troponin C, troponin I, myosin light chain-1 (MLC-1) tropomyosin 1, tropomyosin 2, troponin T, actin, desmin, troponin, C protein, and myosin heavy chain (MHC).

Table 2. The number and total relative concentrations of isolated myofibrillar proteins obtained by capillary gel electrophoresis separation from fresh pork meat

Myofibrillar proteins	Molecular weight range, kDa					
	< 20	20–50	50–100	100–150	> 150	Total
Number of isolated proteins[1]	24–27	15–17	6	1	4	52–53
Relative concentration[1], %	26.75–27.87	50.98–53.34	9.73–10.96	1.11	9.08	100.00

Note. [1]The range shows the results obtained after 6 measurements.

These results are in agreement with the findings of other authors [4, 8, 20, 21].

Sarcoplasmic proteins. The electropherogram obtained by separating sarcoplasmic proteins extracted from the pork meat (*M. longissimus dorsi*) is shown in Fig. 4. The total number of peaks (whose relative concentration was greater than 1%) was 56–58. The proteins were divided into five groups according to their molecular weight. The numbers of peaks identified in the groups were as follows: 34–35 in group I (0–20 kDa), 14–15 in group II (20–50 kDa), 6–7 in group III (50–100 kDa), one peak in group IV (100–150 kDa), and one peak in group V (> 150 kDa) (Table 4).

Based on the obtained molecular weights of sarcoplasmic proteins, the following proteins were identified (Table 5): myoglobin, myokinase, triosephosphate isomerase, phosphoglycerate mutase, lactate dehydrogenase, glyceraldehyde phosphate dehydrogenase, aldolase, creatine kinase, enolase, phosphoglucose isomerase, pyruvate kinase, phosphoglucomutase, and phosphorylase b. The results obtained in this paper are in agreement with the previous studies [4, 21, 23]. Marino et al. [23] used two-dimensional gel electrophoresis (SDS-PAGE) to separate sarcoplasmic proteins from bovine meat and then to identify the proteins by mass spectrometry (with exact molecular weights of some sarcoplasmic proteins determined). In comparison with those results, the data obtained in this study, using CGE, show the same order of separated sarcoplasmic proteins and similar values of molecular weights for most of the identified proteins.

Table 3. Myofibrillar proteins identified after separation from fresh pork meat samples (*M. longissimus dorsi*) by capillary gel electrophoresis

Myofibrillar proteins	Molecular weight[1], kDa	Relative concentration, %
Thymosin	8.14 ± 0.14	1.34 ± 0.01
MLC3	14.93 ± 0.24	3.08 ± 0.01
MLC2	17.28 ± 0.30	12.27 ± 0.23
Troponin C	17.76 ± 0.31	3.25 ± 0.02
Troponin I	20.31 ± 0.37	4.49 ± 0.25
MLC 1	22.07 ± 0.41	8.47 ± 0.31
Tropomyosin 1	31.58 ± 0.63	8.96 ± 0.14
Tropomyosin 2	32.99 ± 0.65	5.70 ± 0.12
Actin	37.35 ± 0.78	14.37 ± 0.20
Troponin T	39.11 ± 0.82	4.70 ± 0.01
Desmin	53.37 ± 1.18	1.68 ± 0.23
Troponin	73.85 ± 1.35	2.51 ± 0.89
C protein	145.65 ± 3.74	1.24 ± 0.13
MHC	217.99 ± 6.04	2.39 ± 0.03

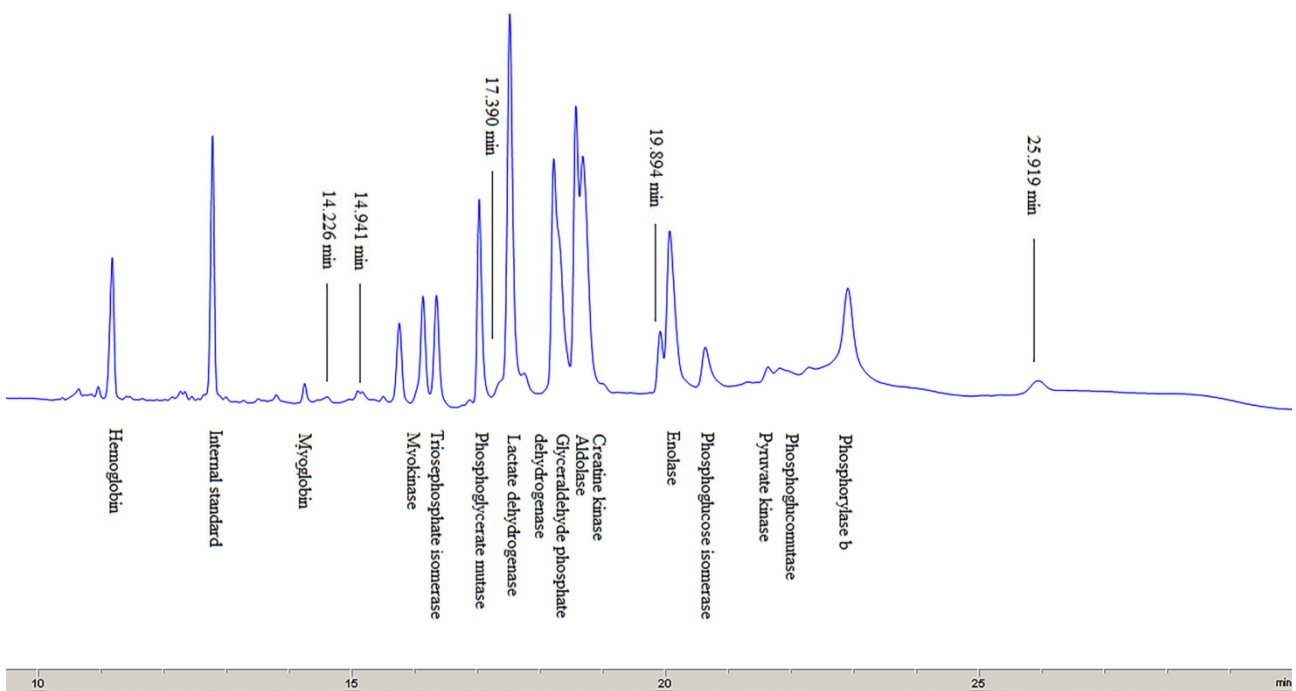*Note.* [1]The average value is determined from 6 repetitions ± standard deviation (SD).

Fig. 4. The electropherogram obtained after separating sarcoplasmic proteins (B) from pork meat (*M. longissimus dorsi*).

Table 4. The number and total relative concentrations of isolated sarcoplasmic proteins obtained by capillary gel electrophoresis separation from fresh pork meat (relative concentration > 0.50%)

Sarcoplasmic proteins	Molecular weight range, kDa					
	<20	20–50	50–100	100–150	>150	Total
The number of isolated proteins[1]	34–35	14–15	6–7	0–1	1	56–58
Relative concentration[1], %	11.25–11.74	59.94–63.23	15.05–27.62	0.00–9.98	0.99–1.19	100.00

Note. [1]The range shows the results obtained after 6 measurements.

Table 5. Sarcoplasmic proteins identified after separation from fresh pork meat (*M. longissimus dorsi*) by capillary gel electrophoresis

Sarcoplasmic proteins	Molecular weight [1], kDa	Relative concentration, %
Myoglobin	16.97 ± 0.320	0.50 ± 0.03
Myokinase	24.92± 0.540	3.62 ± 0.19
Triosephosphate isomerase	26.04 ± 0.560	3.51 ± 0.18
Phosphoglycerate mutase	29.91 ± 0.680	5.73 ± 0.29
Lactate dehydrogenase	33.03 ± 0.770	13.17 ± 0.67
Glyceraldehyde phosphate dehydrogenase	38.11 ± 0.910	12.86 ± 0.49
Aldolase	40.95 ± 1.000	8.47 ± 0.25
Creatine kinase, phosphoglycerate kinase	41.87 ± 1.040	10.68 ± 0.38
Enolase	53.82 ± 1.420	1.75 ± 0.09
Phosphoglucose isomerase	55.53 ± 1.470	8.06 ± 0.16
Pyruvate kinase	62.31 ± 1.700	2.47 ± 0.40
Phosphoglucomutase	79.39 ± 2.313	1.63 ± 0.10
Phosphorylase b, phosphorylase b kinase	98.97 ± 3.020	10.52 ± 1.59

Note. [1]The average value is determined from 6 repetitions ± standard deviation (SD).

In addition to the identified sarcoplasmic proteins, a few more peaks (with a migration time of 14.23 min, 14.94 and 17.725 or 17.390 min) may be observed in Fig. 4. They are likely to be enzymes with a low concentration in the sarcoplasm in pork meat, or they might represent low molecular weight products, resulting from the degradation of myofibrillar proteins. Marino et al. [23] reported that these peaks represented the following proteins: myosin light chain-1 (MLC-1), myosin light chain-2 (MLC-2) and tropomiozin-2. Several authors have studied changes in protein composition of extracts obtained during meat ageing [24], after applying certain meat processing technologies [25], and also to identify or analyse the type of meat in the mixture [1, 9]. Gallego et al. [8] cite molecular weights of multiple sarcoplasmic and myofibrillar proteins, calculated by nLC-MS/MS.

CONCLUSION

Capillary gel electrophoresis (CGE) is used to separate, identify and quantify proteins in pork meat as well as determine molecular weights of separated myofibrillar and sarcoplasmic proteins, ranging from 10 kDa to 225 kDa. After the separation of proteins by capillary gel electrophoresis, the electropherograms contained 53 peaks of myofibrillar proteins and 56 peaks of sarcoplasmic proteins.

The myofibrillar proteins identified on the electropherogram included thymosin, myosin light chain-3 (MLC-3), myosin light chain-2 (MLC-2), troponin C, troponin I, myosin light chain-1 (MLC-1), tropomyosin 1, tropomyosin 2, troponin T, actin, desmin, troponin, C protein, and myosin heavy chain (MHC).

The sarcoplasmic proteins identified on the electropherogram included myoglobin, myokinase, triosephosphate isomerase, phosphoglycerate mutase, lactate dehydrogenase, glyceraldehyde phosphate dehydrogenase, aldolase, creatine kinase, enolase, phosphoglucose isomerase, pyruvate kinase, phosphoglucomutase, and phosphorylase b.

The results obtained in this work are in agreement with the values obtained from the curve for standard protein solutions and with the bands obtained using the classic gel electrophoresis methods.

The advantages of capillary gel electrophoresis (CGE), which justify its recommendation for practical application, include the separation rate, the repeatability of the results, and a simple procedure for determining the content of myofibrillar and sarcoplasmic proteins in meat qualitatively and quantitatively.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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Marbled beef quality grades under various ageing conditions

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Abstract: The Russian beef market is growing, which means that the problem of meat quality is getting more and more relevant. The gradually improving culture of meat consumption raises the demand for beef maturation, or ageing. The current research is the first of its kind in Russia. It features the quality of Russian marbled beef in the process of its open-air and vacuum-packed maturation. The authors studied the changes in the quality grades of dry-aged and vacuum-packed marbled beef during 4, 16 and 28-day ageing and defined the optimal maturation conditions and terms. The study included pH, colour, microstructure, organoleptic properties, the qualitative and quantitative composition of the volatile aroma-forming compounds, and the area of intramuscular fat. The samples were on-the-bone beef cuts obtained from 18-month-old Aberdeen-Angus castrated bulls that had received 200 days of grain fattening. The pH value remained stable throughout the ageing period in the dry-aged and vacuum-packed samples. Approaching the end of the test period, the change in pH reached 0.12 for the dry-aged samples and 0.21 for the vacuum-packed ones. The surface of the dry-aged samples obtained a dark red to burgundy-red dry crust after 16 and 28 days of ageing. The colour was significantly different from the colour of the samples after 4 days of ageing. The colour of the vacuum-packed samples remained unchanged throughout the maturation period. However, the microstructure of the packed beef showed a deeper and more widespread decomposition of muscle tissue on days 16 and 28. The changes in the dry-aged samples were less obvious. All in all, the process of extended ageing improved the organoleptic properties of the beef.

INTRODUCTION

The Russian beef industry is currently experiencing a period of growth. According to the Federal Service for National Statistics, 263,430 tons of beef were produced in 2016, which is 103.3% of the amount produced in 2015. The proportion of fresh-killed, fresh and refrigerated beef was about 80% of the total production in 2016 [1]. The current production growth is connected with such large, vertically-integrated holdings as Miratorg Agribusiness Holding and Zarechnoye Group. Besides, there are numerous state support programs for beef farming and production. However, the share of beef cattle in the total number of Russian livestock hardly exceeds 10%, whereas in the largest beef producing countries it is 40% or higher [2].

The meat consumption in Russia often falls below the recommended level, especially if compared to the United States, Germany and the United Kingdom. However, it is increasing at a significant rate year by year [3]. Hence, the culture of meat consumption is improving. Nowadays, there are specialized restaurants and shops, where consumers can buy various meat products, including aged beef.

The issues of beef ageing became subject of scientific studies in Russia and abroad in the middle of the XX century. For instance, according to a study conducted in 1966, there are three stages of after-slaughter biochemical processes: rigor mortis, rigor mortis resolution and maturation [4]. Still, the problem of meat ageing maintains its relevance. In January 2017, a team of scientists from the United States studied the effect of stepwise dry/wet-aging (10 days in carcass and 7 days in vacuum-packed cuts) and subsequent freezing on beef quality. It was concluded that the stepwise ageing method accompanied by cryogenic freezing can be a good solution for the industry, since it improves such parameters as Warner-Bratzler shear stress and water retention capacity [5].

Another American research featured the ageing of vacuum-packed prefabricated steaks and cuts. It revealed that consumers preferred the strip loin aged as steaks; there were no differences in colour formation and shelf life, but the shear stress was slightly lower in the beef aged as vacuum-packaged cuts [6].

Another team of US researchers studied the effect of open-air and packed extended ageing (14–49 days) on the quality of on-the-bone and boneless beef with low marbling. They discovered that consumers preferred boneless, 28-days wet-aged strip steak, cooked to 71°C [7]. Similar results were obtained by other American scientists who conducted a study of the quality characteristics of *biceps femoris* and *semimembranosus* steaks. Consumer panel results were in favour of the beef aged 14–21 days, while the objective indicators of tenderness, such as Warner-Bratzler shear force values, reached the optimum after 21–42 day ageing [8]. However, a team of Japanese researchers established that for Japanese Aberdeen-Angus cattle the best duration of dry aging for highly marbled beef was 40 days [9]. Researchers from Sweden aged beef samples in a water vapour-permeable dry-ageing bag for 14 days. Compared to ordinary dry ageing, they achieved higher quality characteristics and managed to reduce thawing loss and development of pathogenic microorganisms [10]. G. Lindahl from the Swedish University of Agricultural Sciences found that 5 or 15 days of vacuum ageing was preferable to vacuum ageing in high oxygen modified atmosphere, since it had no negative effect on colour stability [11].

The abovementioned studies prove that the issues of post-mortem changes in meat and proper ageing timing have a significant impact on consumer properties and preferences and are relevant for meat production and sales. Besides, meat ageing studies are important for the current situation in Russia, with its fast development of beef cattle breeding, introduction of new systems for cattle growing and fattening, and new sorts of high-quality marbled beef.

The research objective was a comparative study of the quality of dry-aged and vacuum-packed marbled beef during 4, 16 and 28 days.

STUDY OBJECTS AND METHODS

The on-air and packaged ageing was carried out in a refrigerated compartment at a temperature of 1–2°C and relative humidity < 90%.

The experiment measured the microstructural, organoleptic and technological characteristics of on-the-bone beef cuts obtained from 18-month-old Aberdeen-Angus (Black Angus) castrated bulls that had received 200 days of grain fattening. The slaughter and cutting of the carcasses was carried out in the facilities provided by OOO Bryansk Meat Company (Miratorg Agribusiness Holding), Bryansk Region, Russia.

24 hours after the slaughter, dorsal on-the-bone samples were obtained from the dorsal lumbar cut of 4 carcasses. Each sample was divided into three parts and vacuum-packaged for transportation. A total of 12 samples were obtained, each sample weighed ≥ 3–4 kg. 4 samples (2 open-air and 2 packed) were taken 4, 16 and 28 days after slaughter. The samples from the left side of the carcass were marked with the letter L, those from the right side – with the letter R.

The on-the-bone dorsolumbar pieces were further divided into dorsal and lumbar cuts between the first lumbar and the last (13th) dorsal vertebra. According to the beef cutting pattern established by the interstate standard, the incision continued along the posterior edge of the 13th rib.

Before the beef was put on the shelves of the maturing room, samples 1L and 2L had been unpacked, while samples 1R and 2R remained packaged.

For investigation, the rib eye was excreted from the on-the-bone dorsal cut.

Its degree of marbling is shown in Table 1.

Colour assessment was conducted in the CIE Lab colour space [12] with the help of a Konica Minolta CM-2300d spectrophotometer (Japan).

The measurements involved the following spectrophotometer settings:
− light source D65 (standard daylight);
− viewing angle 2°; and
− exclusive of the mirror component (SCE).

Each measurement was repeated twice, the arithmetic mean of two measurements was taken as the measurement result.

Table 1. The area percentage of intramuscular fat and marbling score

Characteristics	Sample			
	1L	1R	2L	2R
Marbling	Good	Good	Moderate	Moderate
Proportion of fat, % of the loin eye area	23.54	20.43	10.98	11.82
Photos of loin eye samples				

The colour difference (ΔE) was calculated according to the CIEDE 2000 formulas approved by the CIE committee [13].

The potentiometric measurement of pH was conducted with a portable test meter Testo 205. Its electrode was immersed 3 cm in the muscle tissue. The final result was the arithmetic mean of three single measurements; the difference between the limiting values of the three measurement results did not exceed 0.15 pH units.

To study the microstructure, histological samples were taken from intact muscles, which were oriented according to the axis of cutting. Three pieces of $2 \times 1.5 \times 0.5$ cm with longitudinal and transverse orientation of the muscle fibres were selected from each sample. The pieces were placed in a 10% buffered formalin solution for 72 hours at room temperature. After that they were washed under cold running water for 12 hours. Then the material was compacted in gelatine at an ascending concentration (12.5%, 25%) at 37°C for 12 hours [N]. A MIKROM-HM525 cryostat (Thermo Scientific, USA) was used to produce 18-μm slices. The slices were placed on Menzel-Glaser slides (Thermo Scientific, USA) and stained with Ehlich's hematoxylin and 1% aqueous-alcoholic eosin solution (BioVitrum, Russia). An Axio Imaiger A1 light microscope (Carl Zeiss, Germany) and AxioCam MRc-5 camera were used to study and photograph the histological specimens. Image processing involved a computerized image analysis system AxioVision 4.7.1.0 (Carl Zeiss, Germany), adapted for histological studies. Morphometric studies followed the principles of systemic quantitative analysis.

Samples from each cut underwent an organoleptic examination before and after cooking. Before cooking, the appearance and colour of the samples were determined by visual inspection. The colour and consistency of the muscles were evaluated in the deeper layers of muscle tissue on a fresh cut. The odour was defined organoleptically on the surface and inside the sample.

To determine the clarity and odour of the broth, each sample was separately passed through a meat grinder with a 2 mm diameter hole in the grate. 20 g of the minced meat was weighed on a laboratory scale and placed in a 100 cm^2 conical vessel, which then was filled with 60 cm^3 of distilled water, and thoroughly stirred. The vessel was covered with crystal glass and set in boiling-water bath. The odour of meat broth was defined while the vessel was heated up to 80–85 °C at the moment when steam started to emerge from the slightly opened vessel. To determine the transparency, 20 cm^3 of broth was poured into a graduated cylinder (20 mm in diameter, 25 cm^3).

During the cooking process, 1 kg of meat was boiled for 1 hour until the core temperature reached 75°C. The ratio of water and meat was 3:1 (v/v). Salt was added in an amount of 1% to the weight of the meat, 30 minutes before the end of cooking. The meat and the broth were evaluated on a 9-point scale according to the following parameters: appearance, faste, texture, juiciness (for the meat) and appearance, colour, odour, faste and thickness (for the broth).

Instrumental studies of the odour intensity were conducted with the help of the 'electronic nose' multi-sensor system (VOCmeter, Germany). The method was developed in V.M. Gorbatov All-Russian Scientific Research Institute of Meat Industry.

The method of multisensory analysis is based on the ability of the e-nose to sense the volatile components that are released from the surface of the meat sample during cooking. When a volatile component passes over the sensor surface, the physicochemical changes that occur in its sensitive layer are converted into an electronic signal via a converter and transferred to the computer.

The readings of the MOS1-MOS4 sensors of the e-nose were used to determine the freshness of the meat. The signals were transmitted to a PC and recorded in the form of graphs in the Argus programme. Argus was also used to process the signals according to the principal component analysis to obtain the qualitative and quantitative measurement of the volatile components. The principal component analysis is based on the construction of factors, or principal components. Each component represents a linear combination of the original values. The first principal component PC1 defines an axis in the space of initial characteristics with the greatest dispersion of objects (points). The axis of the second principal component PC2 is orthogonal to the axis of PC1 and explains as much of the residual dispersion as possible. Since the separation of the principal components occurs in decreasing order from the point of view of the fraction of variance they explain, the values with large coefficients that enter PC1 exert maximum influence on the differentiation of the objects under study.

The principal components method allowed the team to construct calibration graphs, which made it possible to identify the category of beef freshness.

The indicator of freshness was the boundaries of the clusters that were established in standard samples.

This method has a low detection limit of volatile components that characterize the odour of meat during its ageing.

The surface of beef in the dry-aged samples after 16 and 28 days was characterized by a dry crust of dark red to burgundy-red (Table 3), which was significantly different from the colour of the meat after 4 days of ageing. This is consistent with the results obtained by Aroeira et al., who established that redness (a*) changed during the ageing period [14]. The colour of vacuum-packed samples remained the same throughout the entire ageing period (Table 3).

RESULTS AND DISCUSSION

The pH value remained virtually unchanged, which indicates a stable acidity of meat throughout the ageing period (Table 2).

The histological examination of the structure made on the 4th day of ageing revealed that all the samples had similar microstructural characteristics, with muscle fibres in dense primary bundles. The shape of the fibres was polygonal or slightly rounded on the cross section. Interlayer endomysium and the boundaries between individual muscle fibres were well-defined. The diameter of muscle fibres was 55–60 μM.

Table 2. Value of pHL. dorsi during ageing (mean observation)

Ageing period, days	Dry-aged	Vacuum-packed
4	5.74	5.57
16	5.61	5.81
28	5.62	5.78

Table 3. Colour values (L*; a*; b*; mean)

Ageing period, days	Dry-aged	Vacuum-packed
4	42 ± 2.0; 38 ± 2.0; 31 ± 1.5 Bright red	42 ± 2.0; 41 ± 3.0; 31 ± 1.5 Bright red
16	25.8 ± 1.0; 2.47 ± 1.8; 0.59 ± 1.5 Dark red	41 ± 2.0; 39 ± 0.9; 30 ± 1.5 Bright red
28	23.3 ± 1.0; 1.6 ± 1.2; 0.37 ± 0.9 Burgundy-red	41 ± 1.6; 40 ± 1.4; 31 ± 1.8 Bright red

The longitudinal section demonstrated predominantly rectilinear muscle fibres. However, some fibres were wavy, which is typical of the stage of rigor mortis resolution. Transverse striation was well pronounced. Individual fibres showed areas with longitudinal striation, i.e. zones of contraction. The nuclei in the muscle fibres were well-coloured, oval in shape and located directly under the sarcolemma.

The connective tissues of the perimysium were wavy; they were located snugly against the bundles of muscle fibres. The nuclei in the connective tissue layers were clearly visible. Areas of adipose tissue with a typical histological structure were revealed between the bundles of muscle fibres in perimysium.

The functional state of the muscle tissue was quite homogeneous. There were occasional transverse microcracks and sarcomeres with signs of local decay. No damaged sarcolemma, myofibrils or muscle fibre were detected. The microstructure of the samples corresponds to fresh meat at the initial stage of ageing.

The histological examination of the structure conducted on the 16th day of ageing revealed the following microstructural changes in the dry-aged samples. Along with a well-defined transverse striation, some muscle fibres displayed sites with a smoothed striation. The colour of the fibres was uneven. The nuclei in the muscle fibres were shadowy, not as well-defined as in the corresponding samples on the 4th day of ageing. The muscle fibres showed multiple transverse-slit integrity disorders, occasional ruptures and fragmentations. There were areas of sarcolemma exfoliation and destruction of the internal fibre structure (local decomposition of sarcomeres).
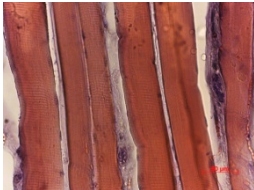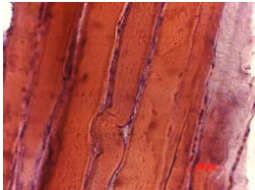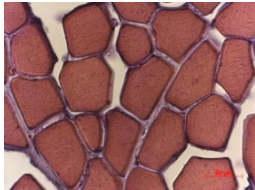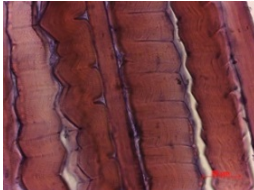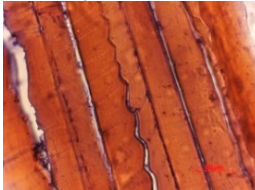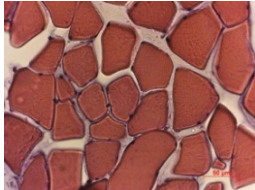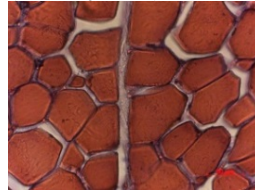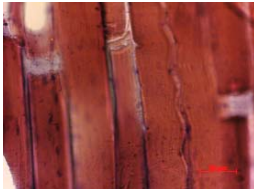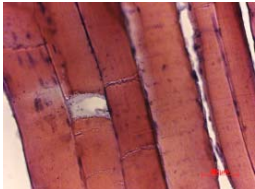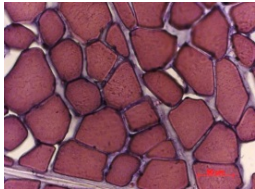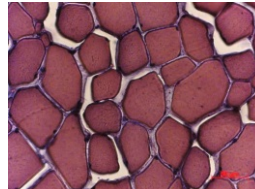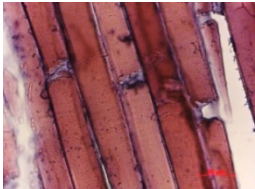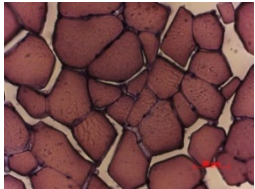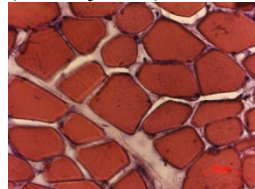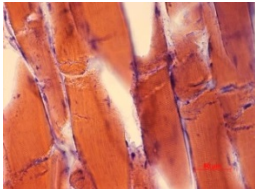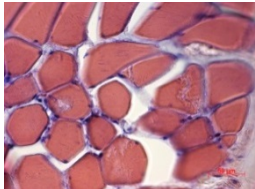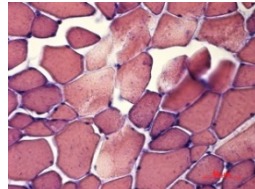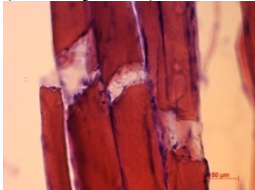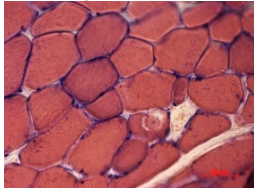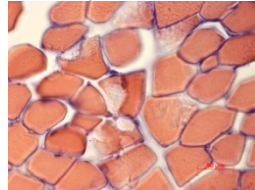
The connective tissue of perimysium showed signs of loosening. The microstructure of the samples corresponded to that of fresh, short-term storage meat at the second stage of ageing.

The vacuum-packed samples revealed more pronounced changes in tissue structure. The muscle fibres showed large areas with a smoothed striation. The nuclei were shadowy; in some fibres they were in a state of complete decay. Multiple transverse-slit integrity disorders and ruptures were present in the muscle fibres. A fine-grained protein mass appeared between the fragments in the areas of fragmentation. There were long sections of sarcolemma exfoliation and destruction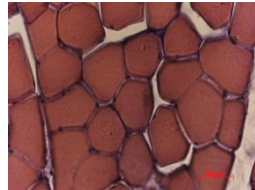 of the internal fibre structure (local decomposition of sarcomeres). The connective tissue of perimysium showed signs of loosening, with occasional detachment from the muscle fibres. The microstructure of the samples corresponded to that of fresh, short-term storage meat at the second stage of ageing.

The histological examination of the structure on day 28 showed that the destructive changes intensified in all the samples. The dry-aged samples revealed a transverse smoothened striation in some areas of muscle fibre. The colouring of fibres was bleak and uneven in the areas infested by microflora. The nuclei in the muscle fibres were either shadowy or in a state of complete decay. The number of areas with muscular fibre ruptures and fine-grained protein mass formation increased in comparison with the corresponding samples on day 16. There were areas with exfoliation and destruction of the sarcolemma, as well as with the destruction of the internal fibre structure (local decomposition of sarcomeres). The connective tissue of perimysium showed signs of loosening, with occasional detachment from the muscle fibres and foci of microflora. The nuclei were poorly visible. The microstructure of the samples corresponded to that of raw meat of the so-called 'dubious freshness' category.

The vacuum-packed samples revealed smoothed striation in most muscle fibres and shadowy nuclei. Individual foci of coccal microflora were identified in the surface layers of muscle tissue. The muscle fibres showed multiple ruptures and fragmentation. Fine-grained protein mass appeared between the fragments in the areas of fragmentation. There were long sections of sarcolemma exfoliation and destruction of the internal fibre structure. A granular mass indicated decomposition of myofibrils on sarcomeres. The connective tissue layers of perimysium were loosened, with detachment from the muscle fibres, which indicated deep destructive changes in muscle tissue.

Table 4. Microstructure of samples at different stages of ageing

Microstructure of samples			
Dry-aged	Vacuum-packed	Dry-aged	Vacuum-packed
Ageing period, days 4			

Sample 1L Longitudinal section (40 × objective)	Sample 1R Longitudinal section (40 × objective)	Sample 1L Cross section (40 × objective)	Sample 1R Cross section (40 × objective)

Sample 2L Cross section (40 × objective)	Sample 2R Cross section (40 × objective)	Sample 2L Cross section (40 × objective)	Sample 2R Cross section (40 × objective)

Ageing period, days 16

Sample 1L Longitudinal section (40 × objective)	Sample 1R Longitudinal section (40 × objective)	Sample 1L Cross section (40 × objective)	Sample 1R Cross section (40 × objective)

Sample 2L Longitudinal section (40 × objective)	Sample 2R Longitudinal section (40 × objective)	Sample 2L Cross section (40 × objective)	Sample 2R Cross section (40 × objective)

Ageing period, days 28

Sample 1L Longitudinal section (40 × objective)	Sample 1R Longitudinal section (40 × objective)	Sample 1L Cross section (40 × objective)	Sample 1R Cross section (40 × objective)

Sample 2L Longitudinal section (40 × objective)	Sample 2R Longitudinal section (40 × objective)	Sample 2L Cross section (40 × objective)	Sample 2R Cross section (40 × objective)

(a)

(b)

—— day 4, —— day 16, —— day 28

Fig. 1. 'Visual footprints' of odour in the vacuum-packed samples during ageing: (a) sample 1R; (b) sample 2R.

Table 5. Areas of 'visual footprints' of odour in the vacuum-packed samples during ageing

Period of ageing, days	Areas of 'visual footprints', c.u. $\times 10^7$	
	Samples	
	1R	2R
4	22.89	24.08
16	26.75	27.79
28	29.82	32.88

The histological studies revealed increasing destructive changes in the tissue. The vacuum-packed beef samples 1R and 2R showed significant destructive changes on days 16 and 28. The dry-aged samples 1L and 2L demonstrated less pronounced changes on the corresponding day of ageing. However, they showed signs of rotting. Therefore, the ageing process was faster and more intensive in the vacuum-packed samples while the beef remained fresh. The dry-aged samples demonstrated signs of meat spoilage by day 28. Similar results were obtained by E. Veiseth-Kent et al., whose research featured 403 beef samples of Norwegian red cattle [15].

(a)

(b)
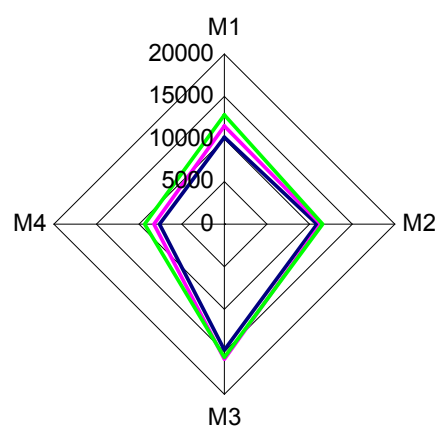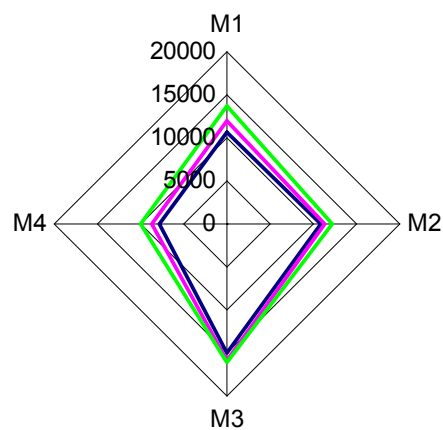
—— day 4, —— day 16, —— day 28

Fig. 2. 'Visual footprints' of odour in the dry-aged samples: (a) sample 1L; (b) sample 2L.

According to the organoleptic research of the vacuum-packed beef on day 28, the samples belonged to the category 'fresh meat' during the entire period of ageing. The organoleptic studies were confirmed by the results of a multisensory assessment of the odour at all stages of ageing.

According to the organoleptic properties of the dry-aged beef, the samples could be placed into the 'meat of dubious freshness' category after 28 days of ageing. The samples possessed a less elastic consistency and emitted an acidic or musty odour, untypical of fresh meat; the broth was opaque.

The multisensory analysis of volatile components during the gaseous phase showed that the odour intensity increased during the ageing in both packed and unpacked beef. According to the multisensory analysis, the packed samples remained fresh without signs of decomposition throughout the entire ageing period (28 days). The odour intensity increased by 1.2 and 1.4 times on day16 and day 28 respectively. During the gaseous phase, there was an increase in the content of volatile fatty acids and free amino acids. Low molecular nitrogen compounds responsible for the aroma of meat and precursor substances of cooked meat odour were detected (Figs.1a and 1b, Table 5).

The multisensory analysis of the gaseous phase on day 28 showed that the odour intensity in samples 1L and 2L increased by 1.9 and 2.2 times respectively. The increase in odour was associated with an increase in the content of volatile fatty acids, ketones, and aldehydes that resulted from the oxidation of muscle tissue fats during the gaseous phase (Fig. 2a and 2b, Table 6).

The multisensory analysis of the dry-aged samples on day 28 showed that the meat remained fresh in the refrigerator for 16 days, which agreed with the results of the organoleptic assessment.

According to the organoleptic assessment made after cooking on day 4, the sample received a high score (8 points) in terms of flavour, juiciness, and tenderness. Its appearance and odour received the maximum score (9 points). The total score was 8 points, which denoted a very good quality. The sample had a fine texture and a very pleasant strong odour. The broth also received a high overall score of 8 points. According to its organoleptic properties, it was evaluated as tasty and thick, with a pleasant strong odour.

After 16 days of ageing, the panellists detected some increase in the tenderness of the vacuum-packed beef. Its quality level remained the same. The overall score of the product was 8 points (very good). The broth also had high organoleptic properties; its overall score was 8 points (very good) (Table 8, 9).

After cooking, the dry-aged samples demonstrated less juiciness – 7 points (juicy enough). The overall score was 8 points (very good). No decrease in broth quality was detected. The overall organoleptic score of the broth was 8 points (very good) (Table 10, 11).

By day 28, the organoleptic properties of the cooked dry-aged samples had not deteriorated. The panellists gave them 9 points for tenderness (very tender) and 8 points for juiciness (juicy). The overall score of the quality level of the cooked meat and broth was 8 points (very good) (Tables 7, 8).

After 28 days of ageing, the organoleptic indices of cooked dry-aged meat (appearance, odour, taste juiciness) and broth started to go down because of foreign smell, off-taste, opaque broth, poor juiciness, etc. The overall score was 7 points for the cooked meat and 5 points for the broth (Table 9, 10).

Table 6. Areas of 'visual footprints' of odour in the dry-aged samples

Period of ageing, days	Areas of 'visual footprints', c.u. $\times 10^7$	
	Samples	
	1L	2L
4	23.63	23.45
16	28.46	30.73
28	45.15	51.26

Table 7. Quality indices of the vacuum-packed beef after cooking, 9-point scale

Ageing period	Appearance	Odour	Taste	Texture	Juiciness	Overall score
4 days	9	9	8	8	8	8
	Very pleasant	Very pleasant, fragrant, strong	Tasty	Tender	Juicy	Very good
16 days	9	9	8	8	8	8
	Very pleasant	Very pleasant, fragrant, strong	Tasty	Tender	Juicy	Very good
28 days	8	9	8	9	8	8
	Very good	Very pleasant, fragrant, strong	Tasty	Very tender	Juicy	Very good

Table 8. Quality indices of broth, 9-point scale

Ageing period	Appearance	Odour	Taste	Thickness	Overall score
4 days	9	9	8	8	8
	Very pleasant	Very pleasant, fragrant, strong	Tasty	Thick	Very good
16 days	9	8	8	8	8
	Very pleasant	Pleasant, strong	Tasty	Thick	Very good
28 days	9	8	8	8	8
	Very pleasant	Pleasant, strong	Tasty	Thick	Very good

Table 9. Quality indices of the dry-aged beef after cooking, 9-point scale

Ageing period	Appearance	Odour	Taste	Texture	Juiciness	Overall score
4 days	9	9	8	8	8	8
	Very pleasant	Very pleasant, fragrant, strong	Tasty	Tender	Juicy	Very good
16 days	9	8	8	8	7	8
	Very pleasant	Pleasant, strong	Tasty	Tender	Juicy enough	Very good
28 days	6	3	6	7	6	7
	Not good enough	Pleasant, strong	Not tasty enough	Tender enough	Not juicy enough	Good

Table 10. Quality indices of broth, 9-point scale

Ageing period	Appearance	Odour	Flavour	Thickness	Overall score
4 days	9	9	8	8	8
	Very pleasant	Very pleasant, fragrant, strong	Tasty	Thick	Very good
16 days	9	8	8	8	8
	Very pleasant	Pleasant, strong	Tasty	Thick	Very good
28 days	6	3	3	6	5
	Not good enough, opaque	Slightly unpleasant, foreign	Slightly unpleasant, foreign	Not thick enough	Medium

CONCLUSION

The method of extended ageing had a significant impact on the quality of the beef.

During ageing, destructive changes developed in the structure of muscle tissue; volatile aroma-forming compounds accumulated in the beef, which improved its organoleptic properties.

The vacuum-packed beef retained the properties of fresh meat, colour and pH value for the entire 28-day period of ageing. However, the destructive changes in the muscle tissue detected on day 16 and 28 were obvious and widespread.

The dry-aged beef retained the properties of fresh meat for 16 days. After 16 and 28 days, its surface obtained a dark red to burgundy-red dry crust. The colour was significantly different from the colour the samples obtained after 4 days of ageing (ΔE from 49.4 to 49.8). The destructive changes in the muscle tissue on the corresponding day were less pronounced.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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Prospects for DNA authentication in wine production monitoring

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Abstract: Wines DNA authentication is a technological process of their authenticity verification by genetic identification of the main plant ingredient by means of molecular genetic analysis of the residual amounts of *Vitis vinifera* L nucleic acids extracted from end product cellular debris. The main aim of the research was the analysis of scientific and methodological approaches to the extraction of residual amounts of nucleic acids in wine raw materials and DNA authentication of wines for their subsequent application in solving the problem of determining wine products authenticity and place of origin. The prior art includes various approaches to the extraction of *Vitis vinifera* L. nucleic acids among which the three methods by Savazzini & Martinelli, Pereira and Bigliazzi can be named basically. Analysis of the effectiveness of different methods of DNA extraction from wines indicates the superiority of the Pereira method over other traditional methods of extraction in terms of DNA yield and quality. Besides, the nucleic acid extracted from wines is characterized as residual since its concentration is significantly reduced in a multi-stage wine production process. The yield of extracted nucleic acid also decreases as the wine ages. The use of microsatellite DNA loci designed for grapes genetic identification is one of the approaches applicable for wine DNA authentication. SSR markers of nuclear and chloroplast DNA, as well as sets of STS primers delighed for special SRS loci (and originally used for identification and certification of grape varieties and hybrids), found partial application in wine DNA authentification. Along with SSR markers, SNP markers, integrated into the system of wine DNA authentification by PCR method in real-time mode, HRM analysis, ad sequencing, have a high identification potential.

Keywords: Wine, grapes, variety, *Vitis vinifera* L, DNA, authentication, identification, marker, SSR, SNP, PCR, HRM analysis

INTRODUCTION

Considering its wide assortment variety and multicomponent chemical composition, wine production belongs to the segment of difficult-to-identify goods whose established authenticity serves as a basic element of consumers and producers' rights protection [1, 2].

The search for objective identification criteria with a high degree of wine products authenticity and place of origin assessment reliability is a strategically important task achievable by multidisciplinary science-intensive approaches [3, 4].

The complex identification scheme based on the approved methods of analysis (documentary, visual, organoleptic, and physicochemical) [5–9] can be expanded by the molecular genetic method [10] applicable for wine DNA authentication [11, 12].

Wine DNA authentication is a technological process of authenticity verification by genetic identification of the main plant ingredient – wine grapes – by means of molecular genetic analysis of the residual amounts of *Vitis vinifera* L nucleic acids extracted from end product cellular debris [11, 12].

Analysis of research and methodological approaches to the extraction of the residual amounts of nucleic acids in wine raw materials, and wine DNA authentication demonstrates the applicability of DNA technologies for the monitoring of counterfeit and adulterated wine products.

RESULTS AND DISCUSSION

Methods of extraction of wine raw materials DNA residual amounts. The prior art includes various approaches to the extraction of *Vitis vinifera* L. nucleic acids [13–21], among which the three methods by Savazzini & Martinelli [14], Pereira [16] and Bigliazzi [17] can be named basically in both the original and modified set-ups [11].

Reagents used at various stages of sample preparation and extraction of *Vitis vinifera* L. DNA in the three namesake methods of nucleic acids extraction from wines are shown in Table 1.

Table 1. Reagents used at various stages of sample preparation and extraction of *Vitis vinifera* L. DNA in the three namesake methods of nucleic acids extraction from wines

Savazzini & Martinelli [14]	Pereira [16]	Bigliazzi [17]
WINE PLANT DEBRIS PRECIPITATION		
NaCl or 2-propanol, or sodium acetate	2-propanol	2-propanol or sodium acetate
RESUSPENSION AND LYSIS OF WINE PLANT DEBRIS SEDIMENT		
Lysing buffer (25 mM EDTA, 1 M Tris-HCl (pH 8.0) , 2 M NaCl, 3% (w/v) CTAB, 1% (w/v) PVP, 0.2% (v/v) 2-mercaptoethanol)	Lysing buffer (20 mM EDTA, 10 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 2% (w/v) STAB, 2% (w/v) PVP, 1% (v/v) 2-mercaptoethanol, 20 mg/ml proteinase K)	Lysing buffer (20 mMm EDTA, 1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 3% (w/v) STAB, 5% PVP, 1% (v/v) 2-mercaptoethanol)
NUCLEOPROTEIN COMPLEX DEPROTEINIZATION		
Phenol:chloroform:isoamyl alcohol (25:24:1)	Chloroform:isoamyl alcohol (24:1)	Chloroform:isoamyl alcohol (24:1)
RNA REMOVAL		
	RNase A (10 mg/ml)	
UNTREATED DNA PRECIPITATION		
	2-propanol	
RESUSPENSION OF PRECIPITATED DNA		
	Elution buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA)	
NUCLEOPROTEIN COMPLEX DEPROTEINIZATION		
	Neutral phenol	Chloroform: octanol (24:1)
SELECTED AQUEOUS PHASE DEPOSITION		
2-propanol	2-propanol	2-propanol
PRECIPITATED DNA WASHING		
70% ethanol	Wash buffer (76% ethanol, 10 mM ammonium acetate)	
RESUSPENSION OF PRECIPITATED DNA		
Double-distilled water	Elution buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)	Elution buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)
RESUSPENDED DNA ADDITIONAL PROCESSING		
		Proteinase K (20 mg/ml)
DEPROTEINIZATION		
		Phenol:chloroform (1:1). Chloroform:isoamyl alcohol (24:1)
SELECTED AQUEOUS PHASE DEPOSITION		
		Precipitator (95% ethanol, 2.5 M ammonium acetate)
RESUSPENSION OF PRECIPITATED DNA		
		Qiagen P1 buffer. Elution buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)

The unifying feature of the methods presented is the precipitation of wine plant debris by centrifugation using precipitators, suchas sodium chloride, 2-propanol, and sodium acetate separately or in combination of the latter two.

Resuspension of the plant debris sediment and its lysis are carried out by a multicomponent lysing buffer consisting of ethylenediaminotetraacetic asid (EDTA), trisaminomethane hydrochloric acid (Tris-hcl), sodium chloride (NaCl), cetyltrimethylammonium bromide (CTAB), polyvinylpyrrolidone (PVP), and 2-mercaptoethanol [14, 16, 17]. The buffer also includes the proteinase K in the Pereira method [16].

The stage of deproteinization of the lysate nucleoprotein complex is carried out by organic solvents (chloroform and isoamyl alcohol), with the inclusion of phenol in the three-component mixture phenol:chloroform:isoamyl alcohol at the ratio 25:24:1 in the Savazzini & Martinelli method [14].

The Pereira method [16] includes the stage of RNA removal by treatment with RNase A followed by untreated DNA deposition by centrifugation, further resuspension of the deposited DNA with an elution buffer and additional deproteinization with neutral phenol. In the Bigliazzi method [17], the additional stage of chloroform-methanol deproteinization at the ratio 24:1 is preceded by the stage of adding 0.1 volume of CTAB to the double centrifugation supernatant.

The selected aqueous phase is precipitated by 2-propanol in all the three methods. The subsequent deposited DNA washing in the Savazzini & Martinelli method [14] is performed with 70% ethanol, while in the Pereira method [16] with a washing buffer containing ethanol and ammonium acetate, and in the Bigliazzi method [17] this stage is unavailable.

However, after the precipitated DNA resuspension with an eluent, the Bigliazzi method [17] incorporates additional resuspended DNA treatment with proteinase K, and subsequent manipulations, including the use of QIAprep Spin Miniprep Kit.

It must be noted that a number of commercial kits selectively binding extractable nucleic acid on spin

columns, such as "Plant Genomic DNA Miniprep Kit" (Sigma), "Dneasy Plant Mini Kit" (Qiagen), "High Pure PCR Template Preparation Kit" (Roche), "Power Plant DNA Isolation Kit" (MO-BIO), "Power Soil DNA Isolation Kit" (MO-BIO), "FastDNA Spin Kit for Soil" (MP Biomedicals), "NucleoSpin Food" (Macherey-Nagel), "Genomic DNA from Food" (Macherey-Nagel), and "Dneasy Mericon Food Kit" (Qiagen) were tested in *Vitis vinifer*a L. DNA extraction, including the use of "DNA Purification System for Food" (Promega) based on DNA extraction with the help of magnetic particles [11].

At the same time, the procedure of DNA isolation by commercial sets from the 2-isopropanol precipitated plant debris had minor modifications associated with the introduction of a set of 100 μl α-amylase (incubation at 80°C for 30 min) and/or 40 μl of proteinase K (incubation at 55°C for 30 min) into the first used buffer [11].

Sequential application of α-amylase and proteinase K is also provided by the procedure of nucleic acid isolation from lyophilized, pre-dissolved in a buffer (0.1 M Tris-HCl (pH 8.0), and 0.1 M NaCl) wine powder whose DNA extraction protocol is described in Nakamura et al. (2007) [15]. In this case, proteinase K is included in the hydrolysis by incubation at 55°C for 60 min together with sodium dodecyl sulfate (SDS). The resulting hydrolysate undergoes stepwise stages of precipitation by centrifugation in a mixture with 2-propanol; nucleic acid precipitate resuspension with an eluent buffer followed by the addition of 70% ethanol; re-deposition in a mixture of 2-propanol with sodium acetate; and deposited DNA resuspension in TE buffer. The extraction procedure also includes the stages of RNA removal by treatment of nucleic acid with RNase A and deproteinization first with neutral phenol and then with phenol:chloroform (1:1), with precipitation of the selected aqueous phase by addition of 0.2 M NaCl and 2 volumes of cold ethanol. The resulting DNA precipitate is washed with 70% ethanol and eluted with TE buffer.

Performance analysis of *Vitis vinifera* L. DNA extraction by different methods indicates the superiority of the Pereira method [16] over other traditional methods of extraction [13–15, 17] in terms of DNA yield and quality [11]. Detailed information on the stages of *Vitis vinifera* L. sample preparation and DNA isolation by the Pereira method [16] is shown in Fig. 1.

The comparative quantitative and qualitative assessment of the isolated nucleic acid preparations pointed to the ineffectiveness of the Bigliazzi method [17]. A number of commercial sets with the exception of "Dneasy Plant Mini Kit" (Qiagen), "High Pure PCR Template Preparation Kit" (Roche), and "Power Soil Isolation Kit" (MO-BIO) with their minor modifications associated with the addition of α-amylase and/or proteinase K providing test systems performance, also proved ineffective. Treatment with α-amylase is prescribed for the set produced by Roche, while for the MO-BIO set [11] it is α-amylase and proteinase K treatment.

1. WINE PLANT DEBRIS PRECIPITATION

Mixing 10 ml wine with 0.7 volume of 2-propanol

Storage of the mix for 2 weeks at –20°C

Wine plant debris precipitation by centrifuging at 4,000 g for 30 min at ambient temperature

2. RESUSPENSION AND LYSIS OF WINE PLANT DEBRIS SEDIMENT

Resuspension of plant precipitation in 750 mcl lysing buffer (20 mM EDTA, 10 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 2% (w/v) STAB, 1% (v/v) 2-mercaptoethanol, 2% (w/v) PVP, 20 mg/ml proteinase K)

Incubation of the resulting mix at 65°C for 60 min

3. NUCLEOPROTEIN COMPLEX DEPROTEINIZATION

Organic extraction by equal volume of chloroform:isoamyl alcohol (24:1)

Aqueous phase extraction by centrifuging at 13,000 g for 15 min at 4°C

4. RNA REMOVAL

Treatment with RNase A (10 mg/ml) at 37°C for 30 min

5. UNTREATED DNA PRECIPITATION

Blending the mix with 0.7 volume of 2-propanol

Incubation at –20°C for a night

Centrifuging at 10,000 g for 15 min at 4°C

6. RESUSPENSION OF PRECIPITATED DNA

Resuspension of sediment in 300 mcl elution buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA)

7. DEPROTEINIZATION

Extraction with an equal volume of neutral phenol

Aqueous phase extraction by centrifuging at 13,000 g for 15 min at 4°C

8. SELECTED AQUEOUS PHASE DEPOSITION

Stage 5 repeated

9. PRECIPITATED DNA WASHING

Precipitated DNA exposed to the wash buffer (76% ethanol, 10 mM ammonium acetate) for 5 min

Wash buffer removal and sediment drying at ambient temperature

10. RESUSPENSION OF PRECIPITATED PURIFIED DNA

Sediment resuspension in 50 mcl elution buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA) at ambient temperature and DNA preparation storage –20°C
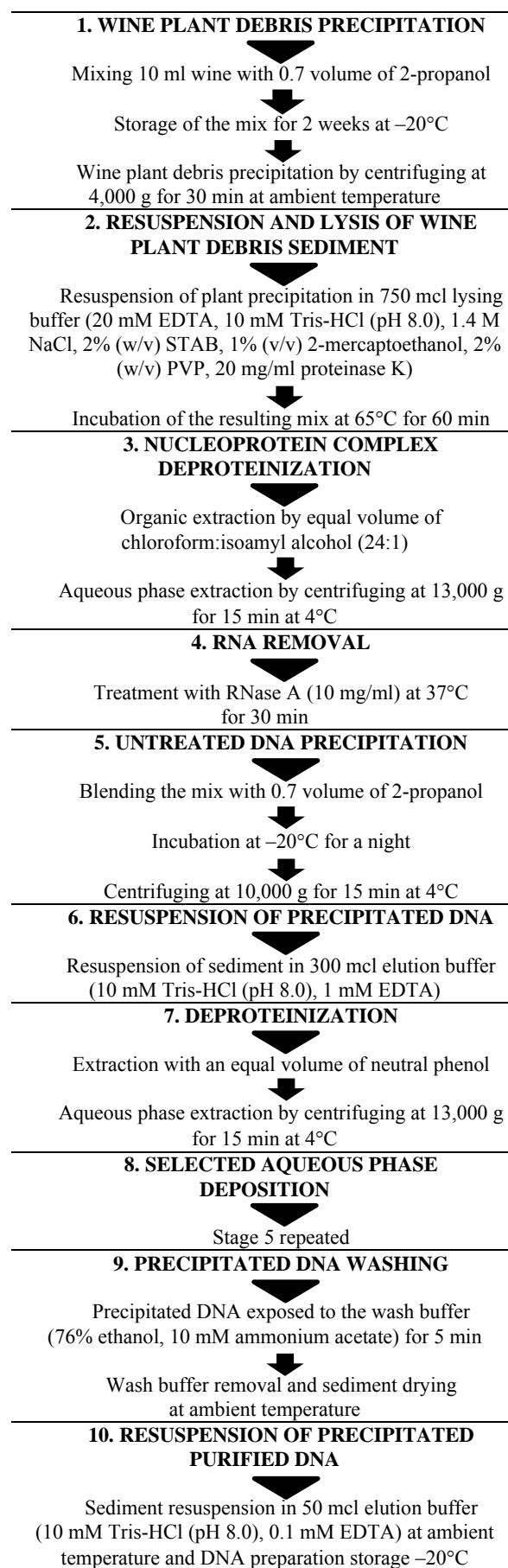
Fig. 1. Stages of sample preparation and *Vitis vinifera* L. DNA extraction by the Pereira method.

Precipitators used for wine plant debris precipitation, such as NaCl, 2-propanol, sodium acetate used both individually and in a complex are the key reagents at the initial stage of sample preparation which involves concentration of the test material by centrifugation. From the point of view of the efficiency of DNA yield and quality, the method is not inferior to concentration by lyophilization though rather time-consuming. For example, the Pereira method [16] regulates storage of wine and precipitator mixture in the freezer for 2 weeks for maximum precipitation effect.

Extracted from wines *Vitis vinifera* L DNA has the status of residual nucleic acid since its concentration is significantly reduced during the multi-stage wine production process including decantation, purification, filtration and other processing methods [15, 20]. In addition, grapes DNA is degraded by the DNase of wine microbiota during fermentation [11].

Wine aging reduces the yield of the extracted nucleic acid [22]. At the same time, it is experimentally proved [11] that the amount of *Vitis vinifera* L DNA isolated from wines with the completed stage of alcoholic fermentation is significantly reduced or practically absent depending on the terms of end product testing.

Methods of wines DNA authentication. The use of highly polymorphic microsatellite DNA loci designed for grapes genetic identification [23–27] is one of the approaches to wine DNA authentication as well [11, 13–17, 21, 22, 28, 29, 30].

Nine highly specific and reproducible polymorphic markers of the nuclear DNA microsatellite (SSR) loci presented in Table 2 form the basic set for identification and certification of grapes varieties and hybrids, where the specified sequences of oligonucleotide primers initiate amplification of SSR fragments of the extracted DNA for subsequent granular analysis in capillary gel electrophoresis [31] of the sequencer with interpretation of results by the genetic analyzer software.

SSR fragments amplification is carried out by multiplex PCR, combining several analyzed loci. This amplification strategy is based on working with DNA extracted from the components of grape plants (fruit, leaf, stem, root) but is not effective while studying extracted residual wine nucleic acid [11, 13, 16, 42]. Therefore, wine DNA authentication is usually carried out by PCR with a set of primers of a single SSR-marker to achieve an analyzable result [11].

While DNA testing monosort and assemblage commercial wines researchers noted identifiable results of the markers specified in Table 2: VrZAG79 [11, 16], VVS2, VVMD27 [14, 17], and VVMD25 [17] These allow for a retrospective assessment of the wine materials varietal identity and actual wine DNA authentication by the interpretation of the fragmented data analysis.

The database of 3675 grape varieties genetic profiles in VIVC interactive catalogue (Vitis International Variety Catalogue) (Fig. 2) is successfully used when comparing the DNA-test-generated SSR markers profiles with the already hosted on the server published information.

Another type of SSR markers, spSSR [43–46], targeted to the chloroplast DNA, has several advantages over the nuclear DNA (nSSR) analysis due to the greater number of representation per cell, greater resistance to exonuclease influence and lower susceptibility to degradation due to its content in double membrane organelles [11, 13].

Table 2. SSR markers of nuclear DNA used for identification and certification of grape varieties and hybrids partially applicable for wine DNA authentication

item no.	SSR locus	Sequence of oligonucleotide primers	Allele length range, bp	Reference
1	VVS2	5$^/$-CAGCCCGTAAATGTATCCATC-3$^/$ 5$^/$-AAATTCAAAATTCTAATTCAACTGG-3$^/$	123–165	[23, 32]
2	VVMD5	5$^/$-CTAGAGCTACGCCAATCCAA-3$^/$ 5$^/$-TATACCAAAAATCATATTCCTAAA-3$^/$	220–268	[24, 33]
3	VVMD7	5$^/$-AGAGTTGCGGAGAACAGGAT-3$^/$ 5$^/$-CGAACCTTCACACGCTTGAT-3$^/$	231–267	[24, 34]
4	VVMD25	5$^/$-TTCCGTTAAAGCAAAAGAAAAAGG-3$^/$ 5$^/$-TTGGATTTGAAATTTATTGAGGGG-3$^/$	243–275	[35, 36]
5	VVMD27	5$^/$-GTACCAGATCTGAATACATCCGTAAGT-3$^/$ 5$^/$-ACGGGTATAGAGCAAACGGTGT-3$^/$	173–223	[35, 37]
6	VVMD28	5$^/$-AACAATTCAATGAAAAGAGAGAGAGA-3$^/$ 5$^/$-TCATCAATTTCGTATCTCTATTTGCTG-3$^/$	216–285	[35, 38]
7	VVMD32	5$^/$-TATGATTTTTTAGGGGGGGTGAGG-3$^/$ 5$^/$-GGAAAGATGGGATGACTCGC-3$^/$	234–272	[35, 39]
8	VrZAG62	5$^/$-GGTGAAATGGGCACCGAACACACGC-3$^/$ 5$^/$-CCATGTCTCTCCTCAGCTTCTCAGC-3$^/$	173–219	[25, 40]
9	VrZAG79	5$^/$-AGATTGTGGAGGAGGGAACAAACCG-3$^/$ 5$^/$-TGCCCCCATTTTCAAACTCCCTTCC-3$^/$	236–270	[25, 41]

Fig. 2. VIVC interactive catalogue grapes varieties genetic profile database interface.

Table 3. SSR markers of chloroplast DNA used for identification and certification of grape varieties and hybrids, partially suitable for differentiation of a limited range of wine products

Item no.	SSR locus	Sequence of oligonucleotide primers	Reference
1	NTcp12	5$^/$-CCTCCATCATCTCTTCCAA-3$^/$ 5$^/$-ATTTATTTCAGTTCAGGGTTCC-3$^/$	[47, 48]
2	NTcp8	5$^/$-ATATTGTTTTAGCTCGGTGG-3$^/$ 5$^/$-TCATTCGGCTCCTTTATG-3$^/$	[47, 48]
3	ccmp10	5$^/$-TTTTTTTTTAGTGAACGTGTCA-3$^/$ 5$^/$-TTCGTCGDCGTAGTAAATAG-3$^/$	[44, 48]
4	ccmp3	5$^/$-CAGACCAAAAGCTGACATAG-3$^/$ 5$^/$-GTTTCATTCGGCTCCTTTAT-3$^/$	[44, 48]
5	ccmp2	5$^/$-GATCCCGGACGTAATCCTG-3$^/$ 5$^/$-ATCGTACCGAGGGTTCGAAT-3$^/$	[40, 49]
6	ccmp4	5$^/$-AATGCTGAATCGAYGACCTA-3$^/$ 5$^/$-CCAAAATATTBGGAGGACTCT-3$^/$	[40, 49]
7	ccmp6	5$^/$-CGATGCATATGTAGAAAGCC-3$^/$ 5$^/$-CATTACGTGCGACTATCTCC-3$^/$	[40, 44]
8	ccSSR14	5$^/$-GGGTATAATGGTAGATGCCC-3$^/$ 5$^/$-GCCGTAGTAAATAGGAGAGAAA-3$^/$	[43, 50]

Despite the weak discriminatory ability of these SSR markers, incapable of wide range of grape varieties certification, the analysis of chloroplast DNA microsatellite loci remains an alternative approach to the varietal genetic identification of *Vitis vinifera* L, although not quite suitable for wine DNA authentication [11, 13, 16, 20, 21, 29] due to the low level of polymorphism of the analyzed loci, suitable only for a limited range of wine products differentiation (Table 3).

In the study of 21 grape varieties by fragmented analysis of 8 cpSSR-loci, whose oligonucleotide primers are listed in Table. 3, in the studied sample selection V. Catalano et al. (2016) [11] discovered 4 chlorotypes, whose results with grouping of the tested *Vitis vinifera* L varieties by their haplotype are shown in Table 4.

At the same time, half of these cpSSR-loci (NTcp12, ccmp2, ccmp4, and ccmp6) had no allele polymorphism, but two analyzed loci (ccmp10 and ccSSR14) were characterized by the presence of three alleles, and two more loci (NTcp8 and ccmp3) – by the presence of two alleles [11], respectively (Table 4).

Although this method shows relatively low resolution [11], it can be used as an additional test for counterfeit and adulterated wine products identification.

Microsatellite DNA is also used as a source of STS (Sequence Tagged Site – sites marked with a sequence) – unbroken unique sequences whose amplified profiles serve as molecular genetic markers [11, 15, 23]. Thus, S. Nakamura et al. (2007) [15] designed experimental sets of STS primers for certain SSR loci of mitochondrial and chloroplast DNA [25, 43, 51–54], having tested them in PCR during varietal genetic identification of *Vitis vinifera* L, and DNA authentication of wines produced from them.

Along with SSR markers, SNP markers [55, 56], applicable for wine DNA authentication [12], both thanks to tracing *Vitis vinifera* L. individual genotypes in monosort and assemblage wines with the potential for the quantitative assessment of plant ingredients and their performance in the analysis of the fragmented nucleic acid bear a high identification potential. Based on SNP markers, test systems can be designed for genetic identification of individual grape varieties [11].

Table 5 shows the sets of primers and probes for real-time PCR with fluorescent hybridization detection used in genetic identification of the Sangiovese variety, and DNA authentication of the wine produced from it by assessing single-nucleotide polymorphism (SNP) in three analytical positions (98, 222 and 244) [11].

Table 4. Grape varieties with 8 SSR-loci of chloroplast DNA discovered haplotype

Wine grape variety	PCR fragments of chloroplast DNA SSR loci, bp								Chlorotype (haplotype)
	NTcp12	NTcp8	ccmp10	ccmp3	ccmp2	ccmp4	ccmp6	ccSSR14	
Canaiolo	167	249	109	103	206	126	106	203	A
Pinot Noir									
Petit Verdot									
Riesling Renano									
Tempranillo									
Ancellotta									
L. Salamino									
Teroldego	167	249	110	104	206	126	106	204	B
Chardonnay	167	249	111	103	206	126	106	205	C
Merlot									
Primitivo									
Montepulciano	167	250	110	104	206	126	106	204	D
Ciliegiolo									
Cabernet Sauvignon									
Sangiovese									
Sauvignon Blanc									
Tannat									
Fortana									
L. Grasparossa									
L. Sorbara									
Malbo Gentile									

Table 5. Primers and probes for PCR-RT for the three SNP positions used in the Sangiovese variety genetic identification and DNA authentication of the wine produced from it

SNP	PCR Round	Oligonucleotide primers and TaqMan probes	PCR product
98	1st PCR round with external primers	5$^/$-TTCAAAGCGAAGAACCAG-3$^/$ 5$^/$-ACCCTTCAACAAACCAAC-3$^/$	790 bp
	2nd PCR round with nested primers and TaqMan probes	5$^/$-GTTAGTGTAAGGTGATGCC-3$^/$ 5$^/$-TTTCCTAATCCTTGTTGG-3$^/$ 5$^/$-FAM-TAGGATTTATGAAGGGAAG-3$^/$-BHQ1 5$^/$-VIC-TAGGATTTATGAAGGCAAG-3$^/$-BHQ2	136 bp
222	1st PCR round with external primers	5$^/$-AGACTGACTTTTGAAACACC-3$^/$ 5$^/$-TTCCTGGATTGGGTATG-3$^/$	889 bp
	2nd PCR round with nested primers and TaqMan probes	5$^/$-AAGACACCCACCAAGTTC-3$^/$ 5$^/$-CCAGGCAAGTAACACAAG-3$^/$ 5$^/$-FAM-AGCAATGTGGGCTGA-3$^/$-BHQ1 5$^/$-VIC-AGCAATGTGGGCCGA-3$^/$-BHQ2	128 bp
244	1st PCR round with external primers	5$^/$-AAACGCAGGAGAATGTC-3$^/$ 5$^/$-TTCAACCTGATGCCTAAC-3$^/$	721 bp
	2nd PCR round with nested primers and TaqMan probes	5$^/$-AATCCCCATCCCGAAGTG-3$^/$ 5$^/$-CCCAGTTCCATTCCTACACC-3$^/$ 5$^/$-FAM-CCTTTCTGGGTTGAACA-3$^/$-BHQ1 5$^/$-VIC-CCTTTCTGGGTTGCACA-3$^/$-BHQ2	136 bp

Sangiovese variety genetic identification and DNA authentication of the wine produced from it, based on the assessment of single-nucleotide polymorphism is established by the presence of allele *C* in the homozygous state (*CC* genotype) in all the three SNP analytical positions [11] (Table 6).

Another type of SNP markers application is the use of knowledge about single nucleotide polymorphism in a number of *Vitis vinifera* L genes integrated into melting curves analysis with high resolution (HRM analysis) on PCR platforms in real time [12, 57, 58].

HRM analysis is an effective technology of genotyping [59] with combined stages of PCR and detection of a high degree of specificity and sensitivity, capable of differentiating between several genotypes within one analysis, and suitable for wine DNA authentication [12, 57].

Table 7 presents the sets of PCR primers with subsequent HRM analysis and/or sequencing by grape varieties genetic identification and DNA authentication of the wines produced from them.

Primers Vv3-Fwd and Vv3-Rev initiate amplification of the *UFGT* gene locus, 119 bp long, with localization of the flanked region in the range of 387–505, covering five SNP analytical positions (424, 425, 442, 459, 483) [12] interpreted by HRM analysis and/or sequencing (Table 8).

F3H_H1fwd and F3H-H1rev primers initiate amplification of the *F3H1* gene locus, 375 bp long, with localization of the flanked region in the range of 5–379, covering two SNP analytical positions (47 and 291) [12, 58]; F3H_H2fwd and F3H_H2rev primers initiate amplification of the *F3H1* gene locus, 532 bp long [30], with the flanked region localization in the range of 975–1506, covering eight SNP analytical positions (1039, 1040, 1065, 1157, 1318, 1381 and 1464) [58], respectively, also interpreted by HRM analysis and/or sequencing (Table 9).

Table 6. SNP genotypes of the three wine grapes analytical positions (98, 222 and 244)

Wine grapes	SNP genotypes		
	98	222	244
Sangiovese	*CC*	*CC*	*CC*
Canaiolo	*GG*	*TT*	*AA*
Cabernet Sauvignon			
Chardonnay	*CG*	*CT*	*AA*
Ciliegiolo			
Pinot Noir			
Sauvignon Blanc			
Teroldego			
Merlot	*CG*	*TT*	*AA*
Petit Verdot			
Riesling Renano			
Tannat			
Montepulciano	*CG*	*TT*	*AC*
Primitivo			
Tempranillo	*GG*	*CT*	*AA*

As can be seen from the considered examples, single gene locus SNP identification does not allow the origin of the analyzed wine material, to be established unambiguously therefore, it is not universal. In order to be able to determine the effectiveness of identification methods, as well as their combinations, the concept of identification distance was introduced for the first time. The identification distance (ID) of grape variety A from grape variety B is the number of polymorphic nucleotide positions (SNP) which allow identifying the presence of grape variety B DNA material impurities in wine material A. As seen from the definition, ID is asymmetric due to the presence of mixed nucleotides. For example, *F3H*-gene locus SNP analysis (Table 9) allows to determine the presence of Chardonnay grape variety in Cabernet Sauvignon wine material while detecting polymorphic nucleotide positions 1157, 1381 and 1464 (ID = 3). At the same time, it is not possible to differentiate Cabernet Sauvignon grape variety in Chardonnay wine material by this approach (ID = 0).

Fig. 3 shows the diagram of identification distances according to *F3H*-gene locus SNP analysis (Table 9), where the varieties with identical polymorphic nucleotide positions are combined into a single group.

Thus, it is urgent to create a complex method of wine DNA authentication, allowing a robust analysis procedure in the context of identification distance (namely, uncovering within the method the ID minimum threshold value for all variety pairs that is acceptable for the purposes of standardization) to identify and differentiate grape varieties in both varietal and assembling commercial wines

The arsenal of molecular genetic markers used for wine DNA authentication can be significantly expanded by the development of genomic and postgenomic technologies, with the introduction of experimental developments in the product quality management system based on the standards developed for the wine industry, especially while identifying wines of protected geographical indications, wines of protected designations of origin, and for the purposes of counterfeit products identification [1, 2, 60].

In the meantime, the existing methods of wine DNA authentication, published in scientific literature, have a recommendatory status, are not regulated by GOST/ISO and other legal documents of the Russian Federation, the Customs Union, the International Organization of Vine and Wine, and the European Union.

Legal and regulatory replenishment of the approved wine identification complex scheme with new DNA authentication methods will increase assessment reliability of authenticity and place of origin of wine products.

Table 7. PCR primers with subsequent HRM analysis and/or sequencing by grape varieties genetic identification and DNA authentication of the wines produced from them

Gene locus	Name and sequence of oligonucleotide primers	PCR product	Reference
UFGT	Vv3-Fwd: 5′-AGCAGAGATGGGGGTGGCTT-3′ Vv3-Rev: 5′-AGCAGGTAAAACCACCTGAA-3′	119 bp	[12]
F3H1	F3H_H1fwd: 5′-AGAGAAAGAAGGCGACGT-3′ F3H-H1rev: 5′-GATGGCTGGAAACGATGA-3′	375 bp	[12, 58]
	F3H_H2fwd: 5′-CTGTTGAAGGAGCTTTCG-3′ F3H_H2rev: 5′-GGCTTGGACTCTAACTTG-3′	532 bp	[58]

Table 8. Polymorphic nucleotide positions of wine grapes *UFGT*-gene

Wine grapes	Polymorphic nucleotide positions (SNP)				
	424	425	442	459	483
Touriga Brasileira	-	G	C	C	G
Chardonnay					
Gouveio					
Donzelinho Tinto					
Tinta Francisca	-	G	C	C	S
Alicante Bouschet					
Tinta Amarela					
Côdega do Larinho					
Fernão Pires	-	G	C	T	G
Tinta Roriz					
Malvasia Fina					
Tinto Cão	-	G	C	Y	G
Pinot Noir					
Merlot	-	K	C	C	C
Tinta Barroca					
Touriga Nacional	-	K	C	C	S
Touriga Franca					
Moscatel Galego	-	G	C	Y	G
Vio Viosinho	-	G	C	Y	S
Ruf Rufete	-	G	M	Y	G
Cabernet Sauvignon	T	G	C	Y	S
Sou Sousão	T	G	C	T	S

Note. A – is adenine, C – cytosine; T – thymine; G – guanine, M – A or C, S – C or G, Y – C or T, K – G or T, - – deletion.

Table 9. Polymorphic nucleotide positions of wine grapes *F3H*-gene

Wine grapes	Polymorphic nucleotide positions (SNP)								
	47	291	1039	1040	1065	1157	1318	1381	1464
Tinto Cão	C	T	A	A	C	S	C	R	W
Touriga Franca									
Touriga Nacional									
Alicante Bouschet	Y	T	W	W	C	G	Y	A	A
Rufete									
Cabernet Sauvignon	C	T	A	A	C	C	C	G	T
Sousão									
Chardonnay	C	T	A	A	C	S	C	R	W
Tinta Amarela									
Donzelinho Tinto	C	W	A	A	M	G	C	A	A
Tinta Barroca									
Fernão Pires	Y	T	W	W	C	S	Y	R	W
Touriga Brasileira									
Malvasia Fina	Y	W	W	W	M	G	Y	A	A
Tinta Francisca									
Gouveio	C	W	A	A	M	G	C	A	A
Merlot	C	W	A	A	M	S	C	R	W
Moscatel Galego	C	T	W	W	C	G	Y	A	A
Tinta Roriz	C	A	A	A	A	G	C	A	A
Viosinho	Y	T	W	W	C	G	Y	A	A

Note. A – is adenine, C – cytosine; T – thymine; G – guanine, M – A or C, S – C or G, Y – C or T, W – A or T, R – A or G.
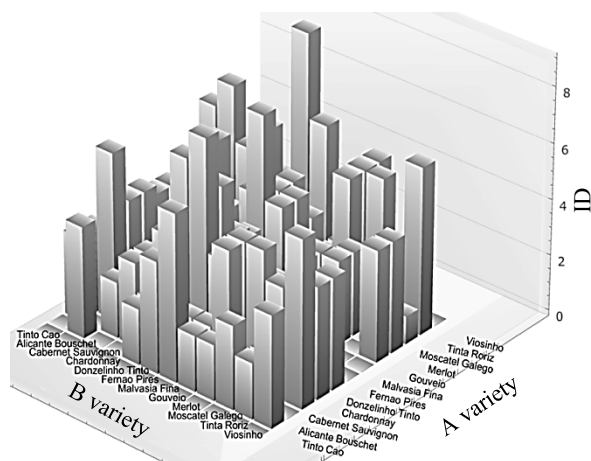

Fig. 3. Identification distance of grape varieties according to *F3H*-gene locus SNP analysis.

CONCLUSION

Analysis of research and methodological approaches to the extraction of residual amounts of nucleic acids in wine raw materials and wine DNA authentication confirms the relevance of this research line and the prospects of its integration into the system of monitoring counterfeit and adulterated wine products. It shows the possibility of determining wine products authenticity and place of origin by DNA technologies, whose use implies ensured traceability of the product's entire life cycle. The concept of identification distance (ID) between grape varieties A and B is introduced. ID stands for the number of polymorphous nucleotide positions (SNP), which allow the presence of grape variety B DNA material in variety A wine material to be identified. The algorithm of ID calculation and analysis of the information obtained is offered.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Ensuring the safety of the lipid fraction of semi-finished products of a high degree of preparation from fatty fish raw materials

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Abstract: The relevance of the studies is caused by the need to improve the safety and extend the shelf life of semi-finished products from fatty fish raw materials. The study was carried out at the premises of the Saratov State Agrarian University. Study objects were fish mixed fodder; two-year-old carps; fish raw materials; carp semi-finished products. Physicochemical, histological, and organoleptic research methods were used in the study. The authors have developed a method for inhibiting the process of fish fat oxidation at all the stages of the life cycle of fish products using CO_2 rosemary extract and milk thistle oil meal as antioxidant components because they contain flavonolignans and carnosic and rosmarinic acids. Technological methods for obtaining complex fish mixed fodder with antioxidant properties were developed and the optimal dosages of the antioxidants added to a feed supplement were determined. A positive effect of an antioxidant supplement on the fish biological characteristics of a reared carp, as well as on the morpho-functional indicators of the obtained raw materials, was shown. The authors developed formulations of the fish semi-finished products made from the raw materials grown using antioxidant fish mixed fodder. The safety and quality indicators of the developed products were estimated. They showed that the use of an antioxidant component at the stage of fish rearing and in manufacturing process of fish semi-finished products makes it possible to significantly improve the stability of the fat phase both in fish raw materials and in the finished products.

Keywords: Fish products, oxidation, fat component, natural antioxidants, semi-finished products of a high degree of preparation, centralized production

INTRODUCTION

The modern market of fish products of a high degree of preparation (fish cutlets, chopsticks, rissoles, meatballs, etc.) is characterized by the use of low-fat varieties of sea fish, mainly imported ones. The manufacturers of these products try not to use fatty fish varieties because of the possible loss of consumer properties of products at the circulation stage due to the rancidity (oxidation) of the fat component [15, 16]. It is known that the determining factor of the shelf life of fish and fish products that causes negative changes in the organoleptic properties and nutritional value, as well as the formation of toxic products, is lipid oxidation [1, 17, 18]. In addition, fatty sea fish varieties are characterized by a rather high cost, which makes it impossible to use them in manufacturing semi-finished products of a high degree of preparation and prohibitive to the majority of the population of our country [7].

Up to date, one of the most important tasks of the food industry is to meet the need and ensure the per capita consumption of the population of our country with quality and safe fish products taking into account the biological norms of consumption and the reduction of import dependence.

With the current pace of import substitution development, there is an increase in carp meat popularity, as the domestic pond fish raw materials are cheap and easy in rearing and in manufacturing.

However, when using it in industrial production, there is a problem to stabilize lipids since the unsaturated nature of carp lipids has a negative effect on oxidative stability during autolysis [11, 13, 19]. A large number of factors that initiate fat oxidation, as well as the chain mechanism of the process and the distinctive features of the technology for manufacturing various types of products, do not allow

for the development of a universal method for preventing the oxidative damage of products with a high proportion of the fat component.

For this reason, it is necessary to estimate the main factors of lipid oxidation and to select the appropriate methods for inhibiting them for a particular food system. An effective way to increase oxidative stability is the addition of antioxidants. Moreover, the approach to the antioxidant protection of each food system should be individual.

In this regard, the development of a method for the oxidative stabilization of carp lipids and the prolongation of the shelf-life of carp semi-finished products and culinary products is an urgent task since the widespread development of the fish production centralized industry is possible in case of the assured safety and quality of the products.

The presented study is aimed at developing a method for the oxidative stabilization of carp lipids and the prolongation of the shelf life of semi-finished products of a high degree of preparation by inhibiting the oxidation of the lipid fraction at all the stages of the manufacturing process.

STUDY OBJECTS AND METHODS

The objects of the studies were:
– standard mixed fodder for feeding two-year-old carps;
– the developed mixed fodder with antioxidant properties on the basis of standard mixed fodder with the addition of milk thistle oil meal (TU 9141-005-46899394-04) and CO_2-rosemary extract (TU 9169-001-10140736-03);
– two-year-old carps of the experimental and control groups;
– fish raw materials obtained after feeding with common feed and cultivated feed; and
– semi-finished products of a high degree of preparation from carp with the specified high antioxidant properties.

The fish were reared in the laboratories of the of Feeding, Zoohygiene and Aquaculture Department of the Federal State Budgetary Educational Institution of Higher Professional Education Saratov State Agrarian University in aquariums with a constant flow and water aeration. Aquariums had dimensions of $100 \times 50 \times 40$ cm and a volume of 200 liters.

The experiment was conducted for 76 days. During the cultivation of two-year-old fish, the thermal conditions were favorable for their growth. The average water temperature was 19.3 and 19.5°C, respectively. The oxygen content was at least 5 mg/l.

The feeding rate was calculated based on the body weight, and water temperature according to the specially developed feed tables [10]. Manual feeding, twice a day during daylight hours, was applied. The daily feeding rate was 3% of the fish body weight.

The fish was weighed according to the recommendations of I.F. Pravdin [20]. All the fish used in the experiment was subjected to control weighing once every 10 days. At the same time, the daily ration was adjusted.

The growth rate was judged by the data of the absolute and average daily gain. The average daily growth rate of the juveniles was calculated using the following formula:

$$A = [(Mк/Mo)1/t - 1] \times 100 \ (\%), \quad (1)$$

where A is the average daily growth rate, %;
Mk and Mo are the mass of the fish at the end and at the beginning of the experiment; t is the duration of the experiment, days.

The absolute gain was estimated using the formula:

$$Pab = Mк - Mo, \quad (2)$$

where Mk is the final mass of juveniles, g; M0 is the initial mass of juveniles, g.

To calculate the mass accumulation coefficient, the following formula was used:

$$Kм = ((Mк1/3 - Mo1/3) \times 3)/t, \quad (3)$$

where Kм is a mass accumulation coefficient, units; Mk and Mo are the final and initial masses of the fish, g; t is the cultivation period, days.

The average daily gain was determined using the formula:

$$P = (Mк - Mo)/t, \quad (4)$$

where P is the average daily gain, g; Mk and M_O are the final and initial masses of the fish, g; t is the cultivation period, days.

The histological analysis of carp muscles was carried out according to the method described by of G.O. Merkulov [6] and M.P. Kokuricheva [5]. The pieces of tissues and organs were immediately placed in a fixing solution, namely a 10% aqueous neutral formalin solution. From the material fixed with the 10% aqueous neutral formalin solution, histological sections 15 μm in thickness were prepared using a model 2515 freezing microtome (ReichertWien). To detect neutral fats and phospholipids, the histological sections were stained with sudan black B.

For biochemical analysis, blood sampling was carried out according to the method of V.V. Limanskiy "Instruction on the physiological and biochemical analyses of fish".

The appearance and color of fish mixed fodder was determined organoleptically according to GOST R 51899-2002 "Granulated mixed feeds. General specifications". The smell of the experimental mixed fodder was evaluated according to GOST 13496.13-75 "Combined animal feeding stuffs. Methods for determinaton of smell, infestation by cereal parasites".

The granule size was determined according to GOST R 51899-2002 "Granulated mixed feeds. General specifications".

To determine the water resistance, GOST 28758-97 "Granular mixed fodders for fish. Methods for determination of water-proofness" was used.

Fats were extracted from the fish raw materials using an extraction-weight method according to GOST 54053-2010 "Confectionery. Methods for determination of fat weight fraction". The mass fraction of methyl esters of individual fatty acids of their total amount was determined by gas chromatography in accordance with

GOST R 51486-99 and GOST R 51483-99 using a Kristall 2000M gas chromatograph. The acidity index of the extracted fat was determined according to GOST R 52110 "Vegetable oils. Methods for determination of acid value". The peroxide number was determined using the method of N.A. Golovkin and R.L. Perkel, and peroxides in fish fats by potentiometric titration. [1.a.i.3].

The organoleptic indicators were estimated in accordance with GOST R 53161-2008. The average score was calculated in accordance with GOST 7631-85, taking into account the weight of individual indicators "Fish, marine mammals, marine invertebrates and products of their processing. Acceptance rules, organoleptic methods of quality control, sampling methods for laboratory tests". To obtain an unbiased evaluation, descriptors, which show the main characteristics of the product and characterize the technological methods that affect the preservation of food and biologically active substances were developed "ISO 11035 Organoleptic analysis. Methodology. General guidance for establishing an organoleptic profile".

RESULTS AND DISCUSSION

The aim of the studies was the development of a method for the continuous inhibition of lipid oxidation at all the stages: from rearing fish raw materials rich in lipids [12, 14] to manufacturing deep-fried semi-finished products of a high degree of preparation therefrom. The study stages included the solution of the following tasks:
– to develop special mixed fodder for fish (carp) with the use of antioxidants to improve the commodity and consumer properties of fish raw materials and increase their shelf life;
– to prove the positive effect of the developed feed on the antioxidant stability of fish raw materials on the basis of the biological studies;
– to develop formulations for fish products of a high degree of preparation for industrial manufacturing with the specified properties using the fish raw materials reared with the use of the new antioxidant mixed fodder.

It is known that some herbs, spices, and their extracts have the ability to slow lipid oxidation down [10, 16].

CO_2-herb extracts are of particular interest. For example, the antioxidant activity of rosemary extract is 10 times higher than that of ionol. Twenty-two substances, among which are phenolic acids, carnosol derivatives, and flavonoids, have been identified in Rosmarinus officinalis extract. In terms of inhibiting lipid oxidation, the most effective are carnosol, rosmarinic acid, carnosic acid, caffeic acid, rosmanol, and rosmadial. Carnosic acid and carnosol are strong lipid peroxidation inhibitors in microsomal and liposomal systems, as well as peroxide radical and superoxide anion absorbers. It was established that rosemary extract is highly resistant to high temperatures [8, 9]. Antioxidant mixtures, rather than individual antioxidants, are advisable for use in manufacturing.

The development of the feed formulation and the balance of the nutrient composition were carried out on the basis of the known carp needs determining the input rates for various components [10]. Milk thistle oil meal rich in antioxidants, flavonolignans and CO_2-rosemary extract, were used [8].

To determine the concentration of CO_2-rosemary extract in the composition of the feed, an experiment was carried out for 4 groups of fish. The study of the behavioral aspects of all the fish groups has revealed that an increase in the concentration of CO_2-rosemary extract results in a decrease in the values of fish-biological indicators (Table 1).

The group with CO_2-rosemary extract in a concentration of 0.05 g per 100 g carp mixed fodder had the best behavioral indicators. The growth rate of this group was equally high throughout the experiment, which confirms the efficiency of this rate of CO_2-extract in carp mixed fodder.

Proceeding from the experiment, to obtain antioxidant experimental feed, the feed mixture was enriched with the above-mentioned antioxidant consisted of 90.8% carp mixed fodder, 9.15% milk thistle oil meal, and 0.05% CO_2-rosemary extract. Table 2 presents the content of nutritional substances of mixed fodder with addition of milk thistle oil meal and CO_2-rosemary extract. The organoleptic and physical indicators of the experimental mixed fodder are represented in Table 3.

Table 1. Behavioral aspects of fish groups that consume various concentrations of CO_2-rosemary extract

Concentration of CO_2-rosemary extract, g	Behavior of fish groups
0.03 (3 drops)	The group ate feed willingly in the morning, less willingly – in the afternoon; and active in the evening; gained weight moderately
0.05 (5 drops)	The group ate feed in the morning and in the afternoon willingly, very active; gained weight well
0.08 (8 drops)	The group eats feed unwillingly, low-active; gained weight slowly
Carp mixed fodder	The group ate feed willingly, active; gained weight moderately

Table 2. Nutrition content of the developed mixed fodder

Nutrients	Content, %	Normal value, %
Crude protein	36.8	30–38
Crude fat	4.2	2–5
Crude fiber	6.9	4–7
Crude ash	4.06	4–7
Lysine	1.82	1.8–2
Methionine	0.98	0.8–1.0
Tryptophane	0.23	0.2–0.3

Table 3. Organoleptic and physical indicators of the experimental mixed fodder

Indicator	Characteristic
Appearance	Cylindrical granules with a matte surface without cracks
Color	Corresponds to the color of bulk mixed fodder, dark brown
Smell	Corresponds to mixed fodder, the smell of CO_2-rosemary extract prevails
Water resistance, min	15–16
Swelling ability, min	27

Note. The choice of indicators is based on GOST R 51899-2002 requirements.

Table 4. Biological indicators of the experimental and control carp groups

Indicator	Groups	
	Control	Experiment
Mass, g:		
initial	390	388
final	469	479
Total gain, g	79	91
Average daily gain, g	1.03	1.19
Average daily growth rate,%	3.94	5.73
Mass accumulation coefficient, U	1.04	1.19
Survivability, %	71.4	71.4

Table 5. Average values of biochemical analysis indicators of blood

Indicator	Units	Control group	Experimental group
Total bilirubin	μmol/l	–	4.6
Direct bilirubin	μmol/l	–	1.3
GOT	U/l	109.9	183.0
GPT	U/l	42.1	15.5
Total protein	g/l	62.6	56
Creatinine	μmol/l	–	49.9
Urea	mmol/l	6.7	2.8
Uric acid	μmol/l	257.8	115.7
Glucose	mmol/l	6.7	6.5
Calcium	mmol/l	2.90	3.25
Phosphorus	mmol/l	1.51	2.23
Magnesium	mmol/l	1.26	1.63
Sodium	mmol/l	–	189.9
Potassium	mmol/l	–	6.5
Iron	μmol/l	–	28.4

The developed fish mixed fodder with antioxidants is balanced in terms of the basic food substances and has good organoleptic and physical indicators.

At the next stage of the experiment, experimental and control groups of two-year-old carps were formed, 14 pieces each.

Table 4 presents the positive dynamics of the live weight of carps in the experimental groups compared to the control.

To study the physiological state of the reared fish groups, biochemical parameters of blood were investigated (Table 4). The results showed that the use of the developed antioxidant feed did not influence its biochemical parameters.

Thus, the use of antioxidant carp feed balanced in the basic nutrition elements positively affects the blood test and the general physiological state of the reared fish.

For detailed investigation of changes in the body, the authors carried out a histological analysis of fish muscle tissue.

The histological sections of fish tissues showed that fats and lipoids in the muscle tissue of fish from the experimental group are larger in size and higher in quantity in comparison with the control group. Thus, one can see from Fig. 1 that fats and lipoids of the control group are presented as single drops, while those in the tissues of the experimental group (Fig. 2) are evenly distributed in muscles; they are larger in size and are contained in larger amounts, which will improve the taste and technological properties of fish.

12 fish from the control group and 20 from the experimental group were involved in the study. Table 6 shows the minimum and maximum sizes of lipoids in both groups. Thus, fat drops (lipoids) in the tissues of the experimental group are bigger than those in the control by 60.385 micrometers, on average.

When manufacturing semi-finished products, the quality of fish raw materials were evaluated.
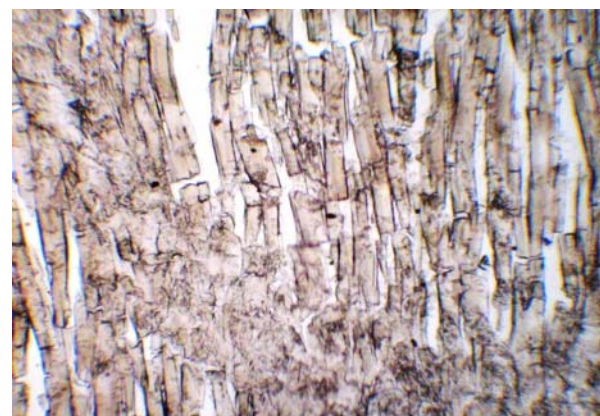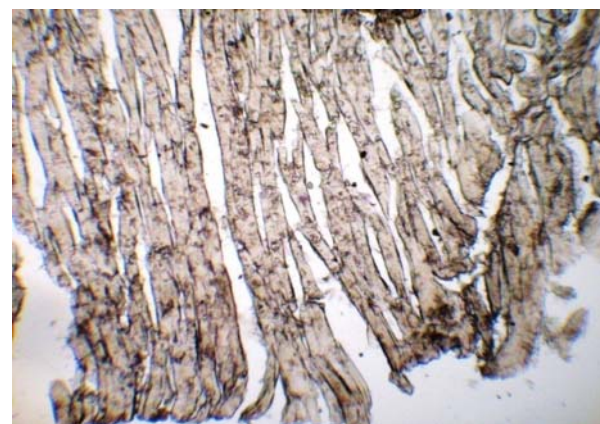

Fig. 1. Microphotograph of the carp tissue section. Small drops of fats and lipoids in the control group.

Fig. 2. Microphotograph of the carp tissue section. Fats and lipoids in the experimental group.

Table 6. Indicators of the linear and geometric values of lipoids in carp

Group	Area, μm^2		Perimeter, μm		Average size, μm	
	Min	Max	Min	Max	Min	Max
Control group 1	5.679 ± 0.003	9.872 ± 0.003	8.486 ± 0.003	11.142 ± 0.003	2.794 ± 0.003	3.694 ± 0.003
Control group 2	0.084 ± 0.003	9.521 ± 0.003	1.828 ± 0.003	11.433 ± 0.003	2.156 ± 0.003	3.688 ± 0.003
Experimental group 1	3.708 ± 0.003	13.279 ± 0.003	7.155 ± 0.003	13.552 ± 0.003	2.344 ± 0.003	4.316 ± 0.003
Experimental group 2	4.376 ± 0.003	16.135 ± 0.003	7.586 ± 0.003	14.604 ± 0.003	2.458 ± 0.003	4.747 ± 0.003

Table 7. Fatty acid composition of the lipid fraction isolated from carp

Fatty acid notation	Fatty acid	Test results		Test method reference	Method error
		Control	Experiment		
C 12:0	Dodecanoic	0.1	0.1	GOST R 51483-99	At the content of the substances sought: less than 5% – 0.28%; equal to or more than 5% – 1.42%
C 14:0	Myristic	1.2	1.6		
C 16:0	Palmitic	15.4	15.9		
C 16:1	Palmitoleic	3.8	4.2		
C 18:0	Stearic	3.8	4.7		
C 18:1	Oleic	31.5	32.4		
C 18:2	Linoleic	41.9	39.0		
C 18:3	Linolenic	0.2	0.3		
C 20:0	Arachic	2.0	1.7		

Table 8. Peroxide number content and acidity index in the fish tissues

Indicator	Unit	Test results		Method error
		Control	Experiment	
Acidity index	mgKOH/g	10.3	6.4	$\pm 7\%$ rel.
Peroxide number	mEq of active oxygen/kg	2.92	2.78	$\pm 14\%$ rel.

The fatty acid composition of the fat phase (Table 7) isolated from the product showed that the ratio and amount of fatty acids slightly varies. In the experimental group, the content of saturated acids slightly increases with respect to unsaturated ones, which favorably influences the storage of fish raw materials. The content of linoleic acid decreases. At the same time, there is an increase in the amount of oleic acid that has a beneficial impact on the maintenance of body immunity.

Thus, changing the diet of carp, it is possible to vary the ratio and the quantitative composition of fatty acids.

Of the indicators that characterize the safety of the product, the peroxide number and acidity index were determined.

During storage, free fatty acids accumulate due to the hydrolysis of muscle lipids under the influence of tissue lipases.

Table 8 shows the content of peroxides and free fatty acids of the fat component in experimental and control groups of fish.

Table 8 shows that the content of free fatty acids in the experimental group is almost 2 times less than that in the control group. This suggests that the hydrolytic processes in the tissues of the experimental group of fish are slower during storage. Nevertheless, the accumulation of free fatty acids in fish lipids is quite intense.

The content of peroxides in both groups corresponds to the norms (no more than 10 mEq/kg) and in the experimental group it is less than in the control group.

Thus, the change in the quality of the lipid fraction of fish after giving them the feed that contain natural antioxidants was characterized by a decrease in the peroxide number and acidity index.

The results of the study testify to the effect of the antioxidants added to the feed on a change in the fatty acid composition and the quality of the lipid fraction of the experimental group of fish.

Deep-fried semi-finished products (fish croquettes) without and with the addition of CO_2-rosemary extract (the control and the experimental sample, respectively) were manufactured in order to develop a formulation for semi-finished fish products of a high degree of preparation with a prolonged shelf life under laboratory conditions for industrial manufacturing.

The purpose of adding vegetable ingredients to the formulation was to improve the safety and organoleptic characteristics of fast food products from fish raw materials, as well as to increase their shelf life.

As a research model was chosen deep-fried fish croquettes, since those are popular among consumers, who prefer quick-frozen fish culinary products, such as fish sticks, cutlets, rissoles, and meatballs [7], as well as because of the expansion of the assortment of this group of products.

The semi-finished products of a high degree of preparation (French croquettes) were prepared from the fish raw materials grown with the use of the antioxidant mixed fodder we had developed to produce fish raw materials with the specified properties according to the formulation given in Table 9. CO_2-rosemary extract was used as an antioxidant.

The formulation considers the following losses: 1% when mixing, 1% when molding, 20% when frying, and 5% the weight gain when crumbing.

The optimum concentration of rosemary extract in mincemeat was selected experimentally. Table 10 and Fig. 3 show the organoleptic characteristics of fish croquettes with the addition of various concentrations of CO_2-rosemary extract.

Table 9. Croquette formulation

Component	Net weight, kg		
	Concentration of CO₂ – rosemary extract in minced meat, %		
	0.03	0.04	0.05
Fish (carp)	80	80	80
Wheat bread	6.2	6.2	6.2
Milk	20	20	20
Eggs	12	12	12
Cooking fat	3.1	3.1	3.1
Yellow onion	6.2	6.2	6.2
Bread crumbs	–	–	–
Salt	2.5	2.5	2.5
Ground pepper	0.02	0.02	0.02
CO_2-rosemary extract	3	4	5
Yield	100	100	100

As one can see from Fig. 3 and Table 9, the sample with a concentration of rosemary extract of 0.05% has the best indicators: a pleasant fresh smell of fish and rosemary, a homogeneous and smooth texture, a pleasant taste, and a homogeneous color.

Fig. 4 shows the process stream of fish croquette production. During the manufacture, CO_2-extract with calculated concentration was mixed with the fish mince. Then, the rest of the ingredients (black pepper and salt) were added and also mixed. The resulting mass was molded into round molds and crumbed twice. The weight of one unit was 60 g. The semi-finished products were fried in a large amount of oil at a temperature of 140–150°C for 5–7 minutes until a

brown crust was formed, then cooled to a temperature of not higher than 15°C.

It is important to note that the developed technology does not require the re-equipment of enterprises, and therefore will not make the manufactured products more expensive.

Table 11 shows the safety parameters of deep-fried fish croquettes after 3 months of storage at low negative temperatures (-18–24°C).
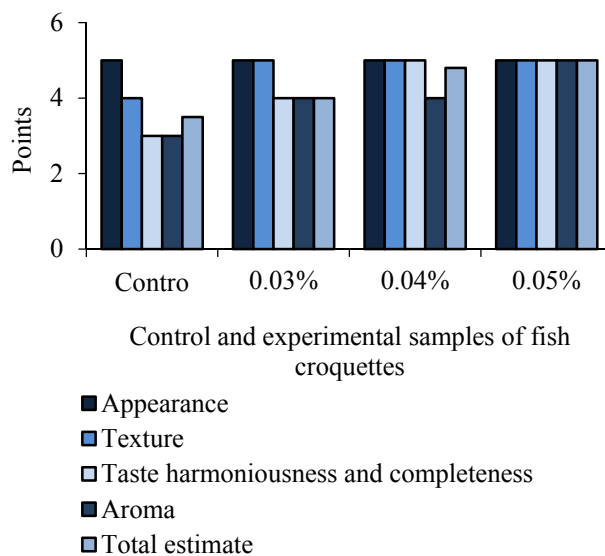

Fig. 3. Organoleptic estimation of fish croquettes with the addition of various concentrations of CO_2-rosemary extract.

Table 10. Organoleptic characteristics of fish croquettes with the addition of CO_2-rosemary extract in various concentrations

Concentrations of CO_2-rosemary extract	Appearance	Color	Smell	Taste	Textute
0.03	The structure is of a regular round shape, the surface is evenly crumbed	A brown crust, white meat on the cut	The distinctive smell of fried fish	A distinctive fish taste	Homogeneous, smooth
0.04	The structure is of a regular round shape, the surface is evenly crumbed	A brown crust, white meat on the cut	A fresh fried fish aroma	Pleasant taste	Homogeneous, smooth
0.05	The structure is of a regular round shape, the surface is evenly crumbed	A gold brown crust, white meat on the cut	A pleasant fresh fried fish aroma	A pleasant taste with a pleasant aftertaste	Homogeneous, smooth

1 – combining components   3 – croquette molding   5 – packing   7 – freezing
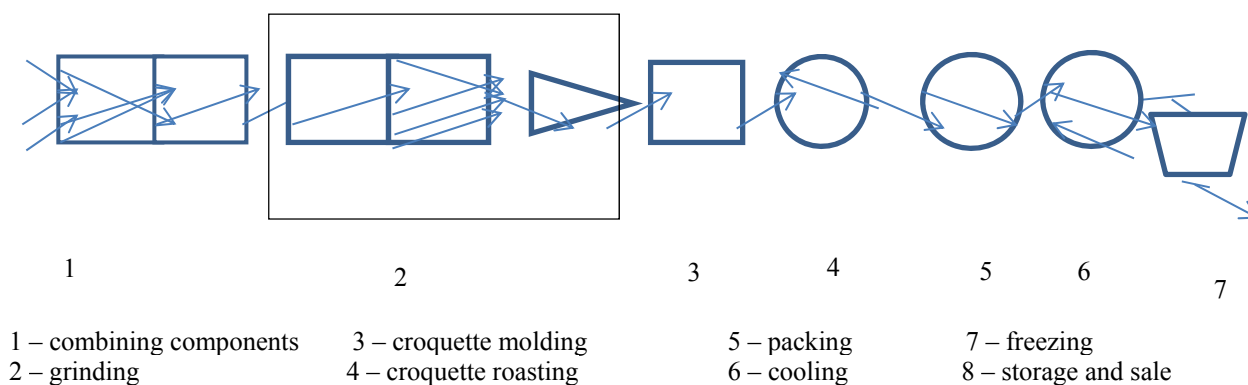2 – grinding   4 – croquette roasting   6 – cooling   8 – storage and sale

Fig. 4. Fish croquette process stream.

Table 11. Safety parameters of the extracted fat component of fish semi-finished products of a high degree of preparation

Indicator	Test results		Concentration of oxidation by-products – petroleum ether insoluble copolymers, %
	Acidity index, mg KOH/g	Peroxide number, mEq of active oxygen/kg	
Croquettes without antioxidants	3.9	23	1.4
Croquettes with antioxidants	2.9	9.9	0.45

In terms of safety parameters, the croquettes produced from unstabilized fish raw materials and mincemeat are more than twice the established norms for a peroxide content and 1.5 times – oxidation by-products – petroleum ether insoluble copolymers. The croquettes for the production of which stabilized fish raw materials and CO_2-rosemary extract were used correspond to the required safety parameters.

During the study, the expediency of antioxidant stabilization of fish lipids for manufacturing industrial products from fish raw materials was proved. Special antioxidant mixed fodder for fish (carp) was developed to improve commodity-consumer properties and to increase the storage and sales terms in the following component ratio: 90.8% of carp mixed fodder; 9.15% of milk thistle oil meal; and 0.05% of CO_2-rosemary extract. The clinical and physicochemical studies of fish and fish raw materials showed that the use of an antioxidant feed supplement had a positive influence on the fish-biological characteristics of fish, as well as on the antioxidant stability of the fish raw materials obtained after providing the developed feed. A number of formulations of fish semi-finished products of a high degree of preparation for industrial manufacturing with functional properties and a prolonged shelf life was developed with the rosemary concentration of 0.05g of per 1 kg of mincemeat for fish croquettes.

CONCLUSION

The technology of antioxidant stabilization of carp lipids has been developed at the stage of rearing and carp raw material processing, which makes it possible to increase the safety the finished product and improve the commodity-technological properties. The use of CO_2-rosemary extract in fish mixed fodder and then in the fish mincemeat produced from the fish reared with the use of antioxidant stabilized mixed fodder (silymarin and carnosic and rosmarinic acids) allows us to significantly improve the stability of the fat phase, both in fish raw materials and in the finished product.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The results of the research are of no commercial interest to legal entities or individuals. The article does not describe objects of patent rights or any other type of rights, except for copyright.

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Criteria for standartization of probiotic components in functional food products

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Abstract: The increasing volume of consumption of probiotics and functional food products requires determination of standardized criteria for cultures and their exometabolites used in functional products manufacturing. The study was aimed at developing criteria for the estimation and standardization of exometabolites and the colony-forming ability of probiotic strains for functional food production. The work included such microbiological and physicochemical methods as GC-MS, GPC, UV, and FT-IR-spectroscopy. Based on the results of the study, the comparative analysis of the microbiological properties of probiotic *Bifidobacterium* strains was provided, the fatty acid composition of the cell wall was described, and the physical and chemical study of the exopolymers produced by them was carried out. According to the data of FT-IR-spectroscopy, the characteristic features of the components of the cell wall of *Bifidobacterium* strains were established. Bifidobacteria form the unique composition of organophosphorus structures of lipoteichoic acids, which determines the adhesive ability of strains. The authors studied the molecular weight distribution of the samples of exometabolites isolated from the nutrient medium after the cultivation of bifidobacteria, under conditions of gel-permeation chromatography. The spectral (UV, FT-IR) characteristics of the produced metabolites and their chromatographic fractions were compared. The fatty acids of the *Bifidobacterium* cell membrane were analyzed using the GC/MS method. The fatty acids were extracted from bacterial cells with different hydrophobicity with a mixture of chloroform and hexane. It has been established that the hydrophobicity is determined by different contents of unsaturated and branched fatty acids in the bacterial membrane. Hydrophobic bifidobacteria are the only that contain the isopentadecane (*iso*C15:0) and methyl-tetradecanoic (13Me-C14:0) acids. With the mean hydrophobicity, a high content of the isopalmitic (*iso*C16:0) and stearic (C18:0) acids was established. Low-hydrophobic strains are characterized by a low content of monounsaturated fatty acids.

Keywords: Bifidobacteria, hydrophobicity, exometabolites, lipoteichoic acids

INTRODUCTION

Probiotics are the promising components of functional food products, as well as of biologically active supplements [1]. The demand for probiotic functional products is growing rapidly due to an increase in the consumer awareness of the effect of food on health and life expectancy.

Most of probiotics are used as the functional food products that are a significant share of the food and pharmaceutical market in the developed countries and

displace a lot of traditional medicines from sales. It should be noted that probiotics maintain the normal intestinal microflora, protect the gastrointestinal tract from colonization by pathogenic microorganisms, showing the antagonistic properties to the latter, improve the functioning of the body's immune system, reduce serum cholesterol, and normalize blood pressure. All these contribute to health maintenance [2–4]. It is also proved that some probiotic strains have an anticarcinogenic activity and contribute to the improvement of metabolism and an increase in the nutritional value of food products. From year to year, the list of pathological conditions extends, which requires the use of probiotics both as part of medicines and in the form of functional food products. Probiotics are used to prevent diabetic diarrhea, urogenital diseases, osteoporosis, food allergies, and atopic conditions, as well as to relieve constipation and to reduce hypercholesterolemia [5].

The creation of functional food products while maintaining the adequate number of probiotic microorganisms is a problem, since there are many factors, both during production and when storing the finished product, which affect the viability of probiotic bacteria [6]. However, it is important to estimate the strains themselves selected for specific functional probiotic properties. First of all, this is the possibility of providing colonization resistance, which is caused by the adhesive activity of microorganisms. Probiotic bacteria should have the ability to competitively adhere to the epithelium through glycoconjugate receptors, closing the attachment points for pathogenic microorganisms. Using the example of Caco-2 colonocyte culture, it was shown that living probiotic strains, adhering to the epithelium due to the induction of the synthesis of TM-5 tropomyosin, actin and occlusin, strengthen the cytoskeleton of enterocyte cells. In parallel with this, the MUC-3 gene is stimulated, the phosphorylation of the protein of the intercellular compounds increases, the synthesis is stimulated, the epithelial growth factor receptor is activated, and the synthesis of the polyamines, which play the role of hormone-like substances, increases. As a result, the permeability of intercellular compounds decreases, the mucin synthesis increases, and the epithelial regeneration processes intensifies [7, 8]. These mechanisms, as a whole, increase the resistance of the intestinal epithelium and its protective functions. However, these effects were obtained and studied *in vitro* using a limited number of probiotic bacteria, whereas in the human body they can be leveled or realized in a low volume therefore approaches are needed to estimate the factors of microorganism adhesion, rather than the process of bacterial adhesion [9]. It was proved that probiotics take part in the formation of free amino acids, organic acids, oligosaccharides, short chain fatty acids, bioactive peptides, and bacteriocins, as well as affect the synthesis of vitamins such as biotin, vitamin K, and the vitamin B complex [10, 11]. Therefore, the estimation of the exometabolites produced by probiotic strains is also urgent and is sought after the producer.

To provide a single scientifically grounded approach to the estimation of the quality and safety of the strains of probiotic microorganisms intended for the production of direct probiotic starters, bacterial concentrates, and the microbial biomass, which is a component of food and biologically active supplements or used to enrich traditional food products, some methodical guidelines were developed [12]. The estimation included a complex of microbiological, biochemical, molecular genetic, and hygienic studies to confirm their safety (harmlessness), as well as the presence of their properties that determine the probiotic effect in the body and are therefore suitable for creating probiotic food products and biologically active food supplements. In this case, it is necessary to take into account a large number of factors and properties of potential strains of microorganisms in order to achieve a further health-saving effect [13]. Classical microbiological methods are routine, time-consuming, cost-intensive and often limitedly characterize the properties of the strain under study. Therefore, when searching for original probiotic cultures meeting the requirements of medical science, dietology, the food industry, and biotechnology, it is promising to use complex approaches to the estimation of their properties. In this regard, the criteria for estimating the functional activity of probiotic strains and their safety, regulated by this methodical document [12], are of special significance. However, this regulatory document does not provide any specific methods for studying the antioxidant activity of bacteria and for determining the composition of exopolysaccharides. The colony-forming abikity of probiotic cultures was proposed to be determined *in vitro* and *in vivo* using biological models (erythrocytes in rabbits), whereas it is more informative to study precisely the factors of specific and nonspecific adhesion in a specific strain.

The study was aimed at developing criteria for the estimation and standardization of exometabolites and the colonization potential of the probiotic strains intended for manufacturing functional food products.

STUDY OBJECTS AND METHODS

The object of the study was the probiotic strains of *Bifidobacterium bifidum 1, B. longum B 379M*, and *B. breve* isolated from commercial preparations. The bifidobacteria were isolated from preparations using a routine bacteriological method. Anaerobic jars (BBL, USA) and gas-generating packets (NPO Novoe Delo, St. Petersburg) were used to create anaerobic conditions. The phenotypic biochemical profile of strains was studied using commercial ANAERO-TEST 23 test systems (Lachema, Czech Republic).

The activity of acid formation of bifidobacteria was determined by alkalimetric titration. To this end, 2 drops of phenolphthalein indicator were added to the daily cultures of bifidobacteria grown in Blaurok's medium in a volume of 5 ml and titrated with 0.1 M NaOH. The volume of sodium hydroxide that was used for titration was equimolar to the amount of the acid produced by microorganisms contained in 10 ml of the

culture liquid. The result was expressed in Turner degrees:

$$T° = V_1 × K × 20, \qquad (1)$$

where V_1 was the volume of a 0.1 M sodium hydroxide solution that was used to titrate 10 ml of the studied liquid, K was the correction factor determined when titrating a 0.1 M alkali solution with a 0.1 M succinic acid, and T° – the value that expressed the amount of 0.1 M sodium hydroxide used to titrate 100 ml of the studied sample.

The hydrophobicity of bifidobacteria was estimated according to Rosenberg et al. in the modification by L-Q Wang et al. To this end, the bifidobacteria were grown for 24 hours in a liquid Bifidum-medium (Obolensk) and then centrifuged at 8,000 g for 10 minutes. The bacterial mass was washed twice with a phosphate buffer and resuspended in the same solution. The optical density (A) of the suspension was determined at a wavelength of 600 nm. Then, 1 ml of dodecane was added to 3 ml of the bacterial suspension. The phases were mixed using Vortex for 2 minutes and left for 1 hour at 37°C to separate them. The optical density (A) of the aqueous phase was determined at 600 nm. The affinity to hydrocarbons was calculated as the percentage of hydrophobicity using the formula

$$H = [(A_0 − A)/A_0)] × 100, \qquad (2)$$

where H is hydrophobicity, %; A_0 and A are the optical density before and after processing the bacterial suspension by dodecane. The strains were considered highly hydrophobic at H = 60% and higher, mid-hydrophobic at H = 40–59%, and low-hydrophobic at H ≤ 39%.

The adhesive properties of microorganisms were studied according to a method by V.I. Brilis (1986). To this end, slant meat-and-peptone agar cultures were grown for 24 hours. The suspension of microorganisms was prepared on the basis of a sterile isotonic sodium chloride solution at a concentration of 10^9 CFU/ml. The cellular substrate was the formalized human erythrocytes 0 (I) of the Rh (+) group, with a density of 100 million/ml. The erythrocytes and the suspension of microorganisms were combined in the equal volumes of 50 μl in test tubes and incubated at 37°C for 1 hour, regularly shaking the mixture. After that, a smear was prepared, dried, preserved in 96% alcohol for 15 minutes, and sent for Romanowsky-Giemsa staining. The adhesion was studied under a light microscope, the counting was carried out using 50 erythrocytes. The results of the experiment were estimated according to the microorganism adhesion index (MAI) that characterized the average number of microbial cells on one erythrocyte participating in the adhesive process. The microorganisms were considered non-adhesive at MAI ≤ 1.75; low-adhesive – from 1.76 to 2.5; medium-adhesive – from 2.51 to 4.0; and highly adhesive at MAI ≥ 4.0.

The lipoteichoic complex was extracted from the cell wall as follows. The bacterial culture grown in a liquid nutrient medium was washed, suspended in ice waterб and destroyed by ultrasonic treatment at 25 kHz. The suspension was centrifuged at an acceleration of 50,000 g for 15 minutes. The cell wall suspension was heated in water at 80°C for 3 minutes, then washed in water at 4°C. The non-covalently bound membrane protein was removed from the suspension by adding the equal volume of 5% (w/v) sodium dodecyl sulfate (SDS) and mixed for 4 hours at room temperature. To remove SDS, the cell walls were washed six times, replacing the volume of water with intermediate centrifugation at 50,000 g for 10 minutes at 20°C. The extraction was carried out using 10% (w/v) trichloroacetic acid with heating the sample at 90°C. After centrifugation at 50,000 g, the precipitation was carried out by adding excess dimethyl ketone in the cold. The carbohydrates were purified by ion exchange gel-permeation chromatography. To this end, the samples dissolved in 2 ml of water were applied to the column (30 cm × 1.5 cm) of DEAE cellulose pretreated with acetate pyridinium (pH 5.3), then equilibrated with water and eluted with a mixture of 50 ml of water and 50 ml of pyridinium acetate (pH 5.3). The fractions were collected in 2 ml by means of a Diafrak-002 fraction collector. The yield of the carbohydrate component in the fractions was controlled refractometrically.

The UV spectra were obtained using a SF-2000 instrument within the range of 190–340 nm with a resolution of 1 nm compared to a cuvette with a pure solvent.

Fourier transform IR spectroscopy was performed by means of a FSM-1202 instrument (Infraspek, Russia) using a MATIR sampler with a ZnSe crystal. The study was carried out in the transmission mode, within the range of 4000–465 cm^{-1}, with a resolution of 4 cm^{-1}, and a number of scans of 20. An empty cuvette was used as an example of comparison, the reference sample had been recorded immediately before each sample was analyzed.

In the gel-permeation chromatography (GPC) mode, Sephadex LH-20 (Pharmacia, Sweden) packed in a chromatographic column d = 6 mm was used as a chromatographic polysaccharide matrix (Pharmacia, Sweden). The volume of the adsorbent gel was 30 ± 1 ml, the elution rate was 0.1–0.15 ml/min, and the volume of the prepared fractions was 0.5 ml. The fractions were detected at a wavelength λ = 265 and 280 nm. Bidistilled water was used as an eluent.

The analysis was performed using the HPLC chromatograph "Tsvet Yauza-04" (with a UV and amperometric detector). The control of the instrument and the processing of the obtained results were carried out using the MultiChrome software, Version 3.1.1550 (ZAO Ampersend, Russia). The following chromatographic columns were used in this work: Gemini 5μm C18, 110A, 250 × 4.6 mm (Phenomenex, USA), Asahipak ODP-40 4E (4.6 × 250 mm). In terms of the trap column, the loop volume was 20 μl, the flow rate was 0.5 ml/min, and the pressure was 45.5 ± 1 bar. The mobile phase was made of acetonitrile for HPLC (Panreac); isopropyl alcohol for HPLC (Panreac); orthophosphoric acid (Panreac); and the bidistilled water prepared on the eve of the analysis. The

components of MP in the volume ratio of 35:10:1:54 or 35:65 were mixed, and degassing was carried out using a vacuum aspirator.

The fatty acid composition of bacterial lipids was determined by gas chromatography. To this end, the lipid fraction was isolated from a 0.9% broth culture of bifidobacteria preliminarily washed with a NaCl solution. The extraction was carried out using a chloroform and *n*-hexane mixture in a ratio of 1:1. The extract was methylated. A sample of 1 ml in volume was placed in a 1.5 ml vial, the solvent was blown away with nitrogen to dryness. 500 µl of a 3% of H_2SO_4 solution in MeOH was added to the dry residue. An internal standard element (10 µg of undecenoic acid) was added to the obtained solution. The sample was then heated at 90°C for an hour. After this, 700 µl of hexane was extracted. The volume of the hexane fraction was concentrated by stripping the solvent to a volume of 200 µl. The resulting sample containing fatty acids in the form of methyl ethers was used for analysis. The methylated samples were analyzed using an Agilent 7000B chromatomass spectrometer. The sample volume was 2 µl, splitless injection. Column: ZB-WAX, 30 m × 0.25 mm × 0.25 µm. Fig.1 presents the study design.

RESULTS AND DISCUSSION

Some of bifidobacteria probiotic species from commercial preparations were used in the study. Various physicochemical characteristics that make it possible to determine the characteristic properties of probiotic species under *in vitro* conditions were compared. In general, there are several approaches that allow for the standardization of probiotic strains. The first approach is biological. It makes it possible to monitor the growth of a species as a whole when introducing a culture into the body, as well as to monitor the joint growth of a culture from a biological object or product/species in a selective growth medium. The count of colonies or turbidimetric measurements are used to determine the degree of inhibition of a pathogenic or conditionally pathogenic microflora using probiotic cultures. An alternative was the diffusion analyses used to estimate the inhibition of antagonistic strains [3]. On the other hand, at present, the physicochemical methods for standardization are being actively developed, which make it possible to supplement or obtain the detailed information on the properties of a probiotic strain and its metabolites.

The most important property of probitics is their ability to adhere to enterocytes. By attaching to the epithelium, microorganisms form a biofilm and thus provide competitive colonization resistance with pathogenic and opportunistic microorganisms. There are two groups of mechanisms of attachment of bacteria to cells or substrates: nonspecific and specific ones. Nonspecific adhesion is reversible and is related to the physicochemical features of bacterial cells – the total surface charge, hydrophobicity and the presence of ionic bonds with ionogenic groups. Nonspecific adhesion determines the primary interaction between a microorganism and the intestinal mucosa. According to modern concepts, hydrophobicity is considered as a property that allows bacteria to interact with organic molecules, form interassociative linksб and contact with the intestinal mucosa and immunocompetent cells.
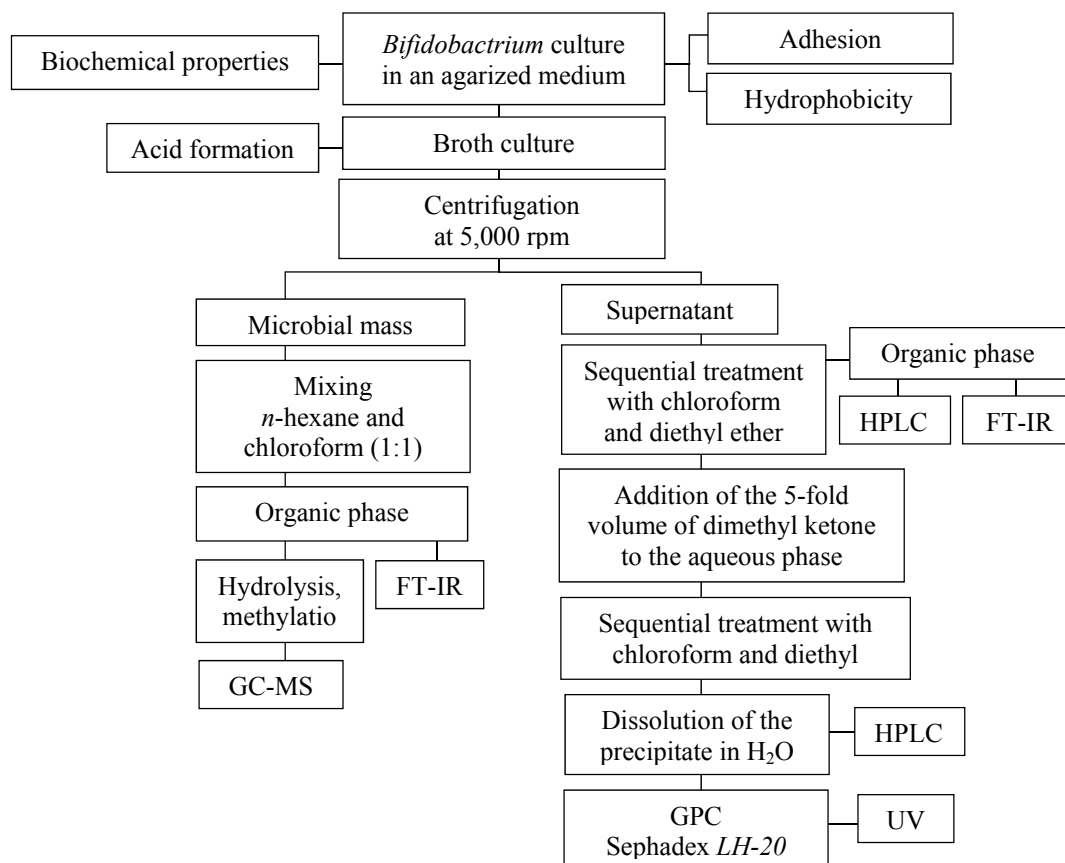

Fig. 1. Study design scheme.

Table 1. Parameters of the fatty acid composition of cell walls in probiotic strains, µg

Group	B. bifidum 1	B. longum B 379 M	B. breve
C13:0	00.79	00.82	00.13
C14:0	05.37	02.34	02.11
C14:1	00.58	00.19	00.36
Me9-C14:0	00.47	00.18	00.18
C15:0	02.55	00.97	01.09
Me*-C15:1	01.44	00.40	00.48
Me*-C15:1	00.78	00.10	00.23
C16:0	00.35	21.78	12.55
C16:1	53.90	01.45	02.08
Me9-C17:0	05.73	00.05	00.09
C17:0	01.12	00.33	00.40
Me9-C17:1	00.15	00.08	00.31
C18:0	00.92	11.92	10.46
C18:1	00.56	03.10	02.53
C18:1	00.40	01.97	03.01
C18:2	15.60	03.08	00.02
C20:0	80.34	00.27	00.29
C22:0	29.24	00.19	00.49
C24:0	00.04	00.40	00.13

Note. p < 0,001

In many respects, the hydrophobicity of a cell is determined by the lipid-like structures and, in particular, the fatty acids (FA) of the phospholipids of the cytoplasmic bacterial membrane. It is known that, regardless of the degree of hydrophobicity, saturated FA predominate in the cell wall of all the probiotic strains of bifidobacteria. In highly and low-hydrophobic strains, the proportion of saturated FA accounted for 64.2% and 71.3%, respectively, whereas, in the case of mid-hydrophobicity, the proportion of these acids was the highest and amounted to 91.1%. Table 1 presents the quantitative content of various FA in bifidobacteria. Approximately 90% of FA is used by microorganisms for the synthesis of phospholipids of the plasma membrane, 10% participate in the formation of lipoic acid, biotin, and in gram-negative bacteria (for the synthesis of lipopolysaccharides). Acyl carrier protein (ACP) plays a key role in the synthesis of fatty acids. Being a carrier that forms an acyl chain, it delivers precursor molecules from one enzyme to another for a condensation reaction. The enzymes of FA synthesis are localized in bacteria between the cytoplasm and the inner side of the plasma membrane. Their activity is determined by external factors, which determines the difference in the ways of FA synthesis. In this regard, the relative content of various fatty acids, the length of their chains, and saturation change. It has been established that in anaerobic bacteria, the precursor of unsaturated FA (C16:1, C18:1) is C_{10}-ACP, and their formation is stimulated by a decrease in temperature and the oxygen content (the way of anaerobic synthesis).

The aerobic way of synthesis of unsaturated FA from saturated precursors is well known and is characteristic of aerobic gram-positive bacteria. In the membrane of gram-positive bacteria, in addition to unbranched saturated FA, there are iso-, anteiso- and ώ-alicyclic FA that promote an increase in the plastic properties of the membrane. The synthesis of branched chain FA with a long carbon skeleton is characterized by the specificity of the enzymes that catalyze the condensation of molecules and the difference in the acyl carrier proteins that are seeds when the chain is elongated. Exogenous branched short chain carboxylic acids and branched 2-hydroxy acids are seeds. It should be emphasized that any changes in the microenvironment of bacteria will lead to differences in the ways of FA synthesis. This fact deserves special attention for study when choosing an optimal technological process when creating products with certain functional properties with a probiotic orientation. At the same time, it is important to determine the fatty acid composition of the membrane of the strains that differ from each other by hydrophobicity. The study of these factors will optimize the mechanisms of the positive effect of probiotics on a macroorganism.

In our study, the following saturated fatty acids were found in bifidobacteria using the GC-MS method (in order of an increase in the length of the carbon skeleton): *n*-tridecanoic (C13:0), *n*-tetradecane (myristic) (C14:0), *n*-pentadecane (C15:0), *n*-hexadecanoic (palmitic) (C16:0), *n*-heptadecane (C17:0) , *n*-octadecane (stearic) (C18:0), eicosanic (C20:0), behenic (C22:0), and lignoceric (C24:0) acids. Their quantitative content of which is presented in Table 1. Methylated acids or branched chain acids were also determined: 12-methyl-tetradecanoic (12CH₃-C14:0), 13-methyl-tetradecanoic (13CH₃-C14:0), isopentadecanoic (*iso*-C15:0), and isopalmitic (*iso*-C16:0). Branched or alicyclic FA, due to their structural features, perform an adaptive function in gram-positive bacteria giving fluidity and plasticity to the membrane [4]. Unsaturated FA, the highest proportion of which was found in bifidobacteria with high hydrophobic properties (35.8%), and, to a lesser extent, the strains with low hydrophobicity (28.7%) had similar properties. A low content of unsaturated FA was determined in the case of mid-hydrophobicity: their share did not exceed 8.9% in the whole FA pool. Among the monounsaturated FA, the bifidobacteria contained myristoleic (C14:1), pentadecenic (C15:1), palmitoleic (C16:1), *n*-heptadecene (C17:1), and oleic (C18:1) acids. There were also found diene FA: hexadecadiene (C16:2). In highly hydrophobic bifidobacteria, branched FA - *iso*pentadecane (*iso*-C15:0) and methyl tetradecanoic (13CH₃-C14:0) acids were identified. With the mid-hydrophobicity, a high content of *n*-hexadecane (*iso*-C16:0) and *n*-octadecane (C18:0) acids were established.

It is known that the presence of unsaturated and branched FA in bacteria increases the fluidity of the membrane and promotes an increase in adhesion [14]. Indeed, the obtained data indicate the increased content and diversity of unsaturated FA with one or two double bonds in bifidobacteria with high hydrophobicity. In addition, in the case of high hydrophobicity, such branched fatty acids as isopentadecanoic (*iso*-C15:0) and 13-methyl-tetradecanoic (13CH₃-C14:0) were found in bifidobacteria. In the strains of bacteria with the mid-hydrophobicity, only two unsaturated acids –

oleic (C18:1) and linoleic (C18:2) – were present in relatively small amounts. In our opinion, the mid-hydrophobicity in the studied strains of bifidobacteria is apparently related to the presence of *iso*-palmitic (*iso*-C16:0) acid, the content of which was 20 times higher than that of the strains with high hydrophobicity. In the case of the low hydrophobicity of bacteria, the liquid-crystal state of the membrane was determined by unsaturated FA, the content of which was reduced in comparison with highly hydrophobic cultures.

A large number of specific adhesion factors is a characteristic property for the representatives of the genus *Bifidobacterium*. These include stack-like structures, surface proteins, as well as lipoteichoic acids. They were found in a lot of gram-positive bacteria and consisted of 1,3-linked chains of polyglycerol phosphate and glycolipid fragments, but these polymers are so unique that they are used for the species identification of microorganisms. Specific adhesion is irreversible and takes place in microorganisms involving adhesin molecules (ligands), which have a high affinity for skin receptors and human mucosa. When studying the indicators of the specific adhesion of probiotic strains *in vitro* on erythrocytes, it was established that, regardless of the species, they had a mid ability for specific adhesion. Thus, MAI for *B. bifidum 1* was 2.8, for *B. breve* – 3.1, and for *B. longum B 379 M* – 3.5. However, it is possible to judge specific adhesion indirectly using red blood cells as a model, since, despite the similarity of the glycoprotein receptors of erythrocytes and the intestinal epithelium, adhesion is tissue-specific. In this connection, the molecular characteristics of explicitly lipoteichoide-type adhesins in bifidobacteria were studied using FT-IR spectroscopy.

For the studied samples isolated from probiotic strains, the bands corresponding to the following structural fragments are determined. The presence of a 2952 cm^{-1} band is due to the stretching vibrations of C−H and alkyl fragments. The asymmetric stretching vibrations ($v_{as}CH_2$) and symmetric (v_sCH_2) stretching vibrations determine the bands of 2,923 and 2,854 cm^{-1} methylene groups. The presence of a 1738 cm^{-1} band characterized by an ether fragment is characteristic for all the samples under study. The strain *B. bifidum 1* was the only that showed a weak intensity band of 1710 cm^{-1}. The presence of this band, in our opinion, is a consequence of the structural features of adhesins in this type of bifidobacteria. These are the electronic effects of the nearest substituents, the compounds with multiple bonds, as well as the presence of inter- and intramolecular hydrogen bonds [15]. On the other hand, the presence of this band is explained by the possible effect of the methyl fragments located in the alkyl fragments, which is confirmed by the results of GC-MS [14]. Considering from these perspectives the shift of the absorption band of the carbonyl group, it is necessary to note the presence of unsaturated fragments in the structure of lipoteichoic acids which

leads to the shift of absorption bands to the segment of the lowest frequencies. However, the effect of the O = P – OH fragment, which is part of the structure of fatty acids and which develops within the range of 1740–1600 cm^{-1}, is the most probable [16]. Considering this band as a result of the effect of a phosphorus-containing fragment, one can find the confirmation of the presence of similar structures in other areas of the IR spectrum. Thus, for the sample of *B. bifidum 1*, the presence of the bands 1217, 1043 and 875 cm^{-1} is characteristic. On the basis of the given data of IR spectra and the established structural features, it should be recognized that the probiotic strain *B. bifidum 1* is distinguished by a rich organophosphorus constituent. This structural feature positively influences the synthesis of highly ergic compounds and the metabolism of bacteria as a whole. Indeed, a wide spectrum of saccharolytic enzymes were determined in the study of the biochemical properties of this strain. *B. bifidum 1* was able to ferment glucose, lactose, melibiose, raffinose, arabinose, xylose, galactose, fructose, and trehalose. The acid-forming capacity of the strain was 185 T°. Thus, the structural features of organophosphorus fragments, fixed under conditions of FT-IR spectroscopy, can be an informative criterion not only for the ability of bacteria for adhesion, but also for the metabolic activity of probiotic strains.

In turn, a relatively low variability of the spectral parameters found in the sample of *B. breve* should be noted (Fig. 2).

Among the positive impacts of probiotics, much attention is paid to exometabolites, which create favorable conditions for the growth and reproduction of one's own human microflora and modulate its metabolic processes. Therefore, it is expedient to develop criteria for the estimation and standardization of exometabolites of probiotic microorganisms.

To study the culture liquid of bifidobacteria, liquid column chromatography was used with Sephadex LH-20 dextran sorbent. The molecular weight fractionation range for LH-20 was 100 for the lower limit and from 2,000 to 10,000, according to some data, from 4,000 to 6,000, for the upper limit [17]. In most cases, the peptides with more than five or seven amino acid residues are soluble only in polar solvents, so it is optimal to perform their separation and preparative accumulation by gel filtration using soft dextran sorbents such as LH-20, stable in organic solvents [18, 19]. Moreover, it should be noted that the pattern of separation using Sephadex LH-20 is determined not only by gel filtration but also by adsorption and distribution chromatography, and the effective fractionation interval depends both on the eluent used and the conformation of the fractionated substances [20]. According to the analytical data obtained in environments of ion-exclusion chromatography and GC-MS, the studied exometabolites did not contain any nucleic and organic acids. There were no pentoses in any of the hydrolysates of exometabolites.
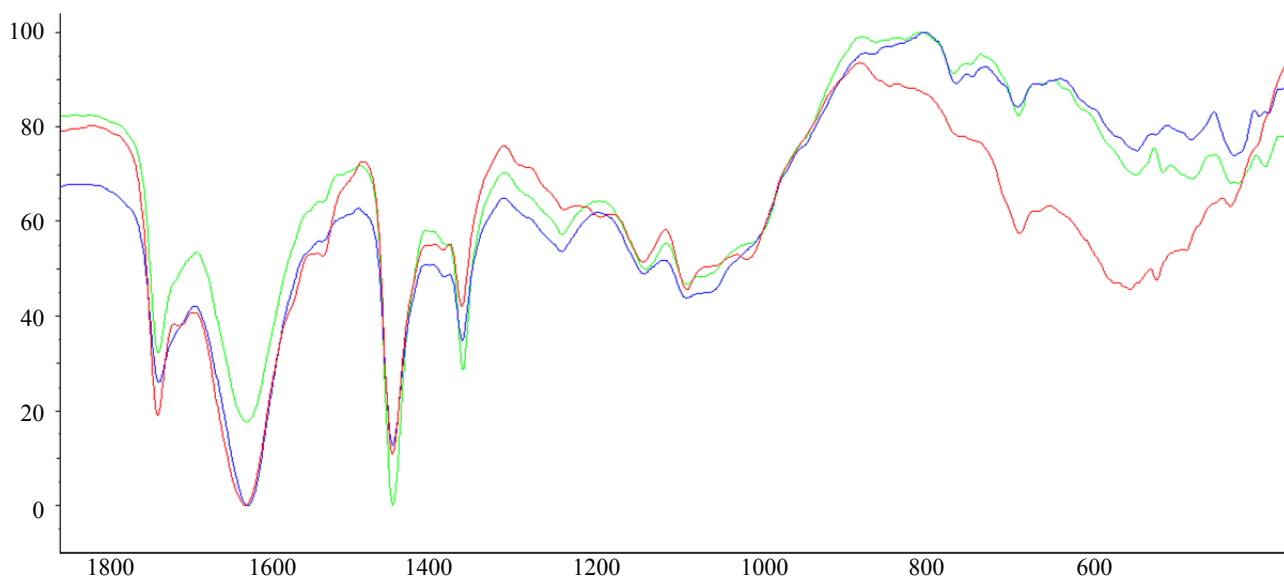
Fig. 2. IR spectrum of the extract obtained by treating *B. bifidum 1, B. longum B 379 M* and *B. breve* samples with the mixture *n*-hexane and chloroform (1:1).
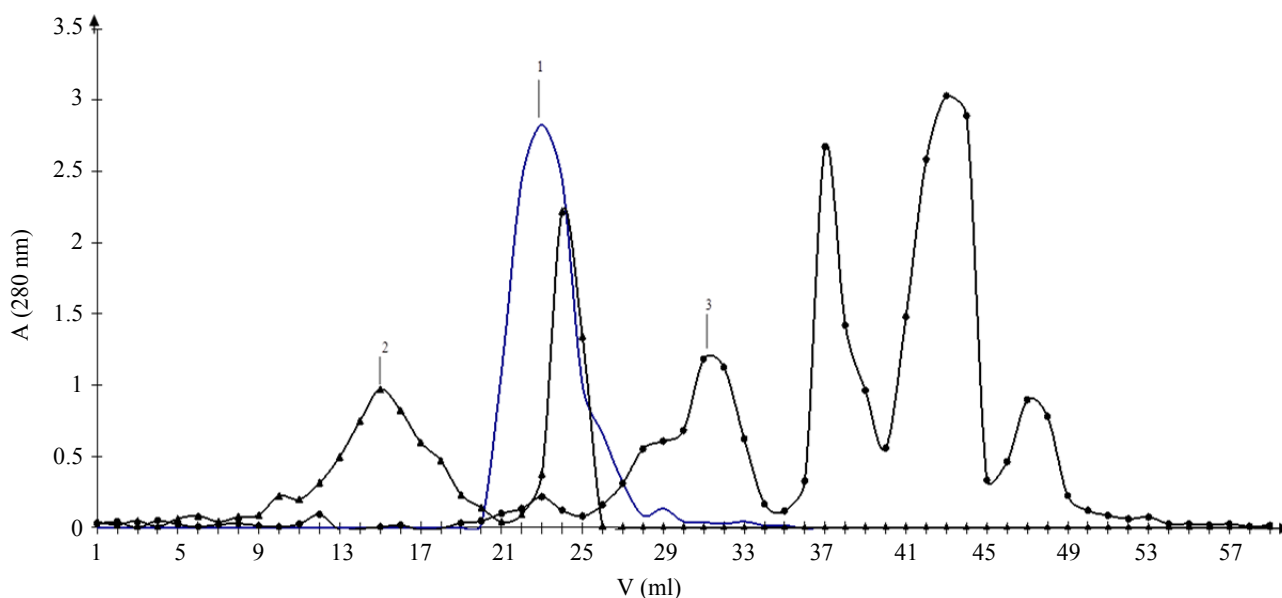

Fig. 3. Chromatogram of exopolymers on the basis of *LH-20* sorbent under conditions of aqueous elution.

Note. 1 is *B. bifidum 1*; 2 – *B. longum B 379 M*; 3 – *B. breve*

The product is free of lipids according to the results of gas chromatography after extraction with chloroform and diethyl ether. The results obtained by high-performance liquid chromatography of HPLC using an Asahipak ODP-40 4E column showed that the product had a glycoprotein (high molecular weight) nature. The exopolymer produced by *B. bifidum 1, B. longum 379 M,* and *B. breve* strains consisted mainly of glucides (measured using the phenolic-sulfuric acid method) and proteins (measured using Lowry's method) in a ratio of 5:1.

The glucid part consisted of glucose and galactose according to the analytical data obtained by ion-exclusion, thin-layer and gas chromatography. Glucose and galactose were present in a ratio of 3:2. According to the results of the gas chromatography of the monomers hydrolyzed by methanolysis and then acetylated with trifluoroacetylation, the polysaccharide part had a variety of bonds, such as 1–2, 1–3, 1–4, and 1–6.

The molecular-mass distribution (MMD) of the exometabolites of the studied strains in the chromatography mode using Sephadex LH-20 was indicated by the following features. The spectrum of the metabolites produced by bifidobacteria was quite wide. In the case of GPC, in the mode of water elution in a volume of 15–17, the yield of the component c in the UV segment λ_{max} = 243 nm was noted in the sample of *B. breve (2)* (Fig. 4c). In our opinion, this spectral characteristic is determined by the presence of steroid-like structures. The yield of the component with a lower MMD of this sample was in a volume of the

fraction 23–25 and has a UV spectrum with a maximum of 265 nm (Fig. 4d). As is known, such spectral characteristics are typical for protein-peptide structures. It is noteworthy that this component of *B. bifidum 1* using Sephadex LH-20 sorbent elutes in the fraction zone 21–27 as a single chromatographic peak. The spectrum in the UV segment had a maximum of 265 nm (Figs. 4a and b). The sample of exometabolites isolated from the culture medium *B. longum B 379 M* had the highest heterogeneity of MMD components. The chromatographic profile of this sample was represented by quite well separated peak fractions (3) (Fig. 3). The UV spectrum of the components eluted in the zone of the fraction 27–31 characterized the presence of a maximum of absorption of 264 nm, whereas this value was 272 nm for fractions 37–45 (Fig. 4e). These features should be considered as the distinctive conformational features of protein-peptide components in the presence of lipoprotein formations.

Thus, in the mode of GPC, using Sephadex LH-20, a fraction with a low degree of MMD and quite homogeneous UV spectra was released from the sample of *B. bifidum 1*. The feature of *B. breve* was the variability of the spectral indices. The effect of MMD under conditions of GPC, in turn, is the significant indicator of the enzymatic activity of the studied strains of microorganisms. As it was established, the strain *B. bifidum 1* had a low enzymatic activity, in turn, the strains *B. longum 379 M* and *B. breve* had a wide set of saccharolytic enzymes and were capable of fermenting various carbohydrates [10]. In the development of probiotic products of functional nutrition, strains with a low enzymatic activity are often used in multicomponent bacterial consortia to create favorable conditions for their joint cultivation and to preserve the viability of all participants in a multicomponent community. Indeed, the *B. bifidum 1* strain was isolated from a two-component probiotic consortium, so GPC allowed estimating not only the enzymatic activity of the strain, but also making an assumption about the number of strains in a functional product. The results of MMD obtained in our study allow us to say that the *B. longum B 379 M* strain has a relatively high enzymatic activity. Under conditions of GPC, using Sephadex LH-20, the analyte of exometabolites could be fractionated into four chromatographic zones, whereas the sample of *B. longum B 379 M* is characterized by the presence of fractions with the distinctive parameters of MMD and/or conformation.
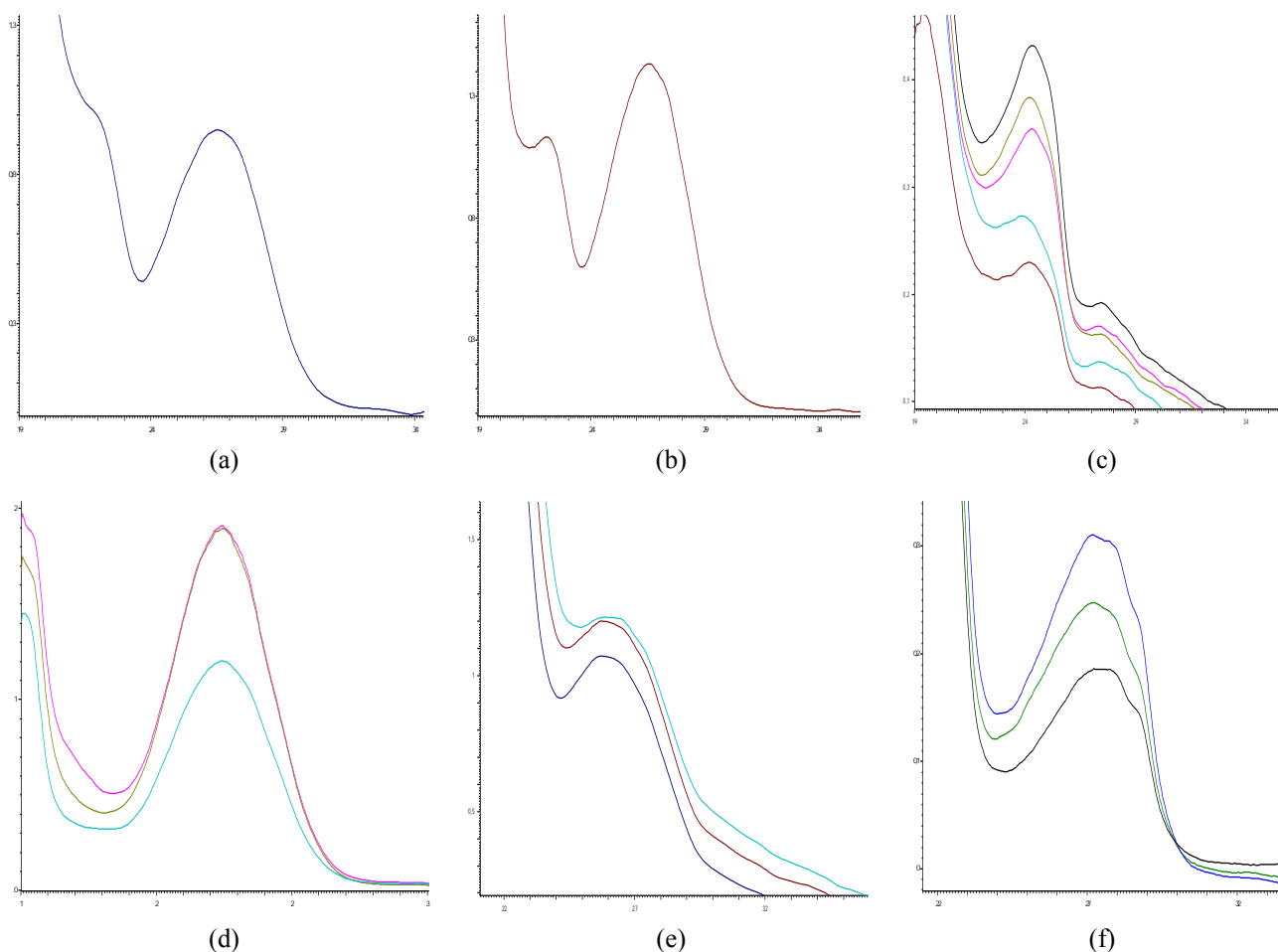

(a)   (b)   (c)

(d)   (e)   (f)

Fig. 4. UV spectra of chromatographic fractions after Sephadex LH-20 of exometabolites of the samples: (a) *B. bifidum 1* , fraction 23, λ_{max} = 265 nm; (b) *B. bifidum 1,* fraction 29 ; (c) *B. breve*, fractions 13–17, $\lambda_{1\,max}$ = 243 nm, $\lambda_{2\,max}$ = 274 nm; (d) *B. breve*, fractions 23–25, λ_{max} = 264 nm; (e) *B. longum B 379 M*, fractions 29–33, λ_{max} 264 nm; (f) *B. longum B 379 M*, fractions 46–48, $\lambda_{1\,max}$ = 272 nm.

The use of the GPC version in combination with the study of the spectral parameters of fractions in the UV segment also reveals the distinctive physicochemical characteristics of exometabolites of bifidobacteria strains. The exopolysaccharides produced by microorganisms are most often the basis for feeding other members of the normal intestinal microflora (*Bifidobacterium, Escherichia coli*), which stimulates its growth, development, and metabolic activity, i.e., the exometabolites of a polysaccharide nature have prebiotic properties. The produced exopolymer matrix also promotes the formation of a full biofilm on the intestinal mucosa. Like a "glove", it covers the intestinal mucosa and mechanically protects the intestinal biotope from being populated by pathogenic microorganisms. In this case, the minor amounts of the produced protein level out the possibility for microorganisms to multiply in the biotope with a proteolytic activity and minimize the development of putrefactive dyspepsia. This feature is especially relevant in the development of specialized probiotic products for high-risk groups [21]. The use of sorption-chromatographic approaches in the analysis and study of biofilm formation in probiotic strains appears to be promising.

Thus, in our opinion, it is expedient to select the strains of microorganisms that are promising for the biotechnological process and also to estimate the functional characteristics of the applied probiotic cultures using a set of classical bacteriological and physico-chemical methods. The most informative chromatography methods for studying the factors of specific and nonspecific adhesion and exometabolites are GC-MS and GPC, as well as FT-IR spectroscopy.

To estimate the non-specific adhesion of probiotic strains, it is expedient to use the data on the fatty acid composition of the cell walls of bifidobacteria. The criterion for the high ability of strains for reverse adhesion is the predominance of the carbon skeleton and unsaturated fatty acids in the cell wall of fatty acids with a branched structure.

The estimation of structural features of lipoteichoic acids using IR spectroscopy allows us to estimate not only the ability of probiotic cultures for specific adhesion, but also their functional potential. The indicator of high biological activity of strains is the presence of a large number of phosphoric acid fragments in lipoteichoic acids.

The most informative estimation of the composition of the exometabolites produced by probiotic bifidobacteria is provided by a complex of such chromatographic methods as GC-MS and GPC, as well as FT-IR spectroscopy. The predominance of bifidobacteria of glucides in the composition of exometabolites is an indicator of the high saccharolytic activity of probiotic strains.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Informational support as an element of state control of agriculture

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Abstract: This article substantiates the importance of informational support in the agricultural sector for controlling authorities and for agro-industrial organisations in enhancing their monitoring and competitive strategies. It identifies specific features of the agricultural sector that determine its needs for information, namely its dependence on climatic conditions and the state of natural resources available to economic entities, a large territorial expanse, the location of major production facilities in rural areas, the development of interregional and international contacts, and an active growth of agricultural exports. By studying the current regulatory and legal database of the Russian Federation, the authors established the regulatory framework of a state information system and identified its key data groups. Finally, the paper looks at those information resources which were created by the federal authorities and regional bodies in the Northwestern and Siberian Federal Districts, reflecting the tasks of state informational support in agriculture.

Keywords: Agriculture, information management, agro-industrial complex, informational support, controlling, sector-specific information system, state regulation

INTRODUCTION

In the context of modernising Russian economy, one of the key tasks for achieving highly competitive development indicators is to ensure a qualitatively new level of informational interaction between various economic agents and enable their integration into a data exchange system based on dynamically developing telecommunications. Today, having instant access to economic information is a prerequisite for effective interaction between economic agents, for making managerial decisions by individual organisations, their associations and state regulators, as well as for economic research.

One of the sectors of the Russian economy that could benefit from introducing information and telecommunication technologies and improving informational support of its agents is the agro-industrial complex, in particular agricultural production.

Informational support in this sector is directly related to management processes, since hardly any managerial decision can be made without having timely access to objective, complete and up-to-date information. It plays an important role in regulating and controlling business processes, establishing criteria for key performance indicators and measuring performance with a view to

further improvement, i.e. in the system of controlling agro-industrial organisations [1]. In this regard, information is especially important for the subsystem of monitoring and developing competitive strategies within the framework of the project controlling system.

STUDY OBJECTS AND METHODS

This article aims to identify those peculiarities of the agricultural production sector which determine its need for informational support and to substantiate the importance of informational support in agriculture from the point of view of management and controlling systems.

Having identified the existing information resources provided by state authorities to the agricultural sector at the federal level and in the Northwestern and Siberian Federal Districts, the authors seek to show their adequacy and necessity not only for controlling, but also for stimulating agricultural production.

We believe that informational support of agriculture should be organised in accordance with its three main features to improve the performance of the agro-industrial complex. Firstly, the sector is dependent on natural phenomena and climatic conditions, the state of

soils and other natural resources affecting its efficiency, and it is therefore vital for agricultural producers to have immediate access to up-to-date information on any possible changes of the above. Secondly, a considerable territorial expanse typical of most agricultural production creates an increased need for information on logistics centres, transport infrastructure, and customer location. And thirdly, it is common practice in agriculture to locate production facilities in rural areas that do not have direct access to the information and telecommunications infrastructure. Those three features of the agricultural sector should be taken into consideration when providing any kind of informational support to agro-industrial enterprises, since it is the information on the state of, and changes in, natural resources and the possibilities to overcome geographical remoteness that can enable agricultural producers to use those resources more effectively and profitably. At the same time, it is important to involve those rural areas that lack the support of information and telecommunication systems.

The on-going development of interregional and international economic relations and an active growth of agricultural exports raise the importance of providing managers of agricultural companies with access to information on prices in various territorial segments of the agricultural market, as well as information on how to reach those segments. According to V.V. Berdnikov, when studying management systems in the agro-industrial complex, it is extremely important for the researcher to 'form an integrated information field for the key groups of stakeholders and develop appropriate analytical tools that would enable them to understand the current situation in the markets, substantiate and support (or monitor) industrial, financial, investment and other kinds of initiatives aimed at achieving the best results in the current conditions' [2].

RESULTS AND DISCUSSION

Providing informational support is an important task in agriculture and, to some extent, it involves all stakeholders in the sector. All agricultural producers – from farms to large enterprises and agroholdings – take part in collecting and processing information in some form. Contractors that supply agricultural producers with goods or buy their products also need access to relevant information. Insurance companies and lenders create their own systems for monitoring sectoral information. Finally, the most important functions in providing informational support to agriculture are performed by state authorities, and a number of these functions are assigned to them by law. The main problem of all the systems for monitoring sectoral information is their inconsistency with each other, as they use different sources of information and therefore pursue different objectives. To ensure better state management of the industry, the system of monitoring indicators and other information resources should be targeted not so much at controlling the use of natural resources as at their redistribution, considering the sector's large territorial expanse and limited resources.

The Federal Law 'On the Development of Agriculture' [3] stipulates the availability of information on the state agricultural policy as one of its key principles. Therefore, a major means of implementing this policy is providing informational and consulting support to agricultural producers and other stakeholders in the market of agricultural products, raw materials and foods. Article 17 of this law sets a regulatory framework for the system of state informational support in agriculture and its functioning. According to the article, this system includes information on:

– federal and sectoral targeted programmes;
– crop and livestock sectors;
– agricultural machinery (quantity and condition), fuel supply, and energy consumption;
– chemicalisation and land reclamation;
– monitoring agricultural land;
– financial and economic standing of agricultural organisations;
– phytosanitary and epizootic situation in Russia and its current measures to identify, eliminate and prevent the spread of animal and plant diseases, agents of infectious animal diseases, and plant pests;
– employment figures and staff structures in agricultural organisations;
– food and processing industry;
– hunting resources and their use; and
– monitoring the prices of basic foods, as well as materials and resources purchased by agricultural organisations, at the markets of agricultural products, raw materials and foods.

According to the law, such information must be free and accessible to all stakeholders on equal conditions. And although it is true, the question is whether this information is really useful to all agricultural producers. Practice shows that this type of informational support is either intended for reference purposes only or it needs to be adjusted.

The RF Government Decree No. 157 of March 7, 2008 'On developing a system of state informational support in the sphere of agriculture' [4], which stipulates a procedure for creating and maintaining a system of state informational support in agriculture, defines this system as an 'integrated database of all information on the state of agriculture and its development trends, as well as information technologies and processing facilities, contained in the databases of the federal executive authorities, constituents of the Russian Federation, other state bodies and local authorities.' The purpose of such a system is to develop state information resources in agriculture, make them accessible to all stakeholders and use them as a basis for providing public services with telecommunication technologies.

The information system is operated by the RF Ministry of Agriculture, with its information resources formed by:

– the Ministry of Agriculture;
– the Federal Service of State Statistics;
– the Federal Customs Service; and
– the authorised bodies in the RF constituents and local authorities.

In addition to the data required by Article 17 of the Law 'On the Development of Agriculture' (see above), the RF Ministry of Agriculture provides information on its decisions and new regulatory acts on state support of agriculture, customs duties, tariff quotas and their application, on federal intervention stocks of agricultural products at year-end and after purchases or sales of intervention stocks, on tenders for purchasing agricultural products, raw materials and foods for public needs, on planned vs. actual production indicators for the main types of agricultural products, raw materials and foods, and their stocks at end-year, and finally on the results of measuring functional performance and efficiency of agricultural machinery and equipment relevant to the provision of state support.

All this information is available on the Ministry's website; however, if we consider informational support as an element of state control, making it accessible per se is not sufficient – it needs to be followed up to ensure that the information does reach the intended user. Unfortunately, the RF constituents are not fully responsive to this need, which leads to a certain contradiction, i.e. the Ministry of Agriculture is ready to provide important information to improve the sectoral performance, but this information gets to intended users either too late or in a distorted form (which is not reflected in their feedback to the Ministry though). We believe that this problem can be solved by making information resources clearly targeted at the intended users and reducing the amount of information open to general use. All the information provided by the Ministry should refer to particular administrative units (regions), while measuring their fulfilment of governmental assignments for food supplies should be based on the same indicators as those provided by the information resources.

At the regulatory level, priority is given to food security. Presidential Decree No. 120 of January 30, 2010 'On adopting the doctrine of food security in the Russian Federation' stresses the need for developing state information resources as part of a mechanism to ensure food security in the country. The document also stipulates the need for monitoring (along with forecasting and controlling) food security indicators at the federal and regional levels, as well as monitoring the unemployment rate and real income of the rural population in an effort to ensure sustainable development of rural areas as one of the key priorities of the state economic policy.

In addition, the decree specifies a system of indicators to be used in assessing food security, including [5]:
(a) consumption:
– disposable household resources by population groups;
– premises available for trade and foodservice (per 1000 people);
– consumption of food products per capita;
– targeted assistance to the population;
– daily caloric intake;

– the amount of proteins, fats, carbohydrates, vitamins, and macro- and microelements consumed by a person per day;
– consumer price index for food products;
(b) production and national competitiveness:
– production of agricultural and fish products, raw materials and foods;
– import of agricultural and fish products, raw materials, and foods;
– state support of producers of agricultural and fish products, raw materials, and foods (per 1 rouble of sold products);
– productivity of land resources used in agriculture; and
– food sales in trade and foodservice;
(c) management and controlling:
– volumes of foods in the state material reserve formed under the RF regulatory acts; and
– stocks of agricultural and fish products, raw materials, and foods.

Furthermore, the decree sets a criterion for assessing food security as a proportion of domestic agricultural products, fish products and foods in the total volume of commodity resources in the domestic market of the corresponding products, in particular:
– grain: at least 95 percent;
– sugar: at least 80 percent;
– vegetable oil: at least 80 percent;
– meat and meat products (in terms of the equivalent amount of meat): at least 85 percent;
– milk and dairy products (in terms of the equivalent amount of milk): at least 90 percent;
– fish products: at least 80 percent;
– potatoes: at least 95 percent; and
– salt: at least 85 percent.

The list of monitoring indicators developed by the RF Government forms a methodological basis for organising informational support. According to the RF Government Order No. 2388-r of November 18, 2013 'On adopting the list of indicators in the sphere of food security of the Russian Federation,' the state automated information system should contain the following sections of information on food security [6]:
(1) Target indicators for:
– food consumption;
– physical accessibility of food for the population;
– food independence of the Russian Federation;
(2) Monitoring indicators for:
– consumption;
– circulation of agricultural and fish products and foods;
– processing of agricultural and fish products;
– production of agricultural and fish products;
– exports and imports of agricultural and fish products and foods;
– stocks and reserves;
– population and employment figures and structure in the RF;
(3) Predictive indicators:
– scenarios for ensuring food security;
– sectoral macroeconomic indicators; and
– food balance sheets.

These sections include 129 indicators, most of which differentiated by commodity group, RF constituent or other characteristics.

These two government documents demonstrate a clear attempt at controlling the state of food security, identifying specific indicators for every activity involved in food supplies, and examining possible risks of weakening the existing positions of our government on this matter. The practical application of these documents, however, necessitates a certain adjustment by correlating information on the above indicators, which is crucial to state control of agriculture, with performance indicators of a specific economic agent and a specific constituent of the RF.

The official website of the RF Ministry of Agriculture contains a section entitled 'The list of information systems of the RF Ministry of Agriculture' including [7]:
– the functional subsystem 'The electronic atlas of agricultural lands';
– the Federal state information system for registration of tractors, self-propelled machines and trailers;
– the System for monitoring and forecasting food security in the Russian Federation;
– the System for providing public services in electronic form by the RF Ministry of Agriculture;
– the Automated information system of registers and regulatory information that includes catalogues of pesticides and agrochemicals registered in the RF, the state pedigree register, a list of seed farms, decision statements attached to applications for state registration of appellations of origin, the register of vine plantations, information on departmental checks, lists of infections and other animal diseases, information on the availability of reclaimed land and crop acreage in the RF, employment figures and staff structures in agricultural organisations, information on young breeding cattle, etc.;
– the Information system for planning and controlling the State programme;
– the Integrated information system for collecting and processing accounting and specialised reports of agricultural producers, preparing summary reports, monitoring, controlling and reviewing subsidies to the agro-industrial complex;
– the Unified automated system of veterinary accompanying documents;
– the Central analytical information system of the State informational support system in agriculture; and
– the Register of federal property of the agro-industrial complex.

The functioning of the information systems used by the RF Ministry of Agriculture can be exemplified by the System for monitoring and forecasting food security in the Russian Federation (hereinafter referred to as 'the System'), whose purpose is to organise the collection, processing and storage of monitoring data concerning:
– the state of food security in the Russian Federation;
– purchase prices for agricultural products, raw materials and foods (daily monitored); and
– imports and exports of the main product groups according to the RF Federal Customs Service.

According to the RF Ministry of Agriculture, the purpose of the System is strategic planning for the development of the agro-industrial complex (AIC), and identifying risks and threats to food security. It is therefore tasked with monitoring and analysis of food self-sufficiency and producing food balance sheets; monitoring and analysis of imports and exports according to the Federal Customs Service; and monitoring producer prices and mapping objects of high importance for ensuring food security, i.e. elevators and vegetable storehouses.

The sources of information for the System are the RF Federal State Statistics Service, which provides statistics on the AIC, the RF Federal Customs Service, which provides data on customs statistics, as well as municipal and regional authorities that control the AIC in the RF constituents and are responsible for entering data based on reports from agricultural organisations. In addition, the system contains data on food balances and prices for agricultural products (Figs. 1 and 2).

An important part of basic information in the System comes from municipal and regional authorities controlling the agro-industrial complex in the RF constituents. However, the forms they use to provide their data are of a general nature and cannot fully reflect the real situation in the sector. Some data are completely invalidated by others and therefore cannot be used by stakeholders for making managerial decisions.

The advantages of the System boil down to providing:
– informational support on food security within the System's functionality;
– real-time information retrieval;
– a possibility of obtaining consolidated information;
– an opportunity for users to develop basic individual skills and accumulate their experience; and
– a possibility of creating user reports (Fig. 2).

However, the System has a few disadvantages, namely:
– lack of information on a number of food security indicators stipulated in the RF Government Order No. 2388-r of November 18, 2013;
– non-compliance with information protection requirements;
– inadequate tools for automated data prediction;
– lack of a tool for creating imitation models to track changes in the state of food security in the current geopolitical conditions;
– low productivity and technological limitations that impede efficient state control;
– inadequate compliance with the current user needs, e.g. a lack of access from mobile devices;
– lack of a tool for publishing materials for users; and
– a need for a more user-friendly interface [8].
Further development of the System should involve taking an inventory of agricultural production, processing and storage facilities; enabling a search for commodity exchange information and data from federal authorities; improving the food safety forecasting model; and ensuring integration with other information systems of the RF Ministry of Agriculture [7, 8].
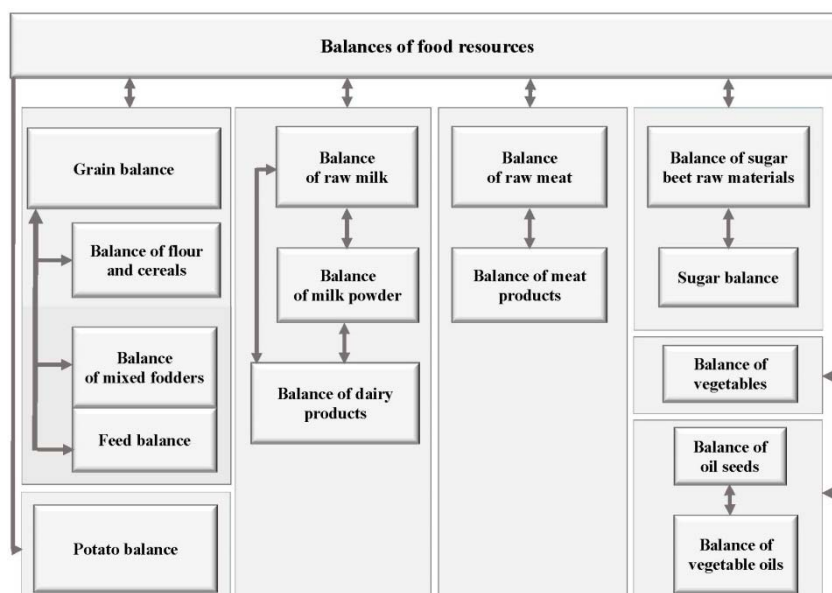
Fig. 1. Balance sheets in the System for monitoring and forecasting food security in the Russian Federation.

Fig. 2. An example of creating a user report in the System for monitoring and forecasting food security.

It is worth mentioning another information system, 'VetIS' developed by the Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor). It is an umbrella system that covers a number of automated information systems, including [11]:

– 'Argus' intended to automate veterinary surveillance on the external border of the Customs Union;
– 'Argus-Phyto' designed to automate registration and maintenance of documents on phytosanitary surveillance;

– 'Mercury' for issuing electronic certificates for, and ensuring traceability of, goods that are supervised by the state veterinary surveillance bodies during their production, distribution and transportation through the territory of the Russian Federation to create a unified information environment for veterinary medicine and improve biological and food safety;

– 'Vesta' intended to collect, transmit and analyse data on laboratory testing of controlled product samples in diagnostic studies and research into food safety, food and feed quality, quality and safety of animal medicines, etc;

– 'Irena' for registering medicines, feed additives and GMO feeds;

– 'Assol' for collecting electronic reports from institutions accountable to Rosselkhoznadzor;

– 'Hermes' designed to automate the licensing of pharmaceutical activities and animal medicines production;

– 'Cerberus' intended to control and keep records of legally significant actions in veterinary surveillance, etc.

Apart from the databases and information resources listed above, the regional authorities responsible for the AIC in the RF constituents provide access to relevant legislation and development programmes, plans and results of state inspections in the field of seed and livestock breeding, as well as financial control. They also provide information on state support at the federal and regional levels, including subsidies and grants to businesses.

Additionally, information portals of the sectoral authorities give access to the registers of economic agents, small and medium-sized enterprises, including state support recipients, to monitoring data on regional markets for agricultural raw materials and foods, details of the progress made by the federal and regional development programmes, and up-to-date information on the AIC performance.

Some regional authorities also provide information on their state-supported crop insurance programmes, wages and vacancies in the regional agricultural sector, and plans for supplying farms with technological support and agricultural equipment.

Moreover, regional agricultural authorities have access to various kinds of information systems in their direct use, such as 1C software and Selax analytical systems designed to monitor and analyse farm performance, herd reproduction, (imported) livestock inventories, land evaluation data, annual reports, ets. [12].

Thus, the current framework of state support offers agriculture a number of functioning tools that can be used by the AIC stakeholders to monitor the sector's performance. However, it is important to understand that providing the regions with a long list of information resources not only helps them in managing the AIC, but, at the same time, hampers its controlling due to poor interrelatedness between numerous flows of information.

The current information system provides agricultural organisations with a considerable amount of disparate data about such sources of fixed and working capital as state subsidies or soft loans.

Monitoring data on agricultural land, its chemicalisation and reclamation, as well as phytosanitary and epizootic information can help the managers of agricultural organisations make informed decisions on the use of biological assets, which are a key element of agricultural production and a major factor in achieving its environmental effectiveness, but they do not provide enough detail on every constituent of the RF and therefore fail to reveal their unique features.

Access to information on producers of agricultural machinery, prices for material and technical resources, livestock and seed farms, and consumer prices for agricultural products, although often poorly updated, helps agricultural organisations build good business relations with the key contractors and make managerial decisions.

Finally, information on agricultural organisations and their financial and economic performance enables managers to use a wide range of business analysis methods, namely to compare their indicators with those of competitors (external benchmarking), analyse their competitive advantages, identify key success factors and strategic economic zones, but such information needs to be verified as financial and economic performance indicators can relate to a particular agricultural enterprise and largely depend on its size.

CONCLUSION

To sum up, the needs of the agricultural sector for information are determined by its specific features, such as its dependence on natural and climatic phenomena and the state of natural resources available to economic agents, a large territorial expanse, and the location of major production facilities in rural areas with inadequate infrastructure. At the same time, some agricultural organisations and their stakeholders need informational support for controlling purposes.

These needs are reflected in the current RF legislation, where informational support is defined as one of the priorities of the state agrarian policy. Moreover, the laws stipulate the key categories of data to be included in the state information system. The main problem, however, is that the information provided by regional executive authorities does not always reflect the reality since it is presented in consolidated groups and in very general terms. As a result, such information can hardly help agricultural producers make managerial decisions.

Today, there are large information resources at the federal and regional levels that are directly related to the agricultural sector. They can be accessed mainly via the Internet, through the official website of the RF Ministry of Agriculture and its specialised information portals, as well as official websites of the Federal State Statistics Service, the Federal Customs Service, the Federal Service for Veterinary and Phytosanitary Surveillance and regional executive bodies controlling the AIC. In addition, there are some automated information systems developed by companies, rather than state authorities, that can be used for some specific purposes, e.g. to monitor livestock productivity, keep records and analyse accounting data, etc.

The abundance of information resources created for different purposes and with different requirements for data presentation leads to numerous inconsistencies in the indicators they use. Another negative aspect is that not all of the resources have a user-friendly interface or adequate integration possibilities. Furthermore, the data provided by these resources are largely collected for authorities to monitor the AIC performance, which sometimes makes them unable to adequately meet the information needs of those who make managerial decisions in agricultural enterprises.

Nevertheless, the current information system formed by the state in the agricultural sector is an extensive database that can help economic agents considerably in searching for sources of fixed and working capital, using biological assets, developing personnel motivation and management systems, and using a wide range of business analysis tools.

CONFLICT OF INTEREST

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Economic effect of innovative flour-based functional foods production

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Abstract: The article presents the analysis of economic effect for the innovative flour-based functional foods production incorporation**.** Based on the analysis of the current state and prospects for the bakery industry development, the authors propose to expand the range of flour-based foods meant for dietary preventive and dietary therapeutic nutrition using diversification methods. For this, they used alternative recipe ingredients of plant origin, such as amaranth seeds, lentil and lupine seeds, chufa, and carob beans. The innovative technologies improve the chemical composition and consumer characteristics of the foods, provide meeting the market requirements, and increase the efficiency of financial and material resources, which, as a result, facilitates the food competitiveness and leads to the bakery industry effective development. We propose to evaluate the economic effect of innovative foods by determining the retail price and profit with a minimum 10% cost effect. The calculations of economic indicators for traditional and new flour-based foods are presented. Estimated economic efficiency of 1 tonne bakery foods for the standard and high-protein diets (according to therapeutic nutrition diet classification) is 5,030–10,740 rbls, flour confectionary foods – 11,022 rbls, gluten-free breads – 7,625–16,990 rbls, depending on the constituents and bakery technology. The results provide strong evidence of economic effect and the advantages of functional flour-based foods introduction.

Keywords: Bakery industry, innovative technologies, functional flour-based foods, diversification, competitiveness, economic effect

INTRODUCTION

In the current unsteady market conditions the world experience shows that product diversification is one of the most effective means to stabilize the bakery business, as well as a tool to eliminate imbalances in production and resource redistribution. Diversification is the process of expanding the activity of the enterprise, which uses its own savings not only to maintain and develop the business, but also to direct them to the development of new types of products, the creation of new types of foods, and the provision of all kinds of services [1]. A reasonable choice of diversification techniques provides companies with a real opportunity to strengthen their economic and financial position, to increase product competitiveness, to guarantee companies' efficient performance, and to meet the people's demand for foods; eventually, it will become the pillar of stability in the market and lay the groundwork for companies' further development.

In the bakery industry the same products are being produced for decades. It does not boost companies' development. Every deviation from traditional recipes, as adding one or more ingredients, is considered an innovative solution. Taking into account consumers' requirements and changes in the diet, bakers change the range of bakery products. Despite the fact that local bakery enterprises can offer a wide range of products (over 700), the total volume of novelties does not exceed 5%. Therefore, bakery product diversification through innovative technologies and recipes seems to be a long-term objective. It is important to develop and introduce alternative foods with higher economic effect [2].

In view of diversification and innovative development, brand new strategies for technological

modernization and product-line expansion are needed. The whole technological cycle should be activated: from a scientific innovative idea to the commercial introduction of the research results, their optimization and effective introduction into the practice. The share of innovative ideas in the bakery industry has, until recently, remained low and ineffective for various reasons, and research that can radically change and improve the situation in many cases remain unwanted or ineffective. Most bakery companies are cautious about the development of new foods, since they do not take into account that product diversification and innovations will give them undeniable advantages in the market expansion, provide lower production costs, extra profit, and more economic efficiency [2].

When planning to diversify and innovate, a detailed demand study and a clear demand forecasting for innovative technologies and new foods, as well as for their introduction are essential.

STUDY OBJECTS AND METHODS

Today consumers are becoming more and more demanding: they are interested in unique and healthy foods. Much attention is paid to product quality characteristics, its storage conditions, esthetics and packaging. In this regard, the aim of the research is the theoretical and economic justification for the development of technologies for functional breads, flour confectionary foods based on the correction of their nutrient composition through the use of alternative products of plant raw materials containing biologically valuable ingredients.

We analyzed the current functional breads market in the Russian Federation. A choice of raw materials for the main recipe ingredients used in functional breads making, namely amaranth seeds, lentil and lupine seeds, chufa, carob beans, lactulose and lecithin is explained and experimentally confirmed. We developed a method for preparing bakery in-process products for the reduced technological cycle for functional bakery products making (activated bakery

yeast, a modified sourdough for liquid yeast making) with improved biotechnological characteristics of yeast biomass, and also reducing the baking flour use.

We developed the recipes for functional breads and buns and provided the overall effect of ingredient properties on the consumer and medical-biological characteristics of the products. Also the cost of production the expected economic effect was calculated.

The creation and production of innovative functional flour-based products is currently a promising direction for the effective development of enterprises in the industry, since it allows them to compete and to occupy a niche in the new or existing markets that are not yet largely filled [1, 2]. Expansion of the flour products range intended for dietary – preventive and therapeutic – nutrition is proposed to be realized through the use of the ingredients shown in Fig. 1.

The analysis of new foods economic effect was done by standard cost estimate and projected retail prices per 1 tonne of ready-made traditional products (control sample) and new foods (experimental samples). The latter included bread with lentil seeds; "Magiya" roll with amaranth and lupine flour and lactulose prebiotic; "Lecitin" bun with a changed glycemic index with amaranth flour; cakes made with chufa seeds flour; gluten-free foods made with amaranth flour (cakes, crisps, honey cakes and breads).

RESULTS AND DISCUSSION

To demonstrate the perspectives for new foods introduction we calculated the economic effect of lentil flour use meant for technological cycle reduction both: on the stage of pressed yeast activation and on that of liquid yeast making.

Economic effect of lentil flour use at the stage of yeast activation includes bakery flour economizing, simplifying process of yeast activation, reducing activation and dough fermentation, and improving nutritional value and physical and chemical characteristics of the foods (Table 1).
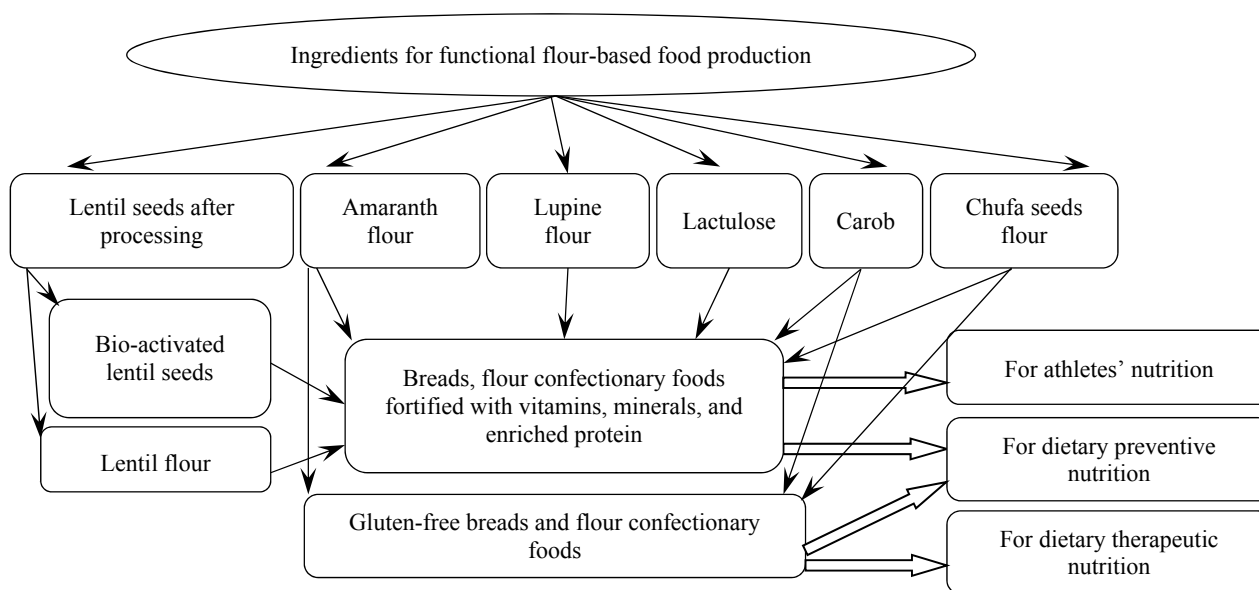

Fig. 1. Components for the production of innovative functional products.

Table 1. Comparison of bakery yeast activation

Criteria	Mixture for bakery yeast activation	
	New	Traditional*
Ingredients	Lentil flour (5% of wheat flour in dough) and non-fermented rye malt (0.5–1.5% of wheat flour)	Dark wheat flour (4% of wheat) non-fermented rye malt (1%), soya flour (1%)
Stages	1. Sourdough with lentil flour making, adding non-fermented rye malt 2. Yeast activation for 20–30 minutes	1. Wheat flour sourdough, adding non-fermented rye malt and more wheat and soya flour 2. Yeast activation for 1–2 hours
Effect	1. 25–30% less use of pressed yeast 2. Improved yeast quality 3. Less bakery flour used for activation, increased bread yield 4. Decreased dough fermentation (1.3 times) due to more intense acid accumulation 5. Increased biological protein value and the whole food value 6. Breadcrumb porosity is 4.3% higher	25–30% less use of pressed yeast

Table 2. Cost estimates and projected wholesale price for lentil breads (0.5 kg)

Calculation items	Unit costs, rbls:					
	Wheat (control)	Native lentil flour	Hydrolyzed lentil flour	Hydrolyzed lentil flour (1/3NaCl substituted KCl)	Lentil flour sourdough	Hydrolyzed lentil flour sourdough
Primary products, basic and supporting materials	13,274	16,130	16,415	16,584	16,130	16,673
Transportation and purchasing costs	1,327	1,290	1,313	1,327	1,290	1,334
Fuel	1,327	806	821	663	645	834
Energy consumption	1,327	806	821	663	645	834
Salary	2,655	2,505	2,505	2,505	2,505	2,505
Social insurance costs	802	756	756	756	756	756
Maintenance expenses	664	626	626	626	626	626
General expenses	664	626	626	626	626	626
Input costs	22,040	23,817	24,154	24,021	23,494	24,459
Selling expenses	170	183	186	185	181	188
Total cost	22,210	23,545	23,883	23,750	23,223	24,188
Cost effect, %	20	10	10	10	10	10
Profit	4,442	2,355	2,388	2,375	2,322	2,419
Wholesale price	26,651	25,900	26,271	26,125	25,545	26,607
VAT	2,665	2,590	2,627	2,613	2,555	2,661
Retail price	29.32	28.49	28.90	28.74	28.10	29.27

The use of lentil flour at the stage of liquid yeast production allows saving basic raw materials, rye flour, as well as improving biotechnological characteristics of yeast: yeast grown on lentil leaven grows 7 minute faster in comparison with yeast grown on rye leaven; the output is 30% higher.

Making liquid yeast with lentil flour promotes significant mix enrichment with digestible monosaccharide and disaccharide, as well as nitrogenous compounds preserving traditional production operations. The nutrients are enough to accumulate biomass and intensify the processes of dough maturation: targeted dough acidity is reached within 120 min., the whole technological process is 30 min shorter.

The new way of liquid yeast making demonstrated that cut-straight flour use 100 t of bread per day on traditional liquid yeast is 414 kg. Lentil flour makes it possible to get 600 kg bread per day. The results are obtained at Bread-baking plant № 1, Voronezh, Russia.

Lentil flour incorporation in wheat dough is possible in a native or hydrolyzed way, as well as a compound of sourdough. We calculated the requirements and costs of raw materials and supporting materials. The results of cost estimates and projected retail price are given in Table 2.

There is a slight increase of expenses share for raw materials and supporting materials (60.23% for control samples vs. 64.92% for experimental samples). Expenses for electricity and fuel are double less, due to the reduction of sourdough making and dough fermentation as a result of microbiological and biochemical process intensification.

Salary costs were fixed for a control sample, as more production staff was not needed. The calculations demonstrate that input costs of the new product and a retail price (20% cost effect) is insignificantly higher than those of a control sample (Table 2, Fig.3).
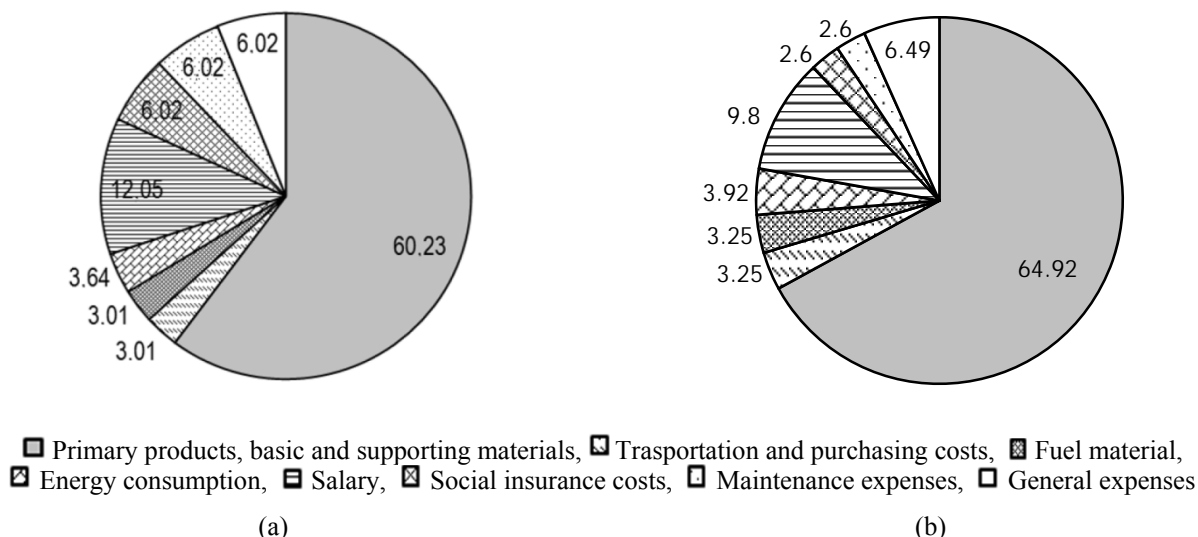
□ Primary products, basic and supporting materials, ▨ Trasportation and purchasing costs, ▨ Fuel material, ▨ Energy consumption, ⊟ Salary, ▨ Social insurance costs, ▢ Maintenance expenses, □ General expenses

(a)   (b)

Fig. 2. Input costs, %. (a) – wheat bread; (b) – and hydrolyzed lentil flour (1/3 NaCl was substituted with KCl).
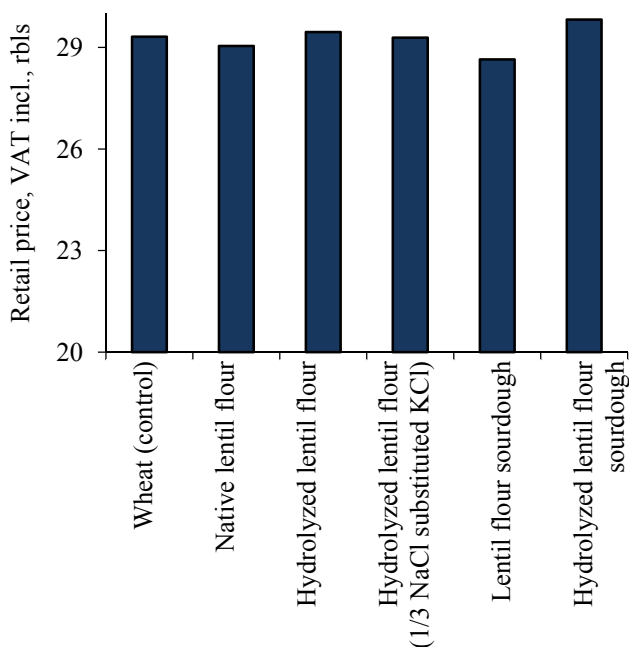

Fig. 3. Retail price for wheat bread with lentil flour (0.5 kg).

Bread with lentil flour does not involve extra expenses for staff and basic resources, which makes it possible to utilize existing equipment. Economic effect calculations offer a gradual market introduction for a new product, as it allows reducing the risks connected with a lack of demand. A manufacturer bears the costs for the in-parallel production of a familiar product and a new one with improved consumer properties and increased productivity though.

The increased output of the product to the level of line capacity allows a manufacturer to improve profitability and profit due to competitive product of a high quality which is in demand with different segments of the population.

Production efficiency of 1 tonne bread sales with lentil flour is 5,030–5,239 rbls (10% cost effect) and

can be significantly increased in case of production volume growth and corresponding cost effect growth.

Valuable properties of amaranth grain make it a unique food in a modern world as the problem of protein intolerance of traditional cereals (wheat, rye, barley, oats) is getting more and more pressing. Besides, introduction of foods with amaranth seeds into antisclerotic diet has been proved effective due to their lipid-lowering effect [3]. The findings in [4] allow concluding that production of functional gluten-free foods from amaranth flour is possible.

The technology of functional flour-based foods allows using sweet lupine flour. Proportions of flours allow changing flour baking properties or/and improving products' nutritional value. A considerable amount of potassium and magnesium in lupine flour makes it a perfect ingredient in foods, suitable for heart disease prevention. Fibre is good for obesity, diabetes and other diseases prevention. Lactulose is beneficial to the whole body; relieves the toxic liver disease, when ammonia is produced by putrefactive bacteria there [5, 6]. Besides, lactulose is best for atherosclerosis prevention by lowering cholesterol level in blood.

The requirements and cost of raw and supporting materials for "Magiya" roll with amaranth, lupine flour and lactulose (per 1 tonne) are calculated. The results of cost budget and projected wholesale price per 1 tonne of end product are given in Table 3.

Production efficiency of 1 tonne "Magiya" roll bread sales is 6,213 rbls (10% cost effect) and can be significantly increased in case of production volume growth and corresponding cost effect growth. Amaranth and lupine flours together with lecithin and lactulose bring a vast improvement: protein content increased by 36.7%, Ca, Mg, K, and P – by 15.1%, 22.8%, 14.8%, and 14.7% respectively; β-carotene – 3.42; B_1 and B_2 vitamins – 7.0% and 4.29%. Energy increased value only – by 3.7%. The roll (100 gr) fills in the need in phospholipids (20.6%), phosphorus (15.1%), β-carotene (22.6%), and lactulose (100%).

Table 3. Cost estimates and projected wholesale prices for "Magiya" roll (per 1 tonne)

Calculation items	Costs per 1 tonne, rbls:	
	Twist roll	"Magiya" roll
Primary products, basic and supporting materials	24,734	43,448
Transportation and purchasing costs	2,473	4,345
Fuel	2,473	2,473
Energy consumption	2,473	2,473
Salary	4,947	4,947
Social insurance costs	1,494	1,494
Maintenance expenses	1,237	1,237
General expenses	1,237	1,237
Input costs	41,069	61,654
Selling expenses	316	475
Total cost	41,385	62,128
Cost effect, %	20	10
Profit	8,277	6,213
Wholesale price	49,662	68,341
VAT	4,966	6,834
Retail price	54,629	75,175
Price for one (0.4 kg), rbls	21.85	30.07

Table 4. Cost estimates and projected wholesale price for "Lecitinochka" bun (per 1 tonne)

Calculaton items	Costs per 1 tonne, rbls:		
	Bun (control)	"Lecitinochka" bun withclactulose	"Lecitinochka" bun with carob
Primary products, basic and supporting materials	47,241	67,003	72,822
Transportation and purchasing costs	4,724	6,700	7,282
Fuel	4,724	4,724	4,724
Energy consumption	4,724	4,724	4,724
Salary	9,448	9,448	9,448
Social insurance costs	2,853	2,853	2,853
Maintenance expenses	2,362	2,362	2,362
General expenses	2,362	2,362	2,362
Input costs	78,438	100,177	106,578
Selling expenses	604	771	821
Total cost	79,042	100,948	107,399
Cost effect, %	20	10	10
Profit	15,808	10,095	10,740
Wholesale price	94,851	111,043	118,139
VAT	9,485	11,104	11,814
Retail price	104,336	122,148	129,953
Price per one (0.2 kg), rbls	20.86	24.43	25.99

Market research conducted by I.B. Krasina [7] shows that flour-based confectionary foods, diabetic-friendly ones in particular, with chocolate taste are in great demand with population. Chocolate ingredients are unadvisable due to increasing blood glucose properties though. The problem can be solved by carob use. It can substitute cacao and sugar (chocolate) for diabetic patients and those with overweight.

Carob has much fibre, insoluble one in particular, and is classified as a food with low glicaemic index [9]. The requirements and cost for raw material suppporting materials for "Lecitinochka" bun (per 1 tonne) are calculated. The results of cost budget and projected wholesale price per 1 tonne of end product are given in Table 4.

Production efficiency of 1 tonne "Lecitinochka" bun with lactulose sales is 10,095 rbls, "Lecitinochka" with carob – 10,740 rbls, (10% cost effect) and can be significantly increased in case of production volume growth. 4% of carob in the recipe (Lecitin bun with carob) results in the following changes in nutritional value: the content of proteins increased by 27.09%, fats – by 11.11% (phospholipids – 49.82 times), and fibres – by 1.8 times, while the content of carbohydrates decreased by 2.78% (monosaccharides and disaccharides – 17.30%). The price of the carob bun is only 6% higher than the one without carob, which is 1.56 rbls for 0.2 kg.

Present-day market necessitates developing innovative functional foods. One of the much-promising market sectors is gluten-free foods, the demand for which is only rising. Statistics shows that annual average growth rate for gluten-free foods over a period of 2009–2014 is 12.3% (based on sales volume analytics in France, Germany, Italy, Netherlands, Russia, Spain, Sweden, the UK, Brazil, the USA, Australia, China, India, Japan, and Korea) [10]. By the year 2020, gluten-free food market is expected to expand and will grow by 1.7 compared to 2015, which means 11% annual increase. Demand growth is driven by the following factors:
– Improved diagnostics allows detecting more cases of celiac and other gluten-sensitive diseases. The only treatment for these patients is strict long-lasting or life-long gluten-free dieting;
– New consumer segment expansion – those people who consider gluten-free foods as an important constituent of healthy lifestyle [12].

Such a situation forces Russian companies to introduce gluten-free foods into production, promoting imports replacement and expanding a customer base [11].

The crucial problem is a search of raw materials with high consumer properties [12, 13]. Our market study and calculations show that chufa seeds and amaranth grain for gluten-free breads and buns are economically viable.

Chufa seeds have a great amount of unsaturated fatty oils and vitamin E, which make them attractive for dietary, disease-preventive food making: they lower blood cholesterol. Besides, chufa is gluten-free, rich in potassium and phosphorus, low in sodium – good for nutrition of those with overweight, cardio-vascular diseases, high blood pressure, and kidney failure [4].

We chose "Stolychny" cake as a sample flour-based food with chufa seeds as it is a low-cost and absolute consumer favourite. We calculated requirement and cost of raw and supporting materials for cake production. "Stolychny" cake with wheat flour was as a control, sample No.1 contained 60:40 wheat and chufa

wheat, sample No.2 – gluten-free chufa flour (per 1 tonne). The results of cost budget and projected wholesale prices are given in Table 5.

The estimated productive efficiency of 1 tonne cake sale a mix of wheat and chufa flour (60:40) is 11,022 rbls, while the sales of sample cakes No.2 reached 16,425 rbls. Retail price of sample cakes No.2 is 2 times higher than that of a "Stolychny" cake and equals to 143,065 rbls per 1 tonne or 7.15 rbls for a 0.05 kg. It is worth noting that it is a gluten-free food, the established price for gluten-free cakes is 364–465 rbls per a 170–250 gr per pack (e.g. http://glutenfree.su/catalog/khleb/dr-schar: the price for "Magdalenas" muffins by *Santiveri* (Spain) is 364 rbls (170 g), gluten-free cakes with apricot jam "Magdalenas" by *Dr. Schar* (Italy) is 370 rbls (200 g), gluten-free cake "Marmorkuchen" by *Dr. Schar* is 465 rbls (250 g).

So, the price of the chufa cake is much lower than that of its import equals. It is worth noting that there are no cakes with chufa seeds flour in the market and it is an additional competitive advantage.

We calculated requirement and cost of raw and supporting materials for gluten-free foods (cakes, honey-cakes, crisp breads, and bread). The results of cost budget and projected wholesale prices are given in Tables 6–8.

The estimated productive efficiency of 1 tonne gluten-free "Vdokhvenie" cakes is 16,211–16,971 rbls. Retail price of "Vdokhvenie" cakes is 1.1 times or by 9.7% higher than that of "Stolychny" cakes (price growth is due to raw materials costs). The price of "Vdokhvenie" cakes is 8 times lower than that of the gluten-free cakes from Italy and Spain, and 2 times lower than the price of "Chudesnitsa" cakes from Belarus. Russian and European gluten-free food markets lack cakes baked with amaranth flour.

Productive efficiency of 1 tonne sale of amaranth gluten-free bread is 16,128 rbls (10% cost effect),

retail price is 58.55–61.67 rbls (0.3 kg) (Table 8).

Gluten-free "Amarantovy" bread has a competitive advantage: its price is 4.6–8.1 times lower (according to http://glutenfree.su/catalog/khleb/dr-schar, the price of Campagnard bread by *Dr. Schar*, 380 rbls for 240 g; "MB Classic" bread by *Dr. Schar* (Italy), 370 rbls for 300 g; gluten-free white bread and low-protein bread by *Bezgluten* (Poland), 260 and 270 rbls for 260 and 300 g respectively.

Table 5. Cost estimates and projected wholesale prices for chufa seeds cakes (per 1 tonne)

Calculation items	Costs per 1 tonne, rbls:		
	"Stolych-ny" cake	sample No.1 (wheat and chufa flour)	sample No.2 (gluten-free chufa flour)
Primary products, basic and supporting materials	42,103	85,783	139,404
Fuel	4,210	4,210	4,210
Energy consumption	4,210	4,210	4,210
Salary	8,421	8,421	8,421
Social insurance costs	2,543	2,543	2,543
Maintenance expenses	2,105	2,105	2,105
General expenses	2,105	2,105	2,105
Input costs	65,697	109,377	162,998
Selling expenses	506	842	1,255
Total cost	66,203	110,220	164,253
Cost effect, %	20	10	10
Profit	13,241	11,022	16,425
Wholesale price	79,444	121,242	180,678
VAT	14,300	21,823	32,522
Retail price, VAT incl.	93,744	143,065	213,200
Price per one (0.05 kg), rbls	4.68	7.15	10.66

Table 6. Cost estimates and projected wholesale prices for gluten-free amaranth flour cakes (per 1 tonne)

Calculation items	Costs per 1 tonne, rbls:				
	"Stolychny" cake	"Vdokhvenie" cake	"Vdokhvenie" cake with molasses	"Vdokhvenie" cake with an emulsifier	"Vdokhvenie" cake with pectin
Primary products, basic and supporting materials	89,344	116,745	116,612	110,798	118,347
Fuel	8,934	8,934	8,934	8,934	8,934
Energy consumption	8,934	8,934	8,934	8,934	8,934
Salary	17,869	17,869	17,869	17,869	17,869
Social insurance costs	5,396	5,396	5,396	5,396	5,396
Maintenance expenses	4,467	4,467	4,467	4,467	4,467
General expenses	4,467	4,467	4,467	4,467	4,467
Input costs	139,412	166,814	166,680	160,867	168,416
Selling expenses	1,073	1,284	1,283	1,239	1,297
Total cost	140,485	168,098	167,964	162,105	169,712
Cost effect, %	20	10	10	10	10
Profit	28,097	16,810	16,796	16,211	16,971
Wholesale price	168,583	184,908	184,760	178,316	186,684
VAT	30,345	33,283	33,257	32,097	33,603
Retail price, VAT incl.	198,927	218,191	218,017	210,413	220,287
Price per one (0.05 kg), rbls	9.94	10.91	10.90	10.52	11.01

Table 7. Cost estimates and projected wholesale prices for gluten-free amaranth honey cakes and crisps (per 1 tonne)

Calculation items	Costs per 1 tonne, rbls:			
	Gluten-free amaranth honey cakes	Crispy bread "Elizaveta" (control)	Crisps	
			sample No.1	sample No.2
Primary products, basic and supporting materials	80,730	24,246	72,008	72,493
Fuel	8,073	2,425	2,425	2,425
Energy consumption	8,073	2,425	2,425	2,425
Salary	16,146	4,849	4,849	4,849
Social insurance costs	4,876	1,464	1,464	1,464
Maintenance expenses	4,037	1,212	1,212	1,212
General expenses	4,037	1,212	1,212	1,212
Input costs	125,971	37,834	85,595	86,081
Selling expenses	970	291	659	663
Total cost	126,941	38,125	86,254	86,744
Cost effect, %	10	20	10	10
Profit	12,694	7,625	8,625	8,674
Wholesale price	139,635	45,750	94,880	95,418
VAT	25,134	8,235	17,078	17,175
Retail price, VAT incl.	164,769	53,985	111,958	112,593
Price for one (per 1 kg), rbls	164.77	53.98	111.95	112.59

Table 8. Cost estimates and projected wholesale prices for amaranth bread (per 1 tonne)

Calculation item	Costs per 1 tonne, rbls:		
	Control (flour mix *Mix B* by *Dr. Schar*)	Gluten-free "Amarantovy"	Gluten-reduced "Amarantovy"
Primary products, basic and supporting materials	333,703	96,393	104,941
Fuel	9,639	9,639	9,639
Energy consumption	9,639	9,639	9,639
Salary	19,279	19,279	19,279
Social insurance costs	5,822	5,822	5,822
Maintenance expenses	4,820	4,820	4,820
General expenses	4,820	4,820	4,820
Input costs	397,361	160,050	168,598
Selling expenses	3,060	1,232	1,298
Total cost	400,420	161,283	169,896
Cost effect, %	10	10	10
Profit	40,042	16,128	16,990
Wholesale price	440,462	177,411	186,886
VAT	44,046	17,741	18,689
Retail price, VAT incl.	484,508	195,152	205,575
Price for one (0.3 kg), rbls	145.35	58.55	61.67

CONCLUSION

In unsteady environment it is of utmost social importance to provide people with high quality local foods. The production of high quality innovative foods is a basis for market development for all manufacturers, including the bakery industry.

One of the reasons for flour-based market contraction is considered to be people's changing nutrition habits: their taste preferences, changing consumer needs, and, as a consequence, a changing list of basic foods. Bread and buns sales are connected with people's prosperity growth: we can trace the transition from their buying cheap, high-calorie foods (usually of foreign production) to more costly, healthy, and high-quality local foods [14, 15].

In view of holding a share of the market, to make commercial breads is not enough, a wide range of such products as functional and dietary ones should be produced taking into consideration consumers'

prevailing habits. Besides, low profitability of bakery businesses objectively slows down their modernization. The problem of technical re-equipment is more pressing than ever before. Low-output, rundown, outdated equipment leads to products high cost prices as well as to low quality products. It is particularly vital today when the output of functional flour-based foods, enriched with vitamins, minerals and bioactive compounds is dramatically low.

Based on the research findings and technological and economical aspects of functional foods production, it may be deduced that our innovation foods do not know local and foreign equals.

We showed the possibility to increase bakery in-process parts production efficiency, meant for reduced technological cycle (blends for bakery pressed yeast activation liquid yeast making are patented) due to the improvement of yeast bio-technological characteristics as well as to the use of lentil flour in the recipe, which

eventually leads to economic performance Product quality characteristics allow defining it as a highly competitive, innovative, in great demand in the dietary market. The foods can be recommended for commercial production at local enterprises.

The use of the above mentioned ingredients for functional flour-based foods will contribute toward product quality improvement, the expansion of product range, the use of local raw materials, the growth of target profit, and companies' economic results improvement and their competitive growth.

CONFLICT OF INTEREST

The authors state that they have no conflict of interest.

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Matrix dynamic models of elements of technological systems with perfect mixing and plug-flow hydrodynamics in Simulink

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Abstract: The dynamic models of elements of technological systems with perfect mixing and plug-flow hydrodynamics are based on the systems of algebraic and differential equations that describe a change in the basic technological parameters. The main difficulty in using such models in MathWorks Simulink™ computer simulation systems is the representation of ordinary differential equations (ODE) and partial differential equations (PDE) that describe the dynamics of a process as a MathWorks Simulink™ block set. The study was aimed at developing an approach to the synthesis of matrix dynamic models of elements of technological systems with perfect mixing and plug-flow hydrodynamics that allows for transition from PDE to an ODE system on the basis of matrix representation of discretization of coordinate derivatives. A sugar syrup cooler was chosen as an object of modeling. The mathematical model of the cooler is formalized by a set of perfect reactors. The simulation results showed that the mathematical model adequately describes the main regularities of the process, the deviation of the calculated data from the regulations did not exceed 10%. The proposed approach significantly simplifies the study and modernization of the current and the development of new technological equipment, as well as the synthesis of algorithms for controlling the processes therein.

Keywords: Mathematical modeling, dynamic systems, sugar syrup cooler, MathWorks Simulink™

INTRODUCTION

The main way to study the regularities of technological heat and mass exchange processes in the food industry is to make a full-scale experiment. In many cases, it results in a number of insurmountable difficulties due to its cost, the ability of engineering implementation of the control parameters, etc. Due to this, the development of adequate and correct mathematical models of technological processes that allow us to replace a full-scale experiment is a relevant task.

The processes of heat and mass transfer in moving media can be simulated on the basis of the Navier-Stokes equation, but the equations obtained are not always suitable for analysis. Therefore, the perfect models of a flow pattern have become widespread in practice: perfect mixing models (they describe a change in concentration, temperature, etc. in compounders), plug-flow models (they describe a change in concentration, temperature, etc. in the case of a plug-flow going through an apparatus), cellular models and others [1–3]. The mathematical models of such apparatus are called perfect mixing reactors (PMR), plug-flow reactors (PFR), etc.

A Simulink interactive graphical simulation environment is widely used for the computer modeling of technological processes that allows using block diagrams in the form of directed graphs to construct dynamic models, including discrete (continuous and hybrid), non-linear models and models with singularities. In addition, the Simulink environment includes the tools for building, modeling and studying control systems which allows for the simulation of a process and control system within a single integrated environment [4].

When modeling perfect reactors, the conversion of the equations to describe a technological process into a set of blocks of the visual programming environment of Simulink is necessary. In the case of systems of ordinary differential equations (ODE), an input signal integration unit and a MathWorks™ numerical methods library are used, as well as the method for representing ODE or an ODE system in the form of a structural Simulink model [5, 6]. In the case of partial differential equations that describe, for example, plug-flows, the heat conductivity equation, etc., there is a problem of bringing PDE to an ODE equation or system. One of the approaches used in the transition from PDE to ODE is the use of integral transformations, for example, Fourier transformations and Laplace transformations [7, 8], and the disposal of spatial derivatives. It is not always possible to obtain a solution to such equations in analytical form. In addition, the implementation of direct and inverse integral transformations by means of Simulink for an arbitrary input signal is difficult. The other approach is to use numerical methods for solving PDE (finite elements, finite differences, etc.) and to integrate program blocks into the structural model of Simulink using the custom functions implemented on the basis of such programming languages as MathWorks and universal ones like C ++ [9]. In this case, it is possible to implement the entire arsenal of available tools for the numerical solution of PDE, but any modification of an object model entails the need for changing the function code and debugging it, thereby violating the object-oriented principle of Simulink – the separation of the internal structure and interface of a model. One of the approaches is based on the discretization of only the spatial variable using the finite difference method, while the derivatives with respect to time remain continuous and PDE is represented as the Cauchy problem for the ODE system [10]. The implementation of each equation of the ODE system results in the formation of rather cumbersome structural models in Simulink, and the compact representation of such systems in a matrix form is relevant [11, 12]. In this case, it is necessary to replace the expansion of spatial derivatives with matrix equations [12]. [13] shows such a representation of a one-dimensional heat conductivity equation for Simulink.

Thus, the relevant task is to develop a method for representing typical perfect reactors using matrix ordinary differential equations (MODE) and to implement structural models therewith in Simulink.

STUDY OBJECTS AND METHODS

To synthesize the matrix dynamic model of a typical perfect reactor, it is necessary to perform the following sequence of actions:
– to select the model of a perfect reactor suitable for describing the structure of the flow in process equipment (this issue is considered in detail in the special literature, for example, in [1–3]);
– to draw up the corresponding differential equation. As a result, for each perfect reactor, one or more ODE or PDE are obtained with the corresponding initial and boundary conditions;

– to solve the resulting equation or a set thereof with respect to the highest derivative with respect to time;
– to discretize spatial derivatives (in the case of their presence) in accordance with the accepted form of finite-difference approximation;
– to replace each PDE by a set of ODE using the replacement of partial derivatives with respect to the spatial variable by the corresponding matrix equation. For example, the first derivative of the temperature $\dfrac{\partial T(x,t)}{\partial x}$ is replaced as follows:

$$\frac{\partial T(x,t)}{\partial x} \approx \begin{cases} \dfrac{T_1(t)-T_0(t)}{\Delta x}, \\ ... \\ \dfrac{T_i(t)-T_{i-1}(t)}{\Delta x} \\ ... \\ \dfrac{T_L(t)-T_{L-1}(t)}{\Delta x}, \end{cases} \approx \mathbf{A}\mathbf{T}(t)+\mathbf{B}(t), \quad (1)$$

where is the decomposition matrix of the first derivative, and

$$\mathbf{A} = \begin{pmatrix} 1 & 0 & 0 & ... & 0 \\ -1 & 1 & 0 & ... & 0 \\ & & ... & & \\ 0 & ... & -1 & 1 & 0 \\ 0 & ... & 0 & -1 & 1 \end{pmatrix}$$

$$\mathbf{B}(t) = \begin{pmatrix} T_0(t) \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

is a vector to define the boundary condition.

The second derivative can be defined as

$$\frac{\partial^2 T(x,t)}{\partial x^2} \approx \begin{cases} \dfrac{T_0(t)-2T_1(t)+T_2(t)}{\Delta x^2}, \\ ... \\ \dfrac{T_{i-1}(t)-2T_i(t)+T_{i+1}(t)}{\Delta x^2} \\ ... \\ \dfrac{T_{L-2}(t)-2T_{L-1}(t)+T_L(t)}{\Delta x^2}, \end{cases} \approx \mathbf{C}\mathbf{T}(t)+\mathbf{D}(t), \quad (2)$$

where

$$\mathbf{C} = \begin{pmatrix} -2 & 1 & 0 & ... & 0 \\ 1 & -2 & 1 & ... & 0 \\ & & ... & & \\ 0 & ... & 1 & -2 & 1 \\ 0 & ... & 0 & 1 & -2 \end{pmatrix}$$

is the decomposition matrix of the second derivative, and

$$\mathbf{D}(t) = \begin{pmatrix} T_0(t) \\ 0 \\ 0 \\ 0 \\ T_L(t) \end{pmatrix}$$

is a vector to define the boundary conditions.
– then, in accordance with the procedure described in [5], to draw up a structural diagram of a perfect reactor model in the form of Simulink blocks that solve MODE taking into account the fact that the signals transmitted from block to block are vectors and matrices and the corresponding multiplication operations will be performed according to the rules of matrix multiplication. In this case, the time derivatives will be integrated using numerical methods from the Simulink library;
– to define the parameters of the model that characterize flow hydrodynamics, the geometry of perfect reactor models and the thermophysical properties of process media;
– to create input and output streams to build process equipment models in the form of a system of perfect reactors and additional subsystems, if necessary; and
– to design a reactor model in the form of a subsystem in the Simulink format.

Let us consider the synthesis process of a dynamic matrix mathematical model using a sugar syrup cooler as an example which is used for fondant sweet processing lines [14]. The cooler is a cylindrical body *1*, inside which a rotating screw *2* (рассмотрите pushes sugar syrup forward (Fig. 1). The body *1* is equipped with a cooling jacket made in the form of a closed spiral canal (CSC) *3* cooling water (a coolant) is fed through. As a result of the heat exchange between the syrup and the coolant through the wall of CSC that separates them, the temperature of the syrup decreases, which results in the crystallization of sucrose from the fondant syrup and the formation of fondant mass.
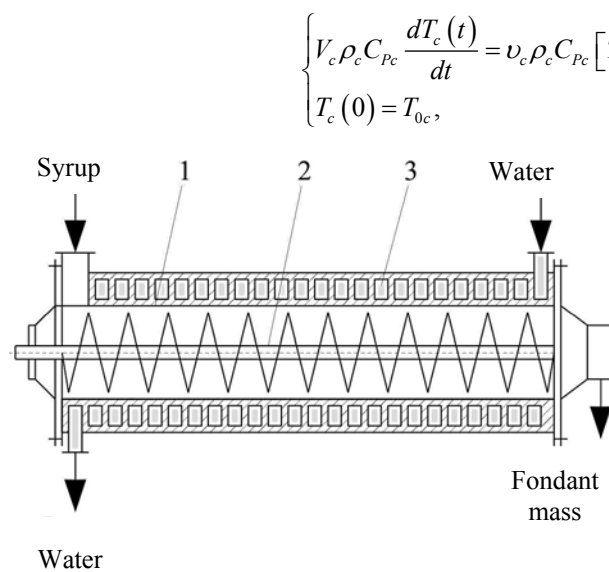
The quality indicators of the finished product (fondant mass) are the size of sucrose crystals and their proportion in the total volume of fondant mass. In turn, the disperse composition of fondant mass depends entirely on both the cooling parameters of fondant syrup–temperature, the intensity of removal of heat from the syrup–and its thermophysical and rheological properties that change during the crystallization sucrose process [14].

To model the heat exchange between the syrup and the coolant, let us select the models of perfect reactors. In view of the intensive mixing of sugar syrup with a rotating screw, the complexity of the mathematical description resulting from the mixing of the three-dimensional temperature pattern, as well as the necessity of conjugation of the geometric coordinates of cooling water and sugar syrup flows, let us estimate in the first approximation the average temperature of sugar syrup according to the length of the cylindrical body 1. Averaging the sugar syrup temperature according to the length of the body by integrating the PMR equation with respect to the variable *x* and dividing it by the length of the body, we obtain an equation that corresponds to PFR in structure. For the cooling water flow, let us neglect the temperature distribution over the cross-sectional area of the canal and choose a PMR model. The perfect reactors are physically separated by a wall through which there is a heat exchange between the coolant and sugar syrup. Let us neglect the thermal effects as a result of the process of crystallization of sucrose from the syrup, as well as heat exchange with the environment. Let us assume that the wall temperature is constant along the length.

Let us consider the synthesis of the PFR model in accordance with the diagram in Fig. 2.

If the structure of the syrup flow corresponds to the PFR model, an equation of a temperature change taking heat transfer into account can be used for the mathematical description of this flow [2]. It will be an ordinary differential equation with respect to the temperature of the syrup T_C

$$\begin{cases} V_c \rho_c C_{Pc} \dfrac{dT_c(t)}{dt} = \upsilon_c \rho_c C_{Pc} \left[T_c^{ex} - T_c(t) \right] + F_c K_{Tc} \left[T_{cm}(t) - T_c(t) \right], \\ T_c(0) = T_{0c}, \end{cases} \quad (3)$$

Fig. 1. *1* – is cylindrical body; *2* – is screw; *3* – is closed spiral canal.

where V_c is the volume of the perfect mixing area, m³; ρ_c is density of sugar syrup, kg/m³; C_{Pc} is the specific heat capacity of sugar syrup, J/(kg × K); υ_c is the volumetric flow rate, m³/s; T_c^{ex} is the flow temperature at the inlet to the perfect mixing area; F_c is the surface of heat exchange between the syrup and the wall of CSC, m²; K_{Tc} is the coefficient of heat transfer from the syrup to the center of the wall of CSC, W/(m² × K); $T_{cm}(t)$ is the temperature of the center of the wall of CSC, °C.
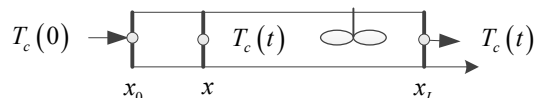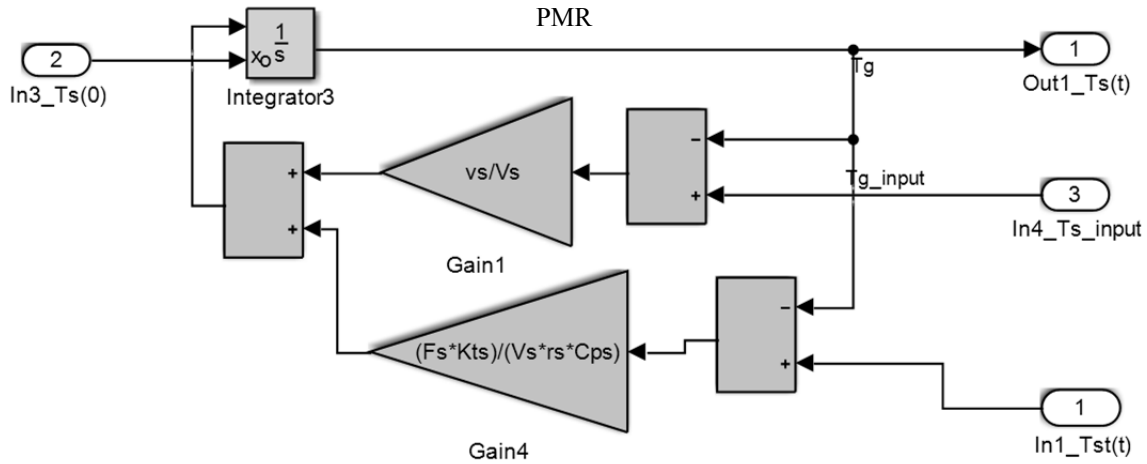

Fig. 2. The syrup flow chart.

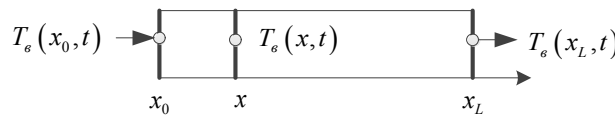**Fig. 3.** Simulink model corresponding to Eq. 3.

Fig. 4. The coolant flow chart.

To solve the equation in Simulink, let us use the technique described in [5] and represent Eq. 3 in the form of Simulink blocks (Fig. 3) having previously solved Eq. 3 with respect to the derivative.

Let us consider the synthesis of the model of a plug-flow reactor (Fig. 4).

If the structure of the flow corresponds to the plug-flow model (the model is based on the assumption that the temperature is constant in the cross section and there is no longitudinal mixing), then for the mathematical description of this flow, an equation taking heat transfer into account can be used [1]

$$\begin{cases} S_{в}\rho_{в}C_{Pв}\dfrac{\partial T_{в}(x,t)}{\partial t} = -\upsilon_{в}\rho_{в}C_{Pв}\dfrac{\partial T_{в}(x,t)}{\partial x} + \dfrac{F_{в}K_{Tв}}{L_{в}}\big[T_{cm}(t)-T_{в}(t)\big], \\ T_{в}(x,0)=T_{в0}(x),\ T_{в}(0,t)=T_{xв0}(t), \end{cases} \tag{4}$$

where $S_{в}=\dfrac{V_{в}}{L_{в}}$; $T_{в}(x,t)$ is the function of spatial and time distribution of temperature of the coolant flow; $V_{в}$ is the volume of the plug-flow area, m³; $\rho_{в}$ is the density of the coolant, kg/m³; $C_{Pв}$ is the specific heat capacity of the coolant, J/(kg × K); $\upsilon_{в}$ is the volumetric flow rate, m³/s; $T_{x0в}(t)$ is the flow temperature at the inlet to the plug-flow area; $F_{в}$ is the surface of heat exchange between the coolant and the wall of CSC, m²; $K_{Tв}$ is the coefficient of heat transfer from the coolant to the center of the wall of CSC, W/(m² × K).

Let us bring Eq. 4 to a finite-difference form and solve it with respect to the derivative

$$\begin{cases} \dfrac{dT_{в1}(t)}{dt} = -\dfrac{\upsilon_{в}}{S_{в}}\left[\dfrac{T_{в0}(t)-T_{в1}(t)}{\Delta x}\right] + \dfrac{F_{в}K_{Tв}}{V_{в}\rho_{в}C_{Pв}}\big[T_{cm}(t)-T_{в1}(t)\big], \\ \dots \\ \dfrac{dT_{вi}(t)}{dt} = -\dfrac{\upsilon_{в}}{S_{в}}\left[\dfrac{T_{вi}(t)-T_{в,i-1}(t)}{\Delta x}\right] + \dfrac{F_{в}K_{Tв}}{V_{в}\rho_{в}C_{Pв}}\big[T_{cm}(t)-T_{iв}(t)\big] \\ \dots \\ \dfrac{dT_{вL}(t)}{dt} = -\dfrac{\upsilon_{в}}{S_{в}}\left[\dfrac{T_{вL}(t)-T_{вL-1}(t)}{\Delta x}\right] + \dfrac{F_{в}K_{Tв}}{V_{в}\rho_{в}C_{Pв}}\big[T_{cm}(t)-T_{Lв}(t)\big] \\ T_{вi}(0)=f_{в}(x_i),\ i=1..N,\ T_{в0}(t)=\varphi_{в}(t),\ T_{вL+1}(t)=\psi_{в}(t). \end{cases} \tag{5}$$

Let us define the vector of the current temperature at the points of coordinate partitioning and the initial conditions

$$\mathbf{T}_{\scriptscriptstyle \theta}(t) = \begin{pmatrix} T_{\scriptscriptstyle \theta 1}(t) \\ ... \\ T_{\scriptscriptstyle \theta i}(t) \\ ... \\ T_{\scriptscriptstyle \theta L}(t) \end{pmatrix} \quad \mathbf{T}_{\scriptscriptstyle \theta 0} = \begin{pmatrix} T_{\scriptscriptstyle \theta 01} \\ ... \\ T_{\scriptscriptstyle \theta 0 i} \\ ... \\ T_{\scriptscriptstyle \theta 0 L} \end{pmatrix},$$

Let us represent the first derivative in the form of a matrix expression [4]

$$\mathbf{A}\mathbf{T}_{\scriptscriptstyle \theta}(t) + \mathbf{C}(t), \qquad (6)$$

where) is the decomposition matrix of the first derivative,

$$\mathbf{A} = \begin{pmatrix} 1 & 0 & 0 & ... & 0 \\ -1 & 1 & 0 & ... & 0 \\ & & ... & & \\ 0 & ... & -1 & 1 & 0 \\ 0 & ... & 0 & -1 & 1 \end{pmatrix}$$

$$\mathbf{C}(t) = \begin{pmatrix} T_{\scriptscriptstyle \theta 0}(t) \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

is a vector to define the boundary condition.

Then let us write the ODE system (5) as a matrix ODE

$$\begin{cases} \dfrac{d\mathbf{T}_{\scriptscriptstyle \theta}(t)}{dt} = -\dfrac{\upsilon_{\scriptscriptstyle \theta}}{\Delta x S_{\scriptscriptstyle \theta}} \big[\mathbf{A}\mathbf{T}(t) + \mathbf{C}(t) \big] + \dfrac{F_{\scriptscriptstyle \theta} K_{T_{\scriptscriptstyle \theta}}}{V_{\scriptscriptstyle \theta} \rho_{\scriptscriptstyle \theta} C_{P_{\scriptscriptstyle \theta}}} \big[T_{cm}(t) - \mathbf{T}_{\scriptscriptstyle \theta}(t) \big], \\ \mathbf{T}_{\scriptscriptstyle \theta}(0) = \mathbf{T}_{\scriptscriptstyle \theta 0}, \end{cases} \quad (7)$$

and represent it in the form of a structural Simulink model (Fig. 5).

Since the statement of the problem implies that the heat capacity of the wall that separates the heat carrier flows cannot be neglected, then these equations will be supplemented by an equation of a change in the temperature of the wall that separates the media $T_{cm}(t)$

$$\begin{cases} m_{cm} C_{Pcm} \dfrac{dT_{cm}(t)}{dt} = F_{c} K_{Tc} \big[T_{c}(t) - T_{cm}(t) \big] - F_{\scriptscriptstyle \theta} K_{T_{\scriptscriptstyle \theta}} \big[T_{cm}(t) - \overline{T}_{\scriptscriptstyle \theta}(t) \big], \\ T_{cm}(0) = T_{cm0}, \end{cases} \quad (8)$$

where m_{cm} is the wall mass, kg; C_{Pcm} is the specific heat capacity of the wall, J/(kg × K); $\overline{T}_{\scriptscriptstyle \theta}(t)$ is the average temperature of the coolant over the entire length calculated as

$$\overline{T}_{\scriptscriptstyle \theta}(t) = \frac{1}{L}\int_{0}^{L} T_{\scriptscriptstyle \theta}(x,t)\,dx, \qquad (9)$$

and when partitioning it by N elements with respect to x it is replaced with the sum

$$\overline{T}_{\scriptscriptstyle \theta}(t) \approx \frac{1}{L}\sum_{i=1}^{N} T_{i\scriptscriptstyle \theta}(t)\Delta x. \qquad (10)$$

Let us represent it in the form of a structural Simulink model (Fig. 6).

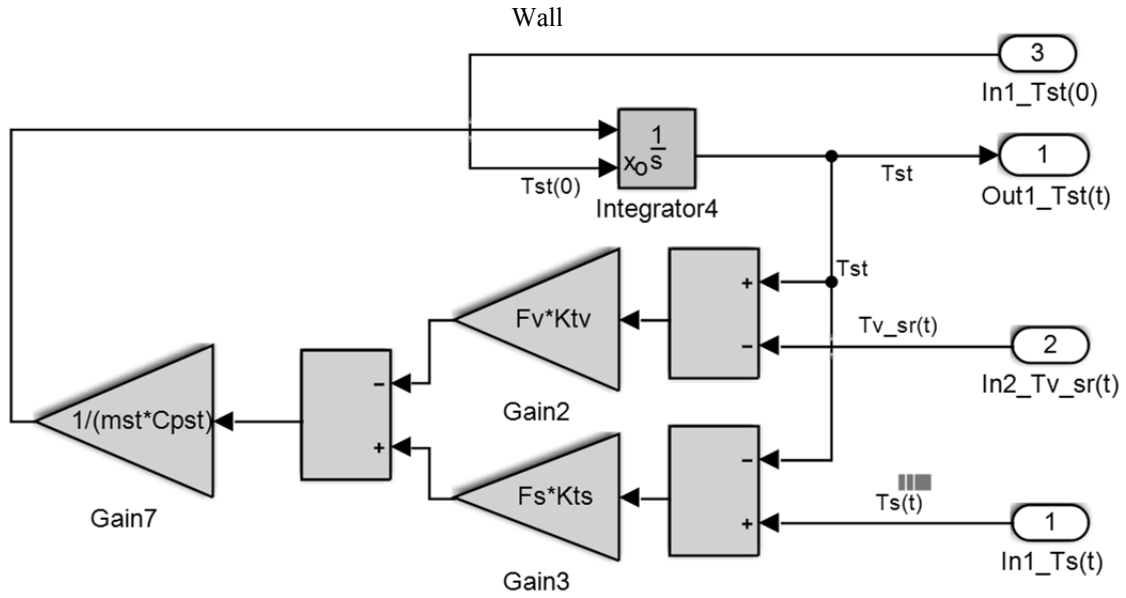**Fig. 5.** Simulink model corresponding to Eq. 5.

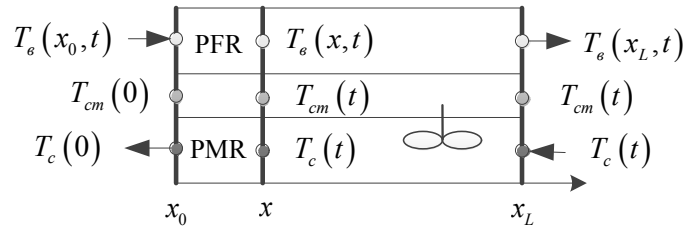**Fig. 6.** Simulink model corresponding to Eq. 8.

Fig. 7. The syrup, wall and coolant flow chart.

As a result, a set of subsystems for modeling perfect reactors is formed. To compile a cooler model, it is only necessary to connect the subsystems into a single design scheme in accordance with Fig. 7 and set the model parameters.

In this case, the mathematical model of the cooler can be described by a system of scalar and matrix ODE

$$
\begin{cases}
\dfrac{d\mathbf{T}_{\text{в}}(t)}{dt} = -\dfrac{\upsilon_{\text{в}}}{\Delta x S_{\text{в}}}\left[\mathbf{A}\mathbf{T}(t)+\mathbf{C}(t)\right]+\dfrac{F_{\text{в}}K_{T\text{в}}}{V_{\text{в}}\rho_{\text{в}}C_{P\text{в}}}\left[T_{cm}(t)-\mathbf{T}_{\text{в}}(t)\right], \\[4mm]
\dfrac{dT_{c}(t)}{dt} = \dfrac{\upsilon_{c}}{V_{c}}\left[T_{c}^{\text{ex}}-T_{c}(t)\right]+\dfrac{F_{c}K_{Tc}}{V_{c}\rho_{c}C_{Pc}}\left[T_{cm}(t)-T_{c}(t)\right], \\[4mm]
\dfrac{dT_{cm}(t)}{dt} = \dfrac{F_{\text{в}}K_{T\text{в}}}{m_{cm}C_{Pcm}}\left[\dfrac{1}{L}\sum_{i=1}^{N}T_{\text{в}i}(t)\Delta x - T_{cm}(t)\right]-\dfrac{F_{c}K_{Tc}}{m_{cm}C_{Pcm}}\left[T_{cm}(t)-T_{c}(t)\right], \\[4mm]
\mathbf{T}_{\text{в}}(0)=\mathbf{T}_{\text{в}0},\ T_{c}(0)=T_{c0},\ T_{cm}(0)=T_{cm0},
\end{cases}
\tag{11}
$$

with the parameters [14, 15]: the heat capacity of the coolant $C_{P\text{в}} = 4190\ \dfrac{J}{kg\cdot K}$; the heat capacity of the wall $C_{Pcm} = 385\ \dfrac{J}{kg\cdot K}$; the heat capacity of the syrup $C_{Pc} = 2500\ \dfrac{J}{kg\cdot K}$; the heat exchange area of the coolant-wall $F_{\text{в}} = 1.3$ m²; the heat exchange area syrup-wall $F_{c} = 1.56$ m²; the wall mass $m_{cm} = 25.465$ kg; the cross-sectional area inside CSC $S_{\text{в}} = 0.000314$ m²; the coefficient of thermal conductivity of the wall $\lambda_{cm} = 400\ \dfrac{W}{m\cdot K}$; the density of the wall material (copper) $\rho_{M} = 8900\ \dfrac{kg}{m^{3}}$; the density of the coolant

$\rho_{\text{в}} = 1000 \dfrac{kg}{m^3}$; the density of syrup $\rho_c = 1500 \dfrac{kg}{m^3}$;

the coefficient of heat transfer from the coolant to the wall $\alpha_{\text{в}} = 643.3 \dfrac{W}{m^2 \cdot K}$; the coefficient of heat transfer from the syrup to the wall $\alpha_c = 820.5 \dfrac{W}{m^2 \cdot K}$; the coefficient of heat transfer from the syrup to the center of the wall

$$K_{Tc} = \cfrac{1}{\cfrac{1}{\alpha_c} + \cfrac{\delta_{cm}}{2\lambda_{cm}}} = 819.672 \dfrac{W}{m^2 \cdot K}$$; the coefficient of heat from the coolant to the center of the wall

$$K_{T\text{в}} = \cfrac{1}{\cfrac{1}{\alpha_{\text{в}}} + \cfrac{\delta_{cm}}{2\lambda_{cm}}} = 642.261 \dfrac{W}{m^2 \cdot K}$$; the discretization interval $\Delta x = 2.07$ m; the number of discretization elements along the length $N = 10$; the thickness of the wall of CSC, $\delta_{cm} = 0.002$ m; the length of CSC $L = 20.7$ m; the volumetric flow rate of the coolant $\upsilon_{\text{в}} = 0.00096 \dfrac{m^3}{s}$; the syrup volumetric flow rate $\upsilon_c = 0.0001 \dfrac{m^3}{s}$; the initial temperature of the coolant $T_{0\text{в}} = 15°C$; the initial temperature of syrup $T_{0c} = 105°C$; the initial wall temperature $T_{0cm} = 25°C$; the volume of the area of PFR $V_{\text{в}} = 0.009$ m^3; and the syrup volume in PMR $V_c = 0.36$ m^3.

The structural model in the form of Simulink blocks is shown in Fig. 8, the simulation results are shown in Figs. 9–11. The studies and computer experiment were carried out at the Voronezh State University of Engineering Technology.

RESULTS AND DISCUSSION

The data in Fig. 9 show that the sugar syrup is cooled from the initial temperature of 105°C to 60°C within 600–800 sec, which is consistent with the technological regulations for fondant mass production. The further cooling of syrup does not result in a decrease in the temperature of the finished product. The deviation of the calculated data from the regulations did not exceed 10%.

The model developed with the help of this approach makes it possible to obtain the estimates of temperatures at the outlet from the cooler in real time (Fig. 9a) which makes it possible to study the dynamics of the technological process and synthesize a control system. It is also possible to estimate the temperature distribution in terms of both the time and length of the heat exchange surface of CSC (Fig. 9b). In addition, by varying the parameters of a mathematical model, it is possible to estimate their effect on the technical and economic indicators of a process, for example, when changing the material which CSC is made of (Fig. 10a), and also to predict a change in the dynamic characteristics of a process (Fig. 10b).

When the syrup is cooled, its viscosity significantly changes which entails a change in the hydrodynamics of the syrup flow and conditions of heat exchange between the coolant and syrup through the wall that separates them. The introduction of a temperature correction in the calculation of syrup viscosity makes it possible to take into account a change in the coefficient of thermal conductivity from the syrup to the wall of CSC. For example, using the data of [16], let us approximate the dependence of syrup viscosity on temperature using the Arrhenius equation

$$\eta_c(T_c) = 3.057 \cdot 10^{-6} \exp\left[\frac{48035.124}{8.31(T_c + 273)}\right] \quad (12)$$

Then, taking into account the dependence of syrup viscosity on temperature, it is possible to estimate the coefficients of heat transfer and thermal conductivity from the syrup to the wall of CSC for each temperature and to clarify the syrup cooling dynamics (Fig. 11). In this case, the heat transfer and thermal conductivity coefficients are calculated with the help of an additional Matlab Function block from the Simulink library that performs the continuous calculation of the heat transfer and thermal conductivity coefficients according to the current values of syrup temperature delivered to the block input.

The further refinement of the cooler model can be due to, first, taking into account the thermal effects resulting from the crystallization of sucrose from the syrup and, secondly, taking into account the design features of a typical fondant beater (feeding cooling water into the shaft of the conveying screw, separating the machine body and cooling water jacket into three sections in each of which the heat is removed from the syrup with various intensity).
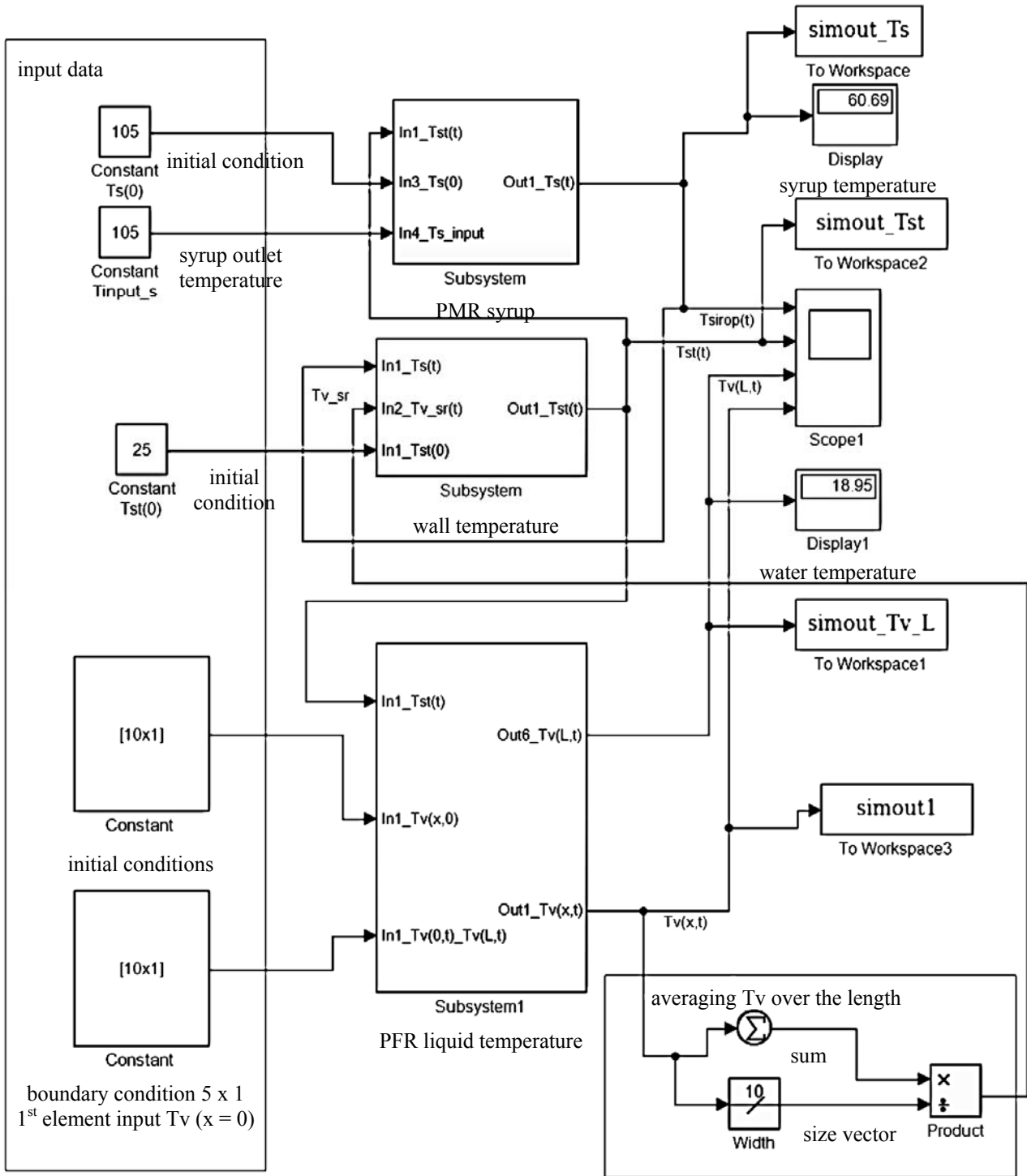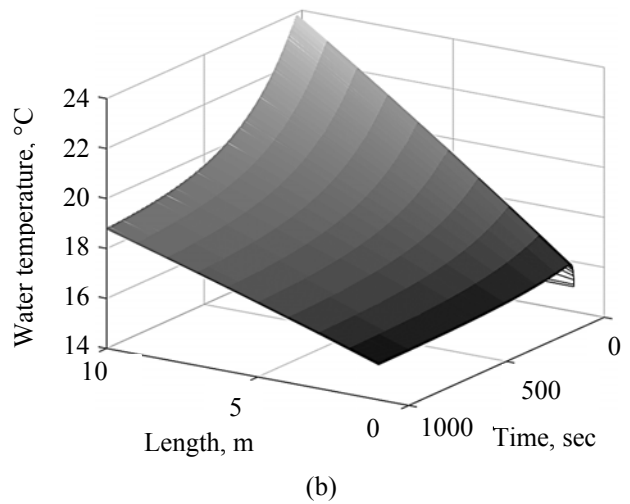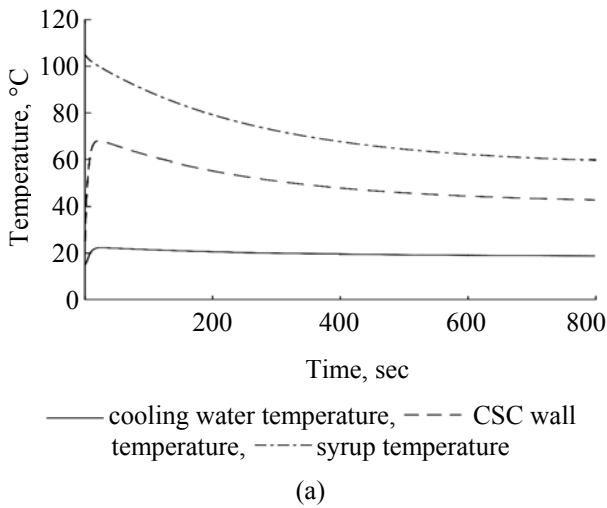
Fig. 8. Simulink model for the syrup cooler.

Fig. 9. The temperatures of: a) cooling water, wall and syrup, b) cooling water distributed along the closed spiral canal.
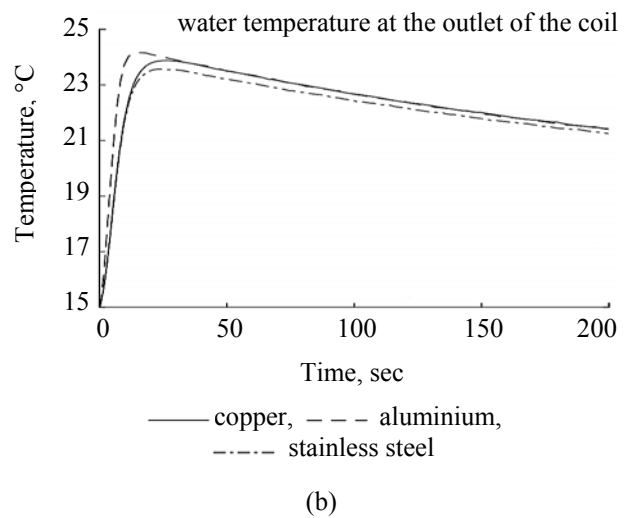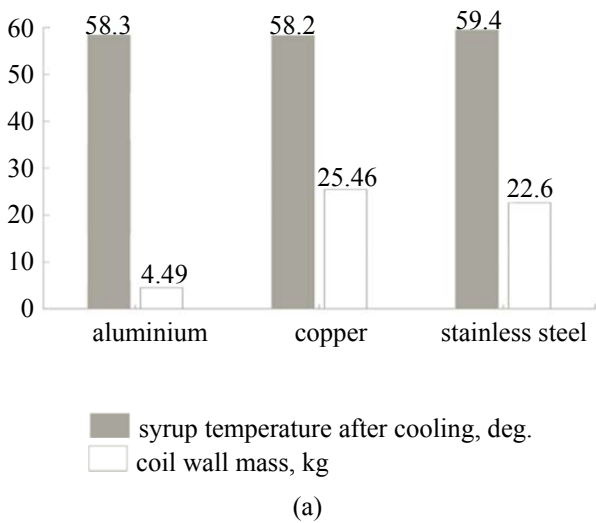

Fig. 10. The CSC material impact on: (a) syrup temperature after cooling and the CSC mass, (b) coolant temperatures.
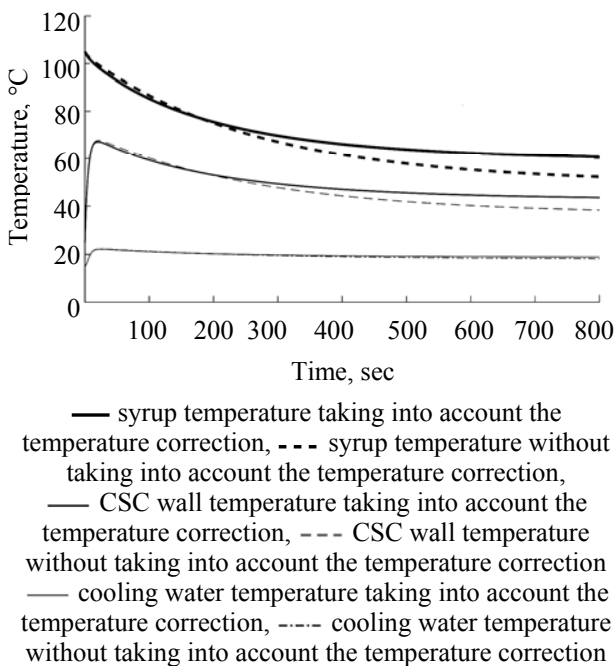

Fig. 11. Syrup viscosity impact on the syrup, coolant and wall temperatures.

CONCLUSION

The presented approach makes it possible to implement the mathematical models of perfect reactors in Simulink by discretizing the spatial variable and to pass over to matrix ordinary differential equations, which makes it possible to convert them into Simulink blocks. The approach is also applicable to other models of perfect reactors, which makes it possible to build the libraries of typical perfect reactors of Simulink for the synthesis of heat and mass transfer equipment that makes it easy to integrate them into a single system of synthesis, study, and debugging of Simulink control systems. Within the framework of Simulink simulation systems, a further refinement of the obtained simplest models based on perfect reactors by introducing variable model parameters, nonlinearities, control circuits, etc. is possible. This significantly simplifies the study and modernization of the current technological equipment and the development of new equipment, as well as the synthesis of control algorithms for the processes therein.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Patents:

Palkin M.V. and Kulakov A.V. *Sposob orientirovaniia po krenu letatel'nogo apparata s opticheskoi golovkoi samonavedeniia* [The way to orient on the roll of aircraft with optical homing head]. Patent RF, no. 2280590, 2006. (In Russ.).

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