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STANDARDIZATION, CERTIFICATION, QUALITY AND SAFETY

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LETTER FROM THE EDITOR-IN-CHIEF

Dear readers,

You are holding the latest issue of the international scientific journal *Foods and Raw Materials*. In 2017, we celebrate the journal's fifth anniversary. Almost 5 years ago *Foods and Raw Materials* was launched at Kemerovo Institute of Food Science and Technology (University) for Russian scientists to exchange their opinions and ideas. Eventually, a number of foreign authors joined us. *Foods and Raw Materials* has risen from a university bulletin to the journal included in the Emerging Sources Citation Index (ESCI), a new index in the Web of Science Core Collection. Since 2016, it is indexed in SCOPUS database. *Foods and Raw Materials* has become a powerful information platform, a reputable scientific journal, and an internationally recognized leader in its academic sphere. Since the publication of the first issue, the editorial board has made it a mission to spread the works on the topics addressed in the journal in the international scientific community, to strengthen the presence of the shared scientific achievements on the international arena, as well as to highlight the results of promising areas of scientific researches in food industry and related industries.

The success of any project is measured by its final results. Coordinated work of our international editorial board allows the readers to always be aware of the results of scientific researches, and theoretical and experimental studies performed in both Russian and foreign organizations, as well as the results of the authors' independent researches. It also allows bringing together the findings of different categories of researchers at academic and non-academic institutions, creating and supporting a common space of scientific communication, and bridging the gap between scientific publications of regional, federal and international levels.

Over the years, the international scientific journal *Foods and Raw Materials* has become a platform for researchers from different countries to share alternative solutions to the problems of the modern society. We hope that our journal will become your reliable guide in the scientific area and will help you to always be aware of the relevant research issues in food industry and related spheres. We are looking forward to welcoming you as our authors!

Yours sincerely,

Alexander Yu. Prosekov
RAS Professor, Doctor of Engineering
Editor-in-Chief of Foods and Raw Materials

FOOD PRODUCTION TECHNOLOGY

INFLUENCE OF PLASTICIZER CONTENT ON ORGANOLEPTIC, PHYSICO-CHEMICAL AND STRENGTH CHARACTERISTICS OF APPLE SAUCE-BASED EDIBLE FILM

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Abstract: Biodegradable edible films are developed as alternative packaging materials. Due to their unique chemical composition, apples can act as a raw material for the production of edible films. The aim of the work is to create an edible film from apple raw materials using the following plasticizers: agar, carrageenan or xanthan gum. Edible films have a yellowish color, characteristic for apple sauce. The film with the addition of xanthan gum as a plasticizer has the most acceptable flavor properties. Microscopic examination of edible film samples was carried out using a conventional and laser microscope. The structure of the edible film with an increase in the proportion of fiber becomes more homogeneous. IR spectra with a Fourier resolution of the analyzed edible film samples were obtained, which make it possible to isolate the presence of free hydroxyl groups. For edible films based on apple sauce with the addition of agar and xanthan gum as a plasticizer, there is a tendency to increase the tensile strength with increasing the amount of plasticizer (from 1.32 to 1.70 MPa for agar and from 1.68 to 3.50 MPa for xanthan gum). With a longer time of exposure to water and a higher water temperature, the edible film samples are destroyed.

Keywords: Edible film, apple sauce, agar, carrageenan, xanthan gum

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INTRODUCTION

Currently, packaging films are widely used to preserve perishable products such as meat, fish, confectionery, cheeses, pasta, fats, etc. Polyethylene films made from synthetic polymers are increasingly used for food packaging because of their low cost, ease of molding and high mechanical and barrier properties [1]. However, they do not decompose and do not recycled that causes serious environmental problems due to waste and the accumulation of environmental pollution [2]. Interest in environmental problems is increasing all over the world and it is especially relevant for the Russian Federation, because for a variety of reasons, 2017 has been declared a year of ecology in Russia. Biodegradable food films are developed as alternative packaging materials and are of great interest to many researchers. Over the past 10 years, the production of biodegradable films has increased tenfold. Research of scientists in this area also increased manyfold. Food films are known, made of polysaccharides, such as cellulose, chitosan and starch derivatives; proteins such as gelatin, corn zein, wheat gluten and soy protein; and lipids, such as paraffins, fatty acids and resins [3–7].

Currently, active research of the chemical composition and physical and chemical properties of apples is underway, as they are a widely consumed fruit, as well as a good source of insoluble (cellulose, xyloglucan) and soluble dietary fibers (mainly pectic polysaccharides) [8].

Apples have a number of advantages as a raw material for the production of edible films:
(1) wide raw material base within the Volga region and the whole territory of the Russian Federation;
(2) presence in the chemical composition of substances necessary for the quality of edible films: cellulose, hemicellulose, pectin, etc.;
(3) developed technologies of processing into various semi-finished products;
(4) ability to vary the chemical composition depending on the variety of apple;
(5) presence of special physical properties of semi-finished products from apples important for the production of edible films: fluidity, pumpability, moldability.

In addition, apples are an important source of phytonutrients with biological activity and health effects, such as vitamin C, potassium, dietary fiber. Due to these components, the content of saturated fats, cholesterol, and sodium in living systems decreases. Among medical research, the most relevant at the moment are studies on the effect of apple components on the development of

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cancerous diseases. In the study [9] of the effectiveness of reducing the occurrence of breast cancer cells by the example of apples of different varieties, important results were obtained: apples of Red Delicious and Golden Delicious varieties prevent the studied disease. Brazilian researchers conducted [10] studies in 2003 on 33 women over 45 years of age and found a correlation in the diet of apples, melons, tomatoes and the risk of breast cancer.

Due to their unique chemical composition, apples can act as a raw material for the production of edible films. As a plasticizer for apple raw materials, such food raw materials as agar, carrageenan and xanthan gum can be used.

Agar-agar (agar) is obtained from marine red algae of *Cracolaria, Gelidium, Ahnfeltia* species growing in the White Sea and the Pacific Ocean. Depending on the type of algae, the composition of the isolated polysaccharides can vary (mixture of agarose and agaropectin) [11]. The agar dissolves slightly in cold water and swells in it. In hot water, it forms a colloidal solution, which upon cooling gives a good, durable gel with a vitreous fracture. In agar, this process is carried out by the formation of double helices and their association, regardless of the content of cations, sugars or acids. Gel-forming ability of agar is 10 times higher than that of gelatin. When heated in the presence of acid, the gel-forming ability is reduced. Gels are stable at pH above 4.5 and thermo-reversible. Agar is used in the production of confectionery products (jelly marmalade, pastille, marshmallow), meat and fish jellies, various jellies and puddings, and also for clarifying juices. All of the above allows to recommend agar-agar as a component of edible films.

The term "carrageenans" refers to a group or family of sulfonated galactans extracted from red algae of *Rhodophyta* species by dilute alkali solutions, usually producing the sodium salt of carrageenan [12]. They are linear chains consisting of D-galactopyranosyl units linked by alternating (1→3)-α-*D*- и (1→4)-β-*D*-glycosidic bonds, most of the saccharide units having one or two sulfate half-ester groups are esterified at the C_2 or C_4 atom to hydroxyl groups. Galactcopyrase units often contain 3,6-anhydride rings. The content of sulfates in such compounds is 15–40%. In this case, the sulphate group giving the chain a negative charge at all pH values is ionized, so that the molecules do not precipitate at low pH values. In modern food products, the stabilizing and gelling properties of carrageenan are used. It is found not only in ham, sausages, poultry products, but also used to make the glaze of cakes and pastries. Carrageenan forms good elastic gels, which allows it to be used in edible films.

By chemical nature, xanthan gum is a heteropolysaccharide consisting of the residues of D-glucose, D-mannose and D-glucuronic acid, and is obtained by fermentation using the bacterium *Xanthomonas campestris*. Films of xanthan gum have the properties of pseudoplastics with rheological behavior in an aqueous medium. This property is favorable for films, since xanthan gum is easily dispersed in cold or hot water, but all this has little effect on its viscosity, regardless of temperature and acidity [13]. Films of gelatin saturated with xanthan gum are transparent with excellent resistance to ultraviolet light, low vapor permeability, improved mechanical properties and thermal stability [14]. Xanthan gum is characterized by formation of high-viscosity stable food systems and it also makes it possible to use it in edible films.

[15] describes the production of biodegradable films from agar-agar, carrageenan and hydroxypropylmethylcellulose and glycerol, which were used as a plasticizer. Various compositions from biodegradable films based on natural polysaccharides were analyzed for their rheological properties and physico-mechanical characteristics, as well as for preservation and ecotoxicity index. It was found that these polymer films are biodegradable and relatively bio-safe. Strength tests showed that the film compositions with carrageenan have a higher strength than polymers containing agar-agar only. Also, biopolymers containing carrageenan have an increased chemical resistance (prolonged time of dissolution in hydrochloric acid).

The use of hydrocolloids of vegetable origin such as, starch, pectin, carrageenan, and agar-agar is promising because of their structural and mechanical properties and micromorphological features [16]. It has been found that with respect to strength and suitability for use in films, the plasticizers can be arranged in ascending order as follows: starch, carrageenan, pectin, agar-agar.

Although edible films are not so widely known to the conventional consumer as synthetic polymeric films, however, a number of scientific papers in recent years have as their object of study the creation, formulation, production technology, the study of physical properties and mechanical strength for edible films, both on the basis of vegetable, and animal raw materials [17].

French scientists produced an edible film based on the hydrocolloid matrix of iota-carrageenan with the addition of glycerol and 30, 60, 90% glycerin monostearate by drying at 100, 150, 200°C [18]. The structure of the film was studied by electron microscopic examination. The data show that it was possible to obtain a two-layer film.

For biscuit and gel from agar, an intermediate edible film of acetylated monoglycerides is proposed [19]. The film was obtained by melting, sputtering or pouring. The larger the film thickness, the better its barrier properties.

In the literature, examples were found on the use of edible films with various plasticizers as packaging materials. For beef cutlets, several types of edible coatings were used from wheat gluten, soy protein, carrageenan, chitosan [20]. In cutlets, moisture loss, oxidation level (thiobarbit ratio, hexanal content) were monitored during storage for 3 days at 4°C. As a control, a polyvinyl chloride film was used. Based on the results obtained, the authors conclude that edible films do not protect against oxidation.

d-limonene and n-hexanal were encapsulated in an edible film of iota-carrageenan with or without lipids [21]. Such film samples were tested for wetting ability at temperature 25 and 35°C of medium composition (water and 0.9% NaCl solution). All the factors considered influence the level of retention of aromatic substances in the film.

Thus, the prospects for creating edible films are obvious.

The purpose of this work is to create an edible film from apple raw material using agar, carrageenan and xanthan gum as plasticizers, to compare the organoleptic, physico-chemical and mechanical characteristics, the water-absorbing capacity of the edible films, depending on the nature and amount of the plasticizer introduced.

OBJECTS AND METHODS OF STUDY

Experimental work was conducted at the Department of Technology and Organization of Public Catering of the Samara State Technical University.

The apples used in this study grow on the territory of the Samara Region, Russia (53 12' N and 50 06' E) of the 2016 crop. Apples of varieties Semerenko, Sinap and Antonovka were used. These varieties were chosen in terms of yield and chemical composition.

Chemicals and reagents. All plasticizers used have "chemically pure" degree of purity: agar, carrageenan, xanthan gum (Sigma Aldrich, Germany).

Preparation an edible film. Edible films from apple raw material using as a plasticizer xanthan gum, carrageenan and agar-agar were prepared as follows: apples were cleared from inedible parts, treated with water vapor, ground to a sauce state, the sauce was concentrated, then a plasticizer (2–4% by weight of apple sauce) was added to the resulting mass and a sheet of film was made by rolling. The resulting sheet of edible film with a thickness of 1–3 mm was dried at 55–70°C for 1–3 hours, and then cooled to room temperature.

Organoleptic analysis of edible film. The organoleptic analysis of edible films was carried out in accordance with GOST R ISO 8586. The analysis involved 14 people, whose average age was from 18 to 50 years old, trained according to GOST ISO 8586-1-2011. The general rules of organoleptic analysis corresponded to GOST ISO 11037–2013. For the analysis, samples of edible films 10x10 cm were taken. Color, taste, flavor, and consistence were used as the evaluated parameters for organoleptic analysis of edible films [22–25]. Table 1 shows the distribution of scores for different indicators. The results of the organoleptic evaluation were treated statistically.

Table 1. Organoleptic properties of edible films from apple raw material with the addition of plasticizers

Parameters	Test results	Number of points
Edible film using 2 agar		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Sourish apple	5
Consistence	Resilient	4
Chewing ability	Good	4
Edible film with 4 agar		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Apple	4
Consistence	Resilient elastic	5
Chewing ability	Medium	4
Edible film with 2 carrageenan		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Sourish apple	5
Consistence	Resilient	4
Chewing ability	Good	4
Edible film with 4 carrageenan		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Apple	5
Consistence	Resilient elastic	5
Chewing ability	Medium	4
Edible film with 2 xanthan gum		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Sourish apple	5
Consistence	Resilient	4
Chewing ability	Good	5
Edible film with 4 xanthan gum		
Color	Pale yellow	5
Flavor	Weak apple	5
Taste	Apple	5
Consistence	Resilient elastic	5
Chewing ability	Medium	4

Photographing samples of edible film. Photographs of edible film samples were made using a camera Sony Alpha ILCE–6000 Kit: film samples having a size of 5 × 5 cm were placed on a sheet of black velvet paper and photographed in daylight.

Microscopic examination of film samples. The film structure was studied using a 400x, Celestron biological laboratory microscope, and JEOL–6390A scanning microscope with a resolution of up to 3 nm and magnification of 30x, 100x and 500x; accelerating voltage 20 kV. A thin section of the edible film was placed on the center of the slide and covered with a cover glass on top.

IR spectroscopy with Fourier resolution. IR spectra were recorded on a Shimadzu IRAffinity-1 instrument in a thin layer with a Fourier transform.

Water-absorbing capacity. The effect of the introduced plasticizer on the water-absorbing capacity of the film is determined. The water-absorbing capacity was determined for all films by the method of Gialamas H. with amendments [20]. Samples of films were placed in distilled water and kept at 23°C for 30, 60, 90 minutes and at 90°C for 30, 60, 90 minutes in the TSO–1/80 thermostat to produce films with different moisture content. The degree of water absorption was determined as the ratio of the film weight after the experiment to the film weight before the experiment in percent. After three consecutive measurements, the average of the arithmetic mean was determined.

Film thickness. The film thickness was measured with a digital micrometer FIT 19909. Five measurements were made for each film: one in the center of the sample and in different parts of the perimeter. The average thickness value w calculated (Table 2).

Tensile tests. Tensile tests of film materials were carried out on a laboratory test complex including a tensile machine INSTRON-5988 (Testing Laboratory for Determining Mechanical Properties and Chemical Composition of Structural Materials, Samara State Technical University, research scientist A.E. Gorbunov) with the rate of application of the load in large ranges from 0.001 mm/min to 508 mm 0.001 mm/min. Samples with a width of 10 mm were tested at a distance between the clamps of 150 mm. Determination of deformation properties of materials with obtaining "load-displacement", "stress-displacement" dependence diagrams and mathematical processing of the results was carried out using the Bluehill 3 software (GOST R 53226-2008).

RESULTS AND DISCUSSION

According to the technology developed by us, six samples of edible film with different types and contents of plasticizers were made. For the prepared film samples, organoleptic characteristics, structure, strength properties and water-absorbing capacity were studied.

Photographs were obtained for all six film samples. Fig. 1 shows that all the films have a yellowish color characteristic for apple sauce. The color shades of the film differ slightly. The structure of all the film samples is dense. For a film with the addition of agar, a decrease in the number of inclusions of air bubbles with an increase in the amount of the additive is observed. The film with the addition of xanthan gum practically does not have inclusions of air bubbles. The surface of the films is smooth and shiny.

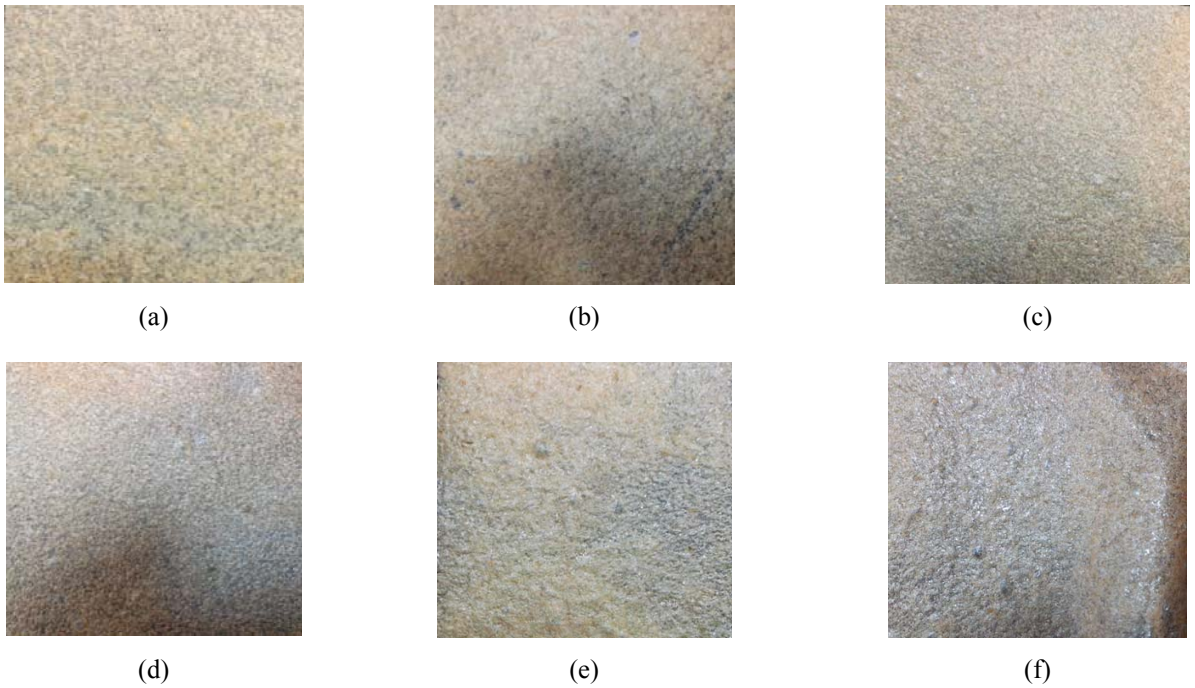


Fig. 1. Appearance of edible film from apple raw material with the addition of plasticizers: (a) 2% agar, (b) 4% agar, (c) 2% carrageenan, (d) 4% carrageenan, (e) 2% xanthan gum, (f) 4% xanthan gum.

For the conventional packaging of food products, appearance and flavor of the material are important. However, the edible film is a very specific type of packaging, for which, in addition to the appearance and flavor, taste and consistency of the edible film are important as well. Therefore, organoleptic testing in the case of edible film has a particularly important role. With all the positive properties, an edible film with poor organoleptic properties will not find its consumer. All films have a weak taste of apple sauce. All films have an attractive natural pale-yellow color. Films have apple flavor, corresponding to the natural flavor of apples. The taste of all films is associated with apples. They have a sourish pleasant taste. A film of xanthan gum has the most acceptable taste properties. All edible films have resilience and elasticity. This is especially pronounced in the presence of plasticizers in the film composition in an amount of 4%. However, with an increase in the amount of plasticizer for all types of films, the chewing ability of films decreases. In general, it should be noted that, despite the fact that all the edible films obtained have an attractive appearance associated with natural fruits – apples, samples of films with a lower content of plasticizers are more appealing in terms of taste and chewing ability. Increasing the proportion of plasticizer to 4% leads to the presence of a "rubber-like" structure. For edible films, criteria for organoleptic tests have been developed and the results are given in Table 1.

For packaging materials, structural properties are extremely important, since the structure largely determines the qualitative characteristics and physical properties. The structure of the film was studied using a biological laboratory microscope – 400x, Celestron and the results are shown in Fig. 2. The mechanical properties of the edible film are determined by the presence of a homogeneous structure. For an edible film made from apple sauce with the addition of agar and an increase in the proportion of plasticizer, the density of the structure increases and the number of air bubbles decreases (Fig. 2a, 2b). A similar dependence is observed for an apple sauce-based edible film with the addition of xanthan gum (Fig. 2e, 2f). Increasing the proportion of plasticizer, although somewhat degrades the eating qualities of edible films, but improves their structure. In this series of experiments, it can be stated that agar, carrageenan and xanthan gum exhibit stabilizing properties, making the film structure more homogeneous.

More detailed data on the structure of the film can be obtained by electron microscopic examination. Fig. 3–5 shows photographs of film samples made with the JEOL-6390A scanning electronic microscope. Studies carried out with the help of an electronic microscope make it possible to obtain more extensive data on the structure of edible films. Interestingly, edible films with carrageenan have a more ordered structure than other film samples studied.

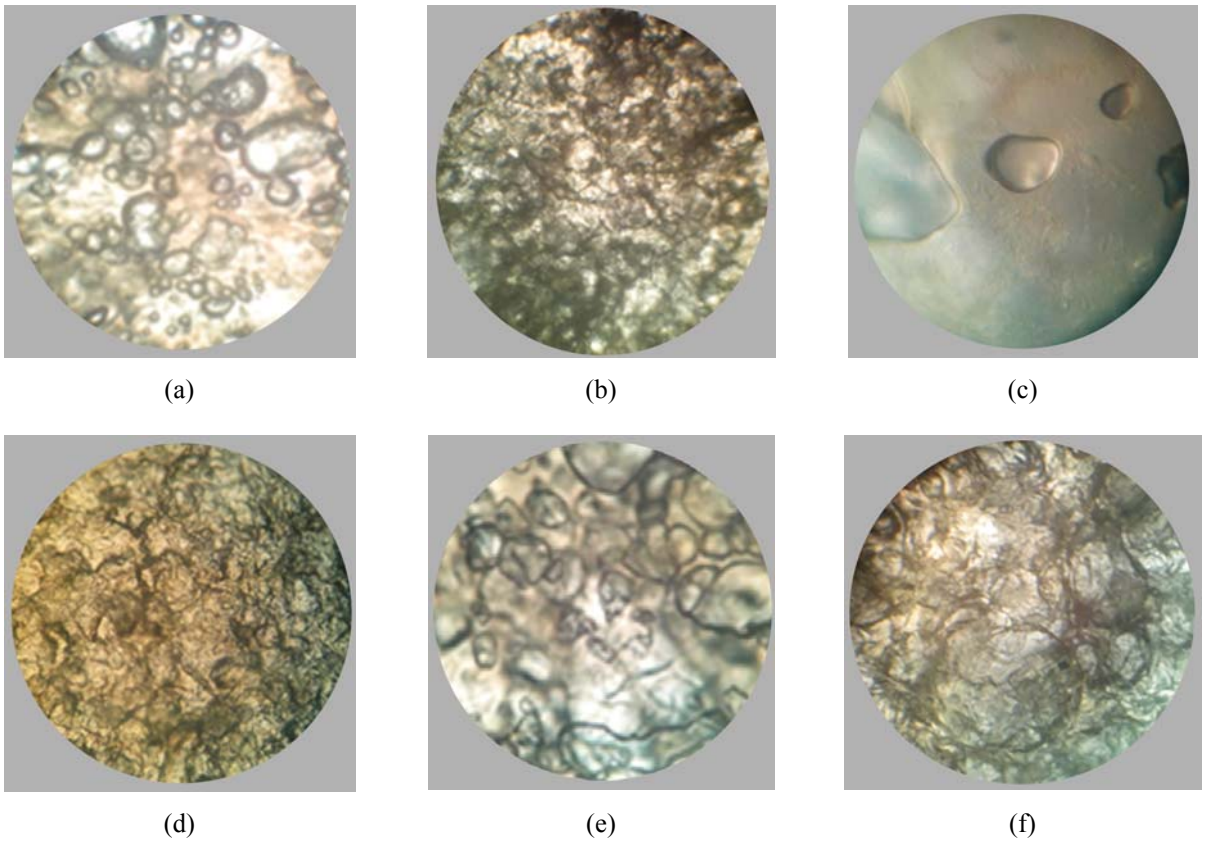
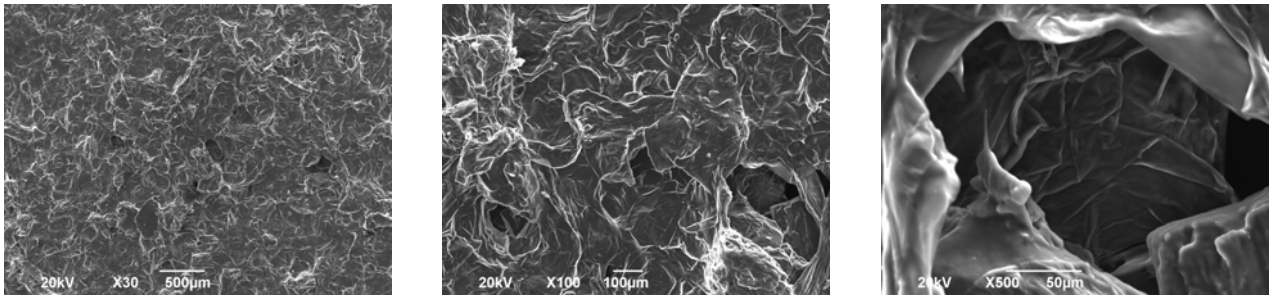
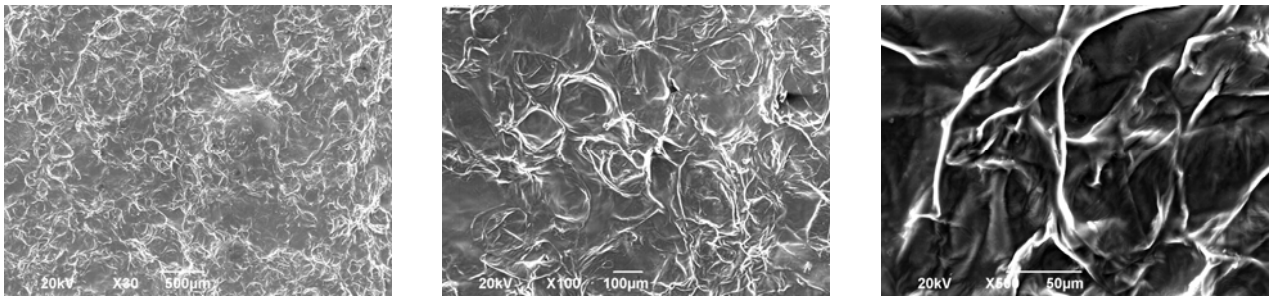


Fig. 2. Microscopic examination of edible films based on apple raw material with the addition of a plasticizer: (a) 2% agar, (b) 4% agar, (c) 2% carrageenan, (d) 4% carrageenan, (e) 2% xanthan gum, (f) 4% xanthan gum.



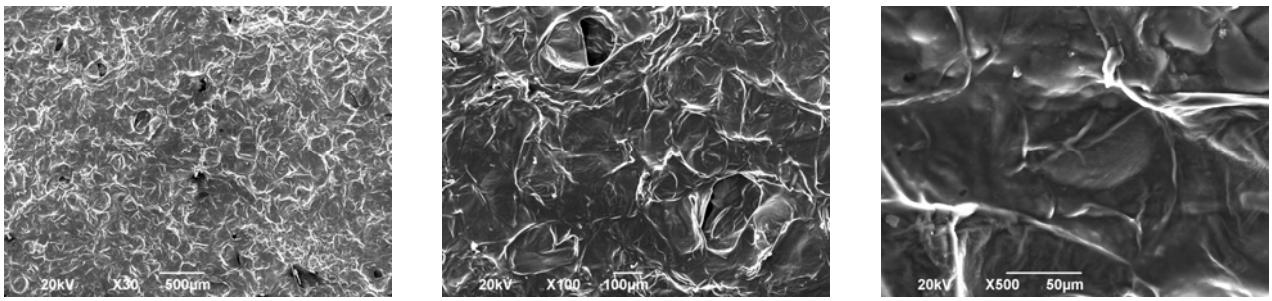
(a) (b) (c)

Fig. 3. Electron microscopic examination of edible films with the addition of 2% agar with magnification of: (a) 30x, (b) 100x, and (c) 500x.



(a) (b) (c)

Fig. 4. Electron microscopic examination of edible films with the addition of 2% carrageenan with magnification of: (a) 30x, (b) 100x, and (c) 500x.

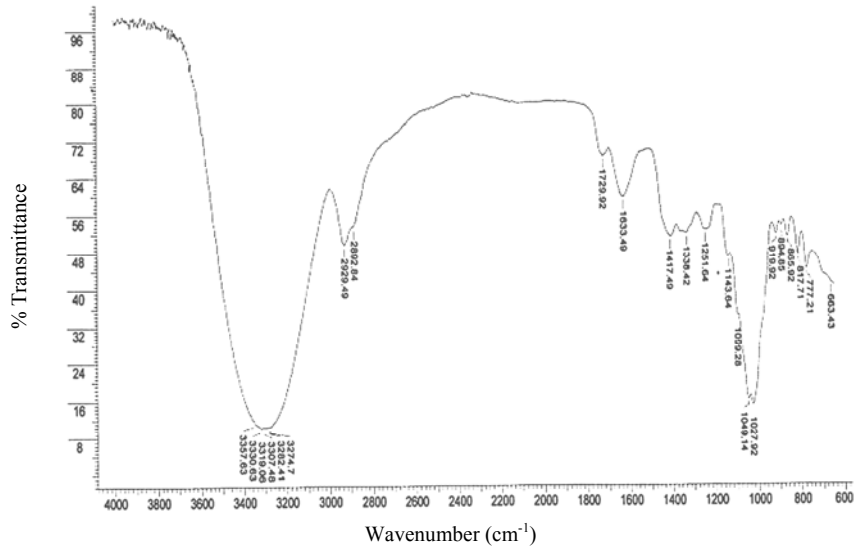


(a) (b) (c)

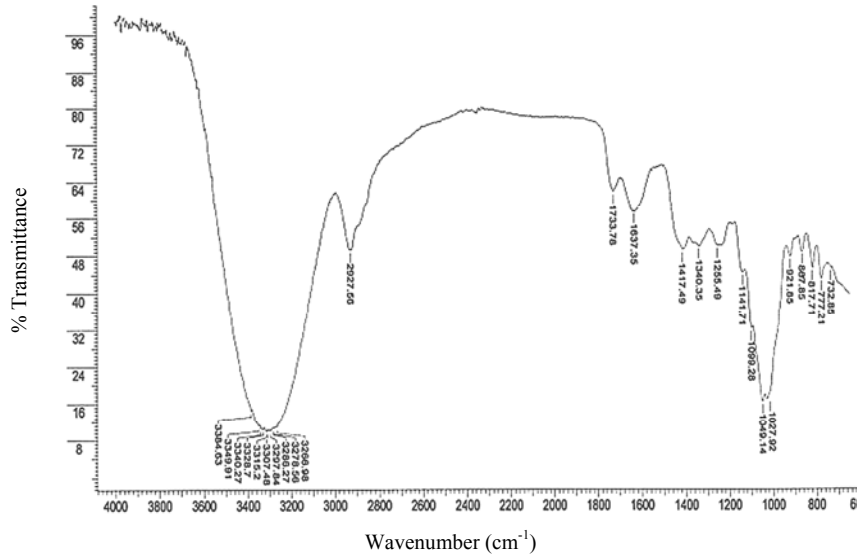
Fig. 5. Electron microscopic examination of edible films with the addition of 2% xanthan gum with magnification of: (a) 30x, (b) 100x, and (c) 500x.

IR-spectroscopy is a method that allows to characterize the chemical composition of the edible film. The IR spectrum of an apple sauce-based edible film with agar, carrageenan, xanthan gum as a plasticizer, is characterized by the presence of a wide absorption band in the region of 3274–3357 cm^{-1}, relating to the stretching vibrations of the hydroxyl group (υ_{OH}); deformation vibrations of the hydroxyl group are observed in the region of 1338 and 1417 cm^{-1} (Fig. 6). A weak peak of the valence vibrations of the carbonyl group in the region of 1729 cm^{-1} describes the presence of agar plasticizer in the film. In the IR spectrum of the apple sauce-based edible film with the addition of carrageenan, there is a broad band in the region of 3266–3384 cm^{-1}, which refer to the valence vibrations of the hydroxyl group (υ_{OH}). The band has a high intensity, because the group participates in the formation of intermolecular bonds. The deformation vibrations δ_{OH} of this group are detected by a peak of 1340, 1417 cm^{-1}. The plasticizer xanthan gum on the spectrum can be described by a weak signal of valence vibrations of the C=O group (1733 cm^{-1}).

Tests of the water-absorbing capacity of the edible film (Table 2) were carried out at two different temperatures: 23 and 40°C, and three time intervals: 30, 60 and 90 min.



(a)



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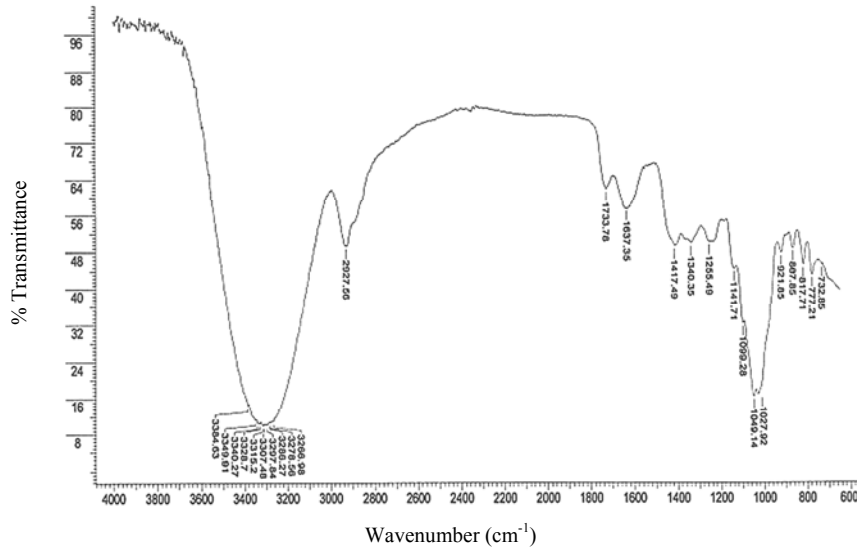

(c)

Fig. 6. IR spectra of edible film samples based on apple sauce with the addition of: (a) agar, (b) carrageenan, (c) xanthan gum.

Table 2. Water absorption capacity of edible films based on apple sauce with the addition of a plasticizer

Conditions (t, τ)	Water absorption capacity, %					
	Agar-agar, 2%	Agar-agar, 4%	Carrageenan, 2%	Carrageenan, 4%	Xanthan gum, 2%	Xanthan gum, 4%
23°C, 30 min	1084.21	895.24	351.43	402.04	894.59	984.38
23°C, 60 min	Sample is dissolved	Sample is dissolved	428.57	477.56	1108.11	1143.75
23°C, 90 min	Sample is dissolved	Sample is dissolved	511.43	473.47	Sample is dissolved	Sample is dissolved
40°C, 30 min	797.14	1016	Sample is dissolved	Sample is dissolved	962.50	826.00
40°C, 60 min	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved	1167.50	916.00
40ºC, 90 min	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved	Sample is dissolved

The characterization of the water absorption capacity is very important, since water absorption on one side characterizes the ability of the edible film to split in the human gastrointestinal tract, and on the other hand describes the barrier properties of the edible film with respect to water. For all films, the general trend is: a higher temperature (40°C) and a longer time of contact with water (60 and 90 min) promotes dissolution of a film. In terms of water solubility, edible films with the addition of plasticizers can be arranged in a row in descending order: films with agar> films with carrageenan> films with xanthan gum.

Strength characteristics of edible films determine primarily the ability of the film to act as a barrier to mechanical influences. But with respect to edible films, another aspect should be considered: The too high strength of the edible film causes a low chewing ability of the film. As for the edible film, one should find the most optimal strength with good chewing ability. The results of tests of thickness, strength characteristics of film edible materials are summarized in Table 3. For edible films based on apple sauce with the addition of agar and xanthan gum as a plasticizer, there is a tendency to increase the tensile strength with an increase in the amount of plasticizer, however, for a film with the addition of carrageenan, the value of the tensile strength varies in the same range, regardless of the amount of plasticizer. This may be due to the fact that the edible films of agar and xanthan gum with the increase of the plasticizing additive change the structure with a tendency to regularity, while the number of air bubbles decreases. Films become more homogeneous and well-ordered.

Undoubtedly, edible films with xanthan gum and carrageenan are more durable. At the same time, their results are more than 2 times higher than the results of edible films with agar. It should be noted that studying the structure of the edible film with a laser microscope shows that the structure of the film with carrageenan is more homogeneous. In this case, films with carrageenan and xanthan gum are more resistant to water absorption. If we summarize these data with the results of an organoleptic analysis of edible films, we can choose two types of films: with carrageenan and xanthan gum as the most preferred.

Thus, on the basis of the data obtained, one can state the effect of the type and amount of plasticizer added on the organoleptic and physico-chemical properties of apple sauce-based edible films. The most durable is a film with the addition of 4% xanthan gum.

The complex of studies of single-layer edible films performed during this work allows us to draw a number of conclusions:

(1) For edible films with addition of 2 and 4% of agar, carrageenan, xanthan gum plasticizers the film with a lower number of plasticizers and have more attractive organoleptic properties;

(2) Electron microscopic examination of edible films allows to choose carrageenan as a plasticizer with a high stabilizing ability;

(3) At room temperature films with carrageenan are more resistant to water, but when the temperature is raised to 40°C, edible films with xanthan gum can be distinguished as resistant to water for a longer period of time;

(4) The strength characteristics of the films also depend on the nature and content of the plasticizer. Films with carrageenan and xanthan gum are more durable;

(5) On the totality of the studied properties and characteristics, films with carrageenan and xanthan gum are promising.

Table 3. The thickness of the edible film based on apple raw material with the addition of plasticizers and its tensile test

Plasticizer added to film		Film thickness, mm	Tensile strength, MPa	Tensile strength load, N
Agar	2%	0.30	1.32	3.95
	4%	0.28	1.70	5.03
Carrageenan	2%	0.24	3.17	6.34
	4%	0.28	3.26	9.07
Xanthan gum	2%	0.31	1.68	5.04
	4%	0.33	3.50	10.48

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NUTRIENT ANALYSIS OF UNDERUTILIZED FISH SPECIES FOR THE PRODUCTION OF PROTEIN FOOD

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Abstract: The fractional composition of proteins of the cod-fish family – polar cod (*Boreogadus saida*) and blue whiting (*Micromesistius poutassou*) which are currently underutilized in the food industry has been studied. A method of multiple extraction of homogenized fish raw material with solutions of increasing ionic strength and pH was used for protein fractionation. Water-soluble (WSP), salt-soluble (SSP) and alkaline-soluble (ASP) fractions of fish proteins have been separated. The content of protein in a whole fish and its aqueous extracts was determined by the Kjeldahl method as well as photometric method. The amino acid composition of the proteins was studied by high-performance liquid chromatography. Protein of blue whiting comprises 92% of WSP and SSP fractions; while polar cod comprises only 60%. All proteins under study contain the full set of the amino acids including essential ones. The content of lysine both in non-fractionated proteins contained in a whole fish and their fractions is considerable (from 59.5 to 121 g kg^{-1}). When evaluating the biological value of proteins, their usefulness is established (there are no limiting amino acids). The data of the rationality coefficient Rc which presents the balance of the amino acid composition of the proteins indicate the high biological value of proteins of the whole polar cod and their ASP fraction. The fish species being studied can be used for production high-grade protein products – fish hydrolysates and isolates. The possibility of using unrefined fish is shown that is peculiarity of novel food technologies for obtaining protein products of high biological value.

Keywords: Amino acid balance, blue whiting, fractional and amino acid composition, polar cod, protein biological value

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INTRODUCTION

The analysis of a range of foods consumed in most countries has proved the growing phenomenon of a protein deficiency in products of animal origin [1, 2]. Currently, industrially processed hydrobionts are a source of high biological value protein that closely resembles that of animal origin.

Fish products play a major role in the rational food intake of millions of people worldwide. Therefore, the creation of new generation food products, based on marine hydrobionts having improved nutritional and biological value, is of great significance to accommodate the increasing demand for high-quality protein fish products. These types of foods, rich in essential components, are intended to replenish the amino acid and bioenergetic deficiencies of the organism.

The World Health Organization and Institute of Nutrition of the Russian Academy of Science have developed the main requirements for a balanced diet and its components [3–5]. When using marine bioresources, the principle task is to develop new technologies for separating organic components, which is linked with the production of new types of fish products that can meet specific biological, medical and technological standards [4–6].

Currently, the physicochemical characteristics and biochemical composition of the majority of traditional, commercially exploited marine species, including the Arctic bioresources, have been studied quite comprehensively. Yet, the decreased fishing volume of the most nutritionally valuable species in the Arctic region, and the changed structure of harvested fish have led to the real need to start using non-traditional and small-sized fish, such as the polar cod (*Boreogadus saida*) and the blue whiting (*Micromesistius poutassou*), for instance. Thus, an abundance of data shows significant cumulative average stocks of blue whiting and polar cod [6, 7]. This suggests the prospect of increasing the production volume of these species and a need to develop new fish processing technologies.

The existing processing methods can be broadly classified into two universal, technological schemes for obtaining non-specific protein. This approach is widely used in processing protein-containing raw materials [8, 9] and manufacturing fish products, including hydrolysates, isolates and concentrated protein [9]. However, development of innovative technologies to separate protein from the whole sum of chemical components of fish raw materials is of vital importance.

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At the research stage, it is important to fractionate the proteins. This protein isolation is based on the solubility principle of various types of proteins in water, salt and alkaline solutions. The necessity of such research is linked with the need to obtain data on the value and technological characteristics of the raw materials used for processing [10, 11, 12]. The qualitative and quantitative protein composition and the protein distribution patterns in the tissue of hydrobionts are important when choosing processing methods for raw materials with the aim of maximizing the output volume of the final products.

In this context, the use of the so-called low-value fishes as raw materials for processing is highly valuable. Recent research devoted to characterizing the chemical and biochemical composition of the abovementioned species exists. However, there has been done no in-depth research into the nutritional value of the blue whiting and polar cod, which are representatives of the cod family available in large quantities.

Therefore, this study aims to examine the protein composition of the raw material obtained from low-value fish species of the cod family, i.e. blue whiting and polar cod, for further utilization and to develop an innovative hydrobiont processing technology.

OBJECTS AND METHODS OF STUDY

Food Samples Analyzed. Both blue whiting (*M. poutassou*) (whole fish) and polar cod (*B. saida*) (whole fish) were analyzed. The blue whiting was caught by the Murmansk Trawl Fleet JSC in the central and eastern part of the Atlantic in spring (March) and in autumn (October) of 2015. The polar cod was caught by the same company in the Barents sea at the same time. Atlantic cod (*Gadus morhua*) fillets (boneless without skin) were used as a reference sample for comparison. The Atlantic cod was caught by the Murmansk Trawl Fleet JSC in the fishing parts of the Atlantic in April 2015. All the fish were frozen and delivered to the port of Murmansk (Russia). Fish was frozen in vertical quick-freezing plate devices VPF-10 produced by "Factory of cold" LTD (Russia), and then it was stored during 1 month at the temperature not higher than minus 18ºC.

Chemicals and Standards. All chemical reagents used were either reagent or analytical grade. All chemical reagents were purchased from “Petersburg’s Red Chemist” LTD (Russia). Standard amino acids for chromatography and crystalline bovine serum albumin for spectrometry were purchased from Sigma-Aldrich (Germany).

Protein Extraction. Fish protein was fractionated by multiple extractions of homogenous fish raw material with solutions of increasing ionic strength and pH [18]. This method is based on the solubility of the various proteins in salt and alkaline solutions.

The frozen samples (see section Food Samples Analyzed) were defrosted with air at 18°C and cut into small pieces. The pieces were homogenized (Ase homogenizer, Nihonseiki Kaisha Ltd, Japan) at 5000 rpm for 5 min. Three protein fractions, i.e. water-soluble proteins (WSP), salt-soluble proteins (SSP) and alkali-soluble proteins (ASP) were extracted. The extraction process is shown in Fig. 1.

The WSP were extracted by soaking 1 g of homogeneous sample in 10 mL of 0.03 M KCl solution. The prepared solution was kept at 1–5°C for 2 h, then centrifuged at 4000 rpm for 20 min, and the solution was separated from the residue by decantation. The residue was 5 times extracted with 0.03 M KCl solutions (1 : 5 v/v) for 1 h, respectively. The supernatant (WSP) and residue were kept for further processing. So, 6 subfractions of WSP were extracted.

The SSP was obtained by soaking the residue left from extracting the WSP with Weber’s solution (a mixture of 0.60 M KCl, 0.0010 M Na_2CO_3 and 0.040 M $NaHCO_3$) at a 1 : 10 v/v ratio, for 20 h. Subsequently, the liquid part was decanted, and the residue was extracted with 0.6 M KCl solution, then with Weber’s solution and finally with 0.6 M KCl solution for 1 h at 1 : 5 v/v, and 1 h infusion time, respectively. The supernatant (SSP) and residue were kept for further processing. This method made it possible to extract 5 subfractions of SSP.

The ASP were obtained by extracting the residue remaining from the isolated SSP with 0.1 M NaOH solution (1 : 10 v/v) twice, for 2 and 20 h, respectively. Then, the whole procedure was repeated 4 times at 1 : 5 v/v and 1 h infusion time, respectively. The protein was extracted by centrifugation at 6000 rpm for 30 min in 0.1 M NaOH solution. This procedure helped to extraction of 6 subfractions of proteins soluble in 0.1 M NaOH. The remaining residue was soaked in 1 M NaOH solution (1 : 10 v/v) at 65°C for 30 min. The supernatant (ASP) and residue were kept for further processing.

Protein Determination. The protein content of the original blue whiting and polar cod (see section Food Samples Analyzed) samples was defined according to the total nitrogen content (TN, g kg^{-1}) determined using Kjeldahl apparatus (JP Selecta, Spain) which contains of two modules: the BLOCK–DIGEST–12 for protein mineralization, and the PRO–NITRO A for ammonia distillation. The amount of protein (P, g kg^{-1}) was calculated by multiplying the TN by a conversion factor (usually of 6.25 for fish protein).

The protein content of the polar cod and blue whiting extracts from fractioning (see section Protein Extraction) was determined by Lowry’s method based on the reaction of the proteins with the copper (II) salts in the alkaline solution followed by reduction using phosphomolybdenum-tungsten reagent (Folin’s reagent). These reactions results in production of colored substances which can be determined by measurement at 750 nm using a T70 UV/visible spectrometer (PG Instruments, UK). Bovine serum albumin (0.05–0.80 mg/mL) was used to establish a calibration curve [13, 14].

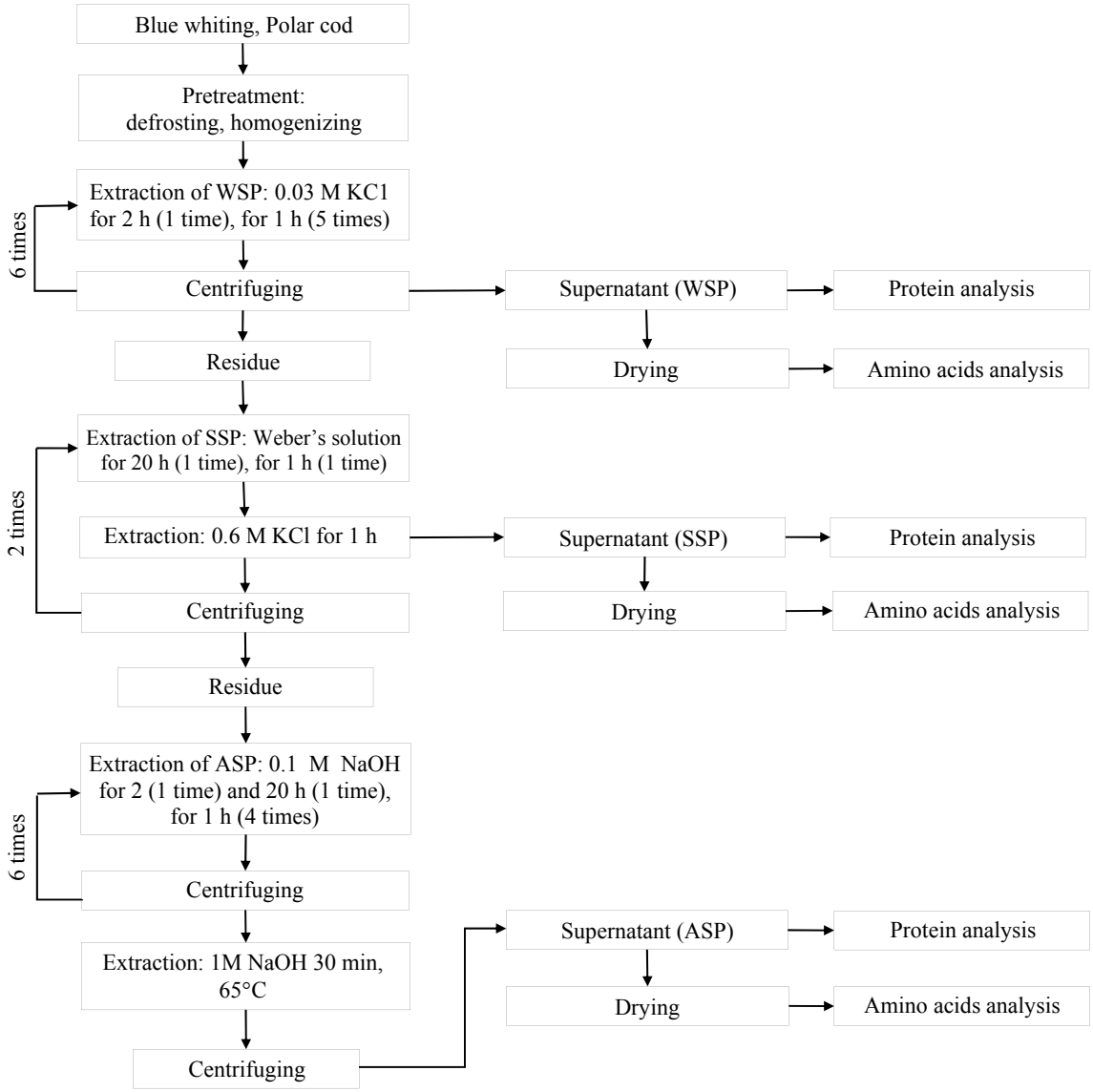

Fig. 1. Flow chart for the extraction of WSP, SSP and ASP from polar cod and blue whiting.

Amino Acid Determination. Samples (10 g) of homogenized whole fish (polar cod, blue whiting) and cod fillet (see section Food Samples Analyzed) were combined with 96% (v/v) ethyl alcohol (1 : 5 w/w). The protein precipitate was degreased with diethyl ether and air-dried. Extracts of protein fractions were freeze and vacuum-dried using a freeze-dryer LSM-07 (Russia) at 45°C. The samples (20 mg) were then hydrolyzed with 4 mL 6 M HCl in glass tubes at 120°C for 24 h under vacuum. The hydrolysates were dried by a vacuum concentrator PE-8920 (Ecros, Russia) at 60°C and then dissolved in citrate buffer (pH 2.2). The amino content was measured [15] by an automatic amino acid analyzer (AAA-88, Ingos Laboratory Instruments, Czech Republic).

The tryptophan content was determined by hydrolyzing the samples in 2% (w/v) KOH at 80°C for 30 min, followed by photometric assay with *p*-dimethylamine benzaldehyde and measurement at 650 nm using a photoelectric colorimeter. Tryptophan (0.02–1.00 mg/mL) was used to establish a calibration curve [16].

Protein Biological Value Determination. The biological value of the proteins and protein fractions was defined by their amino acid contents. The obtained data was compared with the reference protein, etalon, which fully complies with a well-balanced amino acid protein (the reference protein).

Etalon (ideal) protein according to World Health Organization is a hypothetical protein which can completely satisfy the human requirement in all the essential amino acids.

The amino acid score of the i-amino acid (AAS_i, %) was calculated as a ratio of the essential amino acids in the examined protein (A_i, g kg^{-1}) to its content in the reference protein (A_{ie}, g kg^{-1}), according to the following equation (1):

$$\mathrm{AAS}_i = \frac{A_i}{A_{\mathrm{ie}}} \cdot 100\% \cdot \quad (1)$$

The biological value (E/N_e) was calculated as the ratio of the sum of essential amino acids ($\sum A_{\text{essential}}$, g kg^{-1}) to the sum of the non-essential amino acids ($\sum A_{\text{non-essential}}$, g kg^{-1}) in the protein, as follows:

$$\frac{E}{N_e} = \frac{\sum A_{\text{essential}}}{\sum A_{\text{non-essential}}}. \quad (2)$$

The rationality coefficient (R_c), which characterizes essential amino acids against a reference protein, was calculated as follows:

$$R_c = \frac{\sum A_i K_i}{\sum A_i}, \quad (3)$$

$$K_i = \frac{\text{AAS}_{\text{min}}}{\text{AAS}_i}, \quad (4)$$

where AAS_{min} (%) is a minimum amino acid score, and K_i is the utility factor of i-amino acid.

Statistics. Experiments were done in triplicate. The data were analyzed by one-way analysis of variance (ANOVA) using Origin Pro 8.0. Differences among means were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Basic chemical composition of the whole and gutted fish. Chemical composition of the fish is not constant; it depends on its feeding, and it is vary along the year. The feeding intensity of the polar cod and the blue whiting is usually minimal in spring (during spawning period), but it is maximal in autumn (during interspawning period). Table 1 shows the results of researches of the basic chemical compounds in the spring and autumn polar cod and blue whiting (both whole fish and fillets).

The fish caught in spring was used for the further researches.

Protein content of whole, cutted fish and waste. Table 2 presents data on the protein content in the whole fish, fish fillets, and fish waste (heads, fins, viscera, bones). The protein content in the whole fish is from 12 to 14% for the polar cod, and from 16 to 19% for blue whiting. The protein content in the blue whiting and polar cod fillets is somewhat higher (15–17%) than in the whole fish, but even the wastes from fish gutting is significant (from 10 to 16%), which is comparable to the whole fish. Thus, the fish waste produced during cutting, as well as the fillets, is a good source of proteins, and they can be used for extracting water- and salt-soluble proteins.

The analysis of the data presented in the Table 2 shows that a significant part of proteinous raw material can be lost during fish cutting. In case of polar cod cutting the losses are about 64% (of mass), in case of blue whiting they about 41% (of mass). These numbers theoretically correspond the losses of 55 and 37% of proteins contained in the whole fish. The obtained results showed the rationality of processing these fish without cutting which can use the maximal amount of proteins contained in fish for the finished product (hydrolysate or isolate).

Fractional composition of proteins and amino acids content. Table 3 presents the protein fractionation results obtained from multiple extractions (Fig. 1) of polar cod and blue whiting.

Table 1. Basic chemical content in spring and autumn polar cod and blue whiting (whole fish and fillets)

Fish	Sample	Season	Chemical composition			
			Water	Protein	Fat	Ash
Polar cod	Whole	March	82.5	12.0	2.5	2.6
		October	79.0	13.8	4.1	2.8
	Fillets	March	82.7	15.0	0.7	1.3
		October	81.3	15.8	1.3	1.4
Blue whiting	Whole	March	77.7	16.5	2.7	3.0
		October	76.0	16.9	4.2	3.1
	Fillets	March	80.0	17.5	1.0	1.4
		October	78.8	18.5	0.9	1.5

Table 2. The protein content of the samples of whole fish, fish fillets and fish waste from cutting

Fish	Sample	Protein content, %	Proportion from the whole fish, %	Protein proportion of the whole fish protein, %
Polar cod	Whole fish	11	100	100
	– Fillets	14	36	44
	– Waste from cutting (heads, fins, viscera, bones)	9	64	56
Blue whiting	Whole fish	13	100	100
	– Fillets	13	59	59
	– Waste from cutting (heads, fins, viscera, bones)	13	41	41

Table 3. Content of WSP, SSP, ASP (g kg^{-1}) in polar cod and blue whiting (whole and fillets)

Protein fraction	Subfraction	Polar cod		Blue whiting	
		Whole	Fillets	Whole	Fillets
WSP	1	36.8	22.8	34.0	23.0
	2	5.3	3.5	9.1	3.9
	3	2.4	1.4	4.0	1.5
	4	1.4	1.3	3.9	1.3
	5	1.2	1.2	3.3	1.0
	6	0.9	0.1	2.9	0.9
	Sum	48.0	31.2	57.2	31.6
SSP	1	9.6	10.0	23.0	13.7
	2	2.4	2.4	10.7	2.2
	3	2.0	2.1	10.2	2.1
	4	1.5	1.8	7.1	1.6
	5	0.9	1.3	0.7	1.1
	Sum	16.4	17.6	51.7	20.7
ASP (in 0.1 M NaOH)	1	22.4	25.1	4.3	42.8
	2	14.1	13.9	2.4	16.0
	3	2.6	5.2	1.0	10.5
	4	1.5	3.1	0.8	5.6
	5	1.3	2.1	0.6	2.1
	6	0.8	1.6	0.6	0.7
ASP (in 1.0 M NaOH)	1	1.4	3.6	1.0	0.0
ASP (total)	Sum	44.1	54.6	10.7	77.7
Total		108.5	103.4	119.6	130.0

The content of the WSP, SSP and ASP in the whole polar cod and its muscle tissue (fillet) is almost identical (48 and 31 g kg^{-1} of WSP, 16 and 18 g kg^{-1} of SSP, 44-55 g rg^{-1} of ASP), while blue whiting proteins are somewhat different: WSP and SSP in the muscles are almost twice less (57 and 32 g kg^{-1} of WSP, 52 and 21 g kg^{-1} of SSP), but ASP is almost 7 times higher. Analysis of Table 3 shows that the amount of WSP, SSP and ASP yields from the blue whiting were 57, 52 and 11 g kg^{-1}, while the corresponding values from the polar cod were 48, 16 and 44 g kg^{-1}, respectively. Therefore, WSP and SSP comprise 92 and 60% of the blue whiting and polar cod protein, respectively. The blue whiting fillets (muscle tissue) contain more ASP (60% of total), while the polar cod fillets contain almost equal of ASP and sum of WSP and WSP (52 and 47% correspondently).

The amino acid content of the blue whiting and polar cod (whole fishes) protein is shown in Table 4, which also provides the WSP, SSP and ASP amino acid compositions. The research of amino acid composition of ASP of the blue whiting has not been carried because of the low content of the ASP. Data on the sample of Atlantic cod fillet are also included in Table 4 for comparison. Atlantic cod muscle tissue contains about 20% of protein and less than 0.5% of fat, so Atlantic cod is considered a reference fish raw material of high protein content.

All the examined proteins in Table 4 are characterized according to the full range of amino acids, including essential acids. Almost all the samples contained glutamic acid, whose content ranged from 123 to 174 g kg^{-1}. The blue whiting SSP, with the highest content of asparaginic acid at 393 g kg^{-1} but almost complete absence of pyrrolidine carboxylic acid, was an exception. Tryptophan had the lowest content in all the samples, and it ranged from 3.6 to 15.0 g kg^{-1}. A notable content of lysine, which is an essential amino acid, was found in WSP, SSP and ASP of blue whiting and polar cod (from 59.5 to 121 g kg^{-1}). Thus, proteins extracted from polar cod and blue whiting can be recommended as a food supplement to enrich protein foods low in lysine, for example, vegetable food.

The data analysis confirmed that the amino acid content of the proteins from polar cod and blue whiting compare favorably with the amino acids in Atlantic cod fillet proteins, particularly the blue whiting protein samples.

Biological value of underutilized fish species. Table 5 lists the amino acid scores (AAS_i), which characterizes the protein quality of polar cod and blue whiting (whole fishes, as well as their WSP, SSP and ASP fractions). The data analysis revealed that polar cod and blue whiting proteins do not contain limiting amino acids. Therefore, they are complete. However, the polar cod and blue whiting WSP, and blue whiting SSP contained the limiting amino acid, histidine, while blue whiting SSP contained three limiting amino acids, i.e. isoleucine, threonine and methionine.

Fig. 2 reveals the blue whiting had the highest biological value (E/N_e). Among the fractions, the WSP of polar cod and blue whiting had the highest biological value.

It is possible to evaluate biological value of the protein by the sum of essential amino acids; Fig. 3 contains these results.

Table 4. Amino acids content (g kg^{-1})in whole proteins, WSP, SSP, ASP of polar cod and blue whiting, in proteins of Atlantic cod fillet, in etalon protein

Amino acid	Polar cod				Blue whiting			Etalon protein [3]	Atlantic cod fillets
	Whole	Fraction			Whole	Fraction			
		WSP	SSP	ASP		WSP	SSP		
Tryptophan	8.3	8.4	7.0	8.7	6.2	15.0	6.6	6.0	6.9
Lysine	100.0	121.0	117.0	109.0	139.0	128.0	59.5	45.0	120.0
Histidine	34.5	12.0	9.2	33.6	40.1	11.7	42.2	15.0	67.7
Arginine	77.6	90.2	86.6	76.2	72.8	74.6	27.9	–	20.6
Asparagine	102.0	95.1	112.0	91.4	83.3	112.0	393.0	–	106.0
Threonine	30.1	47.2	50.7	48.8	35.9	44.9	16.0	23.0	45.6
Serine	43.7	17.3	16.4	13.5	37.3	15.8	19.8	–	45.0
Glutamic acid	175.0	123.0	154.0	150.0	171.0	135.0	81.4	–	154.0
Proline	45.7	37.7	33.3	42.3	34.6	39.7	traces	–	45.3
Glycine	70.9	42.2	42.0	40.4	48.2	46.6	32.3	–	48.6
Alanine	63.2	81.4	68.7	68.3	58.2	75.9	41.9	–	60.2
Valine	45.9	64.9	52.5	61.0	46.7	53.8	86.8	39.0	51.2
Methionine + Cysteine	23.6	23.7	30.8	30.3	29.5	28.8	16.8	22.0	34.9
Isoleucine	37.0	55.8	47.9	50.2	39.3	49.8	17.2	30.0	47.4
Leucine	76.4	94.6	92.0	102.4	82.8	85.7	90.4	59.0	82.9
Tyrosine	29.3	39.2	47.6	42.0	35.9	38.0	60.2	38.0	36.0
Phenylalanine	37.6	54.4	38.7	40.0	38.8	59.8	14.9		39.7

The rationality coefficient (R_c) considers both deficit and excess essential amino acids in the protein. Therefore, it can be assumed that this parameter provides the most objective characteristic of the protein completeness based on the amino acid composition of the protein and thus, its biological value. The R_c for the whole blue whiting proteins in their entirety and their ASP was the highest, confirming its high biological value (Fig. 4). The R_c value for blue whiting was 18 % superior to that of Atlantic cod fillet. Blue whiting (whole fish) proteins and cod fillet proteins were equally balanced.

Table 5. AASi (%) for the whole proteins and WSP, SSP, ASP of polar cod and blue whiting

Amino acid	Polar cod				Blue whiting		
	Whole	Fraction			Whole	Fraction	
		WSP	SSP	ASP		WSP	SSP
Tryptophan	138	139	117	145	104	250	110
Lysine	223	269	213	243	309	284	132
Threonine	131	205	260	212	156	195	70
Valine	118	166	134	156	120	138	223
Methionine + Cysteine	107	108	138	138	134	131	76
Isoleucine	123	186	160	167	131	166	57
Leucine	129	160	156	174	140	145	153
Tyrosine + Phenylalanine	176	246	227	216	196	258	198
Histidine	230	80	61	224	267	78	282

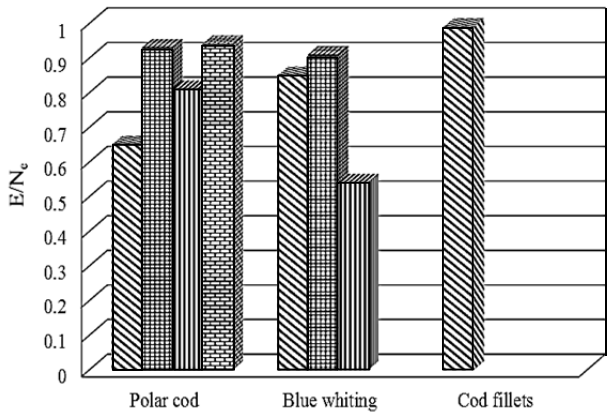

Fig. 2. Essential-to-non-essential ratio (*E/Ne*) for WSP, SSP and ASP from polar cod and blue whiting; and protein from Atlantic cod fillet.

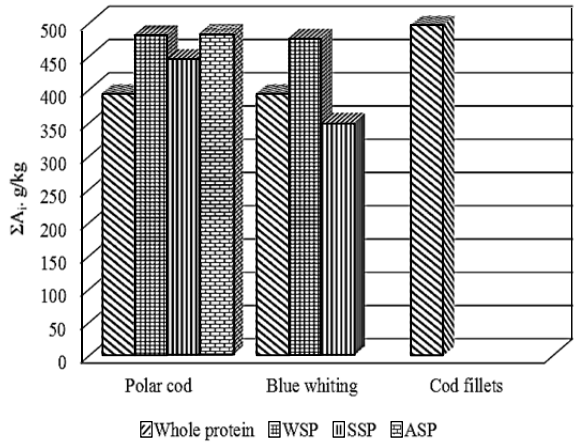

Fig. 3. Summary content of essential amino acids (ΣAi) for WSP, SSP and ASP from polar cod and blue whiting; and protein from Atlantic cod fillet.

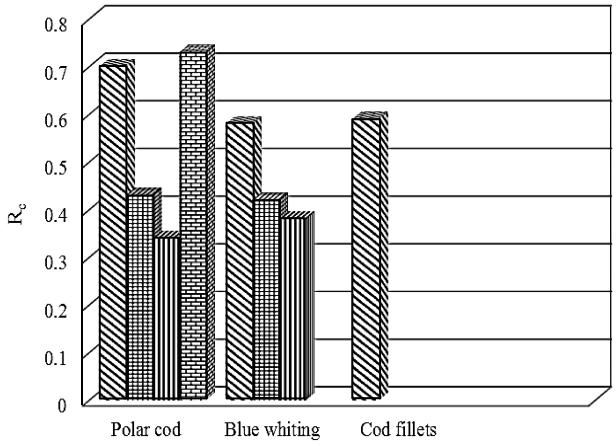

Fig. 4. Rationality coefficient (R_c) for amino acids content of whole protein for WSP, SSP and ASP from polar cod and blue whiting; and protein from Atlantic cod fillet.

The rationality coefficient (R_c) considers both deficit and excess essential amino acids in the protein. Therefore, it can be assumed that this parameter provides the most objective characteristic of the protein completeness based on the amino acid composition of the protein and thus, its biological value. The R_c for the whole blue whiting proteins in their entirety and their ASP was the highest, confirming its high biological value (Fig. 4). The R_c value for blue whiting was 18% superior to that of Atlantic cod fillet. Blue whiting (whole fish) proteins and cod fillet proteins were equally balanced.

When the proteins of all the examined samples were graded according to their biological value i.e. increasing value of R_c, the following order was observed: polar cod SSP < blue whiting SSP < blue whiting WSP < polar cod WSP < whole blue whiting protein < Atlantic cod fillet < whole polar cod proteins < polar cod ASP.

Conclusions of using the raw material for protein hydrolysate and isolate in food production. The fraction composition of the proteins of whole and gutted (for fillets) polar cod and blue whiting has been researched for the first time. The obtained data shows that gutting the polar cod and the blue whiting results in losses of almost a half of the proteins from the whole fish, so it is reasonable to use whole fish while producing hydrolysates and isolates for maximal usage of the protein component. The protein content of the whole fish and its muscle tissue is almost independent from the fishing season, but the fat content of the whole fish is almost twice higher in autumn than in spring. It is found that the whole fish - blue whiting and polar cod – contains the full-grade proteins with the high biological value. So, the explored raw material can (and must) be possible to be used for obtaining such valuable products as hydrolysates and isolates. Fish protein hydrolysates contain from peptides and amino acids. Fish protein isolates contain more than 75% of protein in its native form.

The obtained research data makes it possible to recommend all basic technological methods of processing of small fishes during producing fish hydrolysates and isolates. It is recommended to use the whole fish from spring fishing season for producing proteinous products (hydrolysates and isolates).

It is also recommended to hydrolyze minced whole fish during producing hydrolysates. As for isolates (especially actual for the polar cod because of the high content of ASP), it is better to chop the whole fish, and then to carry out the alkaline treatment of the mince. Thus the alkaline extraction makes it possible to get the whole complex of proteins without substantial losses.

It should be said in the conclusion that it is most rational to use blue whiting and polar cod without gutting to reduce energy and labor costs during such operation as "gutting". Moreover, it will increase the yield of fish hydrolysates and isolates.

Official statistical data of the catch of the blue whiting and the polar cod shows that these fishes have a significant proportion in the total catches. They are not inferior to traditional fishing objects in their protein content. But small sizes and some other disadvantages (for example, high infection with nematodes of the blue whiting) require preliminary gutting using traditional technologies. The possibility to use undressed fish is a feature of the new technologies of producing proteinous products with enhanced biological value.

ABBREVIATIONS

AAS_i – amino acid score; WSP – water soluble proteins; SSP – salt soluble proteins; ASP – alkali-soluble proteins; TN – Total Nitrogen; P – protein.

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PRODUCTION OF VEGETABLE "MILK" FROM OIL CAKES USING ULTRASONIC CAVITATION

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Abstract: The combination of dairy and vegetable raw materials is considered a technological way of mutual enrichment that allows to optimize the composition and content of fatty and amino acids to a certain extent. The ultrasonic effect contributes to an increase the colloidal stability of products of an emulsion and suspended nature based on vegetable and dairy raw materials. The aim of this work was the study of the process of obtaining protein-containing beverages, vegetable-based in conditions of ultrasonic influence. The main object of the study selected the flour from oilcake of nuts of *Pinus sibirica Du Tour* (cedar flour). The beverages were obtained using two methods: 1) by scalding cedar flour with the drinking water heated to a temperature of 70°C; 2) by gradual bringing a model mixture of cedar flour with drinking water to the boil. The mass ratios of cedar flour and drinking water were used in the study at 10 : 90 (10% of cedar flour), 10 : 80 (11.1%), 10 : 70 (12.5%), 10 : 60 (14.3%), 10 : 50 (16.7%) and 10 : 40 (20.0%). The model drinks were processed in a cavitation mode (20 W/cm^2) of the "Volna" apparatus at the frequency of ultrasonic vibrations of 22 ± 1.65 kHz; the processing time was 2 and 5 minutes. The effectiveness of ultrasound exposure was estimated by the content of dry matters, protein and fat, of size of fat droplets and the colloidal stability of the resulting emulsion. The application of an ultrasonic field provides an increase in the degree of transition of soluble dry matters of cedar flour to the water phase up to two times, including fat ten times and soluble proteins up to two times. As a result, the drops of Cedar oil, extracted from the Cedar flour are dispersed effectively, resulting in enhanced stability obtained emulsions. The dosage cedar flour within of 14–16.7% under scalding conditions followed by an ultrasound cavitation for 2 min, can be considered as optimal conditions for producing beverages. The analysis of the resulting beverage (2.6–3.4% of protein and 2.8–3.3% of fat) shows its comparability with cow milk.

Keywords: The combination of raw materials, cedar flour, vegetable milk, ultrasonic cavitation, dissolution efficiency, the nutritional value of beverages, the colloidal stability of beverages.

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INTRODUCTION

The interest in the topic of combined products and beverages has grown in recent years [1–8]. The expediency of such products and beverages is due, first of all, to the possibility of regulating their chemical composition in accordance with the modern requirements of the nutrition science. Under these conditions, the introduction of plant raw materials into the formulation is considered as a partial replacement of animal proteins and lipids, which allows to increase the content and optimize, to some extent, the composition of fatty and amino acids in a new product.

Some types of oil cake, in particular, the oil cake of pine nut kernels can also be considered as the promising food raw materials for the production of combined products [9]. However, under normal conditions, milk protein and polysaccharides of vegetable raw materials are limitedly compatible, which leads to the formation of a sediment or liquid-phase separation, depending on the specificity of the concentration of molecular components [10]. The latter, as is known, is determined by the component composition of raw materials that directly determines the functional and technological properties of polycomponent food systems and substantially distinguishes them from the properties of purified vegetable proteins.

It is well known that the structuring and dispergating of protein and other components in food colloidal systems is determined by a number of physicochemical factors:

– the size of a particle of the processed object (from animal and vegetable raw materials);

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– the ratio of the basic nutrients in the system - proteins, fats and carbohydrates, and their properties;
– the intensity of the physico-chemical effect on the system (temperature, pH, a dispergating method - in the ultrasonic mode, in a pulsing device and others). The first two factors are determined by the raw materials used and initially form the ability of the particles of processed raw materials to form a relatively stable food colloidal system - emulsion, suspension, foam, paste and so on.

The most promising way of processing food environments that allows to give stability to a colloidal system and accelerate the dissolution and structuring processes without the use of specialized food supplements is ultrasonic cavitation.

Most often, the main purpose of applying ultrasound to various environments in the food industry is to increase the extraction efficiency of soluble dietary fibers [11–13] and a variety of biologically active substances of vegetable raw materials - polyphenolic compounds [14–20], carotenoids [21, 22] and some other components that are specific for certain types of raw materials [23], or the total recovery of such components [24]. Extraction under ultrasonic conditions is one of the most environmentally friendly methods of intensifying the release of biologically active substances [25, 26]. This promotes studies on the use of ultrasound to produce finished forms of food [27–29], especially in the dairy industry, for the homogenization of milk and the dairy products derived from dried milk raw materials [30].

In Russia and the CIS countries, the main areas of applied studies in the field of ultrasound in food industries are the production of soft drinks and their semi-finished products [31, 32], the instantization of dry dairy products [33–34] and the homogenization of emulsion and paste-like meat, vegetable and dairy products [35–38]. In the dairy industry, the roleof a factor contributing to the homogenization of fat, a decrease in viscosity and an increase in the emulsion stability of reconstituted milk and serum is assigned to ultrasonic cavitation [39].

In general, the effectiveness of ultrasound in a cavitation mode is proven with respect to the homogenization and structuring of both vegetable and animal food masses (including beverages). Therefore, it can be assumed that the application of effects of ultrasonic cavitation should also contribute to the stability of the beverages produced by combining protein-containing dairy and vegetable raw materials.

The paper aims at studying the conditions for producing protein-containing beverages on a vegetable raw materials basis - the flour from the oil cake of pine nut kernels (cedar flour) - under ultrasonic conditions in the advanced cavitation mode (with an intensity of more than 10 W/cm^2).

Problems to be solved:
– the systematization of literature data to identify the nature and mechanisms of physical and chemical processes in food environments under ultrasonic conditions;
– the study of disperse characteristics of cedar flour, the analysis of transition of proteins and total of soluble dry solids of cedar flour to a beverage when cedar flour is instantized with water without the use of additional methods for physico-chemical effects;
– the study of the general patterns of transition of proteins, fat and total of soluble dry solids of cedar flour to a beverage under conditions of selected ultrasonic modes;
– the estimation of the effectiveness of ultrasonic treatment to produce vegetable analogues of milk from oil cake using the example of cedar flour, the comparative characteristic of the nutritional value of the beverages produced.

STUDY OBJECTS AND METHODS

The following are used as the **study objects**:
(1) cedar flour is flour from the oil cake of nut kernel a Siberian stone pine (*Pinussibirica Du Tour*) produced by the manufacturer through a cold pressing of edible cedar oil, ground to a dispersion of less than 0.5 mm and packed in consumer packaging (a double-layer polyethylene film, a vacuum) under industrial conditions. Cedar flour has a characteristic mild nut flavor and after-taste, of the cream color. Nutritional value of 100 g of the product, according to the manufacturer's label: proteins - 34 g, fats - 20 g, carbohydrates - 25 g; the energy value is 416 kcal. The actual values of components of the chemical composition of cedar flour, determined using standard methods for studying oil raw materials: fats – 21.5 ± 0.2%, proteins – 36.7 ± 0.1%, carbohydrates (in total) – 21.4 ± 0.5%. The shelf life stated by the manufacturer is 12 months at a temperature of no more than 25ºC and a relative air humidity of no more than 75%;
(2) whole milk powder 25% fat, produced and packaged in consumer packaging (a plastic bag and a cardboard box) under industrial conditions. Milky cream fine powder. The taste and smell are typical of pasteurized whole milk, without foreign flavors and smells. Nutritional value of 100 g of the product (according to the label): proteins - 25.4, fats - 25.0; the energy value is 475 kcal. The shelf life stated by the manufacturer is 8 months at a temperature from 0 to 10°C and a relative air humidity of no more than 85%;
(3) the beverages produced from drinking water (pH is 6.7–6.8, the total mineralization is 0.2 g/l) and cedar flour or the combinations of cedar flour and milk powder.

Study methods. When studying literature data, methods of comparative analysis and systematization of information from scientific publications and periodicals were used.

The structure of milk powder and cedar flour was studied using a scanning electron microscope "JSM-840" (Japan, JEJL).

The disperse characteristics of cedar flour and its combinations with milk powder, as well as the sedimentation rate (the stability of beverages), were studied carried out using a method of sedimentation analysis, at a temperature of 20°C, and BT type torsion scales with a unit value of 1 mg.

Fig. 1. The "Volna" device.

The experimental beverages were prepared in glass thermo- and chemically resistant dishes, in two ways: scalding and boiling. When scalding the beverages were produced by pouring into cedar flour (or the prepared combinations of cedar flour and milk powder) drinking water which was preheated to 70°C, the ratio of cedar flour : water: 10 : 90 (10%), 10 : 80 (11.1%), 10 : 70 (12.5%), 10 : 60 (14.3%), 10 : 50 (16.7%) and 10 : 40 (20.0%), bymass. In the case of preparation of a beverage by boiling, drinking water at a temperature of 20 ± 2°C was poured into a weighed portion of cedar flour in a mass ratio of 10 : 90 (10%), 10 : 80 (11.1%), 10 : 70 (12.5%), 10 : 60 (14.3%) and 10 : 50 (16.7%), gradually heating the resulting suspension to boiling (100 ± 2°C).

To improve the efficiency of conversion of cedar flour dry matters to the emulsion of the resulting beverage, the pre-scalded and boiled samples were ultrasonically exposed using an ultrasonic technological device of the "Volna" series of the UZTA-0.4/22-OM model (Fig. 1). The device has been developed at the Laboratory of Acoustic Processes and Devices of the Biysk Technological Institute (branch) of the Polzunov Altai State Technical University and is intended for the intensification of processes in liquid media and also for the intensification of dissolution and dispergating processes [40].

Structurally, the ultrasonic device consists of an electronic unit and an ultrasonic vibrating system connected to it by means of a connecting cable. The frequency of ultrasonic vibrations is 22 ± 1.65 kHz, the maximum power consumption is 400 watts. Manufacturer's recommended operating conditions: at an ambient temperature of 10–40°C and a relative air humidity of no more than 80%.

High-intensity (20 W/cm^2) native ultrasonic modes designated by the apparatus control program were used to process the model liquid media of beverages. An effect of processing time on the composition and colloidal stability of the beverages produced from cedar flour was studied in the course of the studies.

The diameter and number of fat droplets in the beverages produced from cedar flour were determined using a Gorjaev's count chamber.

The content of dry matters in the beverages produced from cedar flour was determined using a gravimetric method (drying of emulsions at 130 ± 2°C to a constant weight), and refractometrically, using an IRF-454B2M refractometer.

The protein content of the beverages produced from cedar flour was determined using a formol titration method.

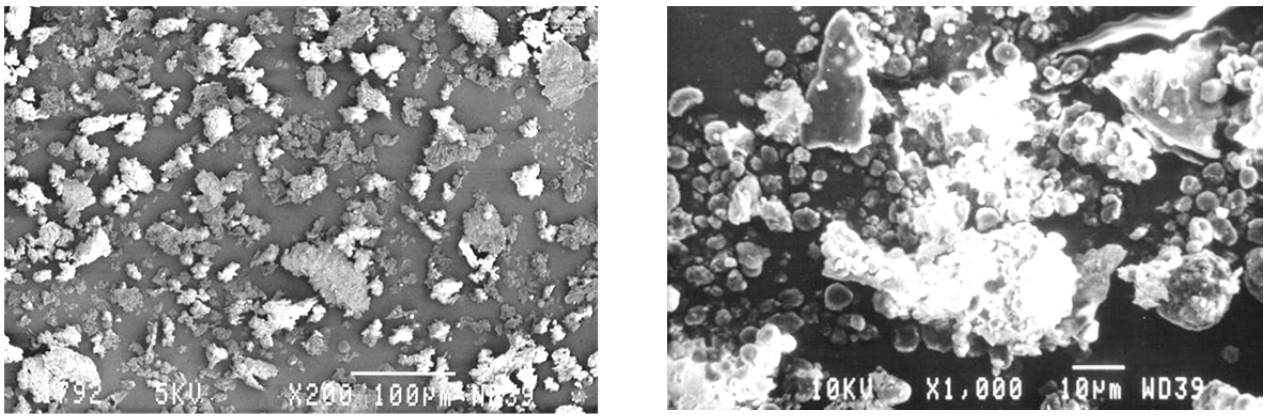
All the studies were carried out with a 3-4-fold frequency. The results of the experimental studies were subjected to statistical processing using a correlation and progressive analysis implemented using standard Microsoft Office software packages.

RESULTS AND DISCUSSION

One of the fundamental characteristics of vegetable and animal raw materials that determine the rate of the processes of wetting, extraction and dissolution is the particle size [41]. Using the example of dried milk instantization conditions, it was shown that the minimum particle size required for their spontaneous immersion in water is about 50 μm [42].

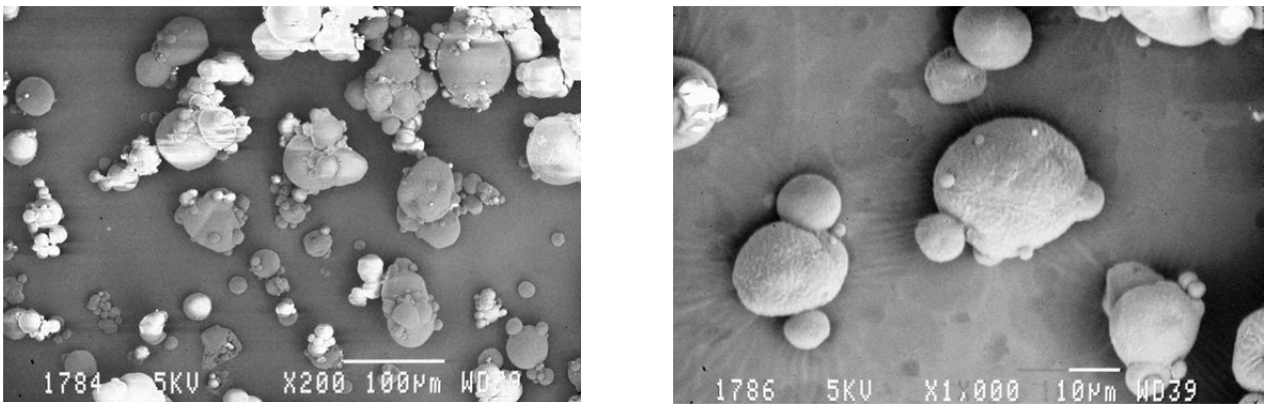
The study of cedar flour by electron microscopy showed that, when in the dry state, its particles are granules of an irregular shape and are characterized by relatively small dimensions: from 0.25 to 14.0 μm; the average particle size is 5 μm (Fig. 2).

For comparison are given micrographs of particles of powder milk made under the same conditions: spheres of an irregular shape, with a diameter from 1 to 30 μm (Figure 3).



(a) (b)

Fig. 2. Micrographs of cedar flour: (a) × 200 and (b) × 1000.



(a) (b)

Fig. 3. Micrographs of milk powder: (a) × 200 and (b) × 1000.

A method for "crushing" casein micelles is widely used in food technologies to improve the homogenization of dairy and vegetable products [43]. According to the results of electron microscopy, cedar flour particles are much smaller in comparison with sufficiently large milk powder particles (micelles). Therefore, it can be predicted that they will dissolve easily, without requiring significant energy costs for disintegration in the preparation of beverages in the form of suspensions or emulsions.

When examining the disperse characteristics of cedar flour and its combinations with milk powder, the following main tasks were solved:

– the determination of the maximum and minimum radii of particles in the system;

– the study of the fractional composition of particles;

– the determination of the average particle diameter in accordance with the mass distribution of particles.

According to the sedimentation analysis, the weight of the agglomerates of water-soaked cedar flour particles is in the range from 14 to 38 mg (Fig. 4). The study of sedimentation stability of colloidal systems based on cedar flour or its combinations with milk powder shows that the suspensions obtained by scalding or boiling are separated, without the use of additional treatment effects, into 2 phases within 30–120 minutes. The upper phase is a sufficiently homogeneous emulsion of a yellowish-cream color, the lower phase is a loose greyish-creamy sediment of the undissolved particles of cedar flour. With the course of time there is a gradual compaction of this sediment.

With an increase in the dosage of cedar flour in the model beverage system, the stability of suspensions decreases, but at the same time reduced and the speed of separation of suspensions, which is due to the satisfactory emulsifying properties of oil cake proteins.

The combination of cedar flour and milk powder in a ratio of 1 : 1 contributes to an increase in the solubility of the raw materials studied.

To characterize the disperse state of the systems obtained on the basis of cedar flour, milk powder and a composition on their basis, integral and differential curves have been drawn. The integral distribution function curves (Fig. 5) show the dependence of the value Q (the percentage of particles with a radius from r_{max} to r) on the particle radius (r), which allows to characterize the fractional composition of particles in a colloid system with a high degree of reliability (the approximation reliability value R^2 = 0.96). The analysis of integral curves allows to conclude that the disperse phase of the resulting suspension, after the particles of cedar flour are wet, is made up of globule particles with a radius from 85 to 355 µm – the particles of such sizes cannot become steady independently in the suspended state.

It is confirmed on the example of the homogenization conditions for pure-like products from vegetable raw materials, that in order to conserve particles in the suspended state, they must be 50 µm or smaller [37]. In our case, when preparing a composition of cedar flour and milk powder in a ratio of 1 : 1, the range of particle distribution becomes from 30 to 170 µm, that is, when producing a beverage in the suspended state, only a small fraction of cedar flour particles will be retained, and the predominant part of the flour will be sedimented.

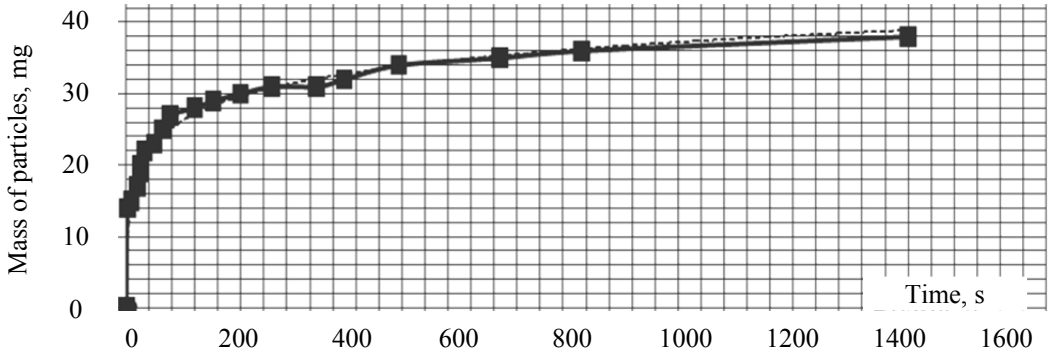


Fig. 4. Sediment accumulation curve in a water dispersion system based on cedar flour.

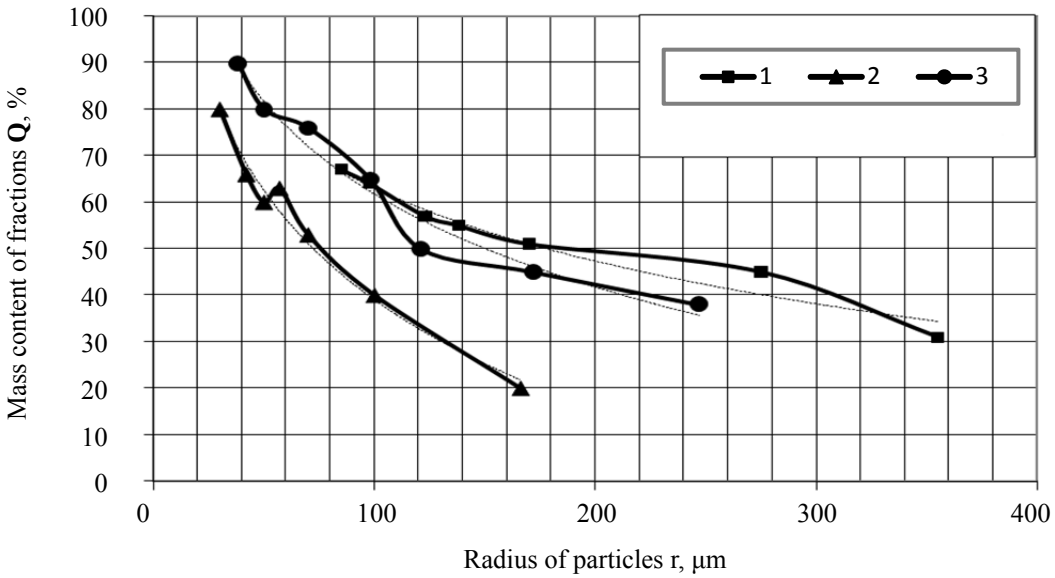


Fig. 5. Integral curves of the distribution of particles in a water disperse system based oncedar flour, milk powder and a composition on their basis: (1) cedar flour; (2) cedar flour: milk powder in a ratio of 1 : 1; (3) milk powder.

Figure 6 shows the differential curves that represent the dependence of the mass function of distribution of particles of the disperse phase on their radius. As is known, the narrower the interval of boundary radii on the differential curve and the higher its maximum, the closer the resulting suspension to the monodisperse one. Such a function is in the case of a colloidal system based on instantized dried milk.

The functions of distribution of particles of the disperse phase in the suspensions based on cedar flour and its combination with milk powder in the relation under consideration are polydisperse. Such a relationship can also be predicted for the milk and vegetable combinations based on cedar flour in other proportions.

Polynomial regression equations that describe the dependence of the mass distribution function on the particle radius in cedar flour suspensions have been obtained by means of the mathematical treatment of the experimental data:

$$y = 3E\text{-}08x^4\text{-}2E\text{-}05x^3+0.005x^2\text{-}0.667x+27.36,$$

for $R^2 = 0.90$,

and a milk and vegetable composition on its basis (at a ratio of 1 : 1):

$$y = 4E\text{-}06x^4\text{-}0.001x^3+0.102x^2\text{-}3.907x+51.92,$$

for $R^2 = 0.91$.

As it has been established earlier [44], cedar flour particles have sufficient hydrophilic and lipophilic properties. Temperature, as a factor that has an effect on a disperse system, in the case of cedar flour itself and dairy and vegetable products therewith, should not have a significant effect on the stability of the system. As the temperature rises, the particles of plant raw materials should swell much more. In this case, they will have larger sizes and have a higher sedimentation rate.

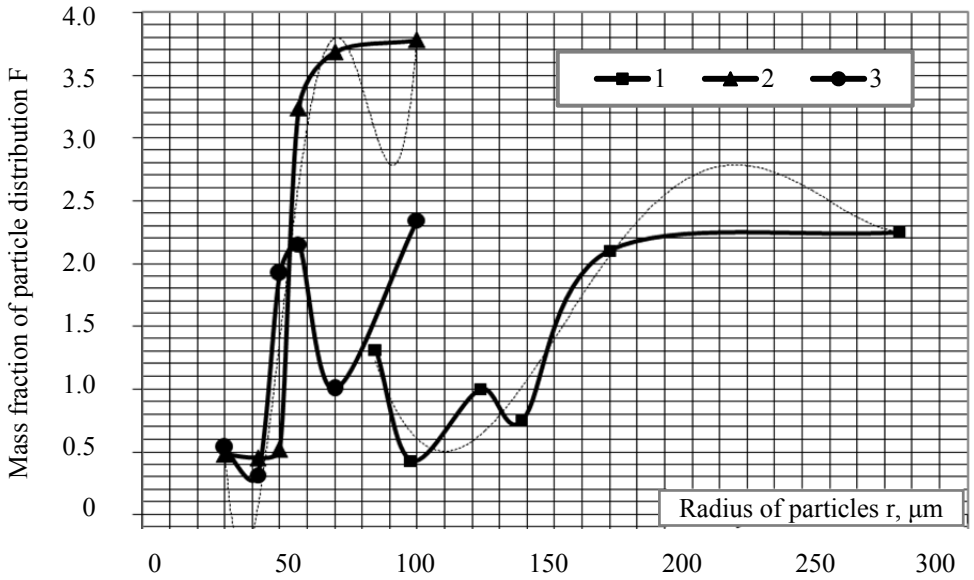


Fig. 6. Differential curve of particle distribution in a water disperse system based on cedar flour, milk powder and a composition on their basis: (1) cedar flour; (2) cedar flour: milk powder in a ratio of 1 : 1; (3) milk pow.

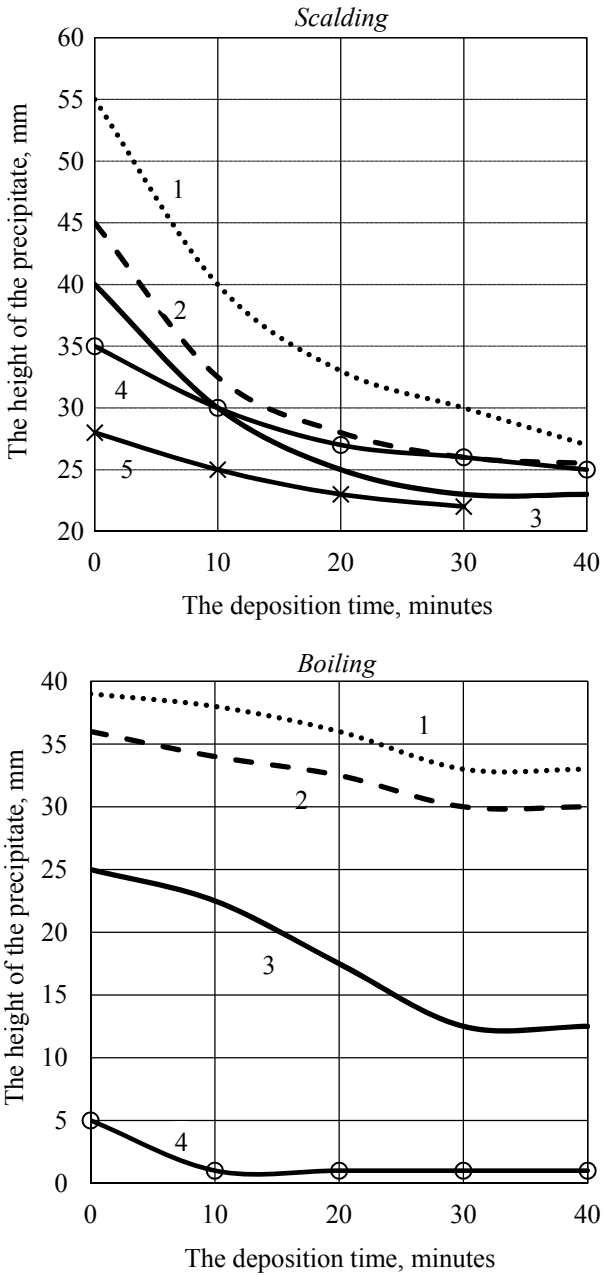


Fig. 7. Dynamics of sediment compaction in the suspensions who obtained from cedar flour (flour) by methods of scalding or boiling: (1) 11.1% of flour; (2) 12.5% of flour; (3) 14.3% of flour; (4) 16.7% of flour; (5) 20.0% of flour.

However, according to the results of a study of the rate of sediment compactionin suspensions, when producing beverages by boiling, the sediments are compactedmore slowly than when producing beverages by scalding cedar flour (Fig. 7). This is probably due to the fact that a looser sediment is formed in the case of scalding.

The sediment is initially formed as a lower and denser layer when boiling and scalding cedar flour in those cases where a higher dosage of cedar flour is used - at a ratio with water of 10 : 60 (14.3%), 10 : 50 (16.7%) and 10 : 40 (20.0%).

The maximum rate of sedimentation of cedar flour particles and sediment compactionis in the first 10 minutes; after 20–30 minutes from the beginning of observations, the process of sediment compactionis inhibited, and by 40 minutes, the colloidal system becomes completely stable.

It is obvious that one cannot expect an effective transition of the most valuable food components of cedar flour - proteins and polyunsaturated fats - into an emulsion without the use of special physical methods for affecting the suspended system of a beverage thus produced, as it is impossible to achieve the necessary colloidal stability of such beverages. In this regard, it can be said that it is necessary to use a powerful physicochemical effect that contributes to destroy or prevent the stratification of the structure of milk and vegetable compositions due to an increase in the solubility of cedar flour particles for finer dispergating of the particles of the considered raw materials and achieving the required stability of such beverages from vegetable raw materials.

Ultrasound in the advanced cavitation mode, like some other powerful physical effects, is an effective way to have an impact on the properties of food systems and the behavior of technological processes. As a result of continuous compression and microflow pulses from movement in various directions, collapse, the fusion of pulsating water bubbles or other solvents, ultrasonic cavitation causes the disintegration of solids (as a consequence of a shock wave produced by the collapse of cavitation bubbles) and accelerates various physical and chemical processes [30, 35, 45]. Physical effects are followed by a change in the viscosity, disperse state and strength of colloidal systems; chemical effects are manifested in the intensification of heat and mass transfer processes, including the processes of dissolution and extraction of the components of the processed raw materials [31, 45–49].

One of the possible consequences of an ultrasonic effect on the organic polymers of raw materials can be the formation of cross-linkages along the broken bonds formed when biopolymers are destroyed, followed by an increase in the viscosity and density of the treated non-aqueous media [50]. Along with this, there is a mechanical destruction of the structure of natural polymers of food raw materials expressed to a different degree, including protein fibers of muscle and connective tissues [37, 51, 52]. The degree and depth of the occurring processes are determined by the conditions of ultrasonic exposure [31, 52–54], including the effects from dissolved gases [55, 56].

The main factors that determine the reaction rate of various diffusion, chemical and other processes in food products include a change in the phase state of water. As a result of hydration, a strong bond of water with vegetable biopolymers such as proteins and complex carbohydrates (that have a basic structuring significance in food environments) is formed. In particular, it is this property of water that the effects of sonochemical water treatment are based on in the technology of instantization of dried milk and dry whey [33, 35].

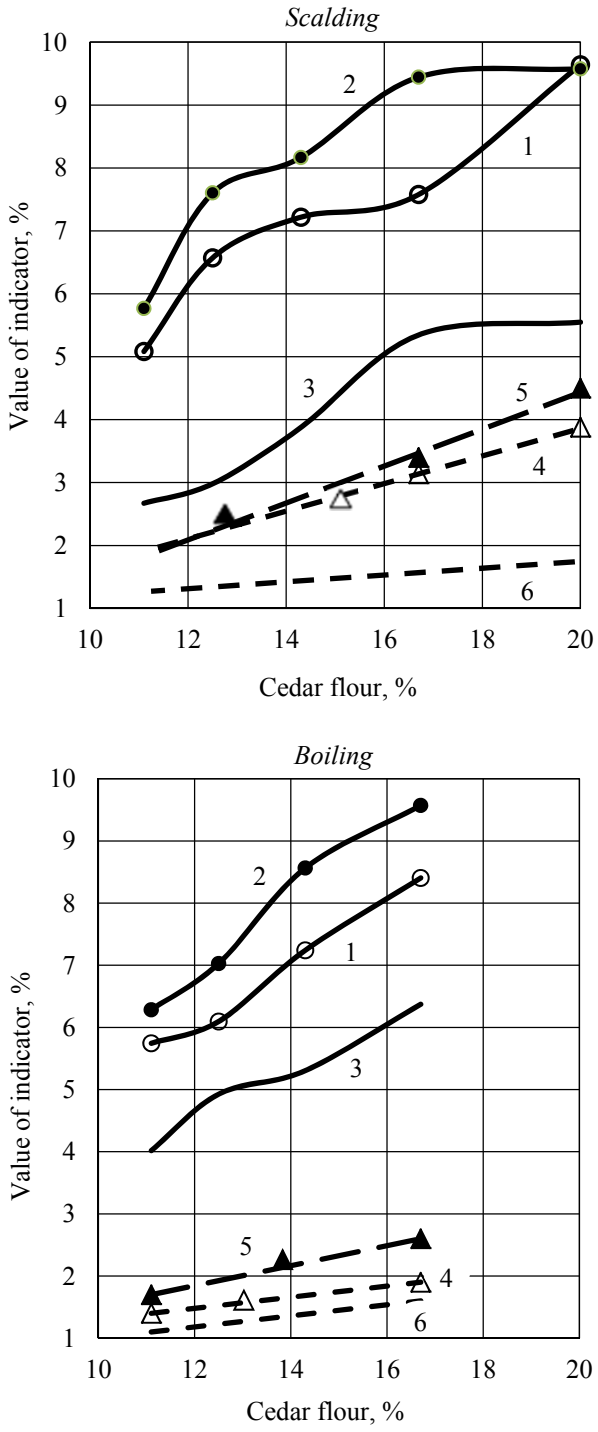


Fig. 8. Content of solids and protein in emulsions depending on the dosage of cedar flour and the processing method: (1) total of dry matters, a 2-minute ultrasound treatment; (2) total of dry matters, a 5-minute ultrasound treatment; (3) total of dry matters without ultrasound treatment; (4) protein, a 2-minute ultrasound treatment; (5) protein, a 5-minute ultrasound treatment; (6) protein, without ultrasound treatment.

When producing emulsion products, the water activated by cavitation effects is more easily bound by a colloidal system as a result of the hydrolysis of the fat molecules that adjoin the cavitating liquid and the appearance of di- and monoglycerides and natural emulsifiers and thickeners in the solution. At the same time, there are data on the coagulating effect of ultrasound related to the destruction of the solvate shell on the particles of the disperse phase [36, 57]. Under real conditions, there can only be a more or less stable equilibrium between the emulsification and coalescence of emulsion phases. Therefore, in each specific case of application of the effects of ultrasonic cavitation to a new object, it is necessary to choose those modes and duration of effect that will provide the production of a stable colloidal system, in this case, - emulsion.

The conditions for producing beverages by milk powder instantization have been studied sufficiently. Therefore, the main task of further studies was to study the conditions for obtaining stable emulsions from cedar flour - vegetable milk analogues.

Unlike milk powder particles, which are dried drops of a homogeneous solution emulsion, cedar flour particles contain about 30% of the substances that cannot dissolve in the aqueous medium, even under cavitation conditions; these are, first of all, the mineral components of ash, fiber and protein insoluble in water. In this regard, the efficiency of dissolution of cedar flour particles was estimated not by the solubility index determined when estimating the efficiency of milk powder instantization and reflecting the degree of protein hydratability, but by the transition of total of dry matters and soluble protein, as one of the most significant components in nutrition, to the resulting emulsion.

Figure 8 shows the dependence of the transition of dry matters and protein to an emulsion on the dosage of cedar flour and a processing method. When processed by ultrasound, the content of dry matters in the emulsions of beverages increases. Consequently, the solubility of cedar flour components, the nutritional value of the beverages produced and their colloidal stability increase. In this case, a high correlation is found between the results obtained using the methods for the gravimetric and refractometric determination of solids.

In the case of the "boiling" of a beverage, the stability of the resulting emulsions is lower than that in the cases of scalding. This is probably due to a more pronounced denaturation of cedar flour proteins as a result of a longer effect of high temperatures on them when boiling.

As it is shown in a lot of papers, the mechanical and chemical effects generated under conditions of high-intensity ultrasonic exposure are manifested primarily in the dispergating and dissolution of components [48, 49, 57, 58]. In this case, the effects that can be seen after 2 minutes of processing of model media (beverages) by ultrasound can be conditionally divided as:

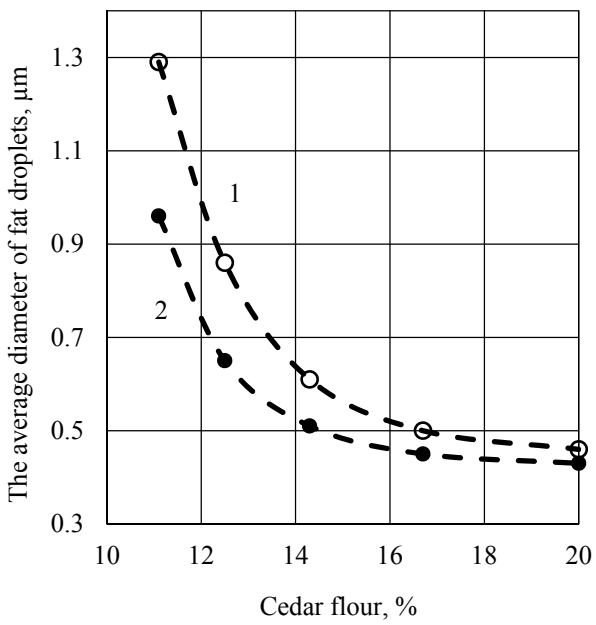
(1) increasing the degree of the transition of soluble proteins and other components that are part of the composition of cedar flour to the aqueous phase of fat;

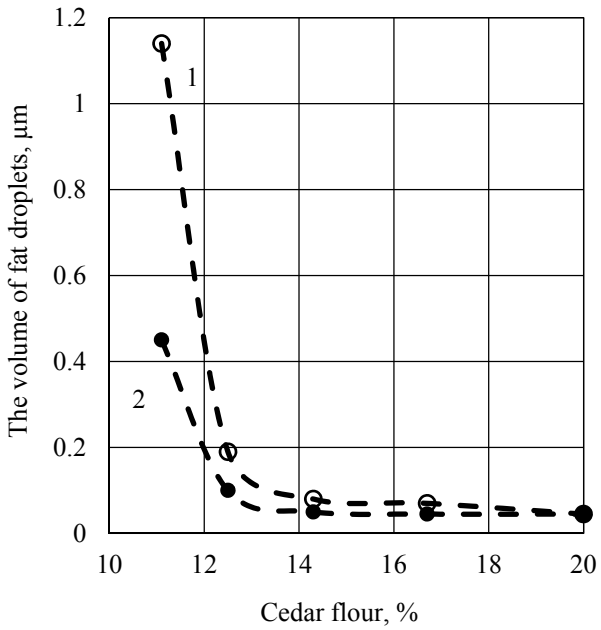
(2) dispergating the cedar oil drops extracted from cedar flour particles, which contributes to an increase in the stability of the resulting emulsions (Fig. 9).

The increase in the duration of treatment of the model media with ultrasonic waves from 2 to 5 minutes does not lead to an increase in the content of dissolved fat in the resulting emulsions. However, with an increase in the duration of ultrasonic treatment, the diameter of fat droplets is reduced, because when affected by continuing cavitation, the large fat droplets extracted from cedar flour break up into smaller ones. Taking into account the fat content of cedar flour established according to the results of physical and chemical studies, the obtained data confirm the completeness of its extraction under ultrasonic treatment.

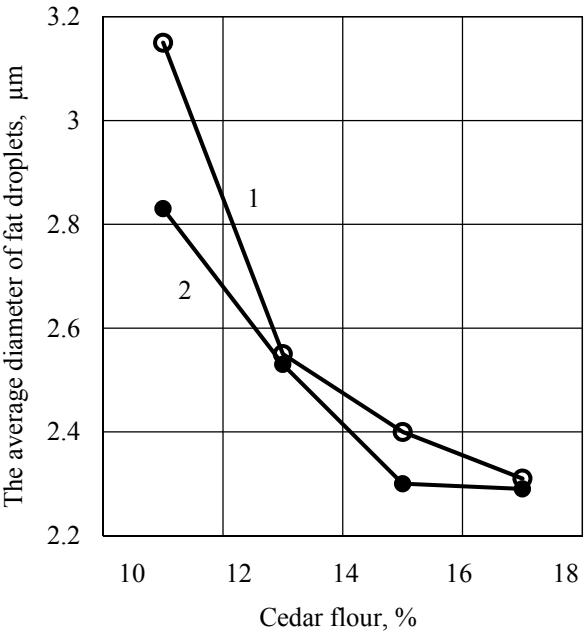
In comparison with the beverages produced by scalding and boiling cedar flour in water without using the effects of ultrasonic treatment, the size range of fat droplets essentially changes in the treated beverages (Fig. 9, Table 1). The effectiveness of ultrasound exposure is illustrated more clearly by the photographs of experimental beverages from cedar flour (Fig. 10).

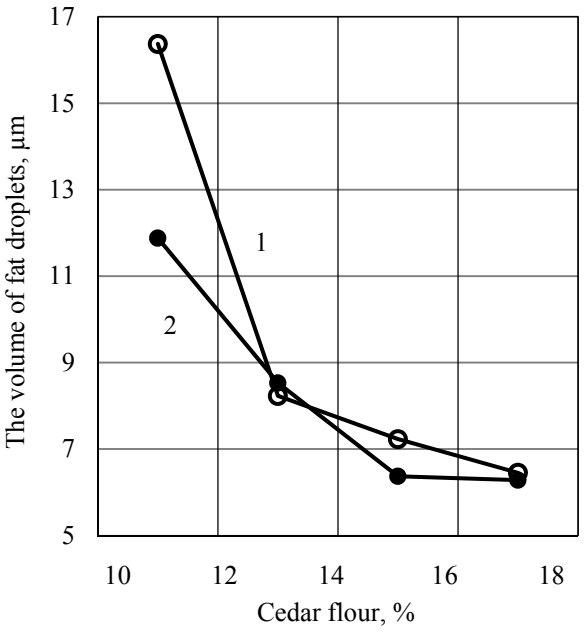
The results obtained demonstrate the apparent traceability of the effect of ultrasound exposure on the composition and colloidal stability of the vegetable milk type beverages produced from the flour from oil cake. The comparative analysis of the data on the content of dissolved proteins and fat in the resulting beverage (Table 1) shows their comparability with whole cow milk (3.3% protein, 3.5–3.9% fat, 12.6% solids).





(a) obtaining the emulsions of beverages by *scalding* cedar flour





(b) obtaining the emulsions of beverages by *boiling* cedar flour

Fig. 9. Dependence of the diameter and volume of fat droplets in emulsions from: the dosage of cedar flour, method of preparation and the duration of the ultrasonic cavitation treatment time: (1) 2 minutes and (2) 5 minutes.

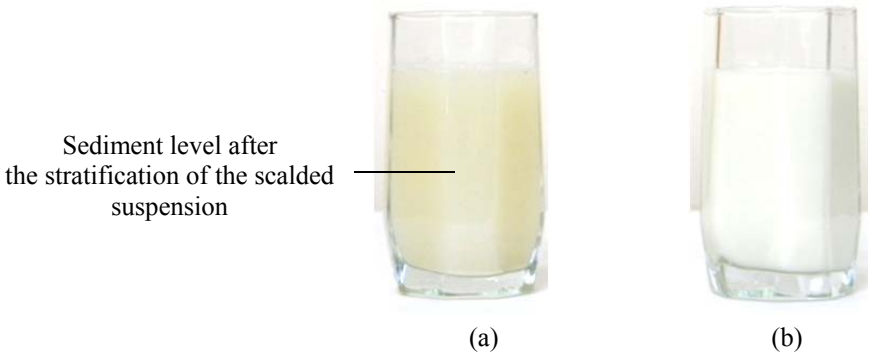


Fig. 10. Photographs of the emulsions of the experimental beverages produced from cedar flour by scalding: (a) with no ultrasonic treatment, with the dosage of cedar flour of 14.3%; (b) with ultrasonic treatment for 2 minutes.

Table 1. Characteristics of the experimental emulsions of beverages from cedar flour

Characteristics of the emulsion	*Scalding* (70ºC)			Characteristics of the emulsion	*Boiling* (100 ± 2°C)		
	with no ultrasonic processing	with ultrasonic processing			with no ultrasonic processing	with ultrasonic processing	
		2 minutes	5 minutes			2 minutes	5 minutes
14.3% cedar flour							
The average diameter of fat droplets, μm	3.11	0.52	0.50	The average diameter of fat droplets, μm	7.50	2.40	2.30
The average volume of fat droplets, μm	15.65	0.08	0.07	The average volume of fat droplets, μm	10.30	7.23	6.37
Mass fraction of solids, %	4.0 ± 0.1	7.2 ± 0.1	8.1 ± 0.1	Mass fraction of solids, %	5.3 ± 0.1	7.2 ± 0.1	8.6 ± 0.1
Mass fraction of protein, %	1.45 ± 0.18	2.65 ± 0.22	2.80 ± 0.20	Mass fraction of protein, %	1.34 ± 0.16	1.70 ± 0.20	2.22 ± 0.23
Mass fraction of fat, %	0.22 ± 0.05	2.8 ± 0.2	2.8 ± 0.2	Mass fraction of fat, %	0.85 ± 0.08	2.8 ± 0.2	2.8 ± 0.2
16.7% cedar flour							
The average diameter of fat droplets, μm	1.25	0.50	0.45	The average diameter of fat droplets, μm	2.46	2.31	2.29
The average volume of fat droplets, μm	1.00	0.07	0.05	The average volume of fat droplets, μm	7.70	6.45	6.28
Mass fraction of solids, %	5.4 ± 0.1	7.6 ± 0.1	9.5 ± 0.1	Mass fraction of solids, %	6.4 ± 0.1	8.4 ± 0.1	9.6 ± 0.1
Mass fraction of protein, %	1.60 ± 0.20	3.17 ± 0.25	3.42 ± 0.20	Mass fraction of protein, %	1.60 ± 0.20	1.92 ± 0.20	2.64 ± 0.25
Mass fraction of fat, %	0.2 ± 0.1	3.3 ± 0.2	3.3 ± 0.2	Mass fraction of fat, %	1.1 ± 0.1	3.3 ± 0.2	3.3 ± 0.2

According to the results of the studies, the dosage of cedar flour in the range of 14–16.7 %, both under scalding conditions and during boiling can be considered the optimal conditions for producing beverages from exclusively vegetable raw materials. The ultrasonic cavitation treatment of the resulting suspensions increases the efficiency and the rate of transition of dry matters to an emulsion up to 2 times during scalding and 1.5 times during boiling. More efficient fat emulsification is achieved under scalding conditions followed by ultrasonic treatment.

As it was established by the results of the preliminary studies, combining cedar flour with milk powder in a ratio of 1 : 1 contributes to an increase in the solubility of the vegetable raw materials studied even without using the effects of ultrasonic cavitation. Therefore, when using ultrasound, it is possible to predict the production of stable emulsions not only from cedar flour, but also on the basis of its combinations with milk powder, which will allow to produce beverages with a higher nutritional value. Revealing the patterns of a change in the composition and properties of the beverage emulsions obtained by the ultrasonic treatment of combined milk and vegetable media requires a further study.

CONCLUSIONS AND RECOMMENDATIONS

Thus, the use of the effects of ultrasonic exposure in the production of new protein-containing beverages such as "vegetable milk" allows to obtain stable emulsions from the flour from oil cake, which do not require the use of emulsifiers and are characterized by an increased content of soluble solids (7.2–8.4%), fat (1.8–3.3%) and proteins (1.9–3.4%).

The recommended limits of the used dosage of cedar flour, depending on a method for producing beverages (scalding or boiling), are 14.3–16.7%. To produce such beverages, 2 minutes of ultrasonic exposure in this mode (20 W/cm^2) are enough, as the processing time increases, the studied characteristics of the emulsions practically do not change their values.

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COMPARISON OF PHYSICOCHEMICAL PROPERTIES AND GENERAL ACCEPTANCE OF FLAVORED DRINKING YOGURT CONTAINING DATE AND FIG SYRUPS

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Abstract: Milk and dairy products have a rich nutritional value and they are the main constituents of a human diet. In recent years, the consumption of dairy drinks containing water, sugar syrups and flavorings has been common. So it is important to find a natural alternative sweetener to remove sugar. Therefore, the purpose of this study is to produce flavored drinking yogurt with natural sweeteners like date syrup and fig syrup as a sugar substitute. The study was made of the comparing physicochemical properties and also of opinions about the general acceptance of flavored drinking yogurt with date and fig syrups. The physicochemical properties such as pH, titratable acidity, viscosity, total solid, syneresis and the sensory evaluation of samples were determined weekly at 4°C for 28 days. The results showed that the samples that contained fig syrup had a lower acidity and syneresis percentage while their viscosity was higher compared to the samples that contained date syrup. It can be concluded that flavored drinking yogurt with 10% fig syrup and 1% pectin was chosen as the best treatment since it was rated the highest in general acceptance compared to other samples.

Keywords: Yogurt drink, date syrup, fig syrup

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INTRODUCTION

Milk and dairy products play a key role in healthy human nutrition and development throughout life. In recent decades, technological advances have supported the development of new dairy-based products. The dairy sector has developed techniques to produce a diverse range of milk-based products and dairy ingredients. Processes such as fermentation are used to produce a variety of dairy products. Between all milk fermented products, yogurt is more popular than others and has a higher acceptability in the world due to its nutritional value [1]. Yogurt, typical fermented milk, is perhaps the most complex and biologically active of all foods consumed all around the world [2]. In the world, the vast majority of the human population suffers from lactose intolerance disease. Lactose intolerance is a condition in which people don't have the ability to digest lactose, the sugar found in milk products, and have the symptoms like abdominal pain, bloating, diarrhea, gas and nausea [3]. Yogurt containing live bacteria may be better tolerated by lactose malabsorbers because of the presence of the bacteria in yogurt that produce β-galactosidase in the small intestine. Furthermore, it takes yogurt longer to pass through the digestive system than milk does, thus allowing for a more effective lactose breakdown [4]. So yogurt should be included in the diet. The Ministry of Health and Medical Education show that people need 2 to 3 servings of dairy products a day but unfortunately people in some countries, such as Iranian, use only 0.7 serving of milk and dairy products [1]. Undoubtedly, one reason for the low rate of dairy products consumption is lack of sufficient diversity. Today, in order to deal with the problem of high soda and low dairy consumption, some dairy-based drinks with high diversity including whole dairy drinks such as fermented milk, drinking yogurt and dairy-based beverages have been placed in the dairy production program. Recently, the consumption of dairy drinks containing water, sugar syrup and flavorings has been common.

Table sugar which is referred to sucrose is a disaccharide consisting of glucose and fructose. Fruit syrups contain some simple sugars like fructose and glucose, however they also contain longer chain and complex carbohydrates that are longer to digest and absorb. Fruit syrups are fantastic health-promoters due to the presence of beneficial plant compounds compared to refined sugars (sucrose). The carbohydrates in fruit are accompanied by vitamins, minerals, protein, fibers, plant pigments and anthocyanins that confer a host of beneficial effects. So if a person doesn't suffer from a health problem such as diabetes, it is better to use syrups instead of sugar [5].

Fig fruit (Scientific name: Ficus carica) is an Asian species of flowering plants in the mulberry family. It is native to the Middle East and western Asia and one of the popular fruits enjoyed since ancient times. Fig is now widely grown throughout the world, both for its

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fruit and as an ornamental plant [6]. Fig is rich in tocopherols, carotenoids, phenolics and vitamins such as vitamin C that can alter the metabolic activation and detoxification/ disposition of carcinogens. These antioxidant compounds affect the processes that modify the development of tumor cells [7], and avoid the neurochemical and behavioral changes related to aging [8]. The studies show that fruits and vegetables are rich in phenolics decrease cardio- and cerebrovascular diseases and cancer death rates [9]. Fig varieties with dark skin have higher amounts of polyphenols, flavonoids and anthocyanins accompanied by a higher antioxidant activity compared with fig varieties with lighter skin [10]. Figs are free of sodium, fat, and, like other fruits, are free of cholesterol. Fig fruit is low in calories since 100 g of fresh fig fruit contain only 74 calories. Fig fruits contain health benefiting dietary fiber and lignin. Fibers and lignin are non-digestible portions of a lot of plants that absorb water, and increase in bulk. They play a major role in preventing constipation by accelerating the passage of material through the large intestine [11]. The chemical composition of fig fruit varies with the type. The average composition of the edible part of a fresh fig with a moisture content of 80.8% is (per 100 gm): 1.3% protein, 0.6% mineral matter, 17.1% carbohydrates, 0.06 mg of calcium, 0.03 mg of phosphorus, 1.2 mg of iron, 270 I.U. of β-carotene, 0.6 mg of nicotinic acid, 50 micro gm of riboflavin (B_2) and 2 mg of ascorbic acid. The total sugar content of fresh figs is 13–20% and that of dried figs is 42–62% which is present mostly in the form of invert sugar. The analysis of fresh and dried figs showed the presence of 15.2% and 45–95% of reducing sugars [12]. Fig syrup is an artisan derivative of fig fruit and is a typical food product made by boiling and concentrating fresh figs in water, without adding any other ingredients. The obtained syrup is a dense product defined by its brown color, sweet taste and smell [13]. Therefore, fig syrup can be replaced by sugar to provide sweet healthy food.

Phoenix dactylifera, commonly known as date or date palm, is a flowering plant species in the palm family, *Arecaceae*. Due to its long cultivation, the place of origin is unknown but it probably originated from the lands around Iraq. The date species is widely cultivated and is naturalized in a lot of tropical and subtropical regions worldwide [14]. It is probably one of the oldest cultivated fruits and has been a part of a staple diet in the Middle Eastern countries. Date is energetic food, and when the body needs more energy, it is considered as the best food. 100 g of dates contain 277 calories [14]. Date production and consumption is increasing continuously because of its therapeutic virtues besides its high nutritive value [15]. The researches show that in a balanced nutrition regime date fruit is an important source of minerals and vitamins [16], so it can be useful in strengthening bones and curing painful diseases like osteoporosis [14, 17]. Numerous studies prove that when dates are eaten alone or in mixed food with plain yogurt, they have low glycaemic indexes [18, 19]. In addition, Miller *et al.* [20] reported that the consumption of dates may benefit in glycaemic and lipid control of diabetic patients. There are the following health benefits of date fruits: curing anemia, constipation; diarrhea; intestinal disorder; allergies and intoxication treatment. Various studies have revealed that dates have an anti-tumor activity [21], antioxidant and anti-mutagenic properties [22, 23]. Date fruit has been recommended in folk curing for the treatment of various infectious diseases and cancer [24]. Furthermore, dry date fruits are consumed in Indian traditional medicine after a child's birth as immunostimulants [25]. Date syrup (date honey or date molasses) is thick dark brown, very sweet fruit syrup extracted from dates and has the same properties of date. It is widely used in the North African and Middle Eastern cuisine. The average chemical characteristics of date syrup with 82 brix degrees are: 16.5% moisture, 1.45% protein, 38.2 glucose, 39.4% fructose and 1.6% ash [26]. Date syrup is rich in such monosaccharides as glucose and fructose. It is therefore highly suitable for people suffering from hypoglycaemia, or for those with sucrose intolerance or those with pancreatic problems who have difficulty absorbing disaccharides. Date syrup is used by women after childbirth to stimulate their immune system [25]. Aqueous date extract also significantly inhibited lipid peroxidation and protein oxidation in a dose-dependent manner [27].

Several studies have been carried out to indicate the potential of dairy- based drinks production. Habibi et al. [28] examined the use of a yogurt starter to produce dairy-based drinks. They tested two species of bacteria (*Streptococcus thermophilus* and *Lactobacillus delb-rueckii* subsp. *bulgaricus*), sucrose syrup, pectin and strawberry flavor to produce a flavored dairy drink. They concluded that they can produce the dairy-based drinks which can be accepted by the customers. The impact of date syrup on kefir physical and chemical properties was examined by Taherian et al. [29]. They found that date syrup has the highest general acceptance according to taste panel opinions. Kazemizadeh and Fadaei [30] used pomegranate peel extract and date nectar to make flavored milk and they got very good results in the general acceptance of the obtained product.

The taste of yogurt is most often enhanced with fruit preserves or other ingredients [31]. Flavored yogurts are produced by adding fruit concentrates or flavored syrups to the cultured milk before or after incubation [32]. Therefore, the purpose of the present study is to optimize the formulation of flavored drinking yogurt with two different sugar substitutes such as date and fig syrups in order to remove sugar and improve the health of the products. In addition, some physicochemical properties of the produced drinks and the general acceptance of two produced drinks was examined and compared.

STUDY OBJECTS AND METHODS

To study the physicochemical properties and the general acceptance of flavored drinking yogurt with date and fig syrups, the materials and the experiments were prepared as described below.

Materials. The commercial yogurt starters YO-MIX 465 and YF-L711 were obtained from the Pardis Roshd Mehregan Company, Shiraz, Iran. The whole cow raw milk was purchased from a supermarket (Fasa, Fars, Iran). Fig syrup and date syrup were taken from a local supermarket (Estahban, Fars, Iran). All the chemicals used in this paper were of an analytical grade.

Preparing flavored drinking yogurt. Raw fresh milk was analyzed by MilkoScan FT1 (Denmark) and consisted of 3.10% protein, 3.1% fat, lactose 4.88 % and 11.79% total solids. Then, milk was preheated to 65°C and homogenized. The homogenized milk was pasteurized at 73°C for 15 seconds and cooled to 42°C. The pasteurized milk was inoculated with a 4% starter and the incubation continued until the pH reached 4.2. Yogurt drink formulation was done by adding Syrups (dates and figs) separately as sweeteners at 5 and 10 percent and 0.5 and 1 percent pectin as a stabilizer. And finally the product was pasteurized at 72°C in a hot water bath for 2 minutes. To prevent evaporation, the dishes were covered with foil, and then they were cooled to a temperature less than 10°C. Finally, they were packed in suitable sterile dishes and they were kept at 4°C for 28 days for further analysis [33].

pH measurement. The pH of each sample was measured at room temperature (20°C) by using a digital pH meter (WTW pH 525 model, Germany) during the storage period. At first, the pH meter was calibrated according to the manufacturer's instructions, using buffer standards of pH 7 and pH 4 and then placed directly into each sample and recorded the number.

Acidity measurement. Titratable acidity was measured for all samples according to the method adopted by the Association of Official Analytical Chemists with 0.1 N NaOH, and phenolphthalein as an indicator to see a pale pink color. The results were calculated in dornic degree.

Dry matter measurement. Total solids of samples were measured using a forced-air drying oven (500UNB model, Germany) at 105°C according to the Iranian National Standard No. 637 during the storage period. Moisture is evaporated from the sample by oven drying. Total dry matter is determined gravimetrically as a residue remaining after drying.

Viscosity measurement. The viscosity measurement was carried out at room temperature of 25°C using a Brookfield Programmable DVE Viscometer (Brookfield viscometer DVII, USA) equipped with a spindle No. 3 and a rotation speed of 30 rpm. The results were recorded in centipoises (cP) after 10 s of shearing.

Syneresis measurement. The syneresis index of different samples was determined according to the methodology proposed by Koksoy and Kilic [34]. Dinking yogurt (40 g) was weighed in sterile scaled plastic containers and the samples were stored at 4°C. After 28 days of storage at 4°C, a clear layer of serum, in case of having serum, was separated by Pasteur pipettes and it was weighed and the percentage of serum separation was calculated [34].

General acceptance. The analysis was performed under normal light, in the sensory laboratory at the Azad University of Fasa by fifteen trained students (eight women and seven men aged 22–28). The test samples, identified by a 3-digit code, were presented to the descriptors in a randomised order, immediately after being removed from the fridge (4°C). Since the samples were unusable on Day 28, so the duplicate samples on days of storage 0, 7, 14 and 21 were tested and the ratings were presented on a 4-point hedonic scale ranging from 4 ("like extremely") to 1 ("dislike extremely") according to the Iranian National Standards [35].

Statistical analysis. The data were analyzed using SPSS 24 software (SPSS Inc., Chicago, IL, USA). One-way ANOVA, Kruskal-Wallis, Mann-Whitney, Repeated Measures and Paired sample t tests were used. The results are expressed as mean ± SD with a significance level of $p < 0.05$. All the experiments were repeated three times.

RESULTS AND DISCUSSION

pH and acidity. The pH and acidity values of flavored drinking yogurt with fig and date syrups during storage at 4 °C are shown in Tables 1 and 2. The data showed that by increasing the percentage of fig and date syrups in the samples, pH increased and the titratable acidity decreased, respectively ($p > 0.05$). This phenomenon is due to the high pH of fig syrup (5.02) and date syrup (4.83) which significantly affects the final pH of flavored drinking yogurt. However, the samples containing date syrup had a significantly higher acidity in comparison with the samples containing fig syrup ($p > 0.05$). The pH and titratable acidity of all the samples decreased and increased, respectively during this period ($p > 0.05$). It seems that bacteria use such monosaccharides as fructose and glucose in fig and date syrups and produce acidic metabolites. These findings are in keeping with those of Milani *et al*. [36], who also reported that the use of date honey along with a stabilizer guar significantly decline the pH of the orange low-fat yogurt dessert during this period.

The different small letters indicate statistically significant differences in columns ($p < 0.05$). The different capital letters indicate statistically significant differences between days in each sample ($p < 0.05$).

The different small letters indicate statistically significant differences in columns ($p < 0.05$). The different capital letters indicate statistically significant differences between days in each sample ($p < 0.05$).

Percentage of dry matter. The percentages of dry matter of samples are shown in Table 3. The results indicated that dry matter of flavored drinking yogurt samples containing fig and date syrups significantly decreased when the percentage of syrup increased ($p > 0.05$). In the sugars, when the molecular weight lowered, the total solid decreased. The monosaccharides like glucose and fructose (make the major part of carbohydrate compounds in fig and date syrups) have a lower molecular weight than sucrose does [37]. Thus, when the percentage of syrups increased, the dry matter of flavored drinking yogurt decreased. We also observed that by increasing the pectin content in samples, the percentage of dry matter significantly increased

($p > 0.05$). The samples containing fig syrup had a higher dry matter compared to the date syrup samples. This is because of a high fiber content in fig syrup that leads to a high dry matter content. During storage, no significant difference was found in the dry matter content of the samples ($p > 0.05$). Similar results were obtained by Gad et al. [19] that a change in the moisture content of the product can significantly change according to the moisture content of the fruit. There were no significant differences between fig and date syrup in all samples and also during storage ($p > 0.05$).

Viscosity properties. Table 4 illustrates the changes of viscosity in the flavored drinking yogurt samples during storage at 4°C. When the percentage of fig and date syrups increased, the viscosity of the samples significantly increased. The presence of hydroxyl group in sugars can lead to hydrogen bonding between sugar and water. By increasing date and fig syrup (glucose and fructose) compared to sucrose, the hydrogen bonds increased and when the mobility of free water decreased, the viscosity increased. The lower molecular weight of saccharides is, they tend to absorb water and so the viscosity increased [36, 37]. Glucose and fructose (the main carbohydrate content in the fig and date syrups) have a lower molecular weight and absorb more water [38]. In addition, pectin had a significant effect at various levels of addition on the apparent viscosity. The data indicate that by increasing the amount of pectin as a thickening agent and stabilizer, the viscosity of the samples significantly increased ($p > 0.05$). We observed that the samples containing fig syrup showed a higher viscosity than the date syrup samples. This may be due to the high levels of fiber along with the high levels of dry matter in the samples containing fig syrup which hold more water and increase the gel strength. On the other hand, the viscosity of all samples declined with time ($p > 0.05$). That can be attributed to an increase in the acidity of samples. The results of this study are consistent with the findings of Milani *et al.* [36]. They found that frozen yogurt containing 50% date honey and 0.3% guar had a higher viscosity compared to the samples without date honey. Also, Gohari Ardabil et al. [37] found that the higher viscosity in frozen yogurt containing date syrup was due to the absorption of water by reducing sugars.

Table 1. Mean ± SEM pH of various flavored drinking yogurt samples during storage up to 28 days

Samples[1]	Time				
	Day 0	Day 7	Day 14	Day 21	Day 28
F1P1	5.81 ± 0.01^{bE}	5.26 ± 0.01^{cD}	4.88 ± 0.01^{cC}	4.59 ± 0.01^{bB}	4.36 ± 0.04^{bA}
F1P2	5.81 ± 0.02^{bE}	5.23 ± 0.04^{cD}	4.91 ± 0.01^{dC}	4.62 ± 0.01^{cB}	4.44 ± 0.01^{cA}
F2P1	6.09 ± 0.01^{eE}	5.56 ± 0.01^{fD}	5.37 ± 0.01^{fC}	5.07 ± 0.02^{fB}	4.83 ± 0.00^{eA}
F2P2	6.11 ± 0.01^{fE}	5.61 ± 0.01^{gD}	5.38 ± 0.01^{fC}	5.11 ± 0.01^{gB}	4.87 ± 0.01^{fA}
D1P1	5.73 ± 0.01^{Ae}	5.17 ± 0.02^{bD}	4.80 ± 0.01^{aC}	4.51 ± 0.01^{aB}	4.29 ± 0.02^{aA}
D1P2	5.72 ± 0.02^{aE}	5.13 ± 0.03^{aD}	4.83 ± 0.01^{bC}	4.50 ± 0.01^{aB}	4.34 ± 0.01^{bA}
D2P1	5.99 ± 0.01^{cE}	5.46 ± 0.01^{dD}	5.27 ± 0.01^{eC}	4.97 ± 0.02^{dB}	4.71 ± 0.02^{dA}
D2P2	6.02 ± 0.01^{dE}	5.49 ± 0.01^{eD}	5.26 ± 0.01^{eC}	5.01 ± 0.01^{eB}	4.74 ± 0.01^{dA}

[1]*Abbreviations:* F1P1 = 5% Fig syrup + 0.5% Pectin, F1P2 = 5% Fig syrup + 1% Pectin, F2P1 = 10% Fig syrup + 0.5% Pectin, F2P2 = 10% Fig syrup + 1% Pectin, D1P1 = 5% Date syrup + 0.5% Pectin, D1P2 = 5% Date syrup + 1% Pectin, D2P1 = 10% Date syrup + 0.5% Pectin, D2P2 = 10% Date syrup + 1% Pectin.

Table 2. Mean ± SEM titratable acidity (dornic degree) of various flavored drinking yogurt samples during storage up to 28 days

Samples[1]	Time				
	Day 0	Day 7	Day 14	Day 21	Day 28
F1P1	53.17 ± 0.47^{dA}	64.78 ± 0.87^{dB}	66.88 ± 0.54^{cC}	72.59 ± 0.65^{bc}	76.66 ± 0.68^{cdE}
F1P2	55.20 ± 0.96^{eA}	65.86 ± 0.72^{dB}	69.80 ± 0.31^{dC}	73.72 ± 1.00^{cD}	78.56 ± 0.56^{eE}
F2P1	44.07 ± 0.43^{aA}	55.00 ± 0.69^{aB}	61.05 ± 0.84^{aC}	65.84 ± 0.36^{aD}	69.30 ± 0.15^{aE}
F2P2	46.17 ± 0.98^{bA}	58.22 ± 0.98^{bB}	62.68 ± 0.46^{bC}	66.98 ± 0.72^{aD}	70.90 ± 0.40^{bE}
D1P1	62.23 ± 0.96^{gA}	72.89 ± 0.72^{eB}	76.83 ± 0.31^{fC}	80.75 ± 1.00^{dD}	85.05 ± 0.47^{Ge}
D1P2	60.29 ± 0.47^{fA}	71.90 ± 0.87^{eB}	74.00 ± 0.54^{eC}	79.71 ± 0.65^{dD}	83.78 ± 0.68^{fE}
D2P1	49.85 ± 0.43^{cA}	61.68 ± 1.76^{dB}	67.29 ± 1.52^{cC}	71.82 ± 0.64^{bD}	76.05 ± 0.91^{cE}
D2P2	52.43 ± 0.98^{eA}	64.48 ± 0.98^{dB}	68.94 ± 0.46^{dC}	73.24 ± 0.72^{cD}	77.16 ± 0.40^{dE}

[1]*Abbreviations:* F1P1 = 5% Fig syrup + 0.5% Pectin, F1P2 = 5% Fig syrup + 1% Pectin, F2P1 = 10% Fig syrup + 0.5% Pectin, F2P2 = 10% Fig syrup + 1% Pectin, D1P1 = 5% Date syrup + 0.5% Pectin, D1P2 = 5% Date syrup + 1% Pectin, D2P1 = 10% Date syrup + 0.5% Pectin, D2P2 = 10% Date syrup + 1% Pectin.

Table 3. Mean ± SEM dry matter (weight percent) of various flavored drinking yogurt samples during storage up to 28 days

Samples[1]	Time				
	Day 0	Day 7	Day 14	Day 21	Day 28
F1P1	23.16 ± 0.22^{cA}	23.22 ± 0.36^{cA}	23.04 ± 0.64^{cA}	23.23 ± 0.48^{bA}	23.19 ± 0.20^{cA}
F1P2	27.04 ± 0.07^{dA}	26.45 ± 1.44^{dA}	26.75 ± 0.56^{dA}	27.17 ± 1.47^{cA}	27.05 ± 0.24^{dA}
F2P1	16.15 ± 0.26^{aA}	15.70 ± 0.39^{aA}	16.17 ± 0.05^{aA}	16.15 ± 0.32^{aA}	16.06 ± 0.15^{aA}
F2P2	18.08 ± 0.14^{bA}	18.01 ± 0.09^{bA}	18.02 ± 1.06^{bA}	17.60 ± 0.62^{aA}	17.71 ± 0.45^{bA}
D1P1	23.11 ± 0.24^{cA}	23.18 ± 0.37^{cA}	23.06 ± 0.54^{cA}	23.27 ± 0.52^{bA}	23.16 ± 0.16^{cA}
D1P2	27.04 ± 0.06^{dA}	26.83 ± 1.71^{dA}	26.78 ± 0.55^{dA}	27.10 ± 1.45^{cA}	27.04 ± 0.28^{dA}
D2P1	16.04 ± 0.18^{aA}	15.70 ± 0.32^{aA}	16.01 ± 0.12^{aA}	16.05 ± 0.33^{aA}	16.07 ± 0.26^{aA}
D2P2	18.04 ± 0.07^{bA}	17.96 ± 0.09^{bA}	18.09 ± 1.02^{bA}	17.56 ± 0.71^{aA}	17.77 ± 0.41^{bA}

[1]*Abbreviations:* F1P1 = 5% Fig syrup + 0.5% Pectin, F1P2 = 5% Fig syrup + 1% Pectin, F2P1 = 10% Fig syrup + 0.5% Pectin, F2P2 = 10% Fig syrup + 1% Pectin, D1P1 = 5% Date syrup + 0.5% Pectin, D1P2 = 5% Date syrup + 1% Pectin, D2P1 = 10% Date syrup + 0.5% Pectin, D2P2 = 10% Date syrup + 1% Pectin. The different small letters indicate statistically significant differences in columns ($p < 0.05$). The different capital letters indicate statistically significant differences between days in each sample ($p < 0.05$).

Syneresis of the flavored drinking yogurt. The results of the syneresis of all the flavored drinking yogurt samples are presented in Fig. 1. The results of syneresis percentage of the samples revealed that by increasing the amount of pectin and syrup, the syneresis of the flavored drinking yogurt samples significantly decreased ($p > 0.05$). The similar results reported by Amerinasab et al. [38] show that low syneresis at an optimum date liquid syrup (DLS) content (6%) in DLS-fortified yogurt can be attributed to a high water binding capacity of fructose monosaccharide in DLS composition. In addition, Kumar and Mishra [39] reported that the use of pectin in mango soy fortified set yogurt significantly reduced the syneresis. It seems that stabilizers like pectin form a hydrocolloid network which entraps casein and water in the network and prevents syneresis. The data showed that fig syrup significantly decreased the syneresis of samples compared to date syrup ($p > 0.05$). This might be due to high fiber in syrups especially in fig syrup that have a higher water-binding capacity and absorb the water.

Table 4. Mean ± SEM viscosity (cP) of various flavored drinking yogurt samples during storage up to 28 days

Samples[1]	Time				
	Day 0	Day 7	Day 14	Day 21	Day 28
F1P1	123.00 ± 1.00^{cE}	112.67 ± 0.58^{cD}	96.67 ± 0.58^{bC}	73.33 ± 0.58^{bB}	42.67 ± 0.58^{bA}
F1P2	166.67 ± 0.58^{fE}	156.00 ± 2.00^{fD}	133.67 ± 0.58^{eC}	112.33 ± 0.58^{eB}	81.00 ± 1.00^{eA}
F2P1	144.67 ± 0.58^{eE}	136.00 ± 1.00^{eD}	121.00 ± 1.00^{dC}	98.33 ± 0.58^{dB}	66.33 ± 1.53^{dA}
F2P2	203.00 ± 1.00^{hE}	186.00 ± 3.61^{hD}	167.67 ± 1.15^{gC}	137.00 ± 1.00^{gB}	96.67 ± 0.58^{fA}
D1P1	101.67 ± 1.15^{aE}	90.67 ± 1.15^{aD}	73.00 ± 0.00^{aC}	50.67 ± 0.58^{aB}	22.67 ± 1.53^{aA}
D1P2	141.33 ± 1.15^{dE}	132.33 ± 0.58^{dD}	117.67 ± 0.58^{cC}	82.67 ± 1.53^{cB}	59.67 ± 1.53^{cA}
D2P1	119.00 ± 1.00^{bE}	109.67 ± 0.58^{bD}	96.00 ± 1.00^{bC}	75.00 ± 0.00^{bB}	44.00 ± 1.00^{bA}
D2P2	181.00 ± 1.00^{gE}	171.00 ± 1.00^{gD}	153.33 ± 1.15^{fC}	121.67 ± 2.08^{fB}	82.67 ± 1.53^{eA}

[1]*Abbreviations:* F1P1 = 5% Fig syrup + 0.5% Pectin, F1P2 = 5% Fig syrup + 1% Pectin, F2P1 = 10% Fig syrup + 0.5% Pectin, F2P2 = 10% Fig syrup + 1% Pectin, D1P1 = 5% Date syrup + 0.5% Pectin, D1P2 = 5% Date syrup + 1% Pectin, D2P1 = 10% Date syrup + 0.5% Pectin, D2P2 = 10% Date syrup + 1% Pectin. The different small letters indicate statistically significant differences in columns ($p < 0.05$). The different capital letters indicate statistically significant differences between days in each sample ($p < 0.05$).

Table 5. Mean ± SEM general acceptance scores of various flavored drinking yogurt samples during storage up to 28 days, n=15

Samples[1]	Time			
	Day 0	Day 7	Day 14	Day 21
F1P1	3.40 ± 0.51^{abC}	3.20 ± 0.68^{aC}	2.60 ± 0.51^{bB}	2.13 ± 0.74^{abA}
F1P2	3.67 ± 0.49^{abC}	3.40 ± 0.63^{aBC}	3.13 ± 0.35^{dfAB}	2.80 ± 0.86^{cdA}
F2P1	3.60 ± 0.51^{abC}	3.33 ± 0.72^{aBC}	3.07 ± 0.70^{cdfAB}	2.67 ± 0.62^{bcdA}
F2P2	3.80 ± 0.41^{bC}	3.53 ± 0.64^{aBC}	3.20 ± 0.68^{fAB}	2.93 ± 0.59^{dA}
D1P1	3.27 ± 0.46^{aB}	3.07 ± 0.59^{aB}	2.13 ± 0.64^{aA}	1.93 ± 0.59^{aA}
D1P2	3.53 ± 0.52^{abB}	3.20 ± 0.56^{aB}	2.73 ± 0.46^{bcdA}	2.40 ± 0.74^{abcdA}
D2P1	3.40 ± 0.51^{abB}	3.13 ± 0.52^{aB}	2.67 ± 0.49^{bcA}	2.27 ± 0.70^{abcA}
D2P2	3.60 ± 0.51^{abC}	3.27 ± 0.70^{aBC}	2.87 ± 0.35^{bcdeAB}	2.53 ± 0.64^{bcdA}

[1]*Abbreviations:* F1P1 = 5% Fig syrup + 0.5% Pectin, F1P2 = 5% Fig syrup + 1% Pectin, F2P1 = 10% Fig syrup + 0.5% Pectin, F2P2 = 10% Fig syrup + 1% Pectin, D1P1 = 5% Date syrup + 0.5% Pectin, D1P2 = 5% Date syrup + 1% Pectin, D2P1 = 10% Date syrup + 0.5% Pectin, D2P2 = 10% Date syrup + 1% Pectin. The different small letters indicate statistically significant differences in columns ($p < 0.05$). The different capital letters indicate statistically significant differences between days in each sample ($p < 0.05$).

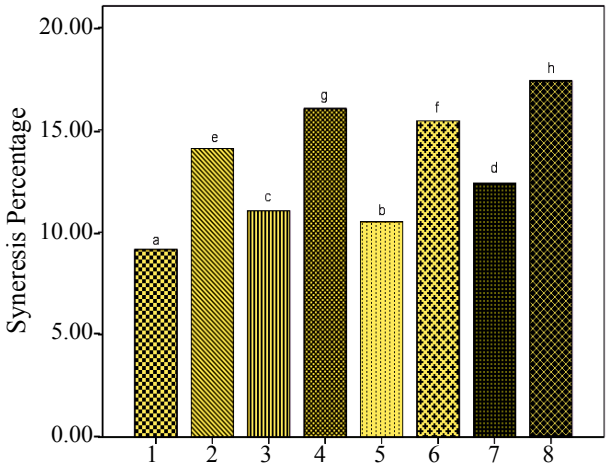

Fig. 1. Syneresis percentage of various flavored drinking yogurt samples at day 28 of storage. (1) 10% Fig syrup + 1% Pectin, (2) 5% Fig syrup + 1% Pectin, (3) 10% Fig syrup + 0.5% Pectin, (4) 5% Fig syrup + 0.5% Pectin, (5) 10% Date syrup + 1% Pectin,(6) 5% Date syrup + 1% Pectin, (7) 10% Date syrup + 0.5% Pectin, (8) 5% Date syrup + 0.5% Pectin. The different letters indicate statistically significant differences ($p < 0.05$).

General acceptance evaluation. However the samples with high syrup and pectin presented higher preference scores, no significant differences were found between them ($p > 0.05$) (Table 5). But there was a gradual decrease in the general acceptance score of samples over 21 days ($p > 0.05$). This phenomenon may be due to an increase in acidity followed by a decrease in viscosity. It seems that panelists prefer samples with a higher viscosity and a better texture. The lowest scores for the overall acceptability of samples could probably be due to their high syneresis in the results of low firmness and viscosity [38]. Keshtkaran et al. [26] reported that any increase in viscosity had a positive effect on the rate of the general acceptance of date milk drink. In addition, Dalim et al. [2] confirmed that the general acceptance of a banana milk drink was higher than a Chico drink because of its higher viscosity.

Today, a variety of additives such as chocolate, honey, strawberries and etc. are used to improve the taste of milk and different types of dairy based healthy drinks. Therefore, the present study used different percentage of fig and date syrups as natural sweeteners to make flavored drinking yogurt in order to remove the sugar. The study was made of the comparing physicochemical properties and general acceptance of flavored drinking yogurt with date and fig syrups.

In general, this study showed that samples contained fig syrup had a lower acidity and syneresis percentage while their viscosity was higher compared to the samples that contained date syrup. Hence, it can be concluded that flavored drinking yogurt with 10% fig syrup and 1% pectin (F2P2) was chosen as the best treatment since it was rated the highest in general acceptance and it had a longer shelf-life compared to other samples.

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NEW APPROACHES TO CREATING FUNCTIONAL PRODUCTS FOR A CLOSED MILK-POLYSACCHARIDE SYSTEM

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Abstract: The development and production of new functional products is a priority for the development of the food industry. At the same time, the quality and biological value of raw materials are of particular importance. From this point of view, when processing milk, the use of the process of membraneless osmosis using polysaccharides is promising. It allows to obtain dairy fractions of a high nutrition value that can be used as the main raw materials in the technology of functional products. The paper aims at developing a functional dessert based on a protein and lipid fraction (PLF) obtained by the fractionation of whole (or normalized) milk using the apple pectin manufactured in Russia. Technological parameters of milk fractionation by pectin have been studied and the conditions under which the process is most efficient have been determined. The most significant parameters were the milk pasteurization temperature (not lower than 85°C) and the concentration of pectin in the system (0.6–0.65 kg per 100 kg of milk). The recommended fractionation method is "cold" at a temperature of 4–6°C. Based on the technological parameters of fractions, depending on the conditions of the process, a method has been proposed for calculating the necessary content of the mass fraction of fat in raw materials to be regulated in PLF. As a practical implementation of the process of fractionation of normalized milk raw materials, a formulation of a new dessert that contains milk fat and protein in the native form has been developed. The functional value of the dairy dessert is provided by a complete replacement of granulated sugar with raw honey, using a starter culture with a probiotic microflora, and concentrating the product with sesame seeds. The study of the biological value of the dessert showed that it fully meets the requirements for healthy food.

Keywords: Pectin, raw honey, fractionation, flocculation, protein and lipid fraction, sesame

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INTRODUCTION

At the present time, functional products are the fastest growing segment of the food industry in the world and dairy products are in the first place among them [1, 2]. In the Russian Federation, for the majority of the population, sweet desserts are the most attractive dairy products, they are widely used and are in great demand with various groups of the population and the market of these products has been growing rapidly in recent years. The necessary sweetness of desserts is normally provided by adding refined sugar in the amount of 10–12% to the formulation of the product. The excessive use of refined sugar leads to the development of hyperglycemia, the development of diabetes, obesity, etc. It is the mentioned negative properties of sucrose that make it necessary to develop new products with a sweet functional taste, but without the negative properties of sucrose [3, 4].

The presented study aims at creating a fermented functional product with a complete replacement of sucrose with raw honey that meets the objectives of the state policy in the field of healthy nutrition. The aim of the study is the development of a functional dessert based on the protein and lipid fraction obtained by fractionation of whole (or normalized) milk by apple pectin.

To obtain the protein and lipid fraction (PLF) of milk, the membraneless fractionation of dairy raw materials was used with the help of the apple pectin manufactured in Russia. The possibility of flocculation (isolation) of casein from dairy raw materials using polysaccharides has been repeatedly proved by the studies of V. V. Molochnikov and other scientists. The main advantages of the process are low energy costs, lack of denaturing changes in the system, both fractions (the whey-polysaccharide and protein fraction) are an excellent basis for obtaining functional products [5–8].

The objectives of the study are to optimize the process of fractionation of whole milk (normalized in fat) by apple pectin, to study the physico-chemical and technological properties of the protein and lipid fraction, to select the formulation components for the production of functional desserts, to study the composition and to estimate the functional value of the product obtained.

The developed product is intended to improve the structure of the population's nutrition, contains probiotics and is enriched with vital natural minerals

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and vitamins. It does not contain refined sugar, the necessary sweetness is provided by raw honey and, due to its high sweetness, twice as little granulated sugar is required. Honey, in addition to the high content of monosaccharides (glucose and fructose in the amount of more than 70% of the mass of honey), contains about 400 different substances - vitamins, enzymes, proteins, minerals, therapeutic and flavoring substances. Thanks to the combination of flavor and sweetness of sugars and the acidity of the organic acids therein, honey is a good combination with dairy components [9].

The original attractive properties of the product will be provided by adding sesame to the formulation. Sesame seeds additionally enrich the cream dessert with a wide range of such mineral elements as silicon, copper, calcium, nickel, iron, phosphorus, magnesium, zinc, selenium and others, as well as polyunsaturated fatty acids, protein and vitamins [10].

The dessert will help to strengthen the health of the population, prevent the diseases caused by poor and unbalanced nutrition, its production is focused on domestic raw materials.

OBJECTS AND METHODS OF STUDY

Study objects are cow raw milk (GOST 31449-2013, TR TS 033/2013), whole and normalized milk with a mass fraction of fat from 0.5 to 2.0%; pectin (TU 9199-012-01014470-04 “Apple pectin, dietary food supplement”), manufactured in Belgorod region, a concentrated solution of protein and lipid fraction (PLF); raw honey (GOST R 54644-2011); sesame seeds (GOST 12095-76); the starter culture “BioMatrix-LB1” (*Str. thermophilus, Lac. lactis subsp diacetilactis, Lac. acidophilus, L. plantarum, L. fermentum, L. caseisubsp rhamnosus, B. bifidum, B. longum, B. adolescentis.* Bioproduct, LLC), the direct-set yoghurt starter culture YO-MIX™ (*Str. thermophilus, Lac. lactissubsp. lactis, Lac. bulgaricus.* Danisco), the direct-set starter culture “Sour cream vivo” (*Str. Salivarius subsp. thermophilus, Lac. Lactis subsp. lactis, Lac.lactissubsp. cremoris, Lac.lactissubsp. diacetylactis.* VIVO, Ukraine).

When carrying out a complex of physical and chemical studies and studying the properties of objects, standard and common methods were used:

– the determination of the mass fraction of moisture and solids using an infrared thermogravimetric method by means of the moisture content analyzer "Evlas-2M" and using a thermogravimetric method by means of the dryer "APS-1" Analit-Servis, and using an arbitration method - drying the weighed quantity to a constant mass in a drying cabinet at a temperature of 102–105°C;

– the determination of the content of solids in the whey-polysaccharide fraction using the refractometer "IRF-454 B2M" (Komz, JSC);

– the determination of the mass fraction of fat using the Gerber method (an acid based method);

– the determination of the mass fraction of protein according to Kjeldahl;

– the determination of the calcium content using a complexometric method;

– the determination of dynamic viscosity using an Ostwald viscometer;

– the determination of active acidity using the pH-meter/ionometer IPL-201 (MULTITEST "Semiko");

– the determination of the composition of raw and pasteurized milk using an ultrasonic analyzer "Laktan1-4" (Sibagropribor, Russia);

– the determination of titrable acidity by titration;

– the determination of the lactose content using an iodometric method;

– the organoleptic indicators of the product were determined by means of a tasting assessment of experts on a common 10-point scale.

The content of protein, vitamins, macro- and micronutrients was determined in the testing laboratory of Belgorod SAU, registered in the Russian state register that meets the requirements of GOST R ISO/IEC 17025-2006, accredited in the system of accreditation of analytical laboratories.

A 5% pectin aqueous solution prepared by dissolving pectin powder in hot water at a temperature of 70–72°C, filtered through a screen filter and cooled to a temperature of 20–25°C was used in the studies.

The content limit of pectin necessary for the flocculation of the protein and lipid fraction was determined using the whole milk pasteurized at 85°C with a pectin content from 0.2 to 0.8 g at a pitch of 0.2 g per 100 g of milk in terms of dry powder. The temperature of the components (milk and a 5% aqueous pectin solution) was 20–25°C when mixing, the mass of the mixture of each sample was 200 g. 30 cm^3 of mixture were dispensed from each sample into two rows of biological test tubes (three replications). The estimation of efficiency of milk fractionation was controlled by the height of the layer of the separated whey-polysaccharide fraction (WPF) expressed as a percentage of the total height of the mixture in a test tube in two cases of process temperatures - 4–6°C and 40°C.

To estimate the effect of the pasteurization temperature and the temperature of the fractionation process, pectin was applied into the samples of the milk pasteurized at temperatures of 75, 80, 85, 90°C, cooled to a temperature of 20–25°C, based on its content of 0.65 g in 100 g of milk (on a dry basis) in the form of a 5% aqueous solution at the same temperature, the mixture was well mixed. The efficiency of flocculation of PLF was controlled according to the procedure described above in two cases of the process temperatures of 4–6°C and 40°C.

The effect of the mass fraction of fat of a normalized mixture on the fractionation process was studied as follows. Whole milk was normalized using skim milk to a mass fraction of fat of 0.5; 1.0; 1.5 and 2.0%. Further on, the samples of the same mass were pasteurized at 85–87°C without holding and cooled to 20–25°C, a 5% pectin solution at a temperature of 20–25°C was applied at the rate of 0.65% of dry powder to the mass of milk. The samples were thoroughly mixed and left for 3 hours at a temperature of 4–6°C. The WPF and PLF obtained in the field of gravitational forces were separated by decanting and the physico-chemical indicators of fractions were analyzed.

To study the process of fermentation of the protein and lipid fraction of milk using a fermented microflora, the samples were prepared according to the following pattern. Raw honey was applied into the freshly prepared PLF solution, the mixture was pasteurized at a temperature of 72–74°C with holding it for 20 seconds and cooled to the fermentation temperature appropriate for each kind of starter cultures: "BioMatrix-LB" - 38–39ºC, YO-MIX™- 40–41ºC and "Sour cream vivo" - 27–28ºC. The samples were thermostated in the same temperature conditions. The intensity of the acid formation process was determined by measuring the titrable acidity at the fixed intervals during the process of fermentation.

The proportion of components in the formulation (PLF, honey, a probiotic starter culture, sesame) was determined with a focus on the organoleptic characteristics of the product.

The biological value of cream dessert was characterized by the content of protein, fat, calcium, phosphorus, vitamins A, E, C and such micronutrients as zinc, iron and copper.

RESULTS AND DISCUSSION

Technology of production, technological and physico-chemical properties of the protein and lipid fraction. The main raw material for the development of a functional dessert is the protein and lipid fraction, isolated from pasteurized milk with various fat contents with the help of apple pectin. The flocculation of PLF is based on the colloidal incompatibility of a highly hydrophilic pectin colloid with hydrophobic milk components - casein micelles, colloidal calcium phosphate and fat globules, which manifests itself in a narrow zone of low pectin concentration. The flocculation process is reversible and is not succeeded by the coalescence process (irreversible changes) - the PLF concentrate holds its homogeneous structure characteristic of solutions.

The optimal content of apple pectin of the indicated brand for an effective flocculation of PLF was determined using the whole milk pasteurized at a temperature of 85°C (without holding) with a pectin content from 0.2 to 0.8 g at a pitch of 0.2 g per 100 g of milk in terms of dry powder. Table 1 presents the effect of pectin concentration on the efficiency of fractionation of pasteurized whole milk.

As shown by the results, the effective fractionation of whole and the previously studied skim milk requires a pectin content in the range of 0.6–0.65 g per 100 g of milk in terms of the dry weight of powder. The apple pectin was applied as an aqueous solution. More technologically advanced in viscosity for industrial use is a 5% solution prepared by dissolving pectin powder in hot water at a temperature of 70 ... 72°C with continuous stirring followed by its cooling and the possibility of using it for 72 hours when stored at 4–8°C. When the pectin content is reduced to 0.4% to the mass of the mixture, the rate and efficiency of the process of PLF flocculation decreases, and fractionation completely deceases when the content is 0.2%. When the content of pectin is 0.8% in terms of dry pectin powder, two processes simultaneously proceed: the flocculation of PLF and the partial peptization of PLF concentrate with distinct WPF globules in its structure.

The technology for isolating PLF for the purpose of optimizing the process was studied using bulk whole milk. The composition of whole milk is the following: fat is $3.6 \pm 0.12\%$, MSNF is $8.25 \pm 0.05\%$, protein is $2.96 \pm 0.05\%$, calcium is 146 ± 2 mg% and pH is 6.68 ± 0.04.

Pasteurized milk was used at temperatures in the range of 75–90°C at a pitch of 5°C. Two temperature fractionation options were studied: at a temperature of 4–6°C (in a refrigerator) and at 40°C (in a thermostat).

Fig. 1 clearly presents the results of the analysis.

Table 1. Effect of the pectin content on the efficiency of fractionation of pasteurized whole milk (n = 3, V < 5%)

Time from the beginning, h	The fractionation temperature is 4–6°C				The fractionation temperature is 40°C			
	Pectin, g per 100 g of milk				Pectin, g per 100 g of milk			
	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8
	% of the segregated whey (WPF)				% of the segregated whey (WPF)			
0.5	0	0	65.0	trace amounts	0		82.6	52.2
1	0	indistinct border	80.0	indistinct border	0	81.8 turbid WPF	82.6	65.2 in PLF WPF droplets
2	0	78.3 turbid WPF	80.0	70.0	0	81.8 turbid WPF	82.6	69.6 in PLF WPF droplets
3	0	78.3	80.0	75.0	0	81.8 turbid WPF	82.6	73.9 in PLF WPF droplets

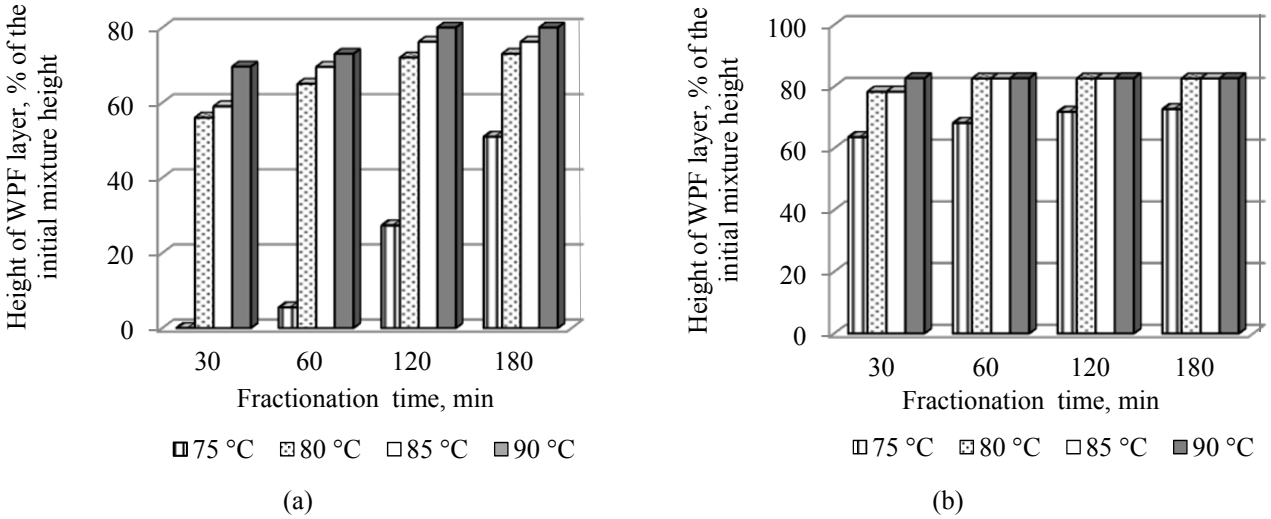

Fig. 1. Effect of the pasteurization temperature of whole milk on the fractionation process: (a) at a temperature of 4–6°C and (b) at a temperature of 40°C.

It follows from the results that with an increase in the temperature of whole milk pasteurization, the efficiency of the fractionation process increases. The mode of milk pasteurization at a temperature of 75°C for the fractionation of whole milk at a temperature of 4–6°C is unacceptable (raw milk is not practically fractionated by pectin). The high-temperature pasteurization of whole milk at a temperature of more than 80°C increases the efficiency of its fractionation with the use of pectin. The observed result can be explained by the denaturation of whey proteins and the transition of part of the soluble calcium phosphate salts into a colloidal form. These processes lead to an increase in the mass of particles, to the compression of the double electrical layer of membranes and a decrease in the electrokinetic potential both of casein micelles and fat globules. It results in a decrease in the aggregative (sedimentative) and kinetic stability of these components. The fat is almost completely displaced by pectin into a protein concentrate without a boundary surface between the lipid and protein layers, a common homogeneous concentrated solution of the rich white color is formed. The process proceeds faster at a fractionation temperature of 40°C due to a decrease in the viscosity of the medium.

The physico-chemical characteristics of the process of fractionation of whole milk can be seen more clearly from the physico-chemical indicators of WPF. Table 2 presents the effect of the pasteurization temperature of whole milk on the physico-chemical indicators of WPF after 3 hours from the beginning of the process for the fractionation conditions at a temperature of 4–6°C. Table 3 presents the physico-chemical parameters of WPF after 3 hours from the beginning of the process for the fractionation conditions at a temperature of 4–6°C.

Table 2. Effect of the temperature of whole milk pasteurization on the physico-chemical indicators of WPF for the conditions of milk fractionation at a temperature of 4–6°C (n = 3, V < 5%)

Milk pasteurization temperature, °C	Physico-chemical indicators of WPF				
	Mass fraction, g per 100 g			Calcium, mg per 100 g	Active acidity, pH
	solids	fat	crude protein		
80	6.41 ± 0.02	0.04 ± 0.01	0.75 ± 0.04	63 ± 3	6.31 ± 0.04
85	6.39 ± 0.02	0.02 ± 0.01	0.60 ± 0.04	55 ± 5	6.30 ± 0.04
90	6.38 ± 0.02	0.02 ± 0.01	0.60 ± 0.04	45 ± 5	6.30 ± 0.04

Table 3. Effect of the temperature of whole milk pasteurization on the physico-chemical indicators of WPF for the conditions of milk fractionation at a temperature of 40°C (n = 3, V < 5%)

Milk pasteurization temperature, °C	Physico-chemical indicators of WPF				
	Mass fraction, g per 100 g			Calcium, mg per 100 g	Active acidity, pH
	solids	fat	crude protein		
80	6.64 ± 0.02	0.03 ± 0.01	0.75 ± 0.04	79 ± 3	6.20 ± 0.04
85	6.58 ± 0.02	0.01 ± 0.01	0.60 ± 0.04	60 ± 5	6.19 ± 0.04
90	6.57 ± 0.02	0.01 ± 0.01	0.60 ± 0.04	52 ± 5	6.19 ± 0.04

It can be seen from the results of Tables 2 and 3 that the fat content in WPF is independent from the fractionation temperature at a level of trace amounts. The milk pasteurization temperature and the conditions for the process of fractionating whole milk using apple pectin effect the calcium content in WPF and the pH of the medium. With an increase in the milk pasteurization temperature, the content of calcium in WPF decreases, which is a consequence of the transition of some of the soluble calcium salts and colloidal calcium hydrogen phosphate caused by a thermal effect on milk into insoluble calcium orthophosphate salt. In case of cold fractionation (the mixture temperature is 4–6°C), in comparison with raw milk (146 ± 2 mg%), the calcium content is reduced by 57–70% within the limits of milk pasteurization standards of 80–90°C. When fractionating warm milk (the temperature is 40°C), the active acidity in WPF is increased by 0.1 U pH and a larger amount of calcium remains in comparison with the WPF obtained by cold milk fractionation. It is known that even a slight acidification of the medium shifts the salt balance of milk towards soluble calcium salts, which leads to an increase in the calcium content in WPF by approximately 10 mg%.

As the pasteurization temperature rises, the residual protein content in WPF decreases, reaching the minimum threshold of 0.6%, which is 20% of the total protein content in milk. At a temperature of 85°C, the denaturation of whey proteins practically ends, which explains the same (0.6%) protein content in the WPF isolated from pasteurized milk at 85°C and at 90°C.

The technological characteristics of the fractions (WPF and PLF), isolated from whole milk with a pectin content of 0.60–0.65 g per 100 g of milk in terms of dry pectin powder, were studied for the process conditions: the milk pasteurization temperature is 85–87°C without holding, the mixing temperature of components (milk and a pectin solution) is 20–25°C, the duration of fractionation is 3 hours. With an increase in the duration of fractionation, the composition of fractions remains practically unchanged. The separation process ends when the osmotic pressure is balanced at the boundary of the layers. Table 4 presents the obtained results.

It follows from the results of Table 4: when fractionating cold (the temperature is 4–6°C) whole milk with apple pectin, the yield of PLF increases by 4.5% when the solids content is reduced by 1.8% in comparison with the fractionation of warm milk (the temperature is 40°C). The degree of fat concentration was 4 times when fractionating cold milk using pectin, and 4.3 times when fractionating warm milk. When fractionating warm milk using apple pectin (the temperature is 40°C), the calcium content in PLF decreases. The WPF indicators change: the content of calcium, the content of solids and the active acidity increase by 0.1 U pH in comparison with milk fractionation at a mixture temperature of 4–6°C. These indicators indirectly show the growth of the thermophilic microflora.

In the further studies, the milk was fractionated using apple pectin at a mixture temperature of 4–6°C. The recommended mixing temperature for milk and a pectin solution is 20–25°C, a "cold" method of fractionation method at a temperature of not higher than 8°C for 3–4 hours is used. The process termination indicators are a clear light yellow WPF solution in the upper part (the density is 1025–1026 kg/m^3, the solids content is not more than 6.4–6.6%) and a bright white layer of a concentrated PLF solution, well separable by decanting, at the bottom.

A study has been carried out on the effect of the normalizable mass fraction of milk fat in the range from 0.5 to 2% at a pitch of 0.5% on the technological and physico-chemical indicators of PLF when fractionating it with apple pectin in the conditions of the parameters specified above.

Table 5 presents the effect of the normalizable mass fraction of milk fat on the technological and physico-chemical indicators of PLF and WPF. The parameters of the fractionation process are the following: the titrable acidity of milk is 16–18°T, the milk pasteurization temperature is 85–87°C, the pectin content is 0.65% in terms of dry powder, the fractionation temperature is 4–6°C and the fractionation duration is 3 hours.

When comparing the results of the study of the presented paper with the earlier studies on the fractionation of skim milk using apple pectin of the same grade, some facts come under notice. The optimal content of pectin per 100 g of milk, regardless of the mass fraction of fat, is 0.6–0.7 g (based on dry pectin powder). The mass of the protein and lipid fraction isolated from 100 g of milk with fat is less (16.8–19.6 g) than the mass of the natural casein concentrate (NCC) isolated from 100 g of skim milk (20–22 g). That is, the fat phase promotes the displacement of water from the PLF layer and an increase in the content of solids therein. The content of solids is 27-28% in PLF and 23–25% in NCC.

Table 4. Technological characteristics of the fractions (WPF and PLF) isolated from the whole milk pasteurized at a temperature of 85–87°C without holding (n = 3, V < 5%)

Fractionation temperature, °C	Fraction type							
	Whey-pectin fraction (WPF)				Protein and lipid fraction (PLF)			
	Mass yield, %	Ingredients in 100 g of WPF			Mass yield, %	Ingredients in 100 g of PLF		
		solids, g	fat, g	calcium, mg		solids, g	fat, g	calcium, mg
4–6	78.1 ± 0.5	6.38 ± 0.04	0.05 ± 0.01	50 ± 5	21.9 ± 0.5	25.2 ± 0.4	14.4 ± 0.5	240 ± 20
40	82.6 ± 0.5	6.57 ± 0.04	0.03 ± 0.01	56 ± 5	17.4 ± 0.5	27.0 ± 0.4	15.5 ± 0.5	200 ± 20

Note. Content in bulk milk: fat is 3.60 ± 0.12%. MSNF is 8.25 ± 0.05%, protein is 2.96 ± 0.05%, calcium is 146 ± 2 mg% and pH is 6.68 ± 0.04.

Table 5. Technological and physico-chemical indicators of fractions (WPF and PLF), depending on the mass fraction of milk fat (n = 3, V < 5%)

Mass fraction of fat in normalized milk,%	Fraction type				
	Whey-pectin fraction (WPF)				
	Mass yield, %	Ingredients in 100 g of WPF			pH
		solids, g	fat, g	calcium, mg	
0.5	83.2 ± 0.5	6.2 ± 0.1	0.02 ± 0.01	65 ± 5	6.26 ± 0.01
1	82.8 ± 0.5	6.3 ± 0.1	0.02 ± 0.01	58 ± 5	6.25 ± 0.01
1.5	81.4 ± 0.5	6.4 ± 0.1	0.02 ± 0.01	54 ± 5	6.30 ± 0.01
2	80.4 ± 0.5	6.4 ± 0.1	0.03 ± 0.01	50 ± 5	6.33 ± 0.01
Protein and lipid fraction (PLF)					
0.5	16.8 ± 0.5	27. ± 0.2	3.2±0.1	290±5	6.22± 0.01
1	17.2 ± 0.5	27.7 ± 0.2	6.5±0.1	250±5	6.22± 0.01
1.5	18.6 ± 0.5	28.0 ± 0.2	8.5±0.1	230±5	6.24± 0.01
2	19.6 ± 0.5	28.2 ± 0.1	11±0.1	200±5	6.25± 0.01

Note. The change in the fat content within these limits did not practically effect the content of the rest of the indicators of normalized milk: MSNF is 8.70 ± 0.05%, protein is 2.96 ± 0.05%, calcium is 146 ± 2 mg% and pH is 6.68 ± 0.04.

Since pectin attracts the aquatic environment and keeps it well, there is a reason to assume that the process of concentrating of the hydrophobic components of milk during the fractionation of milk using pectin proceeds in a similar way to the process of removal of moisture from milk mixtures during canning. To calculate the degree of fat concentration during the fractionation of pasteurized milk with a normalized fat content, we calculated the fat content in 100 g of solid matter in PLF and the fat content in the solid matter of normalized milk. Taking into account that milk fat almost completely transits from milk to PLF, the degree of fat concentration (n) can be determined using the ratio of these indicators ($n = F_{sm}^{PLF} / F_{sm}^{milk}$).

With regard to choosing the type of functional product based on PLF, the ability to whip, the viscosity and the lactose content to identify the possibility of ripening was studied in addition to the normalization of the fat content in PLF. The studies were carried out using the PLF isolated from whole pasteurized milk at a temperature of 87°C with apple pectin. The conditions and parameters of the fractionation process are the same as in the studies above.

The ability of PLF for whipping was checked after preliminary holding it for 10–12 hours at a temperature of 4–6°C in order to stabilize the structure of proteins and fat and for the subsequent absorption of air bubbles when whipping. An effect of the disperser rotations of 500, 1000 and 2000 rpm on PLF was studied, the temperature of the PLF solution was 4–6°C. It should be noted that, in contrast to the natural casein concentrate obtained from skim milk, PLF does not have the ability to whip and foam regardless of the mass fraction of fat (1.5–11%). There is a process of aggregation of fat globules, like whipping cream into butter. We assume that the cause is the dehydration of fat globule membranes caused by pectin and the process of fat hardening within fat globules, leading to the deformation of phospholipid and protein membranes and the displacement of a part of the liquid fraction through the emerged microcracks of mebranes. If combined, it results in the absence of foam and the formation of small particles plastic by touch in the structure of PLF when whipping. The observed effect excludes a possibility of creating a whipped product based on PLF.

Table 6 presents the dynamic viscosity of PLF and the lactose content therein in comparison with raw milk.

The results indicate a high viscosity of the PLF solution, which is important for the development of structured products, and the sufficient lactose content necessary for the production of fermented product variants.

Development of the formulation and technology of the functional product "Cream-dessert" with honey and sesame. To produce PLF, pasteurized milk with the following composition was used at a temperature of 87°C without holding: the mass fraction of fat is 1%. MSNF is 8.6%, protein is 3.1%, pH is 6.72 (16–17°T), calcium is 146 mg% and apple pectin in the form of a 5% aqueous solution. The components were mixed at a temperature of 20–25°C, the amount of pectin to the milk mass was 0.65%, in terms of dry powder. The mixture was left for 3 hours at a temperature of 4–6°C, there was a distinct fractionation during this period: WPF is the whey portion of the mixture in the upper part of the tank and PLF is a concentrated white solution in the bottom of the tank.

We have made an attempt to determine the possible fat content in PLF by calculation. Based on the previous calculations (Table 5), a fact was revealed that the degree of fat concentration in the solid matter of PLF relative to fat in the solid matter of normalized milk ($n = F_{sm}^{PLF} / F_{sm}^{milk}$) during the fractionation of milk by 0.65% apple pectin (in terms of dry powder) is 2.0–2.2 times.

Table 6. Physico-chemical parameters of the whole milk pasteurized at 87°C

Study object	Dynamic viscosity, mPa·s	Relative viscosity of milk	Lactose, g per 100g of raw material
Milk	1.61 ± 0.05	1.0	4.55 ± 0.05
PLF	170.4 ± 0.6	105.8	2.3 ± 0.2

Table 7. Technological parameters of raw materials for a dessert with a mass fraction of fat of 6%

Ingredients in 100 g of raw material									
Pasteurized milk			WPF			PLF			
solids, g	fat, g	calcium, mg%	solids, g	fat, g	calcium, mg	yield, g	solids, g	fat, g	calcium, mg%
9.6 ± 0.1	1.0 ± 0.1	146 ± 2	6.3 ± 0.1	trace amounts	50 ± 5	18 ± 1	28 ± 1	6.0 ± 0.2	250 ± 20

Table 8. Organoleptic parameters of PLF with a different honey content

Indicators	Honey, g per 100 g of PLF				
	4	5	6	7	8
Color	Bright white, uniform throughout the mass				
Taste and smell	Creamy taste, sweetness and honey smells are imperceptible	Creamy taste, sweetness and honey smells are hardly perceptible	Creamy taste, sweetness and a pleasant smell of honey can be felt	Creamy taste, pronounced sweetness, smell and taste of honey	Mawkishly sweet, pronounced taste and smell of honey
Consistence	Homogeneous consistency, opaque bright white liquid similar to cream with 30% of fat in structure				

To calculate the possible fat content in PLF, a degree of fat concentration n = 2.1 has been set. The real composition of the milk used: 1% of fat and 9.6% of solids. The average content of solids in PLF on the basis of numerous studies is 28%. The fat content of the obtained liquid form of PLF should be equal to 6.1% as per calculation. The above calculation was confirmed experimentally, therefore, it is possible to normalize the fat content in raw materials and in the product. To develop the formulation, a use of PLF with a mass fraction of fat of 6% was assumed.

Table 7 presents the technological parameters of raw materials for a dessert with a mass fraction of fat of 6%.

To replace sugar in dessert with raw honey, the possible limits of its content in PLF with a mass fraction of fat of 6% have been determined. The sweetness and structure of the compositions that contain raw honey in the amount from 4 to 8 g of honey per 100 g of PLF at a pitch of 1 g was studied. The honey was kept in a thermostat at a temperature of 40-45°C for plasticity and was dissolved in PLF when stirring. Taking into account the high water-binding properties of carbohydrates and proteins, the obtained compositions for stabilizing the structure were held in the refrigerator at a temperature of 4–6°C for 12 hours. On expiration, the samples were left for 30 minutes at room temperature and were organoleptically estimated. Table 8 presents the organoleptic indicators of PLF with a different honey content.

A mixture with a mass fraction of honey of 7% had the best organoleptic characteristics.

The organoleptic properties of PLF, the high viscosity and sufficient lactose content served as an argument for choosing the type of product - "Cream-dessert" with honey and sesame fermented using a starter culture with probiotic microorganisms. The components of the product formulation were chosen taking into account its functional focus and organoleptic characteristics. The use of starter cultures that contain probiotic microorganisms is the most expedient and convenient way of enriching a dairy product. Probiotics, in case of the natural mode of administration, have beneficial effects on the physiological functions and biochemical reactions of the human body by optimizing its microbiological status [11, 12]. The addition of a plant supplement in the form of sesame to the protein and lipid milk basis allows to increase the nutritional value of the final product, to improve its mineral composition and to enrich it with valuable components [13].

The honey was applied in the amount of 7% to the mass of PLF in the process of gentle heat treatment of the freshly obtained PLF (72–74°C with holding for 20 sec). Two factors served as a basis for choosing a mode of heat treatment: the pre-heating of milk to a temperature of 87°C before the process of fractionation using pectin leads to the complete destruction of lipase and is complete for pasteurization efficiency; secondly, the effect of high temperatures is undesirable for retaining the natural properties of honey. Further on, PLF with honey was cooled to the fermentation temperature and a direct-set starter culture was applied. Fig. 2 presents the dynamics of fermentation using the starter cultures "BioMatrix - LB1", the yoghurt starter culture YO-MIX and "Sour cream vivo".

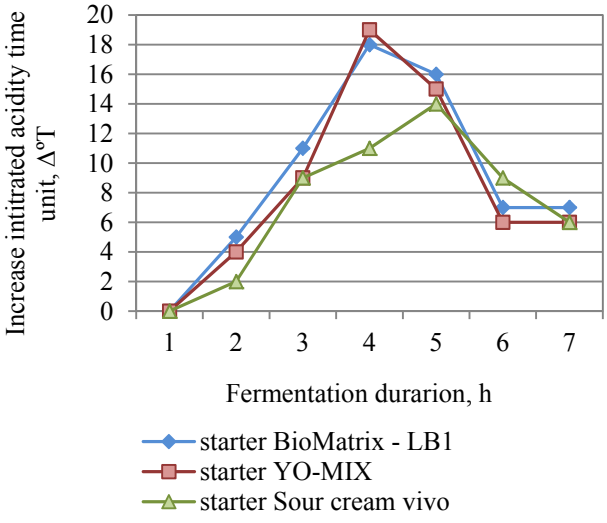

Fig. 2. Dynamics of the ripening process of the protein and lipid fraction of normalized milk.

In general, the process of fermentation of PLF using starters of lactic acid cultures proceeds in a similar way to the fermentation of any other dairy raw material. The presence of the lag phase, the period of active growth of microflora and the phase of extinction of a culture is typical. The formation of a dense curd for a yogurt starter culture and "BioMatrix-LB1" begins on average in 3.5 hours after the fermentation begins. For the starter culture "Sour cream vivo" the curd was formed 4 hours after the fermentation had begun. However, to get a more pronounced sour-milk taste, the duration of fermentation should be increased by 2–3 hours for all the crops. According to the results of the organoleptic estimation, the fermented milk product obtained using the starter culture "BioMatrix-LB1" had the most attractive taste.

The honey performs several functions in the obtained product: a natural effective sweetener, gives the product a special taste and smell, enriches the product with valuable ingredients inherent only in raw honey, and the fructose contained promotes the growth and preservation of the viability of bifidobacteria in the chosen probiotic starter culture.

The mass fraction of sesame was selected for the formulation of the product using a tasting assessment made by experts with respect to 5 approved descriptors: color, taste, consistency, aftertaste and smell. The descriptors were graded on a 10-point scale (Fig. 3).

The sesame was heat treated at 105–105°C for 5 minutes and was applied into the fermented protein and lipid base with honey in a cooled state. The product was stirred for 5 minutes and sent for ripening at a temperature of 4–6°C to provide the final formation of its structure and taste characteristics. The organoleptic estimation of the finished "Cream-dessert" was carried out at a temperature of 20–22°C. It has been found that the sample with a sesame content of 2% to the mass of PLF had the most attractive organoleptic indicators.

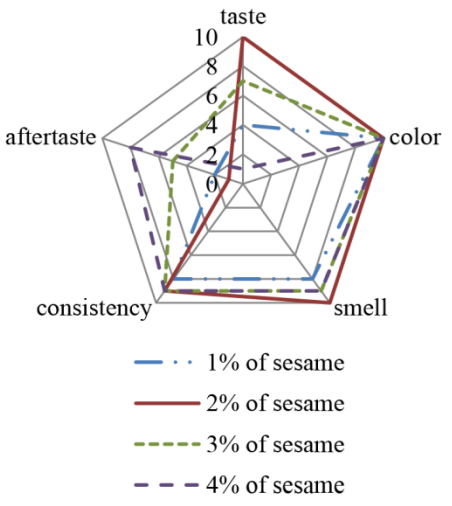

Fig. 3. Taste profile of the formulations of "Cream-Desert" with a different sesame content.

The production technology of "Cream-dessert" in production consists of the following operations. The derivation of a protein and lipid fraction: the normalization of whole milk to a mass fraction of 1% fat, the pasteurization at 87°C without holding, cooling to 20–25°C, applying a 5% pectin solution at a temperature of 20–25°C based on its content of 0.65% to the mass of milk on a dry basis, stirring for 15 minutes, cooling to 4–6°C and fractionating for 3 hours. The indicator of completion of the fractionation process is: a transparent WPF solution with a density in the range of 1025–1026 kg/m^3 and the content of solids in the range of 6.4–6.6%. The process of production of "Cream-dessert": the separation of PLF by decanting from the bottom of the tank, applying honey in the amount of 7% to the mass of PLF, the heat treatment of mixture at a temperature of 72–74°C with holding for 20 seconds, cooling to a fermentation temperature of 35–38°C, applying the starter culture "BioMatrix - LB1", the fermentation to a titrable acidity of 90–100°T, cooling to 20°C, applying sesame in the amount of 2% into the mass of mixture, product packing, biochemical and physical ripening at a temperature of 6–8°C for 12 hours, the technical and chemical control of the composition, quality and safety of the product, organoleptic estimation and implementation.

Based on the results of the organoleptic, physico-chemical and microbiological studies of the product, a shelf life is determined to be 10 days at a storage temperature of 6–8°C. Table 9 presents the resulting formulation of "Cream-dessert" with honey and sesame. Table 10 presents the characteristics of the composition "Cream-dessert" with honey and sesame.

As can be seen from the data obtained, the use of honey and sesame allows to enrich the product additionally with macro- and micronutrients and vitamins.

Table 9. Formulation of "Cream-dessert" with honey and sesame

Component	Mass fraction of the component kg per 100 kg of the product
PLF (the solids are 28–29%, the mass fraction of fat is 6%)	92
Raw honey (GOST R 54644–2011)	7
Starter culture as a bacterial concentrate that contains a complex of probiotic cultures of lactic acid bacteria	"BioMatrix-LB1", (direct-set)
Sesame seeds (GOST 12095-76)	2

Table 10. Chemical composition of "Cream-dessert" with honey and sesame

Indicator:	Ingredients in 100 g	
	PLF without fillers (as the control sample)	"Cream-dessert" with honey and sesame
Solids, g	28.6	30.2
Fat, g	6.0	6.2
Protein, g	12.5	13
Carbohydrates, g	2.3	8.5 incl. honey 6.0
Minerals:		
Ca, mg	250	273
P, mg	163	178
Mg, mg	19.4	25.9
Zn, mg	1.91	2.08
Fe, mg	0.75	1.05
Cu, mg	–	0.11
Vitamins:		
E, mg	0.26	0.33
A, mg	0.18	0.24
C, mg	1.48	6.34

Thus, the developed "Cream-dessert" has attractive organoleptic properties. It has a moderate titrable acidity (not higher than 100°T). It is characterized by a high content of milk protein, which provides the ratio of casein and whey proteins necessary for intake. Its content of carbohydrates is low for a sweet dessert, since the necessary sweetness is compensated by a high content of monosaccharides in honey (glucose and fructose). The portion of 200 g of the product provides 50% of the recommended daily intake of calcium and 34% of phosphorus for a person aged 25–50 [14].

CONCLUSION

The presented paper describes an innovative technology of protein dessert. Protein and fat raw material is concentrated simultaneously being effected by the normalized milk of apple pectin, while the bulk of the calcium of fresh milk is concentrated and transits into PLF, and therefore remains in the product. In the classical protein dessert obtained on the basis of cottage cheese, the bulk of calcium salts transits into whey effected by fermentation lactic acid, and micellar calcium casein phosphate remains practically only within the product.

The protein and lipid fraction is enriched with protein and milk fat in their natural (native) form. The previous high temperature of milk pasteurization (87–90°C) provides the enrichment of casein and fat globules with whey proteins, which significantly increases the protein efficiency ratio (PER). The selective flocculation of milk components by pectin enriches the content of macronutrients in raw materials (calcium, phosphorus and magnesium), micronutrients (zinc, iron and copper) and vitamins A, E and C. The product is additionally enriched with an appreciable quantity of micronutrients and vitamins due to applying raw honey and sesame seeds into it.

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FATTY ACID COMPOSITION OF MEAT FROM VARIOUS ANIMAL SPECIES AND THE ROLE OF TECHNOLOGICAL FACTORS IN TRANS-ISOMERIZATION OF FATTY ACIDS

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Abstract: In assessing the nutritional value of a food product, its fatty acid composition is important. Meat products contain essential fatty acids that positively influence human health, and saturated fatty acids, the high level of which does not allow them to be attributed to healthy nutrition. The article describes the use of raw meat from various species of animals and poultry according to their fatty acid composition; presents the possibility of changing the composition of fatty acids to produce healthy food. The presence of trans-isomer fatty acids (TIFA) in food products is the risk factor for human health. Meat raw materials contain trans fatty acids of natural and industrial origin. The results confirming trans fatty acids formation at the stage of animal growth (biohydrogenation and as the result of the use of growth promoters) and during meat processing (heat treatment at high temperatures, storage, use of sodium nitrite) are presented. At present, meat products are not considered to be a source of health-threatening TIFA, but the results presented in the review confirm the need for further research to identify factors that affect the fatty acids isomerization process in meat and semi- meat products and the normalization of their content in finished products.

Keywords: Meat raw materials, fatty acids, trans fatty acids, safety, trans-isomerization, biological efficacy

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INTRODUCTION

Fat is one of the main components of meat, upon which the nutritional value and functional properties of the finished product largely depend. Food product, depending on the characteristics of the fat contained in it (the level of content in the product, the qualitative and quantitative composition), can be attributed to the consumer product of functional, therapeutic and preventive action, or adversely affecting health. Along with the high level of saturated fatty acids, meat and meat products contain TIFA [1, 2]. Due to the negative influence of some TIFA on health [3, 4], in many countries around the world the issue of limiting their content in food products has been resolved at the legislative level. Various conditions have been determined that affect the formation of TIFA in meat products [5, 6, 7, 8, 9], depending on which the TIFA are divided into natural and industrial. Therefore, the problem of the normalization of natural TIFA, which are synthesized in the animal's organism and have a beneficial effect on human health remains important.

In order to recommend the use of meat raw materials for functional and dietary nutrition, the biological effectiveness of meat lipids of various species of productive animals and poultry was assessed, the indices of the content of TIFA in various types of meat were shown, and technological factors affecting their formation were revealed.

DISCUSSION

The quality of the fat tissue of a productive animal largely depends on the species of the animal, the breed, and the diet. The process of digestion and formation of tissues in ruminant and monogastric animals take place in different ways, as a result of which the composition of the fatty acids in the meat of these animals is not the same. In animals with a single-chamber stomach, fatty acids from the diet undergo slight transformation during digestion and absorption, therefore, the fatty acid composition of the tissues of animals with a single-chamber stomach can be regulated by the fat-acid composition of the diet. It is very difficult to predict the composition of fatty acids in ruminants. Attempts to modify the fatty acid profile require simultaneous study of both the diet of productive animals, especially ruminants, and the mechanisms of bioconversion in the stomach (rumen) of animals [10, 5].

Specialists of V.M. Gorbatov's All-Russian Meat Research Institute (VNIIMP) summarized and systematized the experimental data on the composition of fatty acids in various types of raw materials obtained from farm animals and birds [11]. For the analysis we used *m. longissimus dorsi* muscle tissue samples, selected from adults of sheep, pigs, cows, turkeys and horses. The obtained results showed the presence of significant qualitative and quantitative differences in the lipid composition of muscle tissue of various animal and poultry species: cattle, pigs, sheep, horses and turkeys (Table 1).

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Table 1. Fatty acid content in muscle lipid fraction of various animal species (% of total fatty acids)

Fatty acids	lamb	pork	beef	turkey	horse meat
SFA*	51.99	43.54	58.13	40.40	43.77
UFA*	30.3	47.45	37.80	40.91	45.14
MUFA*	23.55	36.59	30.55	28.63	22.97
PUFA*	6.75	10.86	7.25	12.25	22.17
PUFA/SFA	0.13	0.25	0.12	0.30	0.51
UFA/SFA	0.58	1.09	0.65	1.01	1.03
Elaidic acid (trans-9-$C_{18:1}$)	3.2	2.63	3.1	–	–
Arachidonic acid ($C_{20:4\omega6}$)	1.55	0.90	1.69	0.36	0.66
ω-3 PUFA	0.94	0.91	1.39	0.46	4.07
ω-6 PUFA	5.74	9.74	5.78	11.75	18.10
ω-6/ω-3	6.14	10.76	4.15	25.82	4.45

Note. * SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Saturated fatty acids (SFA) in animal raw materials are mainly represented by myristic, palmitic and stearic acids [11, 2, 12, 13, 6, 14, 15]. The lipid fraction of beef is characterized by the maximum level of SFA (58.13%), the lower content of SFA is in the lipid fraction of lamb (51.99%). Lipids of muscle tissue of pork and horse meat have approximately the same amount of these fatty acids (43.54 and 43.77%, respectively). The lowest content of SFA among the studied meat samples was obtained in the lipid fraction of turkey meat.

Monounsaturated fatty acids (MUFA) are basically palmitoleic, oleic fatty acids and its trans-isomer – elaidic fatty acid [11, 2, 12, 13, 6, 14, 15]. The lipid fraction of pork contains the highest level of MUFA (36.59%), then in descending order – the lipid fractions of beef < turkey < lamb < horse meat (Table 1).

Polyunsaturated fatty acids (PUFA) are mainly: linoleic, linolenic and arachidonic fatty acids [11, 2, 12, 13, 6, 14, 15]. The highest amount of PUFA is contained in the lipid fraction of horse meat (22.17%), almost three times less – in the lipid fraction of beef and lamb (7.25 and 6.75%, respectively) and about two times less – in the lipid fraction of pork (11.49%). In contrast to plant fats, animal fats are characterized by a high content of arachidonic acid (0.36–1.69%).

The balance of the fatty acid composition was evaluated by the ratio of PUFA/SFA, the optimum range of which should be from 0.2 to 0.4 and UFA/SFA – 2.3 : 1 [16]. The fatty acid composition of the lipids of the muscle tissue of pork, horse meat and turkey is more balanced than that of lamb and beef (see Table 1).

The optimal ratio of PUFA of the ω-6 and ω-3 family plays an important role in healthy nutrition. The diet is considered healthy if the ratio of ω-6 to ω-3 PUFA is 10 : 1, is considered to be a therapeutic nutrition with ratios ranging from 3 : 1 to 5 : 1. According to the data of Table 1, horse meat and beef meet the requirements for raw materials for preventive and therapeutic nutrition, all other types – for raw materials for general purpose products. Lipid fraction of horse meat is characterized by a high content of ω-3 PUFA. Belaunzaran et al. associate the accumulation of ω-3 PUFA in horse meat with a unique digestive system of horses [15].

The high content of oleic acid trans-isomer-elaidic acid (trans-9-$C_{18:1}$) [11] in the intramuscular fat of lamb (3.2%), beef (3.1%) and pork (2.63%) deserves attention. In deer fat, this indicator was 2.2%, in chicken – 1.4%, in maral fat – 0.9%, in wild boar fat – 0.4%, in seal fat – 0.3% [2]. In the lipid fraction of turkey meat and horse meat, this isomer was not detected [11]. Some researchers report a low content of TIFA in horse meat, the level of which depends on the diet [15]. In the meat of maral, elaidic acid was detected in two muscles of the three examined (quadriceps muscle – 0%, hip muscle – 1.68%, longissimus muscle – 1.04%). The content of TIFA in meat of kangaroo, african ostrich, mallard, pheasant, sea animals (Lahtak, walrus, seal, gray whale) was not determined [17].

The review, presented by Brazilian scientists, reports that the content of natural TIFA in food products (milk, lamb and beef) varies from 3 to 8% of the total amount of fatty acids [18]. According to Stender S. et al (2008), trans fatty acids of natural origin can account for up to 6% of fat in meat and dairy products obtained from ruminants (cattle, sheep, goats, buffaloes, deers, elks, giraffes and camels) [19].

In this paper, the authors adhere to the following definition of TIFA – a type of unsaturated fats being in the trans configuration, that is, having hydrocarbon substituents on opposite sides of the carbon-carbon double bond.

The studies of recent years in the field of TIFA in food products are focused on determining the conditions for their occurrence and their impact on human health.

Natural and industrial sources of TIFA have different effects on the human body. Natural trans-isomers are not a risk factor for cardiovascular diseases, because, unlike industrial ones, they do not contribute to the reduction of high-density lipoproteins and increase in low-density lipoproteins [20, 21] and are not associated with coronary heart disease [22].

Conjugated linoleic acid (CLA) has anticarcinogenic and anti-allergic properties [23]. CLA is a mixture of fatty acid isomers of the trans and cis configurations, and, therefore, it is difficult to establish the mechanism of action of each of these isomers on the immune function of humans and it is still unexplored. Among

them, some trans-isomer fatty acids have a positive effect on human health, others do not have biological activity, and some, like industrial trans fats, can be harmful to health [24].

In the CLA study, a mixture of cis-9-trans-11-CLA isomers (predominant in milk and beef) and trans-10-cis-12-CLA was used. In experiments on laboratory animals, evidence has been obtained that two isomers of CLA ($C_{18:2}$ cis-9, trans-11 and $C_{18:2}$ trans-10, cis-12) inhibit the carcinogenesis of the mammary gland and large intestine in animals. The mechanisms consist in the anti-angiogenic activity, induction of apoptosis in cancer cells and changes in fat metabolism [25]. In experiments on rats and chickens, CLA's ability to activate the immune system has been proven (no convincing human subject research has been conducted). The results of some studies of the effect of different CLA isomers on the immune function led to the assumption that the isomeric trans-10-cis-12-$C_{18:2}$ has the immunological activity, rather than cis-9-trans-11-$C_{18:2}$ [24]. In young rats, CLA can act as a growth factor and promote the release of energy from fat stores. Human subject research has shown that a diet rich in natural CLA leads to weight loss [5].

Rumenic acid (cis-9-trans-11-$C_{18:2}$) has an anti-cancer effect. The positive effect of its action on different types of cancer has been obtained: breast, skin, and gastrointestinal tract cancer. On the contrary, linoleic acid ($C_{18:2}$) exerts a stimulating effect on cancer cells. Scientists argue that the high level of consumption of CLA, and rumenic acid in its composition, provides antitumor protection due to the fact that this substance replaces linoleic acid in some lipoproteins, which in large quantities increases the risk of cancer in rodents. In experiments on animals, it has been shown that the addition of rumenic acid to feed reduces the formation of plaques in the aorta and reduces existing ones, significantly lowers the level of low-density lipoprotein cholesterol, inhibits the development of atherosclerosis, reduces inflammation [26, 27].

The precursor of rumenic acid - vaccenic acid (trans-11-$C_{18:1}$) also has a positive effect in oncology. Running an experiment one should take into account the fact that vaccenic acid is easily converted into rumenic, and its positive effects can be justified [28]. Final conclusions about the anti-cancer properties of these natural TIFA can be made after studying their influence on cancer risk markers in humans.

The results of studies of the effect of natural TIFA on health do not give an unambiguous answer to the question of the mechanisms of their action in comparison with industrial ones, but negative effects on the functions of the organism have not been established [27, 29], possibly because of their small content in products [20].

Unlike natural TIFA, the consumption of excessive amounts of non-natural, industrial TIFA with food leads to a risk of developing coronary heart disease, and more than any other source of nutrients, abdominal obesity, diabetes, Alzheimer's disease, breast cancer, reproductive harm, endometriosis and cholelithiasis [30, 31].

Consumption of TIFA during pregnancy and lactation period can lead to deviations from normal fetal and postnatal development, which can cause the development of metabolic diseases [32]. People who have low social status, who buy cheap food with a higher content of industrial trans fats fall into the risk group [33, 34, 35].

Based on the clinical trials conducted, WHO experts concluded that consumption of trans fatty acids increases the risk of cardiovascular disease and coronary heart disease due to adverse effects associated with increased levels of low-density lipoproteins, decrease in high-density lipoprotein levels, provocation of inflammation and endothelial dysfunction, and also influence on coagulability of blood, decrease in sensitivity of cells to insulin, replacement of essential fatty acids in cell membranes, which leads to dysfunction of processes associated with prostanoids and key functions performed by membranes [29].

There are several ways of forming TIFA in meat products: natural, as a result of synthesis in the rumen of the animal, and in the process of industrial processing of raw materials (industrial transisomers of fatty acids).

The natural way of forming trans-forms of fatty acids in meat raw materials is the biohydrogenation of unsaturated fatty acids under the influence of the microflora of the rumen. Mapiye C. *et al.* (2012) presented material on the mechanisms of synthesis of trans-isomers and ways to increase their content in beef [5]. In the rumen, the incoming feed lipids are hydrolyzed by plant or microbial lipases, resulting in the formation of free fatty acids. Free fatty acids are toxic to microorganisms, they are detoxified through biohydrogenation (combined isomerization of double bonds and hydrogenation). Rumen microorganisms (mainly bacteria), first of all, biohydrogenate the essential fatty acids (linoleic and linolenic fatty acids) to stearic acid ($C_{18:0}$). As a result of the experiments, it was found that behenic acid ($C_{22:0}$) is the main end product of the biohydrogenation of docosahexaenoic acid ($C_{22:6}$, DHA). Different conditions in the rumen can lead to a series of biohydrogenation intermediate substances with one or two double bonds with different cis and trans configurations.

At present, meat of animals grown using intensive technologies in industrial-scale volumes contains a lower level of natural cis and trans isomers, in contrast to the meat of a pasture grown animal. The feed system can regulate the level of fatty acids in meat. The proportion of linoleic acid isomers in meat raw materials can be increased using pasture management (0.59% for pasture management and 0.28% for stall barn housing). Moreover, the feed system at the final stage of store feeding affects the fatty acid composition of the meat and the level of isomer content of fatty acids more than at the initial stage [6].

To increase the CLA level in meat, linoleic acid-rich vegetable oils, sunflower seeds or safflower are added to the cattle diet in combination with a high concentrate feed (> 75% of the grain in dry matter). Combination of vegetable oils with grain leads to an increase in vaccenic

and rumen acids in beef. When feeding rations with high grain content, trans-10-$C_{18:1}$ (trans-isomer of octadecenic acid) is produced due to an increase in microbial metabolism in the rumen through an alternative pathway of biohydrogenation [5]. The introduction of flaxseed into the diet of small bulls leads to a significant increase in the content of CLA (cis-9, trans-11) [36].

The level of content, the type of concentrate and the degree of its processing affect the mechanism of biohydrogenation. Small grains, such as wheat and barley with a rapidly fermentable starch, stimulate the production of trans-10-$C_{18:1}$ fatty acids. Corn starch is more resistant to fermentation and can stimulate a greater accumulation of vaccenic and rumenic acids compared to starch from other cereals [5].

In the experiment of Uruguayan researchers on the influence of a different ratio of hay and concentrates (corn and soy) in the diet on the quality characteristics of meat and the fatty acid composition of intramuscular fat lambs of the Corydale breed, these data were not confirmed [37]. There was no increase in the trans-isomer content of oleic and linoleic fatty acids from a change in the proportion of cereal concentrates in the diet of lambs.

Regulation of the content of vaccenic acid (trans-11-$C_{18:1}$) is impossible by changing the profile of fatty acids (SFA, MUFA, PUFA). This was the conclusion of specialists of the Leibniz Institute of Animal Biology [38] in the store feeding experiment of bulls of the Holstein breed with a high proportion of linoleic and alpha-linolenic fatty acids in the diet. Regardless of the level of the MUFA and PUFA in the lipid composition of feed, the content of vaccenic acid was constant. Sausage and pate, produced from meat from grown animals, contained the same number of CLA isomers (cis-9, trans-11 and trans-10, cis-12) that meat raw materials. This indicates that the heat treatment, at which sausages and pates were produced, maintains the level of TIFA in finished products.

Work is under way to increase the content of trans-linoleic acids in livestock products, both through the use of vegetable fats, and through synthetic conjugates of linoleic acid. V.A. Matveyev, V.P. Galochkina *et al.* (2010) patented a method for increasing the intensity of growth of Kholmogorsky bull calves raised for meat production, using a biologically active substance (BAS) based on conjugated linoleic acid (CLA, $C_{18:2}$ cis-9, trans-11 isomer). BAS, added to the main diet, changed the biochemical processes in the body of the bull calves (due to the higher activity of the work of the Krebs cycle in the bulls' tissues compared to glycolysis) grown for meat production, and led to an increase in the intensity of their growth [39].

Growth promoters, widely used in modern technologies of growing animals, lead to an increase in both the proportion of trans-forms of fatty acids, and the emergence of mixed cis-, trans-forms [7]. As a result of the study of the effect of the use of the hormonal estrogen regulator diethylstilbestrol introduced into the pig's diet on the isomerization of fatty acids, it was found that animal lipids obtained by the action of forbidden to use diethylstilbestrol contained an increased content of TIFA and an appreciable amount of mixed cis and trans isomers. The authors conclude that a high content of TIFA in meat raw materials may indicate the use of metabolic growth regulators.

The processes of fat oxidation during storage lead to the formation of fatty acid isomers. At the initial oxidation period, the hydrogen atom is stripped from the fatty acid molecule to form a fatty acid radical, which is stabilized by delocalization via double bonds. This leads to a shift in the double bond, in the case of PUFA, by forming conjugated double bonds. Such a shift forms double bonds in both cis and trans configurations, with the predominance of the trans configuration due to its greater stability [40]. The process of storing meat raw materials contributes to an increase in the proportion of TIFA, as well as to the emergence of mixed isomers that contain both cis and trans forms [7]. Significant contribution to the process of cis-trans-isomerization is made by enzymes, and the rate of formation of trans-forms under the action of pancreatin (olienzyme preparation with broad substrate specificity) was higher in comparison with lipase of microbial origin (Candida *rugosa lipase)*. Under natural conditions of storage of raw materials (at room temperature), the rate of accumulation of trans-forms is much higher than at storage at a temperature of + 4°C, and at temperatures below zero the process practically does not develop [7].

A number of studies have shown that sodium nitrite catalyzes cis-trans-isomerization of fatty acids [8]. In order to verify this fact, scientists from Japan evaluated the content of TIFA in ready-made pork cutlets, prepared with the use of a mixture of KNO_3 and $NaNO_2$ in a ratio of 5 : 1. During the production of pork cutlets, a mixture of KNO_3 and $NaNO_2$ in the amount of 0; 100; 1000; 10000 mg/kg of meat was introduced in the salting process. The total content of trans-isomers of oleic and linoleic acids in the finished product was (mg/100 g of product) 0.538; 2.65; 3.38 and 27.6 respectively. As a result of the experiment, there was no evidence that the heat treatment under normal salting and cooking conditions in the presence of sodium nitrite leads to an increase in the proportion of TIFA in finished products. Meanwhile, it was suggested that oleic and linoleic acids in cis-trans meat are isomerized by a large amount of nitrite in the presence of nitric acid. Therefore, the addition of excess sodium nitrite during meat processing should be avoided [41].

Another way of forming TIFA is heat treatment at high temperatures, at which the double cis-bond is subjected to the isomerization process in the trans position. Henon (1999) believes that for the isomerization of alpha-linolenic acid in the trans form, heating to 220–230°C, and for linoleic – heating to 240°C is required. Four hours of heat treatment at 270°C led to isomerization with the formation of trans fatty acids of 80% alpha-linolenic acid and only 13% linoleic acid [42]. High temperature regimes are used in the

production of certain types of meat products (fried, baked) and can lead to isomerization of fatty acids.

The processes of conservation and storing the finished product affect the change in fatty acid composition, including the level of TIFA. The fatty acid composition of the canned food "Stew pork meat" was studied, which was produced in two ways: under a strict sterilization regime with the achieved sterilizing effect of 18 conventional minutes and under a moderate sterilization regime with the achieved sterilizing effect of 12 conditional minutes [9]. It has been established that the strict regimes of thermal processing of meat raw materials and the duration of storage of the finished canned product lead to the formation of trans isomers in the fatty acids of the product: elaidinic ($C_{18:1}$, 9-trans) and brassidine ($C_{22:1}$ -trans).

The regimes of sterilization, strict and moderate, affect the content of brassidic acid in different ways: strict sterilization leads to an increase in the mass fraction of acid in 1.6 times, moderate sterilization reduces the content of this acid by 3.5 times. As a result of long-term storage (7.5 months), brassidic acid also behaves differently, depending on the sterilization regimes. In canned goods made under the strict sterilization regime, its proportion is reduced by 42.9%, and in canned goods, made under a moderate regime, it increases sharply (by 260% from the initial value). Its maximum proportion was 0.18% of the total number of fatty acids and did not exceed the norms established for oils for the content of trans-isomers in food products [9].

The effect of different types of processing and storage conditions on the content of elaidin fatty acid is different from that of brassidine. After 1.5 months of canned food storage, the proportion of elaidic acid in the lipids of a product manufactured under the strict sterilization regime increases dramatically, 5 times with respect to the mass fraction of this acid in the product produced under moderate regime. Its content in the moderate product after 1.5 months of storage is reduced and by the end of 7.5 months of storage elaidic acid is not identified [9].

In the USA, in order to improve the consumer quality of meat raw materials, the methods of growing animals, the procedures for carcass cutting and cooking methods are constantly being improved. J. N. Martin *et al.* (2012) presented the results of a study of the nutritional value of beef cuts to update the reference materials of the US National Database (SR24) on nutrients, indicating the content of TIFA [43]. As a result of the evaluation of the data obtained, 29 beef cuts were considered to be lean because they contained less than 10 g of fat, 95 mg of cholesterol and 4.5 g of saturated fat (per 100 g of product) or had a fat content of no more than 10% (Table 2). As can be seen from Table 2, the content of TIFA depends on the way the meat product is prepared and on the total amount of fat on the cut.

As a result of the evaluation of clinical research data on the effects of TIFA on health, WHO experts stated that the consumption of such fats should be reduced to a minimum level (< 1% of the total energy consumed, i.e. no more than 2 g of trans fats a day with average consumption of 2000 kilocalories per day). Consumption of more than 5 g TIFA per day is a risk factor for health [19].

Denmark was the first European country to take measures that banned all products, including catering products, in which the content of TIFA exceeds 2% of total fat. In the United States, Canada, Finland, Norway, Austria, Hungary, Sweden and other countries, legislative restrictions on the content of TIFA in products have been introduced. The labels of packaged foods must indicate the amount of trans fats. At the same time, when determining the maximum permissible value of trans fat in food products, Denmark did not include natural TIFA (vaccenic, rumenic and other fatty acids) in this list on the labels [18]. Experts recommend consuming about 3 grams of conjugated linolenic acid per day from animal fats or foods enriched with this acid to maintain optimal health [44].

Foreign experience shows that the most effective measure to reduce the consumption of industrial TIFA by the population is the introduction of legislative restrictions on their content in food. In Russia, the content of TIFA is regulated only in dairy products. It is planned to reduce the content of trans-isomers of fatty acids in all food products to 2% by 2018. At the moment, the Russian consumer cannot evaluate their content in food products due to lack of this information on the labels. Unlike the EU, in Russia there is no harmonization of regulatory documentation regulating the content of trans fats in meat products. Meat products are not considered as a source of trans fats, however, under the influence of risk factors (intravital or technogenic origin), initiation of processes of trans-isomerization of lipids to potentially dangerous levels for humans is possible.

Table 2. Effect of heat treatment on the fatty acids profile and the accumulation of trans-isomers in finished beef products

Parameter, (g/100 g)	Rib roast beef (boneless)[1]	Outside skirt steak[2]	Inside skirt steak[2]	Rib steak (ribai) (boneless)[3]	Back ribs[4]
Fat mass fraction	24.0 ± 1.6	18.8 ± 0.8	13.9 ± 0.6	23.0 ± 2.7	30.0 ± 1.9
SFA	10.7 ± 0.9	7.7 ± 0.6	5.5 ± 0.5	10.0 ± 1.4	–
MUFA	10.0 ± 0.7	7.7 ± 0.2	6.2 ± 0.1	9.8 ± 1.0	–
PUFA	0.89 ± 0.04	1.06 ± 0.09	0.59 ± 0.06	0.85 ± 0.08	–
Trans fats (g/100 g)	1.8 ± 0.18	1.05 ± 0.12	0.76 ± 0.11	1.6 ± 0.27	–

Note. [1)] Grilling to an internal temperature of 70°C; [2)] Grilling to an internal temperature of 80°C; [3)] Roasting in a conventional oven to an internal temperature of 60°C; [4)] Stewing in a conventional oven at a temperature of 120°C for 150 min.

CONCLUSION

The content of saturated fatty acids in meat raw materials is not a limiting factor for reducing meat consumption. Horse meat and beef are recommended for dietary and preventive nutrition for their biological effectiveness, and a balanced fatty acid composition of pork, horse and turkey lipids meets the requirements for functional foods. In meat raw materials TIFA are found, which become a matter of concern for an increasing number of consumers in the world and subject to close scrutiny by scientists. Meat raw materials of ruminants (cattle, sheep, goats, buffalo, deer, elks, giraffes and camels, etc.) have a high TIFA level. Trans-isomerization of fatty acids can occur as a result of natural synthesis in the rumen and can be regulated by diets. The use of growth promoters, high-temperature processing of meat raw materials, prolonged storage of a product and high sodium nitrite content during meat processing can contribute to TIFA increase in raw meat. Positive effects on animal health from the consumption of natural TIFA are shown, however, the mechanisms of the influence of TIFA on the functions of the organism are not fully explored. Consumption of foods with a high trans fats content shows adverse effects on human health, as a result of technological processing of raw materials. The necessity to normalize the content of industrial trans fats and to develop measures to reduce their level in food production has been discussed.

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STUDY OF THE COMBINED EFFECT OF PECTIN AND BANANA POWDER AS CARBOHYDRATE BASED FAT REPLACERS TO DEVELOP LOW FAT COOKIES

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Abstract: A carbohydrate based fat replacer is the most important constituent used in a large range of bakery products. It exactly minimizes the role of butter, margarine and fat. It also plays a major role in the reduction of obesity. Carbohydrate based fat replacers also effect in terms of physical and chemical properties. The present study was designed to reveal the combined potential of pectin and banana powder as carbohydrate based fat replacers in cookies. The flour was characterized for a proximate analysis (moisture, fat, fiber, protein, ash and NFE). Moreover, carbohydrate based fat replacers (pectin and banana powder) were mixed with flour at different levels (0%, 5%, 10%, 15% and 20%). Fat replacers of different flour containing levels were studied for rheological properties (farinographic) and were used in baked products such as cookies for enhancing their quality characteristics. Moreover, physical and chemical analyses were carried out. Furthermore, the sensory evaluation of cookies was also carried out. The obtained data from each parameter were subjected to an appropriate statistical analysis for determining the level of significance. The results showed that the content of crude fat was 29.82% in the controlled cookies. The fat content was reduced from 29.82% to 17.07% for T3 by using carbohydrate based fat replacers. The current study revealed that the treatment with T3 better attributes to physical, chemical and rheological properties. The results further affirmed that the cookies prepared from wheat flour from 15% supplementation of carbohydrate based fat replacers showed better attributes regarding color and texture and were declared the best by the panelists.

Key words: Cookies, dough rheology, calorific value, sensory evaluation

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INTRODUCTION

Fat replacers are used in a variety of products ranged from frozen desserts to a number of bakery products. The most appreciable functions of fat replacers are to control calories and provide important nutrients. Fat replacers provide mostly the same functions of fat. Different types of carbohydrate based replacers are pectin, fibers, gums, maltodextrin, cellulose, modified starches and fruits. These can provide 4 kcal/g of energy. When mixed in water, they provide 1–2 kcal/g of energy. Sometimes they provide zero calories. Moreover, the rheological and pasting properties of bakery products can be changed by mixing different starches with hydrocolloids [1].

Fat replacers of a carbohydrate origin may be obtained from modified starches, different types of gels, thickening agents, emulsifying substances and fruits. The amphiteric properties of emulsion are valuable in

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some cases and they can deposit fifty percent of fat in food products. These give a sense of good mouth feel, creaminess of fat and flavor. These can also reduce calories while maintaining the original amount of fat [2].

A fat replacer ingredient can be used to provide part or all of the function in the form of fat. Fat alternatives are proficient to produce all or a number of the purposeful characteristics of fat in foods [3].

It is worth mentioning that the alternative term fat replacer means that it is a particular substance having better physical or sensory qualities of fat without any of the undesirable properties of fat. There are some categories of fat substitutes and there is often misunderstanding about how can they act as alternatives of fat or fat mimetic. Fat alternatives act as material intended to replace fat in a product [4].

A carbohydrate based fat replacer may be derived from starch gums, gels, thickening agents, emulsifying agents and bulking agents. The amphiteric characteristics of emulsions are chiefly valuable and can put back near to 50% of fat in food products. These may provide a sense of mouth feel with a distinctive flavor and creaminess of fat. It also allows for the use of a combination of fat replacers or can be used in combination with other components. Low fat products are also lower in fat and decrease the energy content of fat even when they maintain the percentage of fat in the original product [5].

Carbohydrate based fat replacers are derived from plants, cereals and grains. These ingredients consist both of indigestible and digestible complex carbohydrates. Food industries often utilized microcrystalline cellulose as part of a fat mimetic system. It commonly consists of hydrocolloid starch that can be easily solubilized. Carbohydrate based fat replacers have a wide function and can be used in cakes and cookies. Protein based fat replacers are utilized in baked goods, mayonnaise, butter and cheese. Fat based fat replacers are used mainly in baked goods, salted snacks, confections and cheese [5].

Pectin is one of the most essential polysaccharide which has a lot of applications in pharmaceutical industries, food manufacturing industries and many other industries. The most important function in food industries is that it forms gels in the incidence of lower pH and calcium ions. During gel formation, a lot of interactions are involved, especially that of hydrophobic bonding and hydrogen bonding interactions with calcium ions. There are a lot of other applications of pectin which may include the formation of edible films, act as plasticizers and paper substitutes, etc. It is present in the plant's cell wall, pomace of apple and orange peels [6].

Banana is a fruit and mostly cultivated in the tropical regions. These are transported to cities, where these would be used as fruit or vegetables. If they are not properly transported and marketed, there are a large number of post-harvest losses. In a diet, they would be used as fruit, which can provide protection against chronic degenerative problems such as obesity and acts as fat replacers in bakery products. There are more than 100 different varieties of banana in the world. Banana belong to the genus Musa and the family Musaceae [7]. The most common edible variety is *Musa acuminate*. Gros Michel and Red Skins are some other varieties. Musa banana has an origin in Southeastern Asia [8].

Traditional techniques such as the use of skim milk are used as an alternative of whole milk in frozen desserts. Using traditional techniques such as the use of lean meats in frozen entrées, fat may be replaced in food products [9]. Starch is the main source of energy stored in grains, tubers and other plant parts. Starch provides 70–80% of the calories consumed by people all over the world. Besides, being an important element in a human diet and of nutritional value. Starch and modified starches are the raw materials to modify the physical properties of a lot of foods, such as crystallization thickness, retention, adhesion, humidity and stabilizing. Starch and the products derived from starch are also important in numerous nonfood applications such as pharmaceuticals, paper, textile industries, alcohol based fuels and adhesives. Another distinctiveness of starch is that most starch granules are composed of a mixture of two polymers - amylose and amylopectin [10].

The objectives of this study were to discover the combined potential of pectin and banana powder as carbohydrate based fat replacers and their effect on the rheological properties of flour containing different levels of fat replacers and to develop cookies with reduced fat.

OBJECTS AND METHODS OF STUDY

Procurement of raw material. The commercial wheat flour (white), pectin, bananas and chemicals were purchased from a local market. The study was carried out at the National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

Drying of bananaю Bananas were peeled, dried up to 3–6% moisture and banana powder was formulated for a further study. Drying of banana was carried out by a tray drier dehydrator, Model No. R-5A, Commercial Dehydrator System, Inc. 800-369-4283.

Measurements. Rheological characteristics of white flour with and without the addition of carbohydrate based fat replacers were determined by using Farinograph according to the procedure described in AACC [11] Method No. 54–21.

Farinographic studies. The treatments were run through Brabender Farinograph equipped with a 50 grams bowl of a capacity to evaluate the physical dough behavior of different flour treatments according to (AACC, 2000) Method No. 54–21. The parameter such as water absorption, dough development time, softening of dough, arrival time, departure time, dough stability time and the mixing tolerance index were calculated from the farinograms as described below.

Water absorption. The water absorption capacity of the flour sample was the amount of water required to reach the curve at center, 500 Brabender units (B.U.) line of the graph. It was directly observed from the water level in the burette attach with the apparatus and articulated as percentage.

Dough development time. The dough development time was observed as the time taken by the curve to achieve the point of maximum dough consistency before the first indication of weakening.

Softening of dough. The softening was measured as the difference in BU from the centre of the curve at

the peak and the center of the curve after 12 minutes from the peak.

Arrival time. The arrival time was recorded from the farinogram as a difference in time between the point where the top of the curve first intersected the 500 B.U. line and the point where it started.

Departure time. The resistance of the dough to mixing was noted as the time from the addition of water until the top of the curve declined from 500 B.U. line.

Dough stability time. The dough stability time was calculated using the difference of departure time and arrival time, that is (departure time – arrival time = dough stability time).

Mixing tolerance index. The mixing tolerance index value was derived in Brabender Unit (BU) as a difference in the top of the curve at the peak to the top of the curve after 5 minutes from the peak.

Cookies preparation. Cookies formulation contains the following ingredients: wheat flour; water; sugar; vegetable oil; fat replacers combination; baking powder and eggs. Before the current experiment, a trial was conducted to determine the maximum fat replacers mixture level that could be incorporated in the cookies. The fat replacement was done into cookies at 5 levels (0%, 5%, 10%, 15%, and 20%) by replacing amount of oil for the cookies mixture. The cookies were placed into a tray and baked in a preheated oven at 180°C for 10–12 minutes. The cookies from each type of composite flours were prepared according to AACC (2000) Method No. 10–50D with some modifications as followed.

Analysis of cookies

Physical analysis of cookies. The cookies of different treatments were first prepared and then analyzed for their following physical parameters.

Color measurement. The color of cookies of different treatments was determined according to the methods described using Color Meter (Piga *et al*., 2005) with the help of a hand held tri stimulus colorimeter. The colorimeter was calibrated by using standards (153 CTn for dark and 181 CTn for light). The color of cookies was determined by placing the cookies under the photocell.

Textural analysis. The texture of all the treatment of cookies was determined according to (Piga *et al*., 2005) by using a texture analyzer (Model. TA-XT2, Stable Microsystems and Surrey, UK) with a 5 kg load cell. The Texture Export Program version 4 was used for data analysis. The textural determinations were made by using a 75 mm Compression Platen (P/75) for a compression test. The treatments samples were positioned centrally with the core at the right angles to the direction of force, on the Petri plates. Once a trigger force of 100 g has been achieved, the compression plate proceeds to move down onto the spread and there is a rapid rise in force. During this stage the sample is deformed under the applied force but there was no apparent breakdown of the product. As the compression distance increased, small peaks were seen on the graph profile, each peak indicating a compressive failure of the sample. This stage ended abruptly when the test was completed and indicated a large decrease in force. The greater the distance, the greater is the ability to withstand compression without sample breakage.

Other physical analysis. Cookies of different treatments were analyzed firstly for their physical parameters after preparation. The diameter/width, thickness and spread factor were determined according to their respective methods described in AACC (2000).

Thickness. To determine the thickness (T), five cookies of same treatment were placed on top of one another. The total height was measured in millimeters with the help of a ruler. This process was repeated thrice to get the average value for each treatment and results were reported in millimeters.

Diameter. To determine the diameter (D), three cookies of the same treatment were placed edge to edge. The total diameter of three cookies was measured in mm by using a ruler. The cookies were then rotated at an angle of 90°C for duplicate reading. This was repeated once more and the average diameter for each treatment was reported in millimeters.

Spread factor. The spread factor for each treatment was determined from the diameter and thickness, with the help of the following formula:

$$SF = \frac{D \times CF \times 10}{T},$$

where *CF* is a correction factor at a constant atmospheric pressure. Its value was 1.0 in this case.

Proximate analysis of cookies. Cookies of different treatments were analyzed for moisture, crude protein, crude fiber and crude fat and total ash by using Methods described in AAAC (2000). The nitrogen free extract (NFE) was calculated using a difference method.

Sensory analysis. The cookies of different treatments were rated using a 9-point hedonic score system (9 = like extremely; 1 = dislike extremely) by a taste panel [12]. They were asked to express their opinion about the final product by giving score to the attributes like aroma, taste, crispness, mouth feel and the overall acceptability. During sensorial evaluation, cookies of different treatments were placed in the transparent plates labeled with random codes. Cold water and crackers were supplied to panelists for rinsing their mouths between the samples.

Statistical analysis. The data obtained for each parameter were subjected to statistical analysis to determine the level of significance according to the method described by [13].

RESULTS AND DISCUSSION

Rheological studies

Farinographic studies. The rheological characteristics of white flour containing different levels of carbohydrate based fat replacers were studied for the parameters such as water absorption, dough development time, dough stability time, the mixing tolerance index and softening of dough by using Brabender Farinograph. The data representing the effects of various levels of carbohydrate based fat replacers on the farinographic characteristics of the dough has been given in Table 1.

Water absorption. The mean values of the data regarding water absorption for the flour samples containing carbohydrate based fat replacers are presented in Table 1. The data on water absorption indicated that the values significantly differ from each other. It was obvious from the results that the water absorption was higher in T_4 (61.58%), followed by T_3 (59.59%), T_2 (57.23%), T_1 (55.50%), T_0 (54.62%). The lowest water absorption was observed in T_0 (54.62%). The results revealed that the water absorption increased with the addition of carbohydrate based fat replacers. The results representing the water absorption of dough in this study are in conformity with the findings of Azizi and Rao [13] who found out that the percentage of water absorption increases with the addition of carbohydrate based fat replacers. The mentioned results for water absorption of flour are also comparable with the findings of Ahmad [14] who observed an increase in water absorption with the use of carbohydrate based fat replacers.

Dough development time. Dough development time is defined as the time required for the development of gluten. It was found to be in the range of 4.70–6.70 min. The data regarding the analysis of variance for dough development time is shown in Table 1. The present results indicated a highly significant difference among the treatments. The mean values for the dough development time of different treatments of carbohydrate based fat replacers are given in Table 1. The highest value T_0 (6.70 min) followed by T_4 (4.70 min) was found, T_1 (6.20 min) and T_3 (5.40 min) while it was for T_2 (6.00 min).

The results of this study are in agreement with the findings of Hafeez [16] who observed a decrease in dough development time with the use of carbohydrate based fat replacers. The results are also in concurrence with Asghar *et al.* [17].

Dough stability time. The data indicate a highly significant difference among the treatments for the dough stability time. The mean values of Table 1 showed that the highest value for dough stability time was observed in T_0 (12.50 min) and the lowest value was obtained in T_4 (4.28 min) while the mean values for T_1, T_2, and T_4, were found to be 8.60 min, 7.18 min and 4.28 min respectively. The above findings regarding dough stability time are in close agreement with the results of Ravi *et al.* (2000) who observed that the addition of carbohydrate based fat replacers affects the dough stability time of different treatments. These results are also comparable with the findings of Asghar *et al*. [17] who observed an increased dough stability time with the use of carbohydrate based fat replacers. The results of the present study are also in accordance to the findings of Azizi and Rao [13] who observed a decreased dough stability time by the addition of carbohydrate based fat replacers.

Softening of dough. The results indicate that there is a highly significant difference among the treatments. The mean values for softening of dough in Table 1 showed the maximum value for T_4 (79.00 BU) and the minimum value for T_0 (47.00 BU). The mean values for other treatments were T_1 (63.00 BU), T_2 (71.00 BU), and T_3 (73.00 BU. The mentioned results are in conformity with the findings of Asghar *et al*. [17] who observed an increased BU value for softening of dough with the use of carbohydrate based fat replacers.

Mixing tolerance index. The results indicate a highly significant difference among treatments for mixing tolerance index. The mean values for the mixing tolerance index are given in Table 1. These results show the highest value for T_4 (133.00 BU) followed by T_3 (120.00 BU), T_2 (112.00 BU), T_1 (70.00 BU) and it was lowest for T_0 (60.00 BU). The results indicate that the mixing tolerance index increased with the addition of carbohydrate based fat replacers. These results are in conformity with the values as observed by Hafeez [16] who explicated that the mixing tolerance index varies with the addition of carbohydrate based fat replacers. The current findings are also comparable with the results of Ahmad [14] who observed an increased mixing tolerance index with the addition of carbohydrate based fat replacers.

Table 1. Mean values for the farinograph parameters

Treatments	Water absorption (%)	Dough development time (min)	Dough stability time (min)	Softening of dough (BU)	Mixing tolerance index (BU)
T_0 (0% carbohydrate based fat replacers (control treatment))	54.62^{e}	6.70^{a}	12.50^{a}	47.00^{d}	60.00^{e}
T_1 (5% carbohydrate based fat replacers)	55.50^{d}	6.20^{b}	8.60^{b}	63.00^{c}	70.00^{d}
T_2 (10% carbohydrate based fat replacers)	57.23^{c}	6.00^{b}	7.18^{c}	71.00^{b}	112.00^{c}
T_3 (15% carbohydrate based fat replacers)	59.59^{b}	5.40^{c}	5.20^{d}	73.00^{b}	120.00^{b}
T_4 (20% carbohydrate based fat replacers)	61.58^{a}	4.70^{d}	4.28^{e}	79.00^{a}	133.00^{a}

Note. The means sharing a different letter in a column are significantly different from one another.

Measurement of physical characteristics of cookies

Color determination. The color value was determined by using a color meter. It was first calibrated with the standards having the lower and the upper limits (51–200) respectively. The color value for cookies prepared by using different treatments of white flour supplemented with varying concentrations of carbohydrate based fat replacers. These results indicated a significant difference for color among various treatments. The mean values for color (Table 2) showed that the highest value was observed in T_0 (181.67 CTn) and the lowest in T_4 (154.67 CTn). The mean values for other treatments were T_1 (179.67 CTn), T_2 (177.00 CTn) and T_3 (166.33 CTn). The above mentioned instrumental color values are in agreement with Abu Ghoush *et al.* [19]. These observations regarding color are also comparable to the findings of Azizi and Rao [15] who observed that the color of cookies is affected by the addition of carbohydrate based fat replacers.

Cookies texture. Texture of cookies prepared by using various treatments of white flour supplemented with different concentrations of carbohydrate based fat replacers were measured in terms of hardness (firmness) and fracture ability. The hardness (firmness) was calculated in terms of the maximum force (kg) and the fracture ability was determined in terms of distance (mm).

Table 2 Mean values for the color (CTn) of cookies

Treatments	Color (CTn)
T_0	181.67^{a}
T_1	179.67^{ab}
T_2	177.00^{b}
T_3	166.33^{c}
T_4	154.67^{d}

Note. The means sharing the same letter in a column are not significantly different.

Table 3. Mean values for the hardness (firmness) of cookies

Treatments	Firmness (Kg)
T_0	1.68^{d}
T_1	2.22^{c}
T_2	2.79^{b}
T_3	2.91^{b}
T_4	3.91^{a}

Note. The means sharing the same letter in a column are not significantly different from one another.

Table 4. Mean values for the fracture ability (mm) of cookies

Treatments	Fracture ability (mm)
T_0	22.65^{a}
T_1	21.50^{b}
T_2	21.40^{c}
T_3	20.94^{d}
T_4	20.37^{e}

Note. The means sharing different letters in a column are significantly different from one another.

Table 5. Mean values for the thickness (mm) of cookies

Treatments	Thickness (mm)
T_0	37.80^{a}
T_1	36.03^{b}
T_2	35.93^{b}
T_3	35.43^{c}
T_4	35.11^{d}

Note. The means sharing the same letter in a column are not significantly different from one another.

Hardness (Firmness). Hardness can be defined as the peak force during the first compression cycle (first bite). It is the force required to attain the given deformation. The values for the hardness of cookies ranged from 1.68 to 3.91 kg. The data regarding the analysis of variance for hardness are shown in Table 3. The given values for hardness of cookies differ significantly among the treatments. The mean values given in Table 3 indicated the maximum hardness value for T_4 (3.91kg) and the minimum value for T_0 (1.68 kg) while it showed a significant variation among other treatments. The mean values for T_1, T_2 and T_3 were 2.22 kg, 2.79 kg and 2.91 kg.

The results of the present study regarding the hardness of cookies are in agreement with the observations of Ashwini *et al.* [20]. Different treatments significantly affect the hardness value of product. The above mentioned values are also similar to the findings of Azizi and Rao [15] who also observed a significant effect on the hardness of product with the addition of carbohydrate based fat replacers.

Fracture ability. Fracture ability (also known as brittleness) is a force at a first important break in the curve. As a break is a detectable phenomenon linked to the macro structure of the sample, it is known as changes in the modulation of the curve whose magnitude should be definite. It is the force the material or the product fractures with. These values indicate that there is a significant difference among the treatments for the fracture ability. The mean values for the fracture ability of cookies are given in Table 4. It is obvious from the mean values that the maximum fracture ability was observed in T_0 (22.65 mm) followed by T_1 (21.50 mm), T_2 (21.40 mm), T_3 (20.94 mm) and T_4 (20.37 mm) while the minimum value for fracture ability was observed in T_4 (20.37 mm). The results determined in the present study are similar to the findings of Ashwini *et al.* [20] who observed a significant change in the fracture ability of cookies by the addition of carbohydrate based fat replacers. These results are also in conformity with Azizi and Rao [15].

Cookies thickness. The thickness of cookies is affected by various factors such as the quality of flour, the type of ingredients and processing conditions. Thickness should not be too high or too low as it affects crumb grains. If the thickness of a product is too low, it indicates a very compact and closed grain structure whereas too high thickness results in a very accessible grain structure. The thickness of the cookies prepared by using various treatments of carbohydrate based fat replacers was determined using the following methods described in AACC (2000). The data regarding the

analysis of variance for thickness is presented in Table 5. The data represented a highly significant difference among the treatments. The mean values for the thickness of cookies given in Table 5 indicated that the maximum value for thickness was observed in T_0 (37.80 mm) followed by T_1 (36.03 mm) and T_2 (35.93 mm), T_3 (35.43 mm) and T_4 (35.11 mm). The current findings are in concord with Kaur *et al.* (2000).

Cookies diameter. The diameter of cookies is affected by various factors such as the quality of flour, the type of ingredients and processing conditions. The diameter should not be too high or too low as it affects crumb grains. The diameter of the cookies prepared by using various treatments of carbohydrate based fat replacer was determined using the following methods described in AACC (2000). The data represented a highly significant difference among the treatments. The mean values for the diameter of cookies given in Table 6 indicated that the maximum value for diameter was observed in T_4 (151.00 m) followed by T_3 (145.00 mm) and T_2 (142.67 mm), T_1 (139.00 mm) and T_0 (134.67 mm). The current findings are in concord with Kaur *et al.* [21] and Ashwini *et al.* [20].

Cookies spread factor. The cookies spread factor is affected by various factors such as the quality of flour, the type of ingredients and processing conditions. The spread factor should not be too high or too low as it affects crumb grains. The cookies spread factor prepared by using various treatments of carbohydrate based fat replacers was determined using the following methods described in AACC (2000). The data represented a highly significant difference among the treatments. The mean values for the spread factor of cookies given in Table 7 indicated that the maximum value for the spread factor was observed in T_4 (44.26 mm) followed by T_3 (41.49 mm), T_2 (38.85 mm), T_1 (38.57 mm) and T_0 (35.62 mm). The current findings are in concord with Kaur *et al.* [21] and Ashwini *et al.* [20].

Color. The results revealed that there was a significant variation in the color score for cookies among different treatments. The mean values given in Table 8 indicated that the highest score for color was assigned to the treatments T_3 (7.42) while the lowest score (6.33) was given to T_4. Similarly, the color score of cookies prepared from T_2 and T_1 was 7.08 and 6.75. These findings also revealed that the cookies prepared by using carbohydrate based fat replacers from (T_0) were 6.67. The results are comparable to the findings of Amerine *et al*. [22] and Meilgaard *et al.* [12].

Crispiness. The mean values for this attribute have been presented in Table 9. The highest value for the crispiness score (7.00) was observed for T_3 followed by T_1 having a crispiness score of 6.93. The current results are in agreement to the findings of Amerine *et al*. [22]. The lowest moistness score (6.00) was recorded in the cookies prepared from T_4 containing 20% fat replacers.

Flavor. It is obvious from the results that the cookies showed a significant difference in flavor by the addition of carbohydrate based fat replacers. The scores for flavor of the cookies presented in Table 10 indicated that the cookies prepared from T_2 got the highest scores for flavor (7.08) followed by T_3 obtained (6.92) for flavor. The mean scores for the cookies prepared from T_0 got 6.33 while T_1 and T_4 got 6.58 and 6.25 respectively. The current results are also similar to the findings of Amerine *et al*. [22] who declared that the addition of carbohydrate based fat replacers in any product caused changes in taste, flavor, texture and overall acceptability scores. The current results are also in agreement to the findings of Meilgaard *et al*. [12].

Surface. The addition of carbohydrate based fat replacers significantly affected the surface score of cookies. Mean scores for the surface of cookies showing an effect of carbohydrate based fat replacers has been presented in Table 11. The highest surface score (7.17) was assigned to T_3 and followed by T_2 (7.00). The results further indicated that T_1 cookies got the lowest tenderness score (6.25). The current results are in agreement to the findings of Amerine *et al*. [22].

Table 6. Mean values for the diameter (mm) of cookies

Treatments	Diameter (mm)
T_0	134.67^{d}
T_1	139.00^{c}
T_2	142.67^{b}
T_3	145.00^{b}
T_4	151.00^{a}

Note. The means sharing the same letter in a column are not significantly different from one another.

Table 7 Mean values for the cookies spread factor (mm)

Treatments	Spread factor (mm)
T_0	35.62^{e}
T_1	38.57^{d}
T_2	39.85^{c}
T_3	41.49^{b}
T_4	44.26^{a}

Note. The means sharing the same letter in a column are significantly different from one another.

Table 8. Mean values for the color of cookies

Treatments	Color
T_0	6.67^{bc}
T_1	6.75^{bc}
T_2	7.08^{ab}
T_3	7.42^{a}
T_4	6.33^{c}

Note. The means sharing the same letter in a column are not significantly different from one another.

Table 9. Mean values for the crispiness of cookies

Treatments	Crispiness
T_0	6.33^{bc}
T_1	6.93^{ab}
T_2	6.83^{ab}
T_3	7.00^{a}
T_4	6.00^{c}

Note. The means sharing the same letter in a column are not significantly different fron one another.

Table 10. Mean values for the flavor of cookies

Treatments	Flavor
T_0	6.33^{c}
T_1	6.58^{bc}
T_2	7.08^{a}
T_3	6.92^{ab}
T_4	6.25^{c}

Note. The means sharing the same letter in a column are not significantly different from one another.

Table 11. Mean values for the surface of cookies

Treatments	Surface
T_0	6.33^{b}
T_1	6.25^{b}
T_2	7.00^{a}
T_3	7.17^{a}
T_4	6.33^{b}

Note. The means sharing the same letter in a column are not significantly different from one another.

Table 12. Mean values for the taste of cookies

Treatments	Taste
T_0	6.33^{b}
T_1	6.58^{b}
T_2	6.50^{b}
T_3	7.08^{a}
T_4	6.58^{b}

Note. The means sharing the same letter in a column are not significantly different from one another.

Table 13. Mean values for the texture of cookies

Treatments	Texture
T_0	5.83^{d}
T_1	6.17^{cd}
T_2	6.75^{ab}
T_3	6.83^{a}
T_4	6.33^{bc}

Note. The means sharing the same letter in a column are not significantly different from one another.

Table 14 Mean values for the overall acceptability of cookies

Treatments	Overall acceptability
T_0	4.08^{b}
T_1	4.17^{b}
T_2	4.25^{ab}
T_3	4.42^{a}
T_4	3.67^{c}

Note. The means sharing the same letter in a column are not significantly different from one another.

Taste. The mean values for this attribute given in Table 12 revealed that the taste scores assigned to cookies supplemented with different concentrations of carbohydrate based fat replacers ranged from 7.08 (T_3) to 6.33 (T_0). The results further indicated that the cookies prepared were significant regarding their shape scores. The current results are in agreement to the findings of Amerine *et al*. [22]. The lowest score 6.33 was assigned to the cookies prepared from T_4 .

Texture. It was indicated that the texture of cookies was significantly affected by the increasing concentrations of carbohydrate based fat replacers. The mean scores assigned by the panelists to the texture of the cookies prepared from different treatments of carbohydrate based fat replacers are shown in Table 13. These results revealed that the control cookies T_0 got the score 5.83 for texture. The cookies prepared from T_3 and T_2were considered more acceptable by the panelists regarding the texture scores as 6.83 and 6.75 respectively. The current results are in agreement to the findings of Amerine *et al*. [22]. The results also revealed that the cookies prepared from all the treatments had significant differences for texture. The results are also comparable to the findings of Meilgaard *et al*. [12].

Overall acceptability. It was obvious from the results that the overall acceptability scores of cookies were significantly affected by carbohydrate based fat replacers. The mean values given in Table 14 indicated that the overall acceptability scores of cookies showed a slight variation with the addition of varying concentrations of carbohydrate based fat replacers. The highest score (4.42) for the overall acceptability was assigned to the cookies prepared from T_3. The judges slightly disliked the cookies prepared from T_4 and assigned the overall acceptability score 3.67. The overall acceptability score 4.25 was assigned to $T_2$by the panelists. The current results are in agreement to the findings of Amerine *et al.* [22] regarding the sensory evaluation of cookies for appearance, color, flavor, texture and overall acceptability. The results are also comparable to the findings of Meilgaard *et al*. [11]. The current results regarding the sensory evaluation of the cookies prepared using different concentrations of carbohydrate based fat replacers revealed that the treatments T_3 carbohydrate based fat replacers showed the best results and were assigned maximum scores by the panelists in terms of likeness.

CONCLUSION

The current results regarding the sensory evaluation of cookies prepared using different concentrations of carbohydrate based fat replacers revealed that the treatments T_3 carbohydrate based fat replacers showed the best results and were assigned maximum scores by the panelists in terms of likeness.

In conclusion, the results of this study showed that the total phenolic, total flavonoid, rutin and tannin contents are important components in a 50% aqueous ethanol extract of Bulgarian dry leaves of *Cichorium intybus* L. this plant, and the chicory has some of the pharmacological effects. The results of the present study clearly show that the yield of *Cichorium intybus* L. rises rapidly with time at first, and then less and less quickly as the progress of extraction continues.The Bulgarian dry leaves of *Cichorium intybus* L. are an important component providing a protective/preventative health effect.

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INNOVATIVE TRENDS IN THE DEVELOPMENT OF ADVANCED TRITICALE GRAIN PROCESSING TECHNOLOGY

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Abstract: The study has been carried out at the All-Russian Research Institute of Grain and Its Processing Products. This paper describes the formation of new grades of triticale flour based on the cumulative ash curves the analysis of technological and biochemical indicators of which showed that flour of the grades T-60, T-70 and T-80 obtained from endosperm can be used directly in bakery, flour of the grades T-120 and T-220 obtained from peripheral parts and triticale bran can be limitedly used in bakery, and are mainly raw materials for further processing. On the basis of the study of the kinetics and efficiency of the effect of proteolytic and cellulolytic enzyme preparations (EP) and their compositions, optimal conditions for enzymatic modification (the EP dosage is 0.5–0.75 units of PA/g of flour, 0.3...0.4 units of CA/g of bran, the optimum temperature is 40–50°C, pH is 5.0 and 3.5, the duration of reactions is 1.5 and 2 hours) have been determined. It has been shown using the gel-chromatography method that the use of multienzyme compositions (MEC) of proteases allowed to hydrolyze triticale flour proteins completely and to use the obtained hydrolyzate as a component of hypoallergenic and gluten-free flour products. The use of cellulolytic EP allowed to increase the amount of reducing substances and soluble protein by 1.5–2.5 times in comparison with the control sample. The biomodified bran obtained using the MEC "Shearzyme 500 L" + "Neutrase 1.5 MG" and "Viscoferm L" + "Distizym Protacid Extra" has a high degree of hydrolysis of non-starch polysaccharides and proteins, is characterized by a certain ratio of high-, medium-, low-molecular peptides and amino acids, has different functional and technological properties. They can be used in the production of a wide range of general-purpose, functional and treatment-and-prophylactic food products.

Keywords: Triticale grain, flour, bran, grain processing technology, enzyme preparations, modified grain processing products, functional and technological properties

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INTRODUCTION

The relevant trends in the development of flour technology include both the improvement of traditional methods and the development of technologies of products with a high biological and nutritional value, the use of biotechnological methods in the technology of advanced processing products, the creation of technologies of new, non-traditional products, etc. The final objective of the technologies being developed is to obtain products with the specified composition and properties.

All-Russian Research Institute of Grain and Its Processing Products conducts fundamental and applied studies to develop the basic methods for managing technological processes of the preparation and grinding of grain of various crops in order to obtain products with the specified chemical composition and properties. Thus, using the example of processing of triticale grain into flour and cereals, principles of the formation of stable streams of flour from various anatomical parts of grains have been developed, which allows to form various types of flour with the specified properties. The application of the developed technologies allows to obtain such products from triticale grain as: graded baker's flour, cereals for children's and dietary nutrition and grits for pasta [1, 2, 3, 4].

Triticale is a new crop, this is the first grain crop obtained by crossing wheat (*Triticum*) with rye (*Secale*). The first report on the receipt of a wheat-rye hybrid was published in 1875 [5]. The main manufacturers of triticale in the world are Poland, Germany, France and Belarus, moreover, the cultivation area of this most promising culture expands both in the world and in Russia. The croppage in Russia was 624 thousand tons in 2017, according to Roskomstat. The average yield of triticale in Russia in 2016 is 27.8 c/ha, which is the largest value for the period of 2009-2016, and is also 4.7 c/ha more than in 2015 [6]. 75 grades of winter triticale and 14 grades of spring triticale have been added to the State Register of Selection Achievements approved for use in Russia (2017). All new grades are recommended for food purposes [7].

The biotopotential of triticale grain depends primarily on: varietal features and growing conditions. The nutritional value is related to a high protein content, essential amino acids and a balanced amino acid composition. The biological value of triticale grain depends on the predominance of water and salt-soluble

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protein fractions and, as a consequence, a higher degree of assimilation of triticale proteins, as well as the presence of vitamins, macro- and micronutrients [4, 8, 9].

However, at present, in Russia, triticale is used mainly in the production of mixed fodder and alcohol. Perspective is the application of flour from triticale grain as a component of raw materials in the production of confectionery products: biscuits, cakes and crackers. It is possible to use triticale flour in the production of fast breakfasts or in the production of dietary bread, including multi-grain bread and that from whole grains [9, 10, 11]. There is no production of bread from graded triticale flour currently in Russia.

The use of methods for a biotechnological effect on various crops and their processing products with obtaining general-purpose, functional and treatment and prophylactic food products is a promising and relevant trend of scientific research for the technological development of the milling branch. At present, the use of enzymatic hydrolysis of biopolymers of food raw materials of both animal and vegetable origin is being actively and comprehensively studied and introduced into the practice of food and processing industries [12, 13, 14, 15, 16].

The use of modern biotechnological methods allows to develop methods for enzymatic modification of grain processing products (flour of various types, including that with a high content of peripheral parts, bran) using multienzyme compositions (MEC) based on proteolytic and cellulolytic enzyme preparations; to obtain modified products (protein hydrolyzate, structurally modified flour, biomodified bran) with various values of degree and depth of hydrolysis of proteins and non-starch polysaccharides with various functional and technological properties.

The study aims at developing a flexible technology based on the division of triticale grain into anatomical parts to obtain new general-purpose and special products with a high nutrition and biological value and to obtain components with specific functional and technological properties. The implementation of the taken aim will allow to design food products from grain with the specified composition and properties.

STUDY OBJECTS AND METHODS

The experimental studies have been carried out at the Federal State Budgetary Scientific Institution "All-Russian Research Institute of Grain and its Processing Products". In this paper, flour was used from of triticale grain of new grades formed on the basis of cumulative ash curves. Since the studied samples of triticale grain did not contain any foreign and grain impurities, the technological process of preparing triticale grain for milling included only hydrothermal treatment: the grain was moistened up to 14–15% and softened for 12 hours [3]. The technological process of grain grinding included 4 break, 6 reduction and 2 scratch systems. The parameters and grinding regimes corresponded to the recommended "Rules for the organization and conduct of a technological process at flour mills" for graded wheat milling according to a short process scheme. 6 samples of triticale grain of different grades were isolated for laboratory milling: Topaz (2011, 2012); Skolot (2012); Vocaliz (2012); Tribun (2012) and Donslav (2012). Thus, the range of values of the quality indicators of the studied samples was: glassiness is 55–72%, the natural weight is 715–737 g/l, the weight of 1000 grains is 40–44 g, the ash content is 1.85–1.89%, the crude gluten content is 17–24%, the gluten quality is 46–64 units of GDI, the falling number is 74–175 s and the protein content is 12–13% [1].

Figure 1 presents the process of grinding and forming the quality of flour in the form of cumulative ash curves. The presence of 3 stages of flour formation has been established, which is clearly seen from the graphs of cumulative curves (Fig. 1). In addition, the statistical analysis has shown the reliability of representation of cumulative curves in the form of three linear sections.

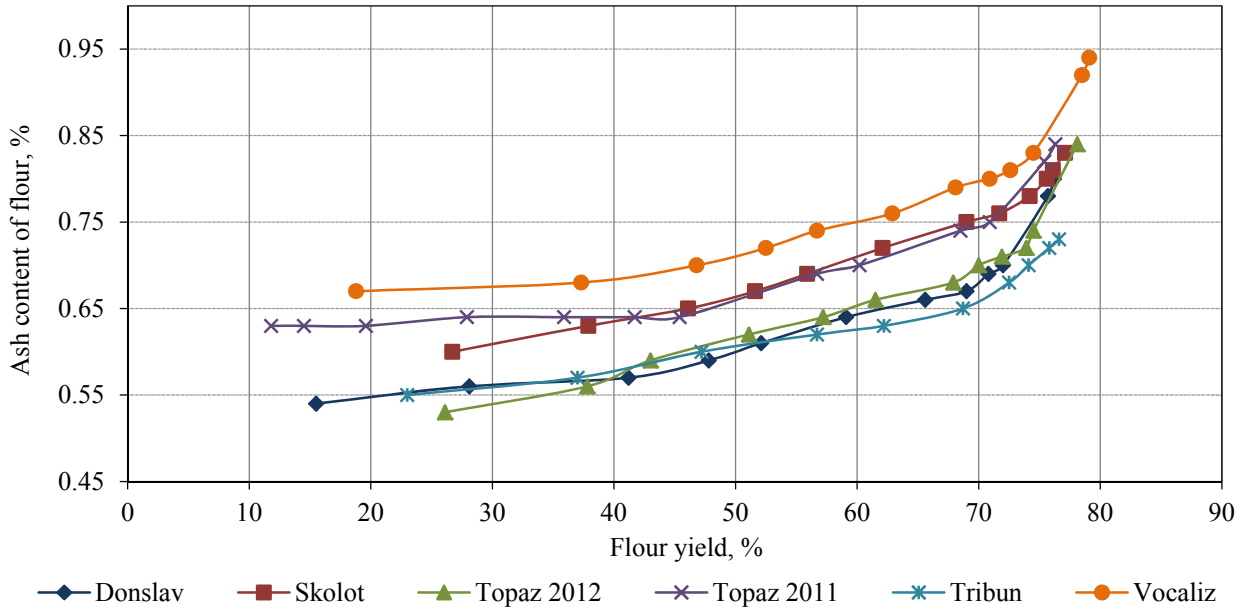

Fig. 1. Ash content cumulative curves.

The first stage of flour formation consisted in extracting the central part of endosperm with a flour yield of 40–45% and an ash content of 0.60% and included the 1st, 2nd, and 3rd reduction systems. The letter designation **A** has conditionally been assigned to the given flour stream. The second stage consisted of 5–7 technological systems and was characterized by a yield of triticale flour in the amount of 25–26% and ash content of 0.91%. The letter designation B has conditionally been assigned to the given flour stream. The third stage consisted of scraping with a flour yield of 5–7% and ash content of 2.20% and included the 6th reduction system and scratch systems. The conventional designation of flour stream is C. Further on, the flour of each of the stages was mixed to obtain individual flour grades, which resulted in obtaining 5 flour grades. The conventional designation of the grades includes the index T which stands for triticale, and a number which stands for the value of ash content × 100. Thus, flour T-60 was the stream A with an ash content of 0.60%, flour T-70 was a mixture of the streams A+B, flour T-80 was a mixture of the streams A+B+C, flour T-120 was a mixture of the streams B+C and flour T-220 was the stream C.

The soluble protein content was determined using the Lowry method [17] and the protease activity - using the modified Anson method [18], bovine serum albumin was used as the standard substrate, amine nitrogen - using the formol titration method, and reducing substances (RS) - using the Bertrand method [19]. Determination of the fractional composition of proteins according to Osborne: albumins were isolated using distilled water, globulins - using a 10% NaCl solution, prolamines - using 70% ethanol, and glutelins - using a 0.2% NaOH solution. The proteins and the products of proteolysis of triticale flour and bran were fractionated by molecular weight using the gel chromatography method with a column with Sephadex G-75 and Toyopearl gel HW-55F [19].

The following were used as proteolytic and cellulolytic enzymatic preparations: "Neutrase 1.5 MG" - a bacterial metalloproteinase (Zn) produced by *Bacillus amyloliquefaciens*, "Alcalase FG" - a bacterial proteinase produced by *Bacillus licheniformis* (Novozymes, Denmark); "Distizym Protacid Extra" - a fungal protease produced by *Aspergillus niger* (Döhler, Germany), "Protease GC-106" - a fungal protease produced by *Aspergillus oryzae* (Genencor, USA), "Shearzyme 500L" - a purified xylanase produced by *Aspergillus oryzae and Aspergillus aculeatus*, "Viscoferm L" - a balanced mixture of xylanase, β-glucanase, cellulase and α-amylase produced by *Aspergillus aculeatus* (Novozymes, Denmark). All the preparations are recommended for the hydrolysis of biopolymers of grain raw materials [20, 21].

The functional and technological properties were determined using the methods described in [22] and in [23, 24]. The water absorption capacity (WAC) was determined as the amount of water adsorbed by the modified triticale bran after centrifugation. To determine the fat emulsifying capacity (FEC), 50 ml of distilled water was added to the weighed amount of 1 g of modified triticale bran and suspended at 4000 rpm for 1 minute. Then 10 ml of refined sunflower oil was added to the mixture and emulsified for 5 minutes at a rate of 8000 rpm. The obtained emulsion was centrifuged for 5 minutes at 2000 rpm. FEC was calculated as a ratio of the emulsion volume and the overall system volume expressed as a percentage. The emulsion stability (ES) was determined by heating the emulsion for 30 min at 80°C, then cooled and centrifuged at 2000 rpm. ES was calculated as a ratio of the emulsion volume and the overall system volume expressed as a percentage. To determine the fat binding capacity (FBC), the weighed amount was put into a pre-weighed centrifuge tube, 5 ml of refined sunflower oil was added and mixed for 1 minute at 1000 rpm, then centrifuged for 15 minutes at 4000 rpm. The unadsorbed oil was drained, the tubes were weighed and the FBC was calculated as a ratio of the weight of the bound oil to the weighed amount. The foaming capacity (FC) was determined by mixing a weighed amount in 25 ml of distilled water in a graduated cylinder and thoroughly mixed, the volume was made up to 300 ml and shaken for 1 min. FC was expressed as a ratio of a foam height (mm) to a liquid height (%).

The analyses were performed in triplicate, presenting the results as average arithmetic ones. The discrepancy between parallel assays did not exceed 3% of the average arithmetic value with the confidence probability P = 0.95.

RESULTS AND DISCUSSION

Starting to develop methods for enzymatic modification of biopolymers of vegetable raw materials, it is necessary to consider the following main factors: first of all, these are the features of biopolymers of the given vegetable raw materials, the heterogeneity of a substrate, the presence of various kinds of effectors capable of activating or inhibiting both endogenous enzymes and enzymes in the composition of enzyme preparations, the presence concomitant enzymes in addition to the basic activity of enzymes, etc.; secondly, the conditions for enzymatic modification, the main kinetic parameters of enzymatic reactions involving the studied enzyme preparations, which may differ from the kinetic characteristics obtained in the studies of purified enzymes using standard substrates.

At the first stage of the study, the main technological and biochemical characteristics of the study objects were studied, namely, the flour samples formed on the basis of cumulative ash curves and triticale bran (Table 1, 2 and 3).

The flour sample T-60, which is a fraction of the central part of endosperm, and is significantly different in whiteness, ash content, quantity and quality of gluten, had the best technological properties, as shown in Table 1. The obtained data allow to estimate the technological properties of new grades from triticale grain flour as high, with the prevalence of a wheat phenotype. It has been established that triticale grain is characterized by the absence of a significant dependence between the content of gluten and protein, both in grain and in single flour streams. The expected tendency of increasing the protein content in the systems of final grain grinding has been revealed.

2 **Table 1.** Quality of the formed triticale flour grades

Flour sample, grade	Moisture content, %	Whiteness, units of RZ-BPL device	Ash content, %	Amount of gluten,%		Quality of gluten, units of GDI
				Crude	Dry	
T-60	12.0	53.75	0.63	22.7	8.24	70 I – sufficient
T-70	12.1	49.75	0.72	21.0	7.96	66 I – sufficient
T-80	12.1	42.2	0.85	21.7	8.20	66 I – sufficient
T-120	11.7	29.95	1.14	15.8	6.10	57 I – sufficient
T-220	11.3	-8.675	1.99	0.4	0.08	89 II – satisfactory weak

Table 2. Chemical composition of new grades of triticale flour

Flour sample, grade	Protein (N×6.25), %	Starch, %	Fat, %
T-60	10.14	82.28	1.00
T-70	12.23	81.11	1.14
T-80	16.84	77.68	1.25
T-120	17.65	75.60	1.60
T-220	24.88	47.34	2.90

Table 2 presents the analysis of the total content of the main grain biopolymers in the formed grades of triticale flour.

The data presented in Table 2 show that the studied samples, especially the sample T-220, despite a high protein content, are characterized by low baking qualities, as evidenced by trial laboratory baking [1], but can be used as valuable food ingredients.

The study of the quantitative ratio and properties of various fractions of soluble grain albumins is, along with theoretical interest, of great practical interest for the technologies that use grain as the main raw material. Despite the fact that the separation of protein substances by solubility is rather relative, nevertheless, it is used quite widely at the present time. However, there are a lot of questions that remain unclear to this day. This is due, most often, to a difference in the methodological approach of different researchers.

The study of the fractional composition of the soluble proteins of the formed grades of triticale flour showed that the samples of T-60 and T-70 differ in the lowest content of albumins and globulins, but the highest content of prolamins and glutelins that are concentrated in endosperm and form gluten. The main part of albumins and globulins is found in the samples T-120 and T-220, this is apparently due to the presence of the refined germ and the aleuron layer in the flour samples. In the sample T-80 flour, the percentage of all fractions is approximately the same and is 20–25%, the given sample has been formed by mixing 3 main flour streams, which are characterized by a different composition of anatomical parts of the grains (Table 3).

Table 4. Proteolytic activity of the formed grades of triticale flour

Flour sample, grade	Protein, mg/ml	Proteolytic power (PP)	
		Acid proteinases, units of PP/mg of protein	Neutral proteinases, units of PP/mg of protein
T-60	0.080	0.60	0.85
T-70	0.080	0.80	1.20
T-80	0.100	1.40	1.80
T-120	0.160	1.40	2.10
T-220	0.400	0.80	1.00

It is known that proteolytic enzymes play an important part in the processes that proceed in grain when stored and processed. The flour obtained by effecting the grain, violating its integrity and, to a certain extent, by destroying the cellular structure, is a completely different object of study from a biochemical point of view. The object in which the oxidative and hydrolytic processes are primarily activated, including the processes related to the proteolysis of endogenous proteins.

The proteolytic enzymes of triticale grain and triticale flour have been studied poorly [25], much less than the parent proteases - that of wheat [26, 27] and rye [28]. The studies carried out at the Federal State Budgetary Scientific Institution "All-Russian Research Institute of Grain and Its Processing Products" on the p0roteolytic enzymes of triticale grain, revealed the presence of three types of proteinases that actively hydrolyze bovine serum albumin (a standard substrate) and self-proteins: acid proteinases with an optimum pH of 3.5; neutral proteinases with an optimum pH of 6.5 and alkaline proteinases with an optimum pH of 9.5 [29].

Table 4 presents data on the activity of acidic and neutral proteinases of the formed grades of triticale flour. The proteases were extracted as described in the paper [29]. Determination of protease activity using the modified Anson method.

Table 3. Fractional composition of soluble proteins of the formed grades of triticale flour

Flour sample, grade	Fractional composition of proteins,% of the total protein content				
	Albumins	Globulins	Prolamins	Glutelins	Insoluble residue
T-60	11.05	17.82	39.25	28.08	3.80
T-70	12.00	18.14	36.78	26.64	6.44
T-80	20.58	22.24	25.68	23.47	8.03
T-120	72.02	12.04	4.08	3.50	8.30
T-220	43.79	28.95	12.53	6.78	7.95

2 **Table 5.** Biochemical composition of triticale grain and triticale bran of different grades

Grade name	Protein (N×6.25), %		Starch, %		Reducing sugars, %		Fiber, %	
	grain	bran	grain	bran	grain	bran	grain	bran
Topaz	7.6	18.11	68.1	24.84	0.20	12.35	2.08	12.35
Skolot	14.5	18.81	62.6	25.25	0.20	14.85	2.40	14.85
Donslav	14.2	15.90	64.8	28.26	0.24	14.42	2.46	14.42
Vocaliz	12.5	17.06	66.4	32.72	0.28	14.68	2.40	14.68

Table 6. Fractional composition of triticale bran proteins, % of the total protein content

Grade name	Albumins	Globulins	Prolamins	Glutelins	Insoluble residue
Topaz	36.8	24.0	9.6	14.0	20.4
Skolot	38.6	22.2	10.2	14.6	19.8
Donslav	34.0	22.6	9.8	14.8	20.4
Vocaliz	38.0	22.4	10.0	14.6	20.0

The analysis of activity of acidic and neutral proteinases in the formed flour grades indirectly indicates that part of the proteolytic activity in triticale grain is related to gluten proteins, but still the highest activity was noted for the samples T-80 and T-120, that is, these are more likely the proteins of the germ and the subaleurone layer. At the same time, the activity of neutral proteases is 1.5–2.0 times higher than that of acid proteases. The value of proteolytic activity in the formed grades of triticale flour is, in addition to other biochemical indicators, of key importance, since proteinases are able to actively hydrolyze their own proteins, including glutens, which ultimately effects the technological process and the finished product. In addition, proteolytic enzymes participate in the regulation of the activity of other enzyme systems, for example, amylases.

The activity of amylolytic enzymes of grain and flour is another important technological and biochemical characteristic that determines along with other indicators the baking advantages of flour. It was estimated using the method for determining a falling number (FN). FN was 294 s for T-60; 266 s for T-70; 272 s for T-80; 245 s for T-120 and 174 s for T-220.

The falling number value for wheat flour at a level of 230–330 s characterizes the normal amylolytic activity of wheat flour, this value for rye flour is about 100 seconds less. The falling number values obtained in the study of triticale flour samples show that the activity of amylases (excluding the flour sample T-220) is similar to the activity of these enzymes in wheat flour, and along with other indicators confirms the predominance of the wheat phenotype in the triticale grain being studied.

Table 5 presents the biochemical composition of triticale bran. The comparative analysis of the main components of triticale grain and bran indicates a regular increase in the content of crude protein in bran - up to 15.90 ... 20.56%, of fiber - up to 10.68 ... 14.85% and a decrease in the starch content up to 32.72 ... 22.62%. The significant increase in sugars in bran fractions compared with whole grains is due, most likely, to the presence of a refined germ [1]. It should also be taken into account that the carbohydrate complex of triticale grain contains a significant amount of insoluble dietary fiber - hemicelluloses (up to 30%) [5].

The analysis of the fractional composition of soluble proteins (Table 6) showed that the proteins of bran from triticale grain differ in a relatively high total content of albumins and globulins, which is generally characteristic of triticale grain proteins, while the number of globulins is 3 to 3.5 times higher than in whole grain (7–8% of the total protein content). When in a dissolved state, they are actively hydrolyzed by endogenous proteolytic enzymes, giving a large number of hydrolysis products with different molecular weights. The content of prolamines is 2–2.5 times lower than in whole grain (23.6–25.0%).

The study of streams of flour from triticale grain allowed to reveal the most promising streams for obtaining advanced processing products [1, 4].

The scheme of advanced triticale grain processing (Fig. 2), which includes the stages of preparing grain for processing, namely: the selection of grain according to certain quality criteria, the formation of mill mixtures, cleaning and hydrothermal treatment and the division into anatomical parts.

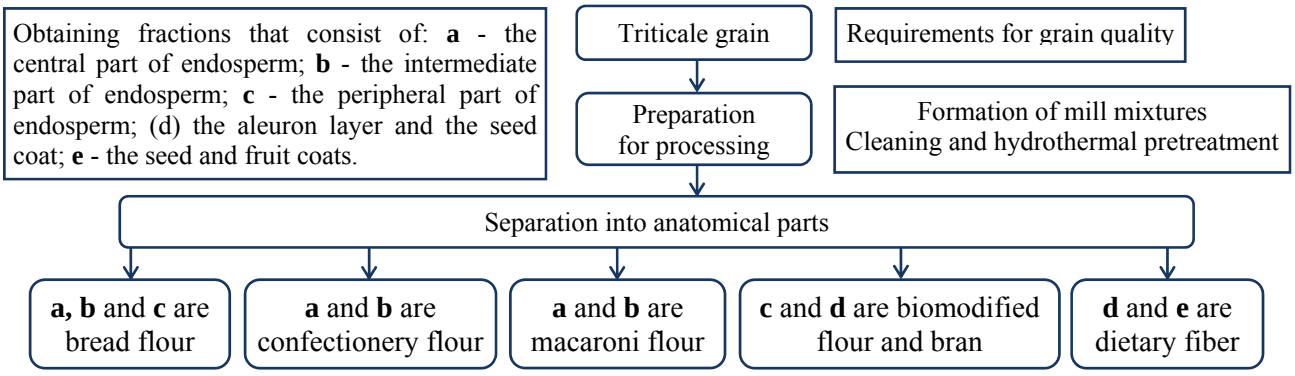

Fig. 2. Scheme of advanced triticale grain processing.

Table 7. Characteristics of the enzyme preparations of proteases during the hydrolysis of triticale flour proteins

Indicator	"Neutrase 1.5MG"	"Alcalase FG"	"Protease GC-106"	"Distizym Protacid Extra"
Initial velocity, V_0 (min)	30	30	30	30
Optimum temperature, °C	50	45	50	40
Optimum pH	5.5	6.5	5.5-6.0	3.5
Optimum amount of enzyme preparation, units of PA/g of flour	0.50	0.5	0.75	0.75
Saturated substrate concentration, mg/cm^3	100	100	100	100

The flour of the samples A, AB and ABC (T-60, T-70 and T-80) was obtained from endosperm and can be used directly in bakery, which was confirmed by trial baking [1], and also after enzymatic modification, as the components of special products that have specific functional and technological properties. The flour of the samples BC and C (T-120 and T-220) from the peripheral parts of endosperm, including the aleurone layer and the seed coat, may be limitedly used in baking, and is mainly a raw material for further processing.

At the second stage of the study, a study was carried out of the effectiveness of proteolytic and cellulolytic enzymatic preparations and the main kinetic parameters of enzymatic reactions in which different types of flour and triticale bran were used as a substrate. The enzymatic modification of proteins of vegetable raw materials, including proteins of grain crops, is an important stage in advanced technologies of advanced processing of grain raw materials. As a result of modification of the protein components of grain and flour with the use of proteolytic enzymatic preparations, hydrolysis products with a certain profile of peptides and a number of amino acids with specific properties can be obtained.

In case of the traditional characteristics of enzyme preparations, the optimum temperature and pH, as well as other kinetic parameters, is detected using a standard substrate [30]. At the same time, in production, in the conditions of a specific food production technology, a complex heterogeneous system acts as the latter, which leads to a change in the basic kinetic parameters of the enzymatic reaction. The composition of the grain substrate can effect the course of the proteolysis process and change the optimum values of temperature and pH [20].

Table 7 presents the main kinetic characteristics of the enzymatic reaction of hydrolysis of triticale flour proteins using bacterial and fungal proteolytic enzyme preparations. The hydrolysis was carried out at the optimum pH and temperature for 30 minutes. It has been previously established that the reaction is zero order for 30 min. The enzyme preparations were added in the amounts from 0.25 to 1.5 units of PA/g of flour, the substrate concentration varied from 20 to 120 mg/ml.

Taking into account the complex structure of the cell wall (the main component of bran), enzyme preparations with a whole complex of activities are required to degrade it and increase the degree of protein extraction: cellulase, hemicellulase and pectolytic activity [31].

Table 8. Characteristics of the enzymatic preparations "Shearzyme 500 L" and "Viscoferm L" when effecting the non-starch polysaccharides of triticale bran

Indicator	"Shearzyme 500 L"	"Viscoferm L"
Initial velocity, V_0 (min)	30	30
Optimum temperature, °C	50	50
Optimum pH	5.5	3.5
Optimal amount of enzyme preparation, units/g of bran	0.3 units of xylanase ability/g of bran	0.4 units of cellulolytic ability/g of bran

Table 8 presents the characteristics of the enzymatic reaction of hydrolysis of non-starch polysaccharides of triticale bran when effected by the enzymatic preparations "Shearzyme 500 L" and "Viscoferm L". The composition of the incubation mixture is the following: milled triticale bran and water (the hydromodule is 1 : 10), a phosphate-citrate buffer 0.1 M (20% of volume) and an enzyme preparation with the activity from 0.1 to 0.5 activity units/g of bran. It has been established that the reaction is zero order for 30 min. The optimum temperature and pH were revealed when studying the activity of the enzyme preparations under study in the range of 20–70°C and pH of 3.0–6.0. The hydrolysis efficiency was estimated by RS accumulation using the Bertrand method.

Similar results were obtained using the flour samples T-120 and T-220 as a substrate. Thus, optimal conditions for the hydrolysis of non-starch polysaccharides of triticale bran and flour with a high content of peripheral parts of grains using the enzymatic preparations "Shearzyme 500 L" and "Viscoferm L" were selected.

The enzymatic hydrolysis of triticale bran proteins using enzyme protease preparations was carried out under the following conditions: the enzyme preparations "Neutrase 1.5 MG" and "Distizym Protacid Extra" were applied in the amounts from 0.25 to 1.5 units of PA/g of bran; the substrate concentration varied from 20 to 120 mg/ml (Table 9).

Table 9. Characteristics of the enzyme preparations "Neutrase" and "Distizym Protacid Extra" when effecting triticale bran proteins

Indicator	"Neutrase 1.5 MG"	"Distizym Protacid Extra"
Initial velocity, V_0 (min)	30	30
Optimum temperature, °C	50	40
Optimum pH	5.5	3.5
Optimal amount of enzyme preparation,units of PA/g of bran	0.50	0.75
Saturated substrate concentration, mg/cm^3	100	100

To estimate the efficiency of the studied enzyme preparations, the enzymatic hydrolysis was carried out under the optimal conditions, which were selected experimentally. The incubation mixture consisted of triticale bran, water (the hydromodule is 1 : 10), the appropriate buffer (20% of volume) and an enzyme preparation based on the final concentration of the corresponding optimum. Sampling was carried out every 30 minutes for 2 hours, the samples were transferred to centrifugal glasses and centrifuged at 6000 rpm for 10 minutes. The supernatant was used to determine the reducing sugars (reducing substances) using the Bertrand method and the amount of soluble protein using the Lowry method.

The hydrolysis efficiency was estimated by the accumulation of RS and soluble protein. The results are shown in Fig. 3 and 4. It has been shown that the enzymatic preparation "Shearzyme 500 L" increases the amount of RS and soluble protein by 2 times; and the preparation "Viscoferm L" increases the amount of RS by 1.5 times and the amount of soluble protein by 2.5 times. The obtained data indirectly indicate the possibility of a significant increase in the nutritional value of secondary products of grain triticale processing.

The flour, obtained from different parts of endosperm, was modified using the multienzyme compositions (MEC) based on bacterial and fungal microbial enzyme protease preparations.

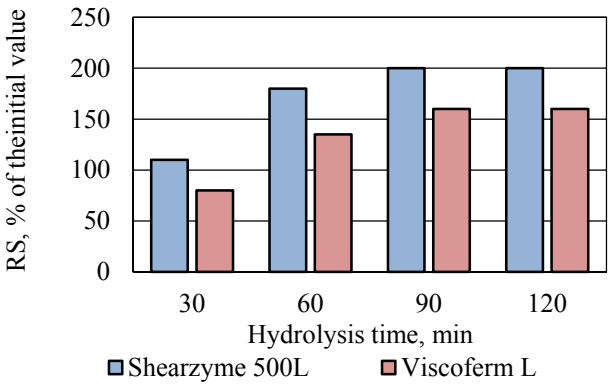

Fig. 3. Accumulation of RS during the hydrolysis of nonstarch polysaccharides of triticale bran using the preparations Shearzyme 500L and Viscoferm L.

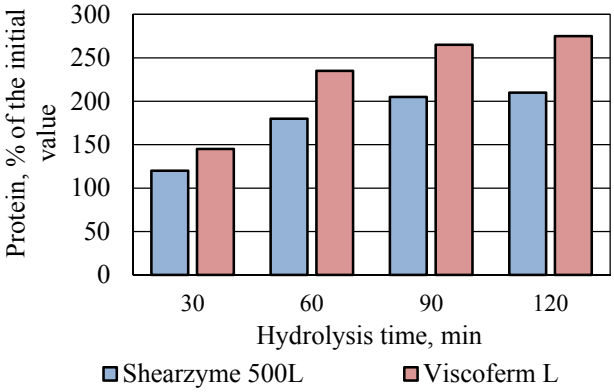

Fig. 4. Accumulation of soluble protein during the hydrolysis of non-starch polysaccharides of triticale bran using the preparations Shearzyme 500L and Viscoferm L.

The enzymatic hydrolysis of triticale flour when effected by the preparations "Neutrase 1.5 MG" and "Protease GC-106" was carried out for 2 hours. The suspension was then centrifuged at 6000 rpm for 15 minutes. 5 ml of supernatant was applied to a column filled with the gel Sephadex G-75. The elution was carried out using distilled water. The volume of aggregated fractions is 4 ml. The optical density of the eluate in the fractions was registered with a wavelength of 280 nm.

A water extract of triticale flour was used as a control sample. The elution profiles are shown in Figure 5.

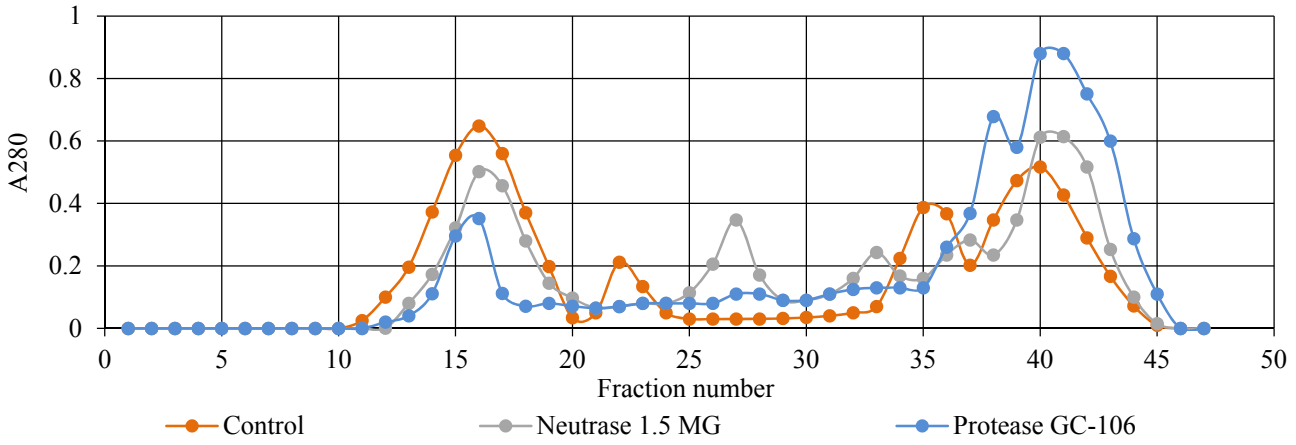

Fig. 5. Fractionation of the products of triticale flour proteolysis using preparations of microbial proteases on a column with Sephadex G-75.

Table 10. Fractionation of the products of proteolysis of triticale flour proteins

Fraction	Molecular weight, Da	% of the total		
		Control	"Neutrase 1.5 MG"	"Protease GC-106"
11–20	≥ 70000	42.71	24.76	19.10
21–25	40000 ÷ 30000	6.49	5.30	4.02
26–32	30000 ÷ 20000	3.36	20.50	7.68
33–36	20000 ÷ 10000	14.18	10.45	6.98
37–45	≤ 3000	34.12	38.91	62.14

Table 10 presents data on the molecular weight, the products of proteolysis of triticale flour proteins formed when applying the preparations of bacterial and fungal proteases, and the percentage of different fractions.

The comparative analysis of the elution profiles presented in Figure 5 and the data of Table 10 shows that the application of preparations of bacterial and fungal proteases does not only change the ratio of high, medium and low molecular weight proteolysis products, but also largely changes the pattern of elution: the nature of distribution of the proteolysis products formed as a result of the use of different preparations is completely different in fractions.

Thus, in case of the enzymatic hydrolysis of triticale flour proteins using the preparation "Neutrase 1.5 MG", there is a decrease in the high-molecular fraction (with a molecular weight of more than 70000 Da) by 42.03%, then, when effected by the preparation "Protease GC-106", - by 55.28%. The increase in the low molecular weight fraction (the molecular weight is less than 3000 Da) is 16.51% and 35.21%, respectively.

When using "Neutrase 1.5 MG", the amount of the formed medium molecular weight peptides with a molecular weight from 30000 to 20000 Da is approximately 2.5–3 times higher as compared to "Protease GC-106"; in turn, when effected by "Protease GC-106", the amount of low-molecular peptides (the molecular weight is 20000 ÷ 10000 Da) is 5.8 times higher than when effected by "Neutrase 1.5 MG".

Table 11. Fractionation of products of proteolysis of triticale flour proteins obtained using MEC

Fraction	Molecular weight, Da	% of the total	
		Control	MEC
11 – 20	≥ 70000	33.56	5.36
21 – 25	40000 ÷ 30000	8.54	4.82
26 – 32	30000 ÷ 20000	14.01	18.94
33 – 36	20000 ÷ 10000	4.57	30.92
37 – 45	≤ 3000	39.29	40.01

On the basis of the studies carried out, multitalzyme compositions have been compiled to obtain products of proteolysis of triticale flour proteins with a different degree of hydrolysis, and, consequently, with various functional and technological functions [32].

The use of MEC, which includes proteolytic enzymes with a different specific effect (the bacterial protease preparations "Neutrase 1.5 MG" and "Alcalase FG" and the fungal protease preparation "Protease GC-106"), allowed to hydrolyze proteins almost completely, as evidenced by this fractionation of products of triticale flour proteolysis using the gel chromatography method on a column with Sephadex G-75 (Fig. 6).

Thus, there are practically no high molecular weight fraction with a molecular weight of more than 70,000 and fraction with a molecular weight of 40,000–30,000 Da, while the amount of low molecular weight peptides and amino acids in the hydrolyzate has increased approximately by 2.5–3.0 times in comparison with the control sample.

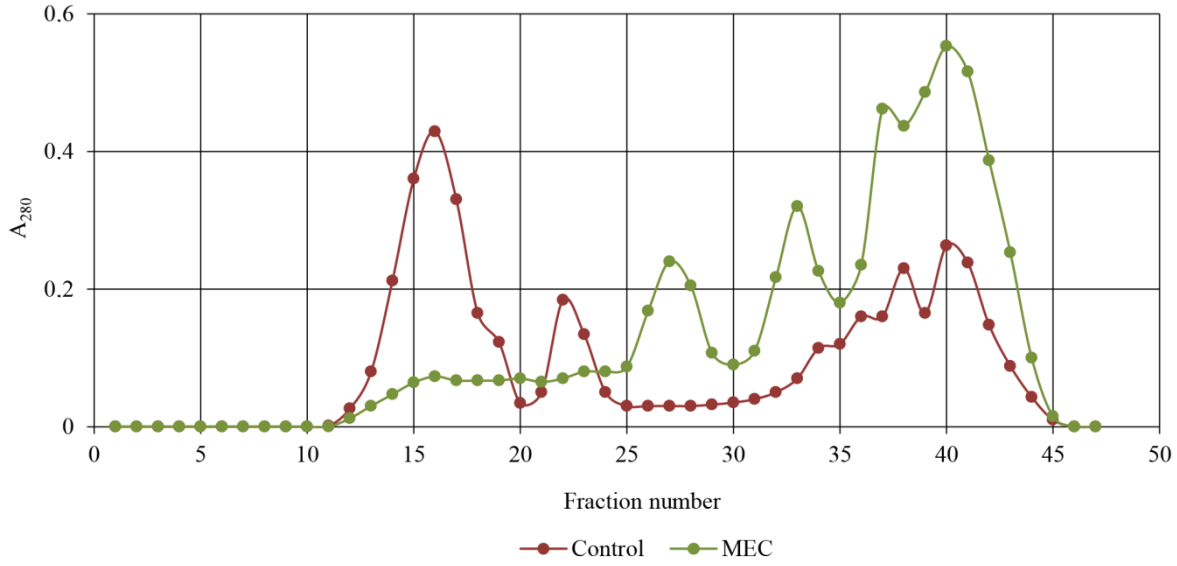

Fig. 6. Fractionation of products of proteolysis of triticale flour proteins obtained using MEC on a column with Sephadex G-75.

The obtained data allowed to position the hydrolyzate obtained with the use of MEC on the basis of enzyme protease preparations as a possible component of hypoallergenic and gluten-free products used for the therapeutic and prophylactic purpose.

Bran and flour with a high content of peripheral parts containing a large number of non-starch polysaccharides, in turn, were modified using MEC based on cellulolytic and proteolytic enzymatic preparations. As a result, products of enzymatic modification of flour and bran from triticale grain with a different degree of hydrolysis of proteins and non-starch polysaccharides and various functional and technological properties have been obtained [21, 31].

The composition of 2 multi-enzyme compositions used for the enzymatic modification of triticale bran and flour with a high content of peripheral parts included: "Shearzyme 500 L" + "Neutrase 1.5 MG" (MEC-1) and "Viscoferm L" + "Dystizym Protacid Extra" (MEC-2). The choice of enzyme preparations is caused by various specific effects and approximately the same effect optima: the optimum temperature is 50°C; pH is 5.5–6.0 for MEC-1 and 40°C; pH is 3.5 for MEC-2. The hydrolysis was carried out in 2 stages. At the first stage, a cellulolytic enzyme preparation was applied. At the second stage, a proteolytic enzyme preparation was applied. The dosage of enzyme preparations, the substrate concentration and the duration of each stage were selected experimentally [4]. Figures 7, 8 and Table 12 present the results of fractionation of the products of proteolysis using the gel chromatography method on a column with Toyopearl gel HW-55F.

The obtained experimental data on the kinetics of enzymatic reactions of hydrolysis of biopolymers of a grain substrate (different types of flour and triticale bran); the degrees of hydrolysis and the ratio of fractions with different molecular weights using the gel chromatography method on a column with Toyopearl gel HW-55F have formed the basis for the development of biotechnological methods for modifying the products of triticale grain processing.

The developed methods for modifying the products of triticale grain processing include the following stages:
– the preparation of a suspension - triticale flour, bran: water (the hydromodule is 1 : 4);
– the preparation of solutions of enzyme preparations; the creation of MEC;
– the enzymatic hydrolysis using MEC under the developed conditions (the substrate concentration, the dosage of enzyme preparations, the optimum temperature and pH);
– the inactivation of enzyme preparations; the product being obtained is hydrolyzed flour or bran (an unclarified hydrolyzate);
– centrifugation;
– the product being obtained is a hydrolyzate (a supernatant) and paste (a precipitate);
– drying;
– the product being obtained is a dry hydrolyzate and hydrolyzed flour and bran;

To estimate the possibility of using the products obtained in food branches, their functional and technological properties have been studied.

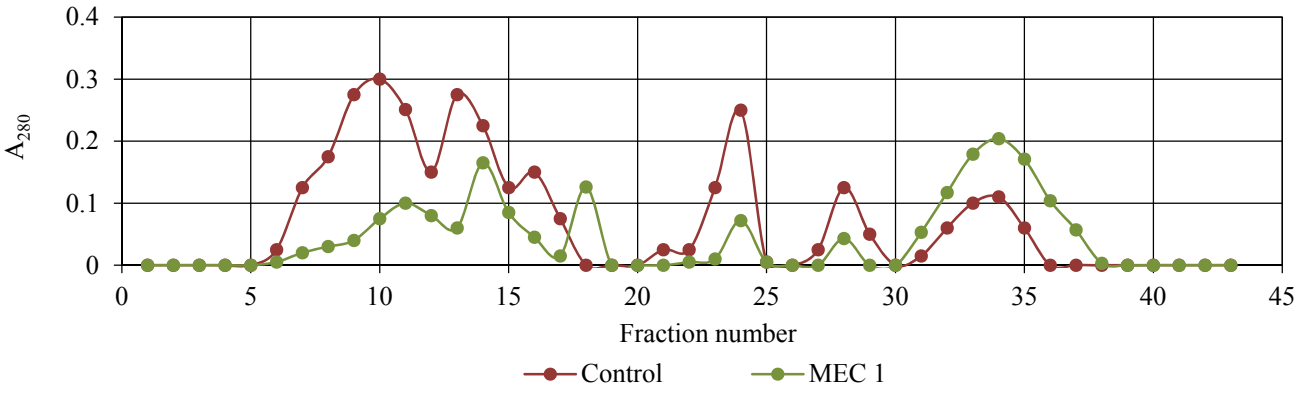

Fig. 7. Fractionation of the products of proteolysis of triticale bran proteins of MEC-1 using the gel chromatography method on a column with Toyopearl gel HW-55F.

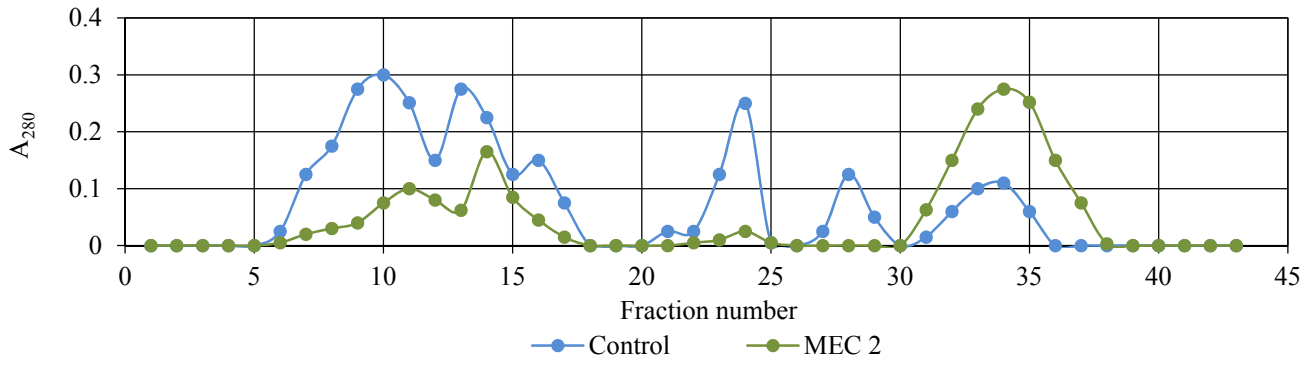

Fig. 8. Fractionation of the products of proteolysis of triticale bran proteins of MEC-2 using the gel chromatography method on a column with Toyopearl gel HW-55F.

Table 12. Fractionation of the products of proteolysis of triticale bran proteins using MEC

Fraction	Molecular weight, Da	% of the total		
		Control	MEC-1	MEC-2
Peak I 6 – 13	≥ 700000 (blue dextran yield)	35.81	2367	19.55
Peak II 14 – 15	450000 ÷ 350000	13.26	14.79	12.62
Peak III 16 – 19	300000 ÷ 100000	9.95	26.04	3.20
Peak IV 20 – 22	100000 ÷ 50000	13.26	0	0
Peak V 23 – 26	50000 ÷ 25000	10.08	5.02	1.77
Peak VI 27 – 30	25000 ÷ 1500	5.31	2.54	0
Peak VII 31 – 36	≤ 1000 (tyrosine yield)	12.33	51.06	62.63

Table 13. Functional properties of the modified triticale bran

Sample*	WBC, g/g	FBC, g/g	FAC , %	ES , %	FFC, %	FS, %
Control - C 1	1.56	1.32	52	58	50	32
Experiment 1 - E1	1.80	1.50	62	53	59	28
Experiment 2 - E2	1.20	1.40	56	46	42	24

Note. * Control C 1 - bran; Experiment 1 - bran + MEC1; Experiment 2 - bran + MEC2

A wide range of physico-chemical characteristics that determine the behavior in heterogeneous food systems during processing, storage and consumption, and also provide the desired structure, technological and consumer properties of food products are to be meant by the functional and technological properties of proteins and protein preparations. Vegetable proteins, as well as proteolysis products with various values of molecular weight, can act as the ingredients of general-purpose, treatment-and-prophylactic and special food products. This is due to the inherent unique functional properties [33]. Depending on the amino acid and fractional composition, molecular weights, the presence of charged and uncharged groups, hydrophilic and hydrophobic groups and other structural features, proteins can serve as gelling agents, foaming agents and form and stabilize suspensions and emulsions, etc. [34, 35].

The requirements for the functional properties of proteins are specific for a certain scope and type of product. For example, when making meat products, the most important are the water- and fat-retaining abilities, gelling, the emulsifying and adhesive properties; in bakery - the water-binding, emulsifying and foaming abilities; the main criterion for choosing a protein preparation in the production of beverages is solubility. To solve the problem of the applicability of specific proteins for obtaining various food products, it is necessary to know how their functional and technological properties change depending on a number of physico-chemical factors: the nature and concentration of proteins in the system, the temperature, pH, the presence and concentration of concomitant biopolymers and low molecular weight substances [33, 36].

In some cases, to improve and regulate the functional properties in order to expand the scope of these or other protein preparations, they are modified using physical, chemical, enzymatic and other methods.

The enzymatic method for the modification of vegetable proteins is preferable to physico-chemical modification, since its advantage are soft reaction modes, the ability to regulate the degree of hydrolysis, its specific directivity and the retention of the biological value [32, 33, 37–40].

Tables 13 and 14 present the water binding capacity (WBC); the fat binding capacity (FBC); the fat emulsifying capacity (FEC); the emulsion stability (ES); the foam forming capacity (FFC) and the foam stability (FS) of the modified triticale bran.

The functional properties of bran from triticale grain and the hydrolyzed samples obtained using MEC1 and MEC2 differ from each other. Thus, the water-binding capacity of the hydrolysed bran in the first option increases by 16%, in option 2 - on the contrary, it decreases by 12.6% with respect to the unhydrolyzed triticale bran. The similar pattern can be seen with respect to the foam forming capacity (Experiment 1: an increase of 18.0%; Experiment 2: a decrease of 16.1%). The fat binding and fat emulsifying capacity increases in both experimental options by 13.6% and 6.1% and by 19.2% and 7.7% respectively.

The stability of the emulsion and foam of the modified triticale bran is reduced: ES - by 8.7%; FS - by 12.5% (Experiment 1) and ES - by 20.7%; FS - by 25.0% (Experiment 2).

Similar studies were carried out using flour samples with a high content of peripheral parts (Table 2).

There is a tendency for samples of the flour modified using MEC1 of an increase in WBC by 21.3 ... 26.0%; in FBC by 13.8 ... 16.0%; in FEC by 74 ... 9.0%. There is, on the contrary, a tendency for samples of the flour modified using MEC2 of a decrease in these functional characteristics: in WBC by 11.8 ... 18.3%; in FBC by 6.7 ... 22.3%; in FEC by 3.8 ... 4.0%.

The stability of the emulsion and foam of the modified flour from triticale grain is also reduced, as in the case of the modified triticale bran: ES - by 8.7%; FS - by 13.4% (Experiment 3) and ES - by 20.7%; FS - by 26.7% (Experiment 4); ES - by 9.1%; FS - by 27.3% (Experiment 5) and ES - by 8.0%; FS - by 30.2% (Experiment 6).

Table 14. Functional properties of the modified flour from triticale grain with a high content of peripheral parts

Sample	WBC, g/g	FBC, g/g	FAC, %	ES, %	FFC, %	FS, %
Control - C2	0.56	0.52	50	52	80	65
Experiment 3 - E3	0.67	0.59	54	50	83	55
Experiment 4 - E4	0.54	0.48	48	42	55	43
Control - C3	0.64	0.54	52	55	86	63
Experiment 5 - E5	0.80	0.62	57	50	98	58
Experiment 6 - E6	0.52	0.41	50	46	64	44

Note. * Control C2 - Flour T-120; Experiment 3 - T-120 + MEC 1; Experiment 4 - T-120 + MEC2; Control C3 - Flour T-220; Experiment 5 - T-220 + MEC 1; Experiment 6 - T-220 + MEC2

It is known that the functional properties of the products of enzymatic hydrolysis of protein raw materials depend on the physico-chemical properties of the initial protein, the specificity of the proteases used, the composition of MEC used, the conditions for hydrolysis, the degree of hydrolysis and the ratio of the fractions of proteolysis products with different molecular weights [36, 37].

The revealed differences in the functional properties in the initial and modified products of triticale grain processing are related, first of all, to the conditions for enzymatic modification (of the pH medium), the composition and specific effect of the enzymes that are part of the composition of MEC; obtaining products of various degrees of hydrolysis, and the number of high-, medium- and low-molecular compounds; an increase or decrease in free polar (charged) aggregations, hydrophilic and/or hydrophobic groups, providing interactions with different types of substances.

The obtained results indicate that the use of MEC on the basis of cellulolytic and proteolytic enzyme preparations allows for an advanced destruction of proteins of the products of triticale grain processing; to obtain products with various degrees of hydrolysis and the ratio of components by molecular weight, which leads to a change in the functional and technological properties of the initial flour and will allow to find its new scopes in food products. Thus, the samples with the pH values close to the neutral ones (modified using MEC1), taking into account the values of the foam forming and fat emulsifying capacities, can be used in foam-emulsion systems, bakery products, cakes and biscuits. The samples with low pH values (modified using MEC2), taking into account their functional properties, can be used to enrich fruit beverages, fermented milk products, salad dressings, sauces, etc. At the same time, it should be taken into account that with low pH values the rate of the Maillard reaction significantly decreases, which can have both negative and positive effects depending on the specific food technology, namely: the retention or reduction of the amount of amino acids and reducing sugars; the formation of melanoidins and a complex of aromatic compounds.

CONCLUSION

In general, the proposed technology allows to form various grades of triticale flour (bread, confectionery, macaroni flour, etc.) and cereals such as "semolina"; to carry out advanced processing of triticale bran and flour, including that with a high content of peripheral parts, using biotechnological methods (enzymatic modification); to receive valuable components for the enrichment and creation of new products with the given properties and composition, contributing thereby to the expansion of not only the raw material base, but also the range of the output products.

The studies carried out have shown that the functional and technological properties of the modified products of triticale grain processing finally depend on the specificity of enzyme preparations and the composition of MEC. The use of MEC on the basis of preparations of microbial proteases allows to hydrolyze triticale flour proteins almost completely, and to position the obtained hydrolyzate as a possible component of hypoallergenic and gluten-free flour products.

The use of cellulolytic and proteolytic enzyme preparations in the hydrolysis of biopolymers of triticale bran allowed to increase the amount of reducing substances (reducing sugars) by 1.5–2.0 times, soluble protein - by 2.0–2.5 times, and the use of MEC on their basis showed that the obtained hydrolysates have a high degree of hydrolysis of non-starch polysaccharides and proteins, a specific ratio of high-, medium- and low-molecular weight peptides and amino acids.

To solve the issue of the applicability of specific products whose proteins are modified, it is necessary to know in various food technologies not only a chemical composition, but also functional and technological properties. The obtained experimental data on the study of the water binding, fat binding, fat emulsifying and foam forming capacities, as well as the emulsion stability and foam stability of the modified triticale bran and flour with a high content of peripheral parts with the use of 2 multi-enzyme compositions showed that enzymatic modification leads to certain changes in the functional and technological properties of the initial flour and bran from triticale grain; and allow to find new and more rational scopes of the modified products as enrichers and as functional and technological components. Thus, the samples with the pH values close to the neutral ones (modified using MEC1), taking into account the values of foam forming and fat emulsifying capacities, can be used in the production of bakery products, cakes and biscuits. The samples with low pH values (modified using MEC2), taking into account their functional properties, can be used to enrich fruit beverages, fermented milk products, salad dressings, sauces, etc. The results of the studies have formed the basis for the development of methods for the enzymatic modification of triticale flour and bran. Hydrolysates, structurally modified flour and biomodified bran, which can be used in the production of a wide range of general-purpose, functional and treatment and prophylactic food products have been obtained.

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MICROPARTICULATION OF CASEIC WHEY TO USE IN FERMENTED MILK PRODUCTION

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Abstract: One of promising methods for caseic whey processing is the production of whey protein microparticulate and its consequent application in food technology. This work is aimed at the development of fermented milk product technology using the whey protein microparticulate based on caseic whey. The researches were carried out at the Chair of Animal Product Technology of the Voronezh State University of Engineering Technology, at laboratories of Kombikorm Scientific and Production Complex, Mollab LLC (Voronezh), at the laboratory and pilot shop of the Voronezh Dairy Plant JSC. Standard and generally accepted physical, physico-chemical, chemical, microbiological, physiological and technological research methods were applied by investigators in the course of the study. The sequence of technological operations to obtain the microparticulate covered pre-preparation of curd whey, ultrafiltration, heating to denaturation temperature and dispersion. This is to ensure protein particle formation average sized 3 μm and formation of unique properties of the microparticulate. Valuable properties of the caseic whey microparticulates are applied in the technology of milk product fermentation. The chosen ferment allows preservation of the required fermentation time (5–6 hours) and to obtain the viscous, creamy consistency of the product. The microparticulate is efficiently proportioned, that is, 10%. We studied the option to use the caseic whey microparticulate in the technology of kefir production. It is found that microparticulate facilitated intensification of process fermentation (10 h long) and maturation (6 h), enhancement of kefiran synthesis by kefir fungi microorganisms, intense formation of carbon dioxide and other osmophoric compounds. Formulations of kefir and sour milk drink are suggested that allow using 10% of microparticulate. Production schemes have been developed and customized to the HACCP system that consider introduction of supplementary operations to obtain the caseic whey microparticulate. The technological solutions developed are known for the following key advantages: realization of the complete production cycle; increase in food biological value; reduction of technological process length due to souring stimulation.

Keywords: Caseic whey, whey protein microparticulate, fermented milk products

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INTRODUCTION

Dairy products are of key importance to ensure food security in the Russian Federation. However, consumption of dairy products by the Russian population is below the national medical standards (it was 230 kg in 2015 at 320–340 kg/year/person (Order № 614 of the Ministry of Health of the Russian Federation dd. 19.08.2016 on "Approval of Recommendations for rational norms of food consumption to meet current requirements of healthy nutrition")). Major problems that restrain the dairy industry development in Russia include the factors like insufficient volume of high quality raw milk and seasonal production variability. Use of milk whey is of high concern as the important source of raw materials. The increase in cottage cheese production volume results in the increase in caseic whey resources, where its share as the processed product is not adequate for food products [1, 2]. Caseic whey is the complex raw stock for food industry. It is widely used for food purposes compensating its short shelf life, low mass fraction of solids, high titrated acidity, specific flavor that converts to final products when processed. A significant portion of caseic whey is not subject to industrial processing; it is discharged to water bodies causing the great environmental damage.

The development of new promising ways to modify the composition and properties of caseic whey enable using its biotechnological potential in food production and meet current concepts of healthy food, refers to urgent task of dairy industry in the Russian Federation [3]. Caseic whey is the important source of functional nutrients. The protein composition of caseic whey is efficiently balanced by amino acids; whey proteins widely function in the human body as follow: stimulate the immune system, lower the cholesterol content in the blood, participate in hormone and enzyme synthesis, and are the source of biologically active substances [4–6]. One of promising directions for caseic whey processing is the production of whey protein microparticulate and its further use in food technology.

The microparticulation technology is known in the Russian Federation [7–10] and in other countries [11–14].

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The method to obtain the microparticulated product titled Simpless was first patented in '80s of the last century by Nutra Sweet Company. The microparticulation technology has been improved to enhance the quality and engineering and technical properties of the product. Whey protein microparticulates have the creamy taste, smooth consistency and they imitate the flavor of milk fat. Microparticulates are used to produce a wide range of food products: sour-milk drinks, cheese, sour cream, ice cream, etc. [15, 16] as the effective fat substitutes. At the same time, commercial products to be sold (dry microparticulate) are of high cost and are normally produced based on cheese whey. For milk processing plants based in Russia, the use of caseic whey to obtain native microparticulates is of particular interest as well as their implementation in process technology of dairy products, including national food products.

This work is aimed at the development of fermented milk production using whey protein microparticulates based on caseic whey.

OBJECTS AND METHODS OF STUDY

The caseic whey obtained during the production of cottage cheese with the mass fraction of 9% fat and skim cheese produced by the Russian GOST 31453–2013 "Cottage cheese. Technical specifications", whey protein microparticulate, and sour-milk drinks produced based on that were used as the research objects. Cottage cheese was produced at the Voronezh Dairy Plant JSC (Russia). The production technology included the milk acceptance, its separation, normalization, pasteurization, cooling, fermentation (mesophilic lactate streptococci were used), ripening, clot slicing, whey separation, clot pressing, cooling and packaging.

The caseic whey was microparticulated at the machinery shop of Voronezh Dairy Plant JSC (Russia) based on the pilot plant by Kieselmann. The sequence of process operations to obtain the microparticulate included preliminary preparation of caseic whey. For this purpose, the whey was cleaned of fat and casein, pasteurized and subje-cted to ultrafiltration to get the concentrate with 14–18% mass fraction of solids. Further on, organoleptic, physical and chemical properties of the obtained UV concentrate were modified using the EcoProt + heat exchanger equipped with the dispersing device.

Two types of fermented milk were considered: with lactic fermentation and mixed lactose fermentation. Lactic fermented drink was produced based on yogurt technology with sugar added by the reservoir method by ripening the normalized mixture at 39 ± 1°C until the dense cluster was formed at the 85–90°T acidity. The normalized mixture was the combination of whole and skim milk with sugar added to make 2.6% mass fraction of fat. To produce the drink, we considered mixed starter cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus* produced by Christian Hansen as follow: YF L 901, YF L 706, YF LX 700, YF L 702.

The drink with the mixed lactose fermentation was produced by kefir technology. Kefir is the fermented dairy product produced by lactic and ethyl-alcohol fermentation using kefir grains-based fermentation starters. The technology of kefir production is known for the standard sequence of operations: skim milk fermentation using the kefir yeast starter (5–10% of the milk weight) at 20–25°C until the dense cluster of 85–100°T in acidity is formed (fermentation length was 8–12 h). Upon fermentation, kefir was exposed to maturation at 14–16°C. The skim kefir was used as the reference sample obtained by the standard technology with no microparticulate.

Sampling and preparation of research objects for testing complied with ISO 707: 2008 (IDF 50: 2008) Milk and milk products. Guidance on sampling. Organoleptic properties were evaluated as per ISO 22935-2: 2009 Milk and milk products. Sensory analysis. Part 2: Recommended methods for sensory evaluation. Composition parameters of research objects, their physical, chemical and microbiological properties were determined in line with Russian standards. The dry solids weight ratio was evaluated by the test sample weight loss in percentage during the sample product drying at the constant temperature. The mass fraction of total protein, whey proteins, non-protein nitrogen was determined by the Kjeldahl method. This method is based on organic matter mineralization of the test sample product with the catalyzing concentrated sulfuric acid to form the ammonium sulfate, convert it to ammonia, distill it to boric acid solution, evaluate the ammonia by quantity by titrimetric method, and calculate the protein mass fraction in the test sample. The lactose mass fraction was determined by the method of Bertrand based on the property of reducing sugars to reduce the divalent copper to copper oxide (I) in alkaline medium, that is oxidized with iron ammonium alum, followed by titration of the reduced ferrous iron with the potassium permanganate solution. The fat mass fraction of was determined by the acidic method that consisted of fat isolation of the research object under the action of concentrated sulfuric acid and isoamyl alcohol, followed by centrifugation and measurement of the volume of released fat in the graduated part of the oleometer. The active acidity was determined by potentiometric method based on measurement of potential difference between two electrodes (measuring and reference electrode) immersed in the test sample. The titrated acidity was found by the titrimetric method. The density was assessed by the densimeter. The method to determine the amount of mesophilic aerobic and optionally anaerobic microorganisms QMAFAnM is based on the count of colonies of microorganisms growing on the solid nutrient medium QMAFAnM at the temperature (30 ± 1)°C for 72 hours.

To evaluate the antagonistic activity of kefir fungi microorganisms in presence of microparaticulate against pathogenic microorganisms, the volume displacement diffusion method (in vitro) was applied. *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* cultures were used as testing cultures. A well of 5–7 mm in diameter was drilled in the agar layer with the test strain using the test drill; the kefir fungi starter was placed inside the well. The Petri dish was placed in refrigerator, then in the thermostat to incubate; the inhibition area of the test strain around the well was measured. The dynamic viscosity of

cottage cheese samples was determined using the Brookfield RVDV-II + Pro rotary viscometer. For purposes of microstructural study, the native preparation of fermented milk drinks was obtained by applying a thin layer of the sample onto the test slide. The prepared specimen was examined under conditions equal to those in the "wet chamber" followed by micro-pattern photofixation using the microphotosystem installed similar to BIOMED-2 microscope and CANON digital camera. The amino acid composition was determined by capillary electrophoresis using the Kapel-105 device. Samples were centrifuged to find the syneretic ability of clots followed by determination of the isolated serum portion. For quantitative analysis of total kefiran content, we used the colorimetric method as samples were pre-test treated as per [17–19].

The content of carbon dioxide in kefir was evaluated by weight gain using the Liebig potash bulb. Toxic properties of fermented milk products were measured in experiments on laboratory animals (white rats). 25 white rats weighing 150–200 g were selected. They were monitored to record the health state considering clinical symptoms of intoxication. The number of died and survived animals was counted for 14 days upon exposure. The preparation was administered to rodents intragastrically, the starting dose was 2 ml/kg, the interval between doses was 1 ml/kg. The following doses were administered: 2, 3, 4, 5, 6, 7, 8, 9, 10 ml/kg per body weight. Allergenic properties of fermented milk drinks were studied on rabbits by taking conjunctival samples. One drop of 1, 5, 10% aqueous solution of fermented milk was put under the upper eyelid of the right eye of three rabbits. One drop of control saline solution was put in the left eye of same animals. Results were measured at 5 minutes, 24 and 48 hours. Such factors as the state of eye and eyelid mucous membrane, presence of vascular injection, and tear secretion were considered accordingly. The effect of absorption through skin was studied on white rats by the tail dipping method. 10 rats were fastened in the special device so that their tails were immersed in 2/3 of the test tube height filled with 50% aqueous suspension of the drink. The animal response was measured at 4 hours by appearance of local changes in the tail, lethal case, extent of intoxication and changes in the body weight of animals.

Test results were processed by mathematical statistics methods based on data obtained in 5 to 10 studies repeated three times. Graphical forms of dependencies are presented as the experimental data were processed by the method of least squares. The data were calculated, plotted and described using Microsoft Office 14 and Excel 14 for Windows 10.

RESULTS AND DISCUSSIONS

We studied the options to obtain the whey protein microparticulate from the caseic whey. This raw material is highly acidic (Table 1) and has specific organoleptic properties that impede its processing and use for alimentary purposes. The caseic whey was pre-concentrated at the ultrafiltration unit. Then, the resulting concentrate was exposed to microparticulation. When the concentrate was heated, disulfide bridges disintegrated that were responsible for the molecular structure of whey protein. SH groups within the tertiary structure were released.

The exposure of reactive groups was followed by the enhancement of the protein molecule tendency to aggregate. They formed new bonds with other denatured molecules of whey proteins. As a result, occurring molecule unions exceeded colloidal state in size and proteins collected in compact aggregates. It is known that all whey proteins denaturate within the temperature range 62–78°C. However, the lactose with the mass fraction of over 4% in the UV concentrate protects the globular proteins against solubility losses during heat treatment, stabilizing their structure against thermal expansion. The high lactose content in the concentrate slows the denaturation response. In this view, more severe heat treatment of UV concentrate is required for microparticulation, that is, from 85 to 110°C.

Table 1. Composition and properties of caseic whey and microparticulate

Parameter	Value	
	Caseic whey	Microparticulate
Dry solids weight ratio, %	5.9	14.2
Mass fraction of total protein,%	0.48	7.46
Mass fraction of plain protein,%	0.33	7.33
Mass fraction of whey proteins,%	0.23	5.67
Content of total nitrogen,%	0.08	1.23
Content of non-protein nitrogen, %	0.0271	0.063
Mass fraction of lactose,%	4.3	5.2
Mass fraction of fat, %:	less than 0.01	0.4
Ash,%	0.65	0.67
Viscosity, 10^{-3} Pa·s	2.7	22.3
Titrated acidity, °T	63	87
Active acidity, units pH	4.7	4.5
Density, kg/m^3	1024.5	1043
Appearance and consistency	Homogeneous liquid	Homogeneous, opaque, moderately viscous liquid
Taste and smell	Typical to milk whey - sourish	Pure milky taste with slight flavor and smell of boiling
Color	Pale green	White with cream tint

To stop the spontaneous aggregation of protein particles and precipitate formation, the UV concentrate was exposed to shearing force of applicable intensity when heated at the same time. This resulted in formation of spherical particles of the microparticulate. By regulating modes of thermomechanical treatment, protein particles sized 3 μm on average formed along with unique properties of the microparticulate. Based on methods of mathematical modeling, the best process parameters for caseic whey microparticulation were identified using the equipment by Kieselmann Company to produce microparticulates of various composition and properties.

The flavor of the caseic whey changed during procedures above. Its specific odor was adjusted by high-temperature treatment of the UV concentrate. The melanoidin reaction resultants contributed to generation of the pleasant "nutty" taste and smell of the microparticulate.

Valuable properties of caseic whey microparticulate were used to produce fermented milk products. For these purposes, whey protein microparticulate was selected with the protein concentration ratio of 15. The microparticulate composition was characterized by the increase in the valuable protein part fraction, in particular, whey proteins (casein/whey protein ratio was 25/75) (Table 1). The content of "plain protein" in the microparticulate was 7.33% which is essential to increase the yield of milk-intensive dairy products and, consequently, increase the production efficiency. The amino acid composition was analyzed to conclude on saturation of the caseic whey microparticulate with essential amino acids. Their ratio is close to the FAO/WHO scaling to prove the high biological value of the microparticulate.

The use of microparticulates is practiced in yogurt production that proves the favorable effect on the rheological and sensory properties of the resultant, as well as the increase in exopolysaccharide synthesis by starter cultures [20–25]. High acidity of caseic whey microparticulate significantly changes properties of the normalized mixture for fermented milk drinks. In this view, of certain concern is the impact of the microparticulate obtained based on the caseic whey on production process and qualitative parameters of fermented milk drinks.

Selection if starter cultures is highly emphasized for products with the modified ratio of components [26]. For this purpose, cluster organoleptic and rheological parameters, dynamics of acid formation were studied when fermenting the drink with the lactic acid fermentation. The cultures YF L 901, YF L 706 that committed to obtain the dense cluster for 5–6 h were identified as cultures with the best acid-forming property in normalized mixtures with the microparticulate added (Fig. 1).

Sucrose is one of components of the fermented milk drink produced with lactose homofermentation. Use of sucrose in formulations of sour-milk products increases the synthesis of exopolysaccharides by starter cultures to be the strain-specific property. In presence of microparticulate as the component with prebiotic properties due to the high content of lactose, protein and free amino acids, exopolysaccharide production is enhanced. And the quality of the finished product is improved: the density and viscosity of the lactic acid cluster increases, the syneresis slows down. This is specifically important when producing fermented milk drinks with lower fat content that is to exclude or significantly reduce the mass fraction of stabilizers and thickeners. The use of YF L 706 starter did not extend the process of normalized mixture ripening and helped to produce the fermented milk drink with viscous, creamy consistency (Fig. 2).

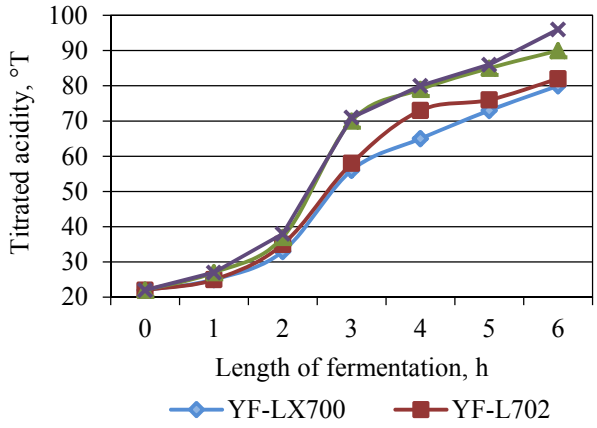

Fig. 1. Dynamics of acid formation of normalized mixture with caseic whey microparticulate.

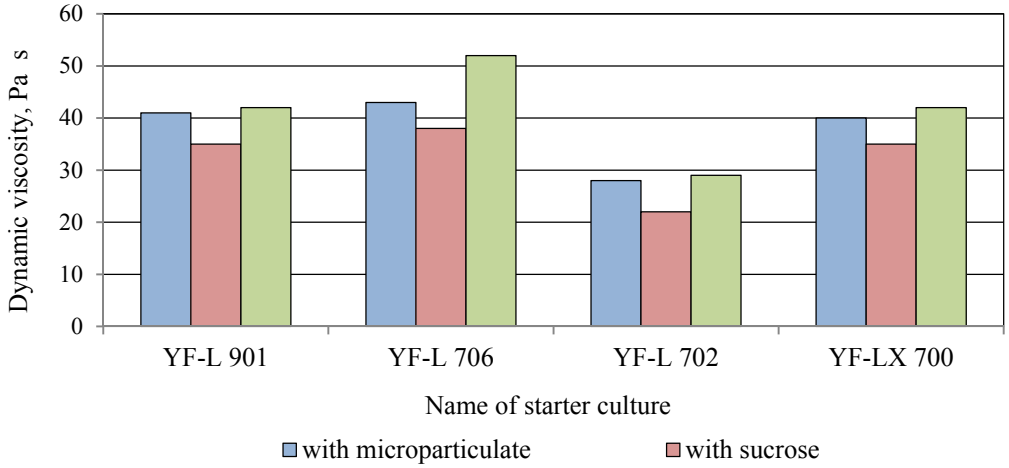

Fig. 2. Influence of whey protein microparticulate and sucrose on the finished product viscosity.

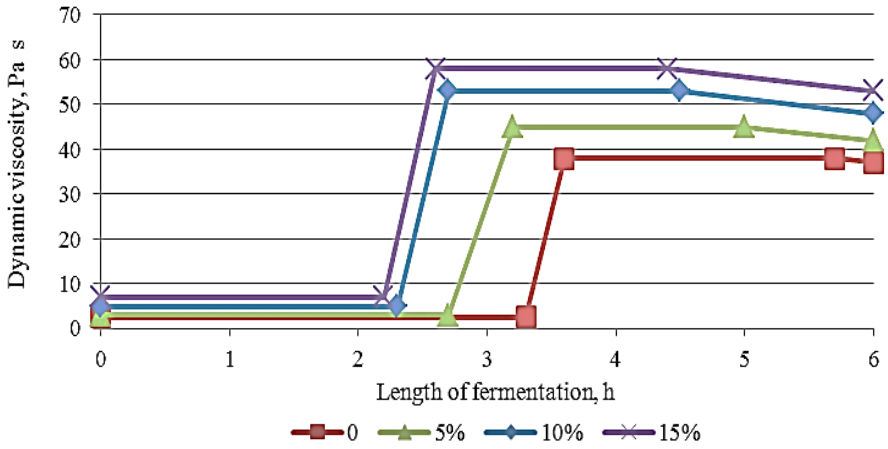

Fig. 3. Rheogram of fermented cluster with microparticulate.

The use of microparticulate accelerated the process of normalized mixture fermentation. The required acidity value was reached at 5 to 6 hours with all test doses of the microparticulate. An increase in the mass fraction of caseic whey microparticulate contributed to the increase in the portion of synthesized exopolysaccharides that affects rheological and syneretic properties of the cluster.

The use of caseic whey microparticulate influenced coagulation and gelling processes and facilitated the formation of stronger bonds between the cluster structural elements and increasing viscosity. The normalized mixture viscosity remained practically the same during the induction period. The microparticulate introduction facilitated an increase in the normalized mixture acidity which affected the rate of casein micelle destabilization and cluster formation. The induction period duration was shortened (Fig. 3). The introduction of caseic whey microparticulate shortened the process of cluster solidifying.

The formed acid gel with the reduced mass fraction of casein has a larger pore size unlike the control one. The microparticulate particles were mechanically captured by the cluster and filled structural pores like fat globules. The high density and strength of bonds of the acid cluster with the microparticulate reduced its ability to syneresis (Fig. 4). A softer cluster formed which is especially important for low-fat gels known to be coarser and stronger than full-fat gels.

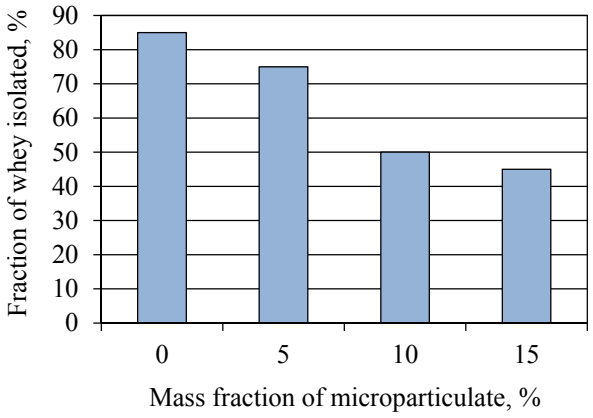

Fig. 4. Influence of the microparticulate mass fraction on the cluster syneretic ability.

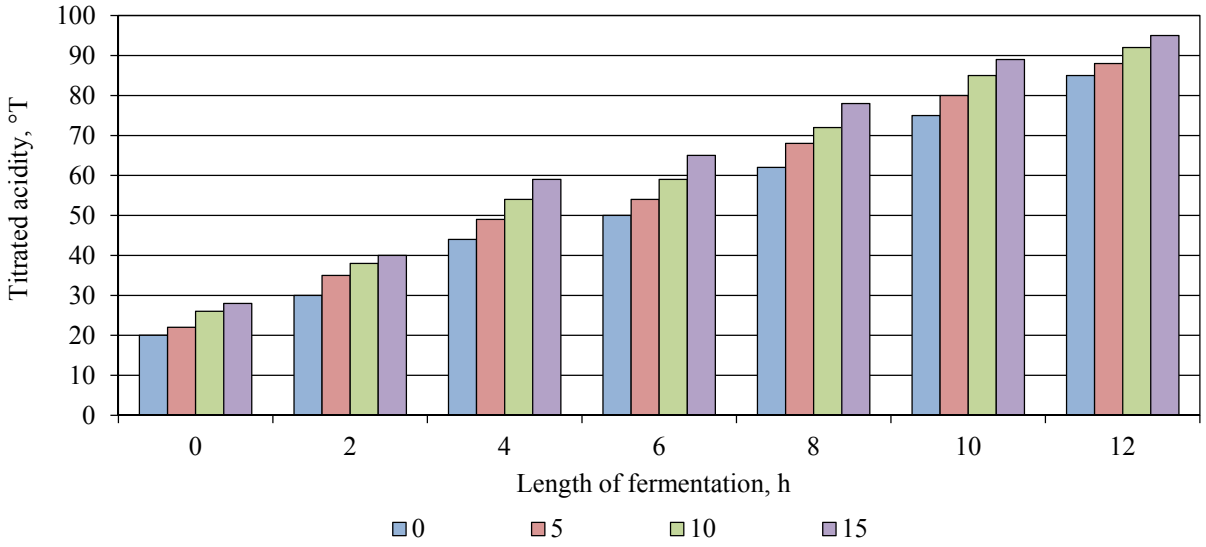

Fig. 5. Influence of microparticulate mass fraction (%) on the dynamics of change in the titratable acidity of kefir during fermentation.

To produce kefir, the whey protein microparticulate was added to the skim milk at 5–15%. The complexity of kefir fungi microbiological symbiosis specifies difficulties to achieve the stable and optimal kefir quality produced using the normalized mixture of modified composition.

It was found that the increase in the mass fraction of the microparticulate facilitated intensification of kefir fermentation process. The standard cluster acidity was achieved for 9 to 11 hours at various microparticulate doses (Fig. 5).

One of factors responsible for the kefir dietary properties is the presence of kefiran, the specific polysaccharide [27]. An increase in the microparticulate mass fraction contributed to the enhancement of kefiran synthesis by kefir fungi microorganisms (Fig. 6) due to an increase in their nutritional factors.

Introduction of the microparticulate intensified the growth of the kefir titrated acidity (Fig. 7) and resulted in lactic acid microorganism and yeast development during maturation (Fig. 8).

The microparticulate intensified carbon dioxide formation that is responsible for the refreshing taste of the drink (Fig. 9). At the same time, its content did not exceed the standard values with no flaws in taste and consistency of the finished product.

Formulations of kefir and fermented milk drink are proposed with lactose homofermentation using 10% caseic whey microparticulate. The developed products have standard quality parameters that meet requirements of the Technical Regulations of the Customs Union 033/2013 "On Safety of Milk and Dairy Products" (Table 2). Organoleptic properties of fermented milk drinks developed are similar to products with high mass fraction of fat; they are creamy and thick in consistency which is achieved without any stabilizers. The developed fermented milk products are characterized with the balanced amino acid composition and high biological value (Fig. 10).

The death of rodents on samples of fermented milk with caseic whey microparticulate during chemical and toxicologic tests at 14 days of monitoring was not registered. Thus, the developed fermented milk products refer to low-toxic chemicals of Class 4. No eye disorders were reported during the study of allergenic properties for the entire observation period in test animals. When assessing the absorption through skin, test white rats did not show any change in the tail skin, no lethal cases and signs of intoxication were reported. The obtained results indicate that fermented milk drinks with the caseic whey microparticulate do not have skin-resorptive and allergenic properties. No cases of negative effect on certain organs, systems and, in general, the body of test animals were reported during the tests.

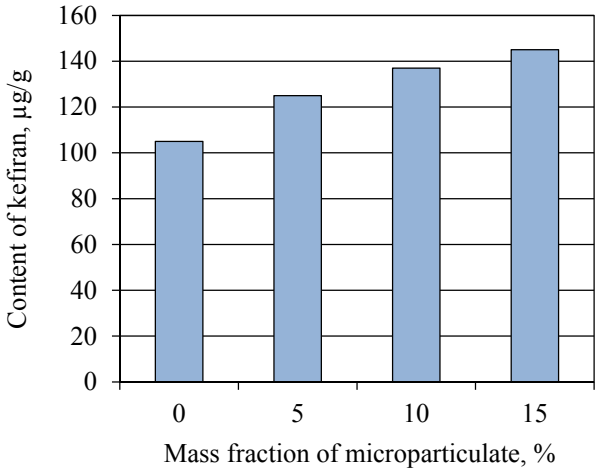

Fig. 6. Influence of the microparticulate mass fraction on kefiran synthesis

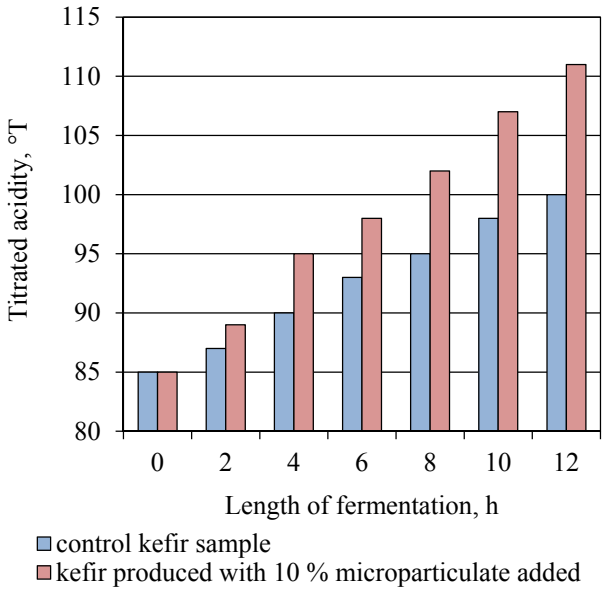

Fig. 7. Change in the titratable acidity of kefir during maturation.

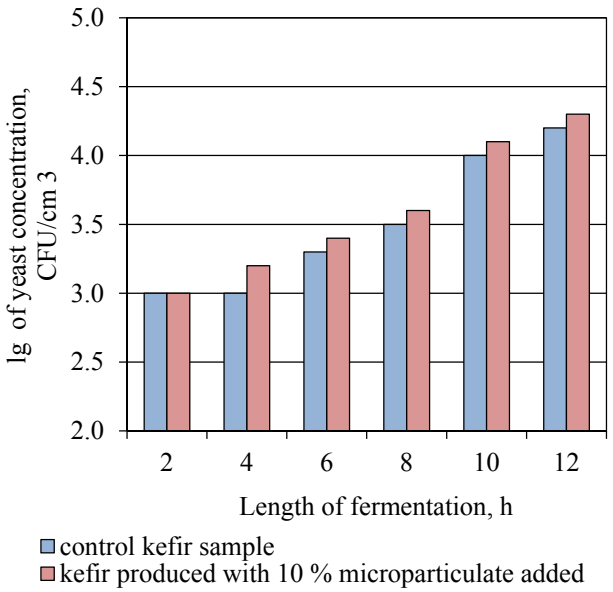

Fig. 8. Change in yeast content during kefir maturation.

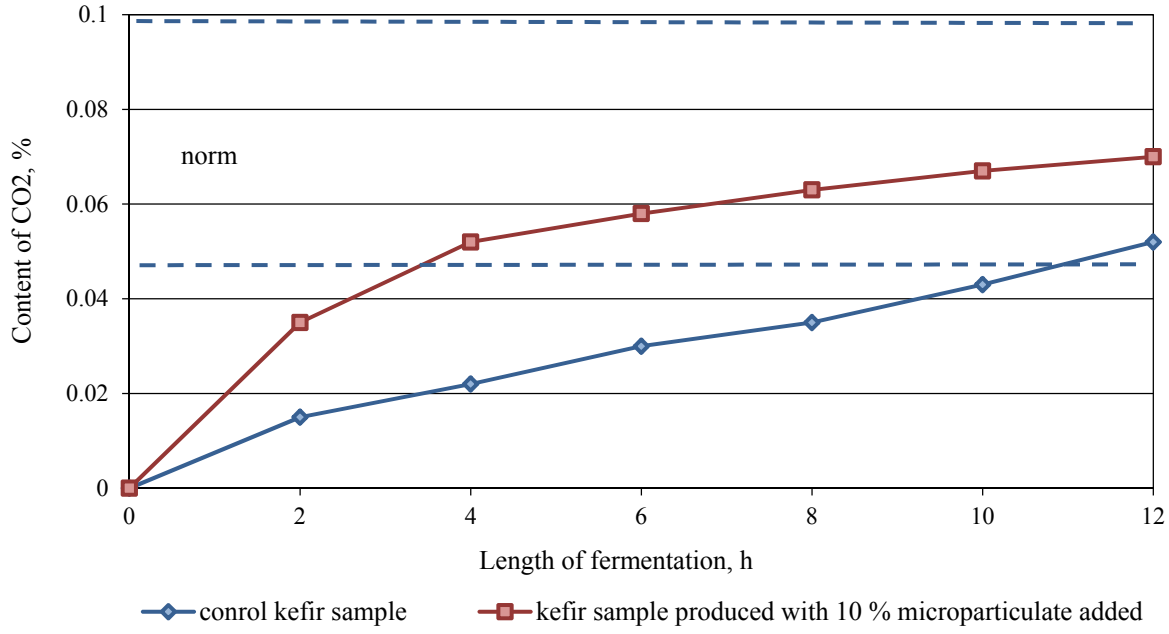

Fig. 9. Influence of the caseic whey microparticulate on carbon dioxide formation in kefir.

Table 2. Physical, chemical and microbiological properties of fermented milk drinks

Parameter	Parameter value for fermented milk drinks		
	Requirements of TR TC 033/2013 "On Safety of Milk and Dairy Products"	with lactic acid and ethyl-alcohol fermentation of lactose (kefir)	with lactic acid fermentation
Mass fraction of fat, %	0.1–9.9	0.1	2.6
Mass fraction of protein, %:	at least 2.8	3.3	3.1
Total mass fraction of skim solids, %	not less than 7.8 (at least 8.5 for dairy component products)	8.9	15.2
Lactic microorganisms, CFU/cm^3	at least $1 \cdot 10^7$	$5 \cdot 10^7$	$8.5 \cdot 10^7$
Yeast (kefir), CFU/cm^3	at least $1 \cdot 10^4$	$2 \cdot 10^4$	–

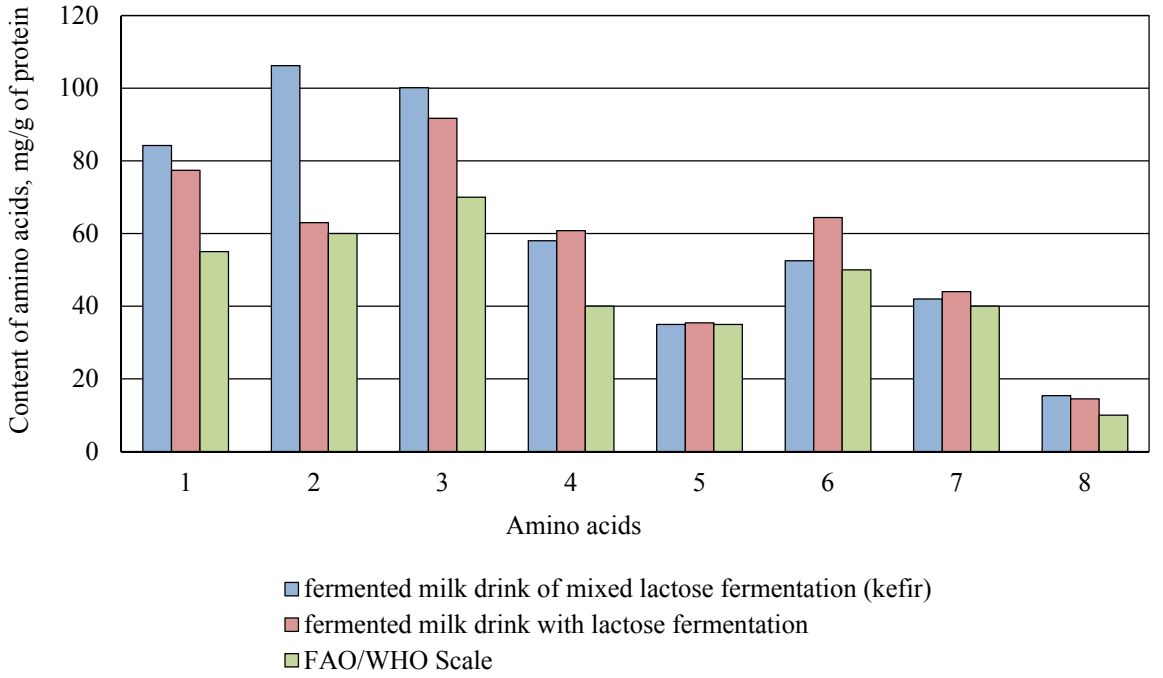

Fig. 10. Content of amino acids in developed fermented milk drinks: (1) lysine, (2) phenylalanine + tyrosine, (3) leucine, (4) isoleucine, (5) methionine + cystine, (6) valine, (7) threonine, (8) tryptophan.

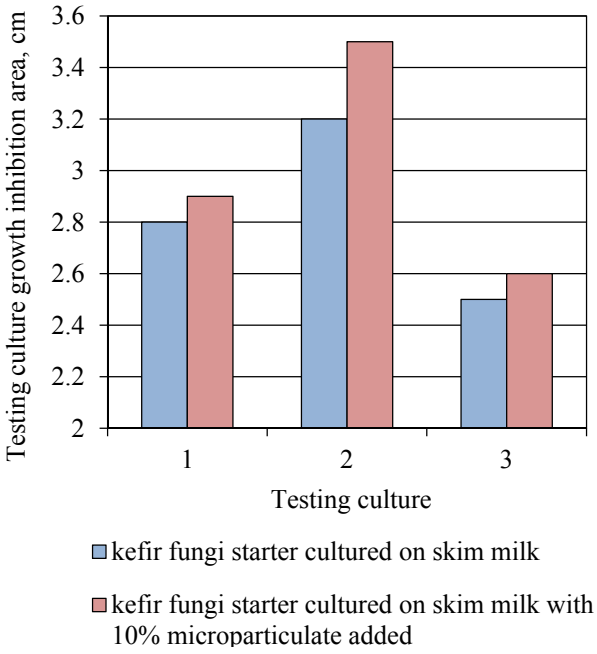

Fig. 11. Antagonistic activity of kefir fungi microorganisms to pathogenic microflora: (1) *Escherichia coli*; (2) *Staphylococcus aureus;* (3) *Salmonella*.

The presence of curdled whey microparticulate slightly increased the antagonistic activity of kefir starter against pathogenic microorganisms (Fig. 11).

This action of the microparticulate is likely explained by the increased fraction of lactic acid, the known antagonist of pathogenic microflora.

Ethanol, propanal, acetone, diacetyl, ethanol were identified in the gas phase of the developed drinks. The typical odor of the fermented milk with lactic fermentation is due to the content of ethanal and diacetyl. Ethanol adds the pleasant refreshing taste to the mixed fermented drink.

A typical feature of fermented drink microstructure with the caseic whey microparticulate is the protein material broadly available as clusters or flakes of various sizes and microorganisms that make up ferments. So, typical representatives of the kefir starter microflora were identified at medium increases in kefir (Fig. 12). The group was lactate streptococci prevailed consisting of active acid formers and aroma-forming bacteria. Their number in test sample preparations is visually greater than in the control ones. Single yeast cells of round and oval form were found almost in all fields of vision.

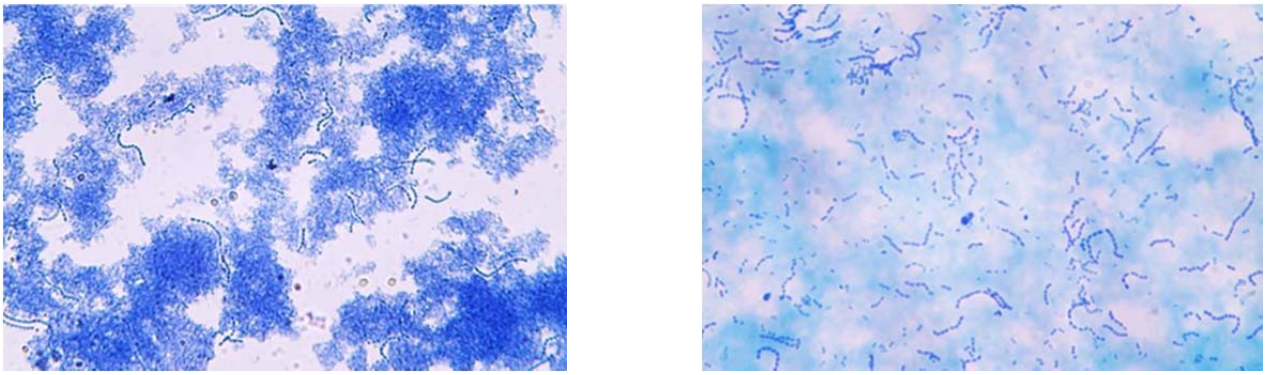

Fig. 12. Microstructure of the control and test sample of kefir with 10% microparticulate added (at X300 magnifying).

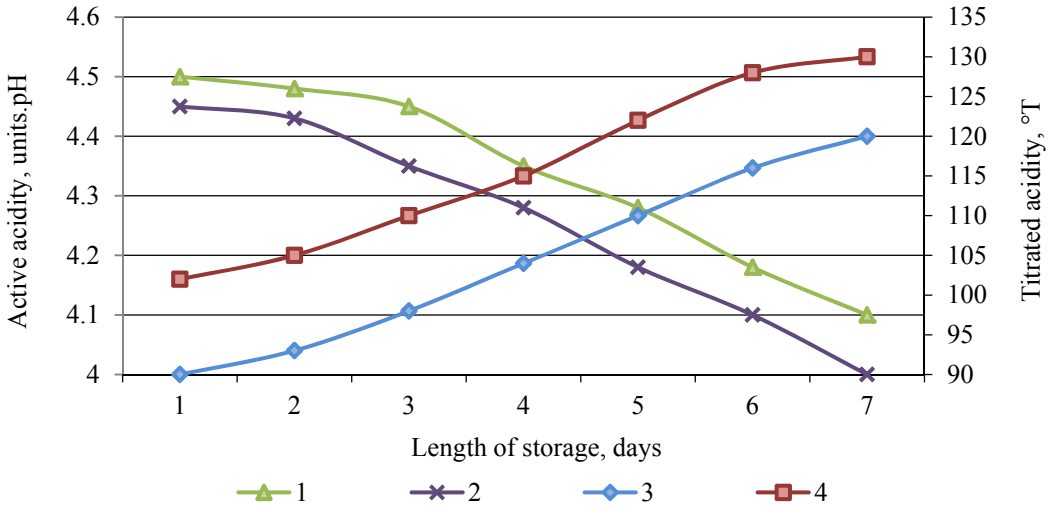

Fig. 13. Effect of storage term on: (1) active acidity of fermented milk drink with lactose homofermentation, (2) active acidity of kefir, (3) titratable acidity of the fermented milk drink with lactose homofermentation, (4) titratable acidity of kefir.

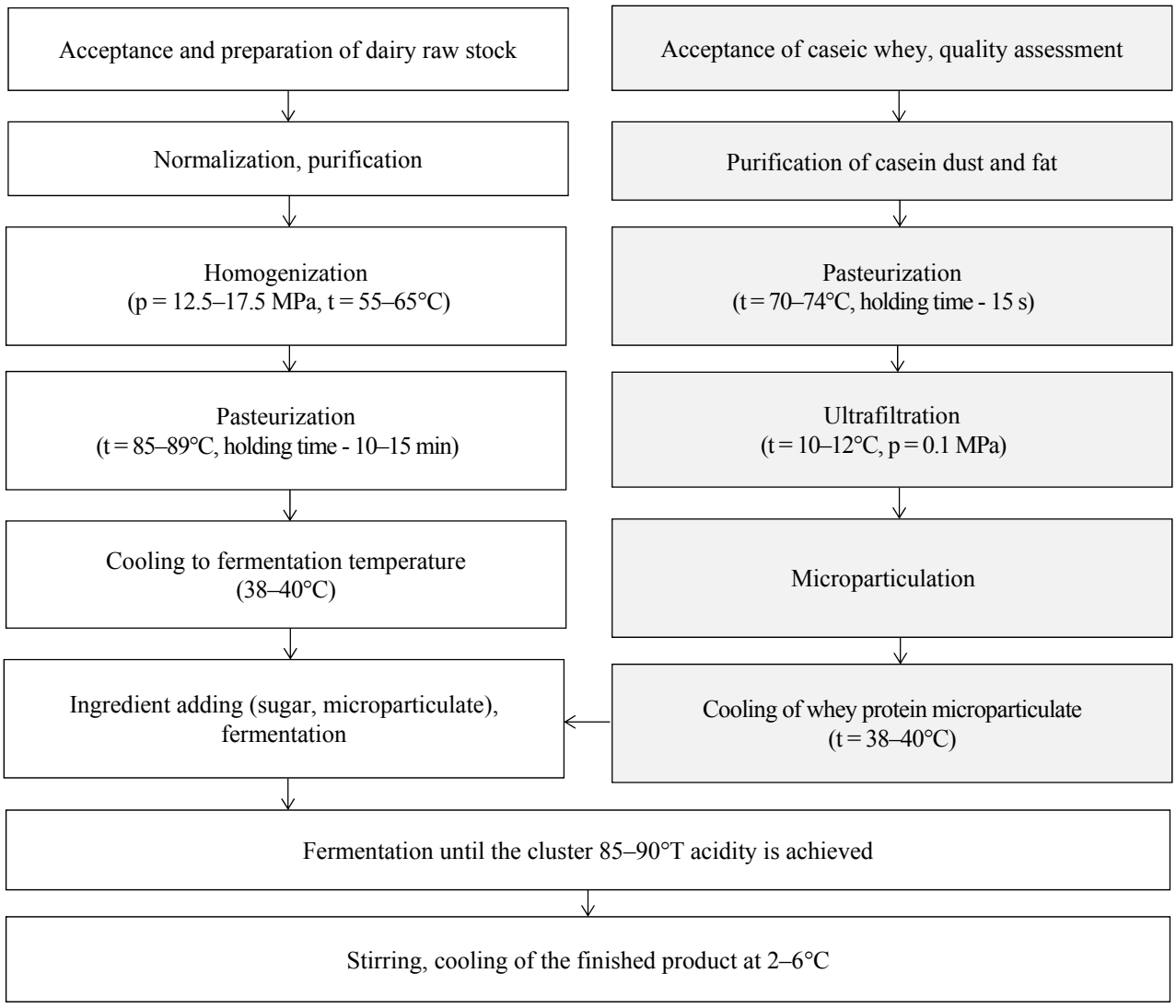

Fig. 14. Technological scheme to produce the fermented milk drink "Snezhok".

To define the expiration date, organoleptic, physical and chemical (microbiological) properties were determined in samples of developed fermented milk drinks assigned for storage. It was found that the guaranteed shelf life of fermented milk drinks at the temperature of (4 ± 2)°C is 5 days.

Production schemes for fermented milk drink have been developed and customized to the HACCP system that consider introduction of supplementary operations to obtain the caseic whey microparticle (Fig. 14).

The hardware design includes the standard equipment of milk processing plants and does not result in confusing of drink production processes.

CONCLUSIONS

The process of microparticulation has been studied to be one of the most complicated raw stock for the dairy industry in the Russian Federation, that is, the caseic whey. The microparticulate regimes have been chosen to allow production of whey protein microparticulates of different composition and properties. Starter cultures have been selected for the fermented drink with lactose and sucrose fermentation to allow active acid formation, high viscosity and good organoleptic properties of the finished product. We managed to identify the rational mass fraction of the microparticulate for the drink with lactose homofermentation during the study of the gelling process with acidic coagulation, as well as the properties of the resulting cluster. We evaluated the impact of the microparticulate on production process and the quality of the fermented milk with mixed lactose fermentation. Its rational mass fraction was 10%. The use of caseic whey microparticulate in the composition of developed fermented milk products increases their biological value, adds to shorten the process length due to fermentation acceleration; improves organoleptic properties of fermented milk drinks, including fat-free ones. We studied of composition, quality and safety parameters of fermented milk drinks with the caseic whey microparticulate to be able to conclude on their nutritional value for the Russian population. These technologies developed in the course of researches allow addressing the important dairy industry issue, namely, the use of by-product (caseic whey) as the full-value stock.

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IMPROVEMENT OF BREWER'S YEAST VIABILITY BY ADJUSTING WORT COMPOSITION

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Abstract: Along with necessary substances for yeast, wort also contains extraneous impurities and technologically important components that affect the cells adversely. The effect of these compounds on yeast can be mitigated by adding adsorbents and stabilizers into a fermentation medium. In this article, methods of improving brewer's yeast viability by removing phenols and high-molecular-weight proteins from wort are considered to accelerate the fermentation process and improve the final product quality. Both traditional materials (natural zeolite-containing tuffs from different fields of Siberia) and new ones (carbon-containing fibers and cyclodextrins) were suggested to transform wort composition. It has been revealed that introduction of 5% of zeolites into a mash at 70°C, or filtration of hopped wort through a mineral or carbon fiber layer at 0.10 l/min reduces haze-forming components content by 12–25%. Without significant effect on other characteristics of wort, polyphenols tend to be absorbed more than proteins. Addition of cyclodextrins in amount of 0.1–5.0% (w/v) into wort before fermentation contributes to clathrate formation with proteins and phenolic compounds. The materials used provide favorable conditions for yeast: biomass increases by an average 20%, yeast growth and extract fermentation intensify, and by-products formation decreases. This allowed us to shorten the fermentation process by 1–1.5 days, produce beer with desired organoleptic parameters, and increase its resistance to colloidal hazes formation.

Keywords: Brewer's yeast, adsorbents, stabilizers, polyphenols, proteins, fermentation, beer

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INTRODUCTION

A culture medium composition has significant effect on both the physiological state of yeast culture and its characteristics, such as yeast growth rate, fermentation activity, flocculating power, and formation of accessory substances [1, 2, 3, 4].

Apart from organic and inorganic compounds needed for yeast, wort may also contain harmful impurities and extraneous substances, such as mycotoxins, pesticides, nitrates, heavy metals, etc. They affect the culture adversely and may come into wort with raw materials (water, malt, hop, or grain unmalted materials), supporting materials, or from equipment [2, 4, 5]. This leads to brewing process breakdown and beer quality reduction. Also, wort contains substances that play a huge role in technology and, at the same time, affect yeast adversely. In particular, they include polyphenols and high-molecular-weight proteins [2, 6].

The role of phenols in beverage manufacturing, including beer, is diversified [2, 3, 6, 7]. On one hand, phenols define color, aroma, flavor, and antioxygenic properties of the product. On the other hand, their excess in beer causes haze formation, insoluble aggregates formation with proteins, and undesired changes in organoleptic properties, such as flavor and color. The important property of plant polyphenols is microorganism growth inhibition – which both extraneous microflora and microorganism cultures need for brewing. This increases the biological stability of finished beer, but has a deleterious effect on the physiological state of yeast, on culture growth, and on the fermentation process. This fact is of special importance for yeast cultures in media with high content of ethanol and extractives. The permeability of cell membranes increases and makes it possible for phenols to penetrate easily into cells with the result that fermentation slows down and yeast viability decreases. An example is high-gravity brewing technology [2, 8, 9].

In a number of cases, wort composition influences yeast mechanically. Insufficient precipitation of cold break which consists of 50% protein from wort at the stage of its clarification and cooling leads to adsorption of cold break by yeast cell walls. This makes the

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fermentation process complicated. Moreover, some components of the precipitation in cold wort degrade the filterability and colloidal stability of beer [2, 6, 7].

Such factors as long-time storage, incorrect storage, low quality of fermentation medium, and a number of stress factors affect the physiological and biochemical characteristics of yeast culture negatively. It is possible to increase yeast activity both at the inoculum preparation stage and during fermentation, in particular, by adjusting wort composition [1, 10, 11]. Two methods can be used for this: supplementation of the fermentation medium with needed chemical compounds and the removal of components inhibiting the culture.

It is known that removal of polyphenols from the culture medium has a favorable effect on yeast growth, and hence, on the fermentation process. Thorough wort clarification gives the same result. However, removal of more than 35% of polyphenols and 30% of polypeptides leads to degradation of such beer characteristics as foam stability, palate fullness, and taste [6, 7].

Current methods of reducing the number of polyphenols and high-molecular-weight proteins are mainly based on their adsorption at final stages of beer production using specific, expansive agents [2, 3, 6, 7].

The aim of the research was to develop methods of improving brewer's yeast viability by removing primarily polyphenols from wort using various adsorbents and stabilizers that intensify fermentation and also improve beer quality.

As adsorbents, natural aluminum silicates (zeolite-containing tuffs) and carbon-containing fibers were used; α-, β- and γ-cyclodextrins were suggested as stabilizing agents.

Natural zeolite-containing tuffs are promising sorbents being used in beverage production to ensure hygienic safety and improve the quality of raw materials, semi-finished, and finished products [5, 12]. Advantages of using these minerals compared to other sorbents are accessible price, availability of extraction, considerable reserves, simplicity of regeneration and utilization, ample possibilities of improvement of properties and processing, and unique properties (ion-exchange, adsorption, and molecular-sieve) [5, 13]. All the properties of zeolites may be used in food industry. First of all, they are of interest as naturally-occurring filtering materials and sorbents in brewing for detoxification and improvement in quality of semi-finished and finished products [5, 12, 14, 15, 16].

Natural zeolite resources in Russia amount to nearly 4.8 billion tons; more than 20 zeolite deposits are located in Siberia and in the Far East. The largest deposits in Siberia are Pegasskoe (Kemerovo region), Khonguruu (the Sakha Republic), Shivirtuyskoe (Chita region), Kholinskoe (the Buryat Republic), and Sakhaptinskoe and Pashenskoe (Krasnoyarsk Krai) [5, 17, 18].

Precursors for carbon fiber are chemical or organic materials, such as polyacrylonitrile, rayon, and pitch [19, 20]. After heat treatment, carbon fiber composes nearly 99.9% of carbon atoms; hence, it allows us to consider it a graphitized carbon material. Each strand of the fiber is 3–15 microns in diameter.

The variety of the properties of carbon fibers obtained at the present time depends on their structure that, in turn, is dictated by the nature of the precursors, their properties, and processes at different stages of fiber manufacturing. The fibers are characterized by high yield strength in tension, low thermal expansion coefficient, low specific density, high chemical inertness, high thermal resistance, and specific properties. In this connection, carbon fibers are widely used in composite materials fabrication, in electrical and radio engineering industries, for purification of liquids and gases from dispersed impurities, as catalysts in the synthesis of organic and inorganic substances, as a source of drug and biologically active substances in medicine, and as sorbents for purification of blood and other biological fluids, etc. [19–24]. The results of new researches allow the usage of carbon fibers to be expanded.

Cyclodextrins (CDs) are a special type of enzymatically modified starch that belong to non-reducing oligosaccharides. Their reactivity is dictated by the presence of one primary (C_6) and two secondary (C_2 and C_3) hydroxyl groups which can participate in a combination reaction with formation of branched and polymeric forms of CDs, chemically modified CDs, and CDs bound covalently to different matrices [25, 26].

Cyclodextrins and their derivatives are used in pharmaceutical, chemical, food processing industries, and in other industrial fields. Their ability to form complexes with different substances makes it possible to improve tastiness and rheological properties of foods, increase their food value and shelf life, stabilize flavoring agents and biologically active substances, remove unpleasant odors and tastes, enhance vitamin stability and solubility of lipophilic components, prolong the effect of drugs, reduce their harmful effect, purify wastewater from oils and toxic impurities, etc. [25]. However, the field where cyclodextrins are used most is food industry. Mixtures of β- and γ-cyclodextrins are used as a complexing agent to improve taste and aroma of juices, whisky, protein hydrolyzates, to make soft beverage powders, juice powders, in manufacture of bread, baked goods, confectionary products, pastas, in brewing (to stabilize the beer composition), etc. [25–31]. Technologies, at which cyclodextrin complexes are formed during the process, are promising [32].

The given list of natural zeolites, carbon fibers, cyclodextrins properties – mainly, the ability to sorb and bind large organic molecules – makes it possible to consider these materials showing potential for using them in brewing to adjust yeast metabolic activity and, as a consequence of this, to intensify the wort fermentation process and improve the finished beverage quality.

OBJECTS AND METHODS OF STUDY

The subject of the research was lager yeast of the *Saccharomyces cerevisiae* strain 11.

The hopped wort with the original gravity of 11 and 12% was used as a fermentation medium.

The natural zeolite-containing tuffs of different deposits of Siberia, such as kholinskiy zeolite, shivyrtuin, and pegasin, were used to remove polyphenols and high-molecular-weight nitrogenous compounds from the fermentation medium. The minerals were preliminarily washed off, dried at 120°C, and crushed into particles of 1.2–1.5 mm in size. In addition to tuffs, carbon-containing fiber in the form of non-woven fabric being obtained by pyrolysis of rayon was used as an adsorbent. In order to stabilize wort composition, chemically pure cyclodextrins («Novodex», Ufa, Russia) in the form of a mixture of homologies (α, β, γ) were used.

To study the fermentation process, yeast was added into wort ($20x10^6$ cells/ml) taking into account the number of non-viable cells. Fermentation was carried out in an enclosed vessel with a hydraulic lock at the temperature of 6–8°C. Secondary fermentation was carried out at 2–3°C for the time needed for a particular beer.

Solids content, acidity, pH, color, viscosity, bitterness, amino nitrogen, and reducing substances in the wort were determined before and after wort treatment, and during fermentation, as well as by means of widely used standard methods [33].

The ethanol content in fermenting wort was determined by distilling, after which the relative gravity of the distillate was determined. Determination of the diacetyl content was carried out spectrophotometrically using EBC technique [33]. Higher alcohols and acetaldehyde were determined by gas chromatography in the FGBU laboratory «Kemerovo Center of Standardization and Metrology».

The total concentration of polyphenols, anthocyanogens, and high-molecular-proteins were analyzed by photocolorimetry. Jerumanis method [6] based on their reaction with ferric ammonium citrate (III) in alkaline medium was used to determine the total amount of polyphenols. The anthocyanogens content was determined by Steiner-Stocker method in Pfeffer modification [6], being based on changing anthocyanogens to anthocyanidins by treating beer with the mixture of concentrated chlorohydric acid containing ferrum, and butanol. The ability of high-molecular weight proteins to react with tannin in acidic medium was used to calculate the protein A fraction [33]. The finished beer was tested for tendency of chill haze formation by measuring beer haze after 24 hours at 40°C, then at 0°C. The test characterizes the colloidal stability of beer indirectly [6].

The yeast physiology state during growth of the culture was analyzed according to the concentration of suspended cells, the number of budding cells by direct counting method, and the concentration of glycogen-containing cells using Lugol's iodine solution [34].

The analyses were performed three of four times, and the results were processed statistically.

RESULTS AND DISCUSSION

At the first stage of the work, the possibility of using zeoilte-containing tuffs to remove components that affect yeast adversely with the aim of intensifying metabolic process of the culture was studied.

Zeolites have unique adsorption and cation-exchange properties due to porous structure and the ability to ion exchange. Sorption properties allow zeolite-containing tuffs to remove polyphenols and high-molecular-nitrogenous substances and their compounds from a media. Using tuffs to remove these compounds is possible at various stages of wort production, such as mashing, filtration of mash, boiling of wort with hop, and clarification of chilled hopped wort.

In the beginning of the research, zeolites were introduced into chilled hopped wort with the original extract of 11%. Wort was filtered through the layer of mineral placed in a column (75 mm in diameter, 230 mm in height). Tuff mass needed to fill column volume was 250 g for pegasin, 245 g for kholinskiy zeolite, and 255 g for shivyrtuin, respectively.

Filtration was carried out at different rates (l/min): 0.05, 0.10, and the maximum, where wort flowed under gravity (0.40 for pegasin, 0.39 for kholinskiy zeolite, and 0.38 for shivyrtuin, respectively). Rate was regulated with a clip; the head of the wort supply into the column was constant. The wort temperature was 8–10°C. The wort volume for filtration was 1 liter; five samples (each of 200 ml) were taken during the filtering and evaluated according to main quality factors. The results are in Table 1.

Filtration of hopped wort through the zeolite layer leads to removal of phenols and polypeptides. The lower the filtration rate, the greater the number of removing substances; however, other characteristics of the medium do not change considerably.

Depending on the mineral used, the minimum filtration rate ensures the reduction of high-molecular-weight proteins amount by 16–21%, polyphenols by 39–51%, and anthocyanogens by 36–40% in comparison to the initial value, while at the maximum rate the number of these components decreases by only 10–12%, 15–37%, and 10–20%, respectively. The filtration rate in the 1.10–0.05 l/min range ensures better clarification of wort as well.

It should be noted the negative effect of zeolites on such important characteristic as bitterness. When filtering wort using tuffs of different deposits, the amount of bitter substances decreases by 2–3% at the maximum filtration rate and by 16% at the minimum rate compared to the initial value.

Among the studied minerals, pegasin and kholinskiy zeolite have demonstrated the highest adsorptive capacity to proteins and polyphenols due to high content of clinoptilolite, which has the sufficient number of active centers acting on large polar molecules [5, 18]. On the other hand, shivyrtuin has a considerable impact on wort clarification; it is because the mineral contains more than 50% of the clay fraction – montmorillonite – which is able to be easily dispersed as extremely small-sized particles in an aqueous medium.

In order to remove the sufficient amount of polypeptides and polyphenols (about 20%), samples of hopped wort were passed through columns filled with the minerals at the rate of 0.10 l/min. Then, the samples obtained were subjected to fermentation. Zeolite-untreated hopped wort was used as a control sample.

Table 1. Quality factors of zeolite-treated hopped wort at different filtration rate

Wort sample	Filtration rate, l/min	Solids content, %	Reducing sugars, g /100 ml	Amino–nitrogen content, mg/100 ml	Color, units	Bitter substances, mg/l	pH	A–protein fraction, mg/100 ml	Anthocyanogens, mg/100 ml	Polyphenols, mg/100 ml	Degree of clarification*, nominal units
Pegasin											
Before treatment	–	11.0	8.40	24.0	2.00	28.0	5.57	18.3	13.2	16.0	1.00
After treatment	0.40	11.0	–	–	2.00	27.2	5.56	16.1	11.8	13.7	1.10
	0.10	11.0	8.27	23.7	1.96	26.4	5.53	15.4	9.4	10.2	1.15
	0.05	11.0	–	–	1.94	23.4	5.55	15.2	7.9	8.5	1.20
Kholinskiy zeolite											
Before treatment	–	11.0	–	–	2.00	29.2	5.55	13.9	12.5	16.0	1.00
After treatment	0.39	11.0	–	–	2.00	28.4	5.44	12.9	11.7	12.8	1.10
Before treatment	–	11.2	8.40	24.0	2.00	28.0	5.65	15.4	13.0	17.0	1.00
After treatment	0.10	11.2	8.23	23.1	1.97	27.1	5.60	13.2	9.8	11.2	1.20
Before treatment	–	11.2	–	–	2.20	28.5	5.55	24.1	13.7	16.3	1.0
After treatment	0.05	11.2	–	–	2.10	26.3	5.46	21.0	8.6	9.1	1.30
Shivyrtuin											
Before treatment	–	11.0	8.91	24.7	2.00	28.7	5.60	19.0	12.6	15.0	1.00
After treatment	0.38	11.0	–	–	2.00	28.0	5.55	17.5	10.0	11.4	1.20
	0.10	11.0	8.64	24.3	1.95	27.7	5.57	16.9	9.2	10.5	1.30
	0.05	11.0	–	–	1.92	24.2	5.56	16.0	8.1	9.0	1.45

Note. *The degree of clarification was calculated as a ratio of the optical density value of wort before treatment to that after treatment. Measurements were carried out by means of photoelectric colorimeter at the wavelength of 650 nm and the layer thickness of 10 mm.

The analysis of the data presented in Table 2 shows that yeast in the study samples grew so rapidly that the maximum number of cells was reached 24 hours sooner than in the control sample. In comparison with the control sample, the greatest increase in biomass was observed in wort treated with pegasin (by 33%) and kholinskiy tuff (by 14%), which contains polyphenols and high–molecular–weight proteins in small amounts.

It should be noted that concentration of suspended yeast in the study samples of green beer by the end of fermentation was lower by a factor of 1.1–1.6 than in the control variant. This fact had a positive effect on the degree of beer clarification and, in a sequel, would allow the service life of filters to be prolonged.

The intensive growth of yeast in study samples led to the increase of fermentation rate of wort by an average of 20% in comparison to the control sample that made it possible to reduce fermentation duration by 24 hours.

Stimulation of yeast metabolic processes has influenced other characteristics of the fermentation medium as well. The study samples of wort are characterized by intensive acid formation and reduction of pH that create favorable conditions for precipitation of proteins and protein–phenolic complexes that, in turn, increase the beverage colloidal stability.

It is seen from table 3 that physical and chemical parameters in finished beer samples changed more significantly than those in the control sample (Table 3). The content of high–molecular–weight proteins and polyphenols in study samples is higher than in the control that indirectly indicates the beer colloidal stability. Beer obtained using tuffs contains less volatile components (which affect taste and favor of beer negatively) than the test.

Table 2. Characteristic of wort treated with pegasin (O_1), kholinskiy zeoilte (O_2), and shivyrtuin (O_3) under dynamic conditions

Parameter	Wort (duration of fermentation in days)				
	C (7)	O_1 (6)	O_2 (6)	C (7)	O_3 (6)
Solids content by the end of fermentation, %	5.5	4.2	4.5	5.6	4.7
Concentration of suspended yeast, x10^6 cells/ml:					
– maximum (in three days for the control sample, in two days for the study samples)	45.5	60.4	52.0	46.0	50.1
– at the end of primary fermentation	11.0	8.5	9.8	11.5	7.4
Maximum concentration of budding cells, % of total	37.0	59.0	54.0	32.3	49.8
Titratable acidity, units	2.30	2.60	2.50	2.06	2.20
pH	4.55	4.35	4.42	4.60	4.50
Color, units	1.00	0.60	0.70	1.55	1.43

Table 3. Parameters of finished beer made from wort treated with zeolite–containing tuffs (pegasin (O_1), kholinskiy zeolite (O_2), and shivyrtuin (O_3)) at clarification stage

Parameters	C	O_1	O_2	C	O_3
Alcohol, % vol.	3.43	3.71	3.63	3.40	3.58
Degree real of attenuation, %	48.6	55.4	50.7	48.1	49.9
pH	4.48	4.33	4.38	4.54	4.44
Titratable acidity, units	2.35	2.60	2.53	2.12	2.28
Color, units	0.96	0.56	0.64	1.45	1.36
Polyphenols, mg/100 ml	12.4	8.8	9.8	13.5	11.8
Antocyanogens, mg/100 ml	8.9	6.7	7.5	9.6	8.1
Tannin index, units	0.38	0.30	0.34	0.35	0.29
Colloids, g/l	120	56	45	98	32
Test 2/1, un. EBC	0.51	0.45	0.46	0.48	0.45
Higher alcohols, mg/100 ml	11.0	9.8	9.0	8.9	8.2
Acetaldehyde, mg/100 ml	0.30	0.26	0.25	0.38	0.35
Diacetyl, mg/100 ml	0.018	0.012	0.015	0.021	0.014

The revealed dependencies of studied medium parameters from different minerals arise from properties and composition of used tuffs. Tuffs not only reduce the amount of polyphenols in wort but also probably adsorb some other (foreign) components which came from raw materials [5, 12, 17].

In addition, it should be taken into account ion–exchange properties of zeolite–containing tuffs [5, 13, 14, 18]. The minerals differ in content of silicon dioxide, calcium, magnesium, potassium, sodium, iron, and other chemical elements. Potassium and sodium in kholinskiy zeolite and shivyrtuin, and calcium and magnesium in pegasin play a dominant role among exchangeable ions [5, 17, 18]. Consequently, they will take part in ion exchange with ions in the yeast culture medium and have an influence on enzyme activity, yeast growth, and their ability to flocculate which was reflected in the results obtained. In addition to basic elements, cuprum, plumbum, zinc, cobalt, molybdenum, vanadium, strontium, beryllium, and others are presented in the tuffs of different deposits.

Micro– and macro elements in natural minerals are in quite available form. Some minerals perform structural function in a cell while others are able to influence the activity of enzyme system [1, 2, 4].

In particular, magnesium and potassium ions enhance fermentation of maltose and maltotriose and relate to fermentation rate. Calcium and zinc cations which activate many enzymes (especially alcoholic fermentation enzymes), have an effect on yeast growth, prevent the culture from degeneration, and take part in the cell flocculation process [1, 2, 3, 4]. An importance of potassium for reducing oxalate content in wort which increases the beer colloidal stability should be noted [6, 7].

Sodium, along with enzyme activation, plays a significant role in transportation of substances through a cell wall.

Manganese enhances the fermentation activity of yeast and, along with calcium and magnesium cations, stimulates sterol formation [4] which has a positive effect on cell membranes structure and penetration of nutrients into the cell.

Being in small quantities in the fermentation medium, iron and cuprum exert a favorable effect on the yeast budding. Cobalt contributes to better assimilation of proteins by yeast and the increase of culture fermentation activity. As the main structural component of natural zeolites is silicon dioxide, this fact allows us to assume that it has an effect on the mineral composition of the fermentation medium. Silicon is present in yeast cell membranes, accelerates carbohydrate metabolism, contributes to better assimilation of nitrogenous substances by yeast, including amino acids [1, 2, 4].

Thus, removal of endo– and exogenous substances inhibiting the yeast culture by the zeolite–containing tuffs allows us to optimize micro– and macro–elements content in the medium. It can be considered as a means to adjust and improve the mineral composition of semi–processed materials and finished beverages.

The use of zeolites is possible not only at the clarification stage but also in mashing. The latter appears advisable in order to optimize the polyphenols content, high–molecular–weight proteins content, and bitter principles content, and to preserve components necessary for yeast feeding.

Based on the above, in our research we used pegasin to make wort. For this, infusion mashing using 100% pale malt was used to make wort in laboratory setting. The mineral in the amount of 5% to cereal product mass (the optimum parameters were determined in earlier researches [5]) was added at the saccharification stage at the temperature of 70°C. Wort made without pegasin was used as the control sample. Samples obtained were subjected to hopping under identical conditions. The parameters of study and control samples of hopped cooled wort are presented in Table 4.

It is revealed that the zeolite adsorbs proteins and polyphenols from wort so that their content is by 18% less than in the control sample. Additionally, the study sample had lower viscosity and contained less phenols which affect wort and beer color as well as color index of the medium. The amino nitrogen content also decreases (by 4%). Bitter principles were found to be the same in both of samples.

Hopped, cooled 11% wort was subjected to fermentation. The parameters characterizing the primary fermentation process are presented in Table 5.

Table 4. Parameters of wort made using pegasin

Parameter	Control sample	Study sample
Solids content, %	11.0	11.0
Reducing sugars, g/100 ml	8.10	8.15
Amino–nitrogen content, mg/100 ml	35.1	33.6
pH	5.60	5.55
Titratable acidity, units	1.80	1.80
Color, units	1.81	1.74
Bitter principles, mg/l	27.0	26.5
Polyphenols, mg/100 ml	22.0	17.8
Tannin index, units	0.58	0.49
Viscosity, mPa·s	1,63	1.58

Table 5. Parameters of yeast physiology state and physical–chemical parameters of wort during fermentation process

Parameter	Sample	Duration of fermentation, days			
		0	3	5	7
Concentration: –of suspended yeast, x10^6 cells/ml	control	20.0	73.5	51.3	33.4
	study	20.0	78.1	57.5	31.7
– of budding cells, % of total	control	43.2	67.9	52.3	45.1
	study	43.2	73.2	56.8	46.2
– of yeast with glycogen, % of total	control	35.7	33.2	39.1	44.4
	study	35.7	33.9	40.6	45.7
Solids content, %	control	11.0	8.9	6.1	4.5
	study	11.0	8.3	5.7	4.3
Alcohol, % vol.	control	–	0.67	2.03	343
	study	–	0.88	2.38	3.54
pH	control	5.60	5.35	5.13	4.51
	study	5.55	5.29	5.05	4.45
Titratable acidity, units	control	1.80	2.05	2.23	2.42
	study	1.80	2.11	2.35	2.50
Color, units	control	1.81	1.52	1.34	1.15
	study	1.74	1.44	1.28	1.05

It is seen from the data obtained that in wort made using the zeolite at the mashing stage, intensive yeast growth during the fermentation process occurred. The maximum amount of budding cells was achieved by the third day of fermentation; however, the value in the study sample was 8% greater than in the control one.

Basic parameters of the fermentation medium (color, pH, titratable acidity, extractives content, and ethanol) in the study sample change faster than that in the control one. The amount of the visible extract in the study sample is 4% lower, and the ethanol content is 3% higher than in the control one.

After primary fermentation, green beer was subjected to secondary fermentation. According to the data from Table 6, the study beverage is characterized by higher degree of attenuation, increased ethyl alcohol content, and lower concentration of diacetyl and acetaldehyde that has a favorable effect on its flavor and aroma.

Table 6. Parameters of beer made using pegasine at the mashing stage

Parameter	Control sample	Study sample
Alcohol , % vol.	3.96	4.15
Degree real of attenuation, %	56.6	57.3
pH	4.44	4.35
Titratable acidity, units	2.60	2.70
Color, units	0.95	0.87
Tannin index, units	0.38	0.30
Content, mg/100 ml – of the A–protein fraction	15.4	12.5
– of polyphenols	19.3	14.5
– of antocyanogens	13.3	9.9
Test 2/1, un. EBC	0.51	0.40
Higher alcohols, mg/100 ml	7.9	7.6
Acetaldehyde, mg/100 ml	0.92	0.81
Diacetyl, mg/100 ml	0.027	0.016

Moreover, the study sample of beer contains less high–molecular–weight proteins (by 19%) as well as polyphenols and antocyanogens (by 25 and 26%, respectively) that obviously will ensure the great stability of beer against colloidal hazes formation.

The parameter called «test 2/1» gives more accurate information about the colloidal stability of beer and its tendency to form hazes. The value is 22% less in the test sample than in the control.

As for organoleptic parameters, beer made using the zeolite at the mashing stage had intense hoppy flavor and pure malty taste.

Therefore, the use of natural zeolite–containing tuffs both at the mashing stage and at the clarification allows polyphenols and high–molecular–weight nitrogenous substances to be removed partially from the medium. As a result, the yeast culture growth intensifies, biomass grows, and the uptake of the extract accelerates. This makes it possible to reduce the primary fermentation duration by 24 hours and to improve the beer quality at the same time.

Along with nature minerals, we used carbon fiber obtained by pyrolysis of rayon as an adsorbing agent to remove components inhibiting the yeast growth from the fermentation medium. Carbon–containing fiber in the form of non–woven fabric was placed between Zeiss filter plates. Filtration of hopped cooled wort was carried out at different rates.

During filtration, several samples of wort were taken (0.1 liter in volume); the total volume of treated wort was 0.7 liter. For each filtration rate, a new filtering material was used. The mass of fiber was 0.22 g (the parameter was determined in previous researches as the optimum one). The results are in Fig. 1.

The amount of polyphenols removed from wort is greater by a factor 2.0–2.5 than that of proteins. A characteristic feature of carbon–fiber adsorbents is high sorption activity due to the presence of microscopic pores in their top surface, which play a dominant role in the molecular adsorption process. Carbon–containing fiber is able to arrest coarse particles as well [19, 20, 22, 23, 24].

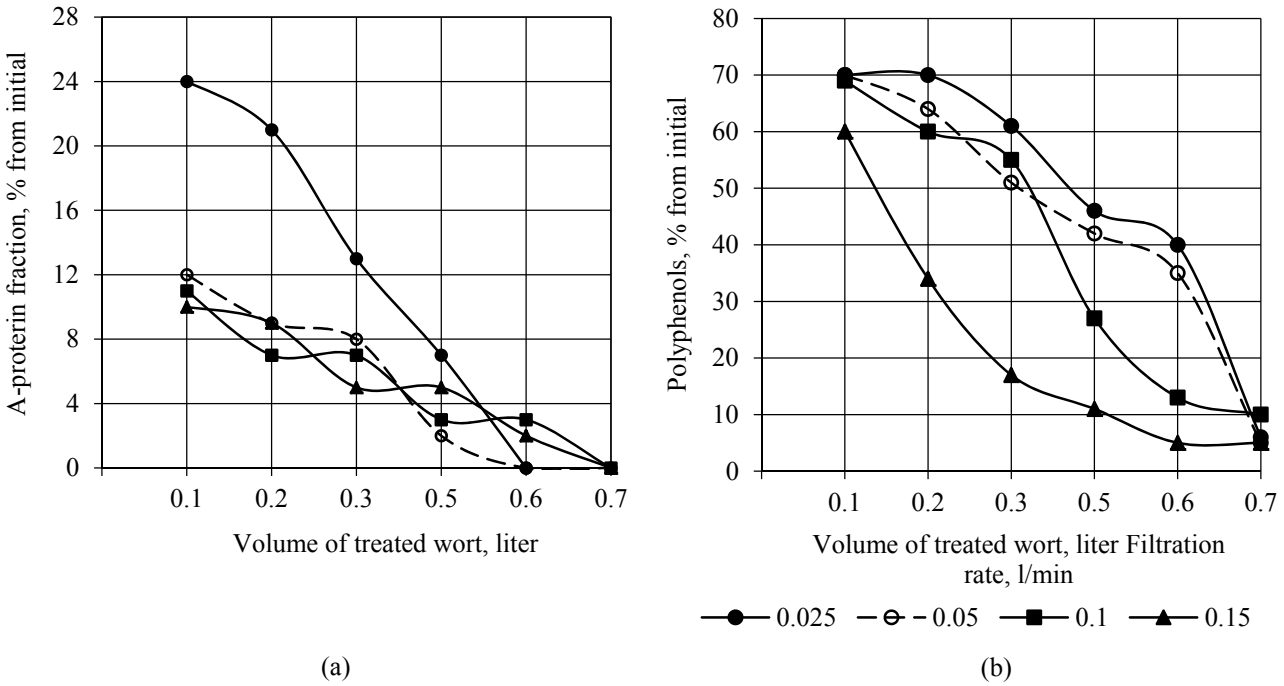

Fig. 1. Dependence of an amount of (a) high–molecular–weight proteins and (b) polyphenols removed using carbon fiber on the volume of treated wort and the filtration rate.

The reduction of filtration rate from 0.15 up down 0.025 l/min leads to the increase in the amount of removed substances (from 6 up to 16% for polypeptides and from 27 up to 54% for phenolic substances.

The adsorption of the studied substances is maximal at the filtration rate of 0.025–0.10 l/min and wort volume of 0.4–0.5 liter; then the degree of their removal from the medium decreases. Some parameters of wort after the treatment with carbon fiber at the rate of 0.10 l/min are given in table 7.

It is seen from the data obtained that high–molecular–weight nitrogenous substances fraction and the total content of phenols and antocyanogens have decreased by 12% and by a factor of 1.4 respectively.

Table 7. Parameters of wort after the fiber treatment

Parameter	Wort	
	before treatment (control sample)	after treatment (study sample)
Solids content, %	11.0	10.9
Content, mg/100 ml – of the A–protein fraction	14.7	1.9
– polyphenols	10.5	7.5
– antocyanogens	5.3	3.7

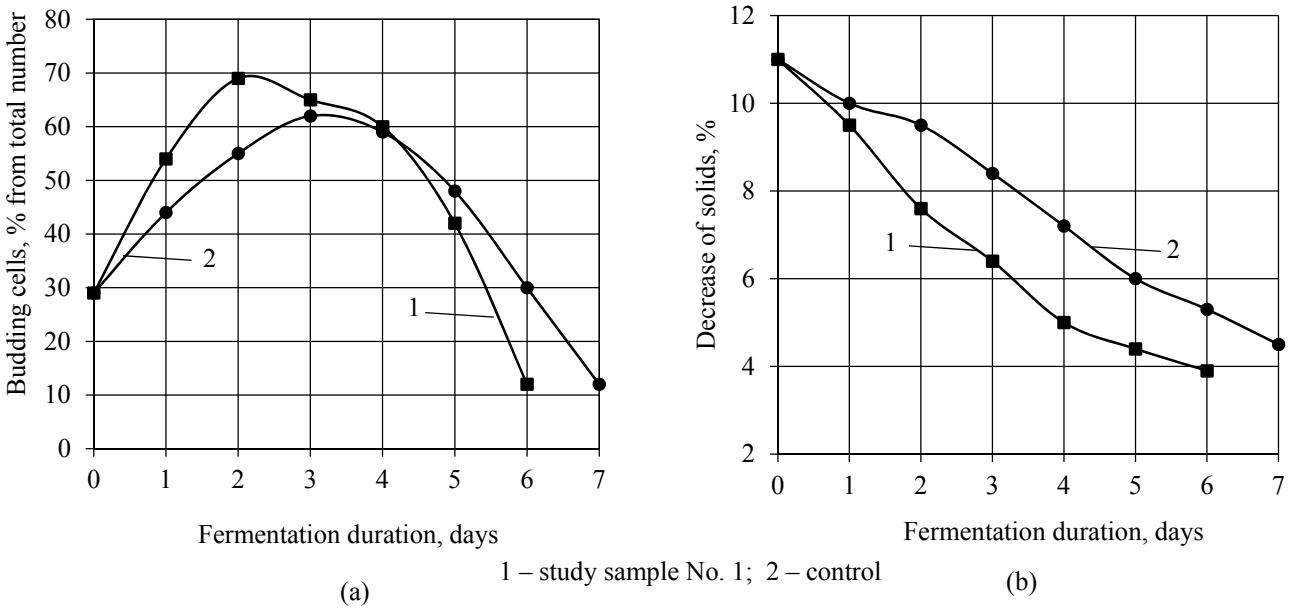

Fig. 2. Effect of treatment of wort with carbon fiber on: (a) yeast growth and (b) attenuation of extractives.

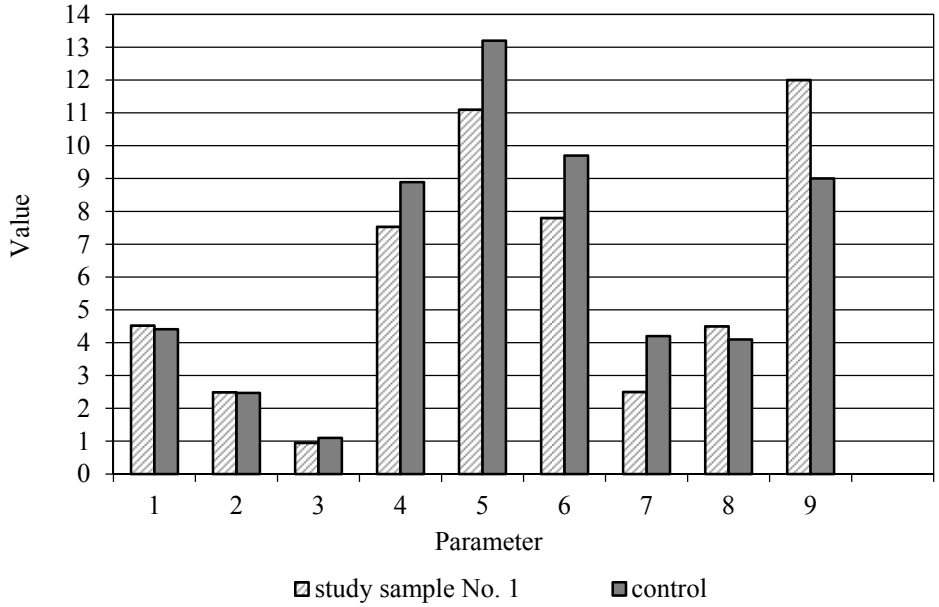

Fig. 3. Parameters of the finished beer made using carbon fiber: (1) alcohol, % vol.; (2) titratable acidity, units; (3) color, units; the content (mg/100 ml) of: (4) polyphenols; (5) of the A–protein fraction; (6) higher alcohols; (7) diacetyl $x10^{-2}$; (8) head retention, min; (9) the stability of unpasteurized beer, days.

The next step of the research was the study of the fermentation process of wort treated with carbon fiber. According to the data presented in Figure 2, the use of hopped cooled wort filtered through the carbon fiber layer made it possible to intensify yeast growth due to partial removal of polyphenols. As a result of that, the maximum number of budding cells in the study sample was achieved 24 hours sooner than in the control. The study sample was characterized by higher growth of yeast cells (by 20%) and higher assimilation rate of extractives from the medium in comparison to the control sample. All this allowed us to reduce the fermentation time without deterioration of the finished beer quality (Fig. 3).

The results presented in Figure 3 demonstrate the improved beer stability against colloidal hazes; this is due to lower content of phenolic compounds which are able to react with proteins. This is the reason why removal of antocyanogens, which play a dominant role in the haze formation, has more influence on beer shelf life than that of proteins.

Apart from that, the content of higher alcohols and diacetyl in the study beer is lower (by 19 and 45%, respectively) than in the control sample that allowed organoleptic parameters of beverage to be improved.

The results obtained allow us to consider carbon fiber as promising material for removing haze–forming components from wort to create favorable conditions for yeast viability and fermentation process.

Along with adsorbents, we proposed to use stabilizing agents (α–, β– and γ– cyclodextrins) to adjust the medium composition, stimulate metabolic processes, and improve the beer attenuation process.

The cyclodextrins (0.1–5.0%, w/v) were introduced into hopped cooled wort, then yeast was added, after which wort was subjected to fermentation. The cyclodextrin concentrations were chosen on the basis of preliminary researches.

The mixture of the α–, β– and γ–cyclodextrins (0.1; 2.5; 5%, w/v) was added into the study samples. In the control sample, cyclodextrins were not used.

Basic parameters of the fermentation process are presented in Table 8. Figure 4 shows quality parameters of the finished beer.

Table 8. Some parameters of wort attenuation with the use of cyclodextrins

Parameter	Sample			
	control	with cyclodextrins, % (w/v)		
		No. 1 (0.1%)	No. 2 (2.5%)	No. 3 (5.0%)
Increase of yeast biomass ($C_{max.} - C_{initial}$), $x10^6$/ml	34.6	43.0	52.4	59.8
Yeast cells content, $x10^6$/ml: – at the end of primary fermentation	8.4	6.6	5.2	4.3
– at the end of postfermentation of beer	2.2	1.3	0.8	0.6
Fermentation duration, days	7.0	6.5	5.5	6.0
Degree real of attenuation, %	52.4	53.9	58.2	56.5

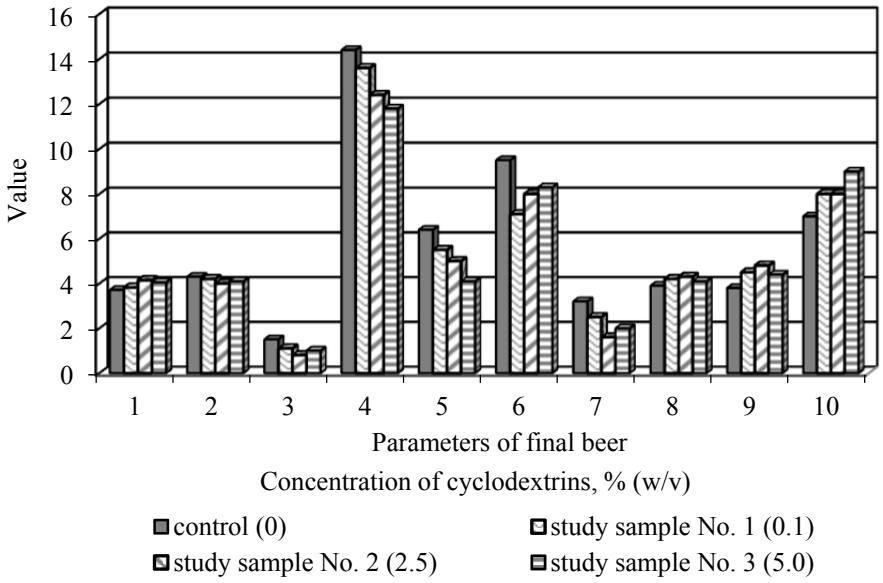

Fig. 4. The influence of the mixture of the cyclodextrins on the parameters of final beer ((1) alcohol, % vol.; (2) pH; (3) color, units; the content (mg/100 ml) of: (4) of the A–protein fraction; (5) antocyanogens; (6) higher alcohols; (7) diacetyl x10^2; (8) the foam height, cm; (9) head retention, min.; (10) the stability of unpasteurized beer, days).

According to the data from Table 8, addition of the cyclodextrins into wort leads to yeast growth; the values of biomass increase in three study samples were 14; 32, and 28% higher than in the control variant. Apart from that, at the end of primary fermentation the concentration of suspended yeast in experimental samples were 21; 38, and 49% lower than in the control beer, which contributes to better beer clarification. The real degree of fermentation value was 3–8% higher in the study samples and was achieved 0.5–1.5 days sooner than in the control variant.

The study variants of finished beer are characterized by high ethanol content, good composition of flavoring agents (higher alcohols, diacetyl), and components ensuring the colloidal stability of the beer (antocyanogens, of the A–protein fraction) (Fig. 4).

The changes can be explained as follows.

It is known that phenolic substances (antocyans, chlorogenic acids, resveratrol, etc.) are able to form complexes with cyclodextrins [35, 36, 37]. In particular, antocyans, being in semi–acetal form, form stable complexes with cyclodextrins, and their stability decreases with the increase of glycosidic radical. In addition, the ability of cyclodextrins to cooperate with gluten proteins is used in baking industry: they enhance the gas retaining ability of dough and improve its adhesive properties [32].

Cyclodextrins are able to form clathrates with both phenolic substances and high–molecular protein fraction as well as with polyphenols–protein complexes of the fermentation medium, and, thereby, reduce a negative impact of these components on yeast. As a result, fermentation time reduces, and the finished beer quality improves due to smaller amount of by–products formation.

The change in color of the study samples were 36–47% in comparison to the control variants should be noted. According to the research results presented in the literature, both the reduction and the improvement of the color stability occur [38]; the latter is due to the complex formation of cyclodextrins with cyanidine and pelargonidin [29]. The changes depend on the type of cyclodextrins (α–, β– or γ–), the complex forming mechanism, and the complex strength.

It is seen from Figure 4, the foaming ability of the study beer samples increases as well. Most likely, cyclodextrins being used as stabilizing agents prevent foam bubbles from coalescence; in other words, they decrease surface tension and contribute to foam formation.

Polyphenols, lipids, and a number of other substances are able to reduce the head retention [2, 3, 7]; therefore, their binding to cyclodextrins during the process has a favorable effect on the foam stability.

The formation of complexes from high–molecular nitrogenous compounds and cyclodextrins prevent the cleavage of proteins which stimulate foam formation by proteolytic enzymes (proteolysis), in particularly, by A–protease. The proteolysis occurs when using a weakened yeast culture or under unfavorable conditions for the fermentation process and beer maturation [1, 2, 3, 4, 7].

The carbon dioxide concentration in the study beer sample was higher than that in the control sample. Taking into account the physical–and–chemical theory of binding carbonic acid, it can be suggested that cyclodextrins themselves and their complexes with high–molecular–weight proteins as well work as «a protector», which covers CO_2 bubbles with adsorption films and does not allow them to form larger aggregates followed by deflocculation.

On the other hand, from the standpoint of the chemical bond theory, dissolved carbon dioxide can form with OH–groups of cyclodextrins mono- and diesters of carbonic acid. The latter has a high chemical stability.

Addition of cyclodextrins into wort at the fermentation stage can reduce the haze–forming components content – high–molecular–weight proteins and antocyanogens – thereby increasing the finished beer colloidal stability. In our research, the study samples of unpasteurized beer remained clear at 20°C by 1–2 days longer than the control beer.

Not only does a colloidal aspect have a stability effect on the beer but also a biological aspect. It is known that the use of antimicrobial/antioxidant complexes with cyclodextrins increase the microbiological stability of foods, which increases their shelf life [27, 39]. Compared to pure cyclodextrins, their complexes with preservatives/antioxidants are able to increase their activity, the compound solubility, the stability of chemical reagents, and the pH range of the medium where the complexes can be used. This allows us to make a conclusion that preservatives/antioxidants are able to protect foods from enzymic degradation, photochemical reactions, and physical factors (such as temperature and pressure).

Beer contains substances having bacteriostatic and antioxidant properties, such as ethanol, acids, carbon dioxide, polyphenols, hop resins, tanning substances, reductones, melanoidins, etc. It may be assumed that the presence of cyclodextrins in the fermentation medium strengthens their effects on both microorganisms and easily oxidizable compounds.

Beer made using cyclodextrins has mild flavor, slight hop bitterness, and pleasant aroma. Obviously, this is due to the ability of cyclodextrins to hold volatile components forming during fermentation and maturation.

Addition of cyclodextrins in an amount of less than 0.1% (w/v) did not reduce fermentation duration. Addition of more than 5% (w/v) of cyclodextrins caused adverse changes in organoleptic and physical–and–chemical parameters of beer.

Therefore, the results have demonstrated the possibility of application of cyclodextrins to intensify the fermentation process by means of adjusting the medium composition in order to create favorable conditions for yeast viability and improve the beer quality.

CONCLUSIONS

The use of natural adsorbing and stabilizing agents (such as zeolite–containing tuffs, carbon fiber, and cyclodextrins) for partial removal of inhibiting yeast components from the fermentation medium stimulates metabolic processes of the yeast culture, which makes it possible to reduce fermentation time by 1–1.5 days and improve the beer quality. The methods of beer wort fermentation with the use of carbon fiber and cyclodextrins are patented in the Russian Federation [22, 30].

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THE STUDY ON EFFECT OF SODIUM CHLORIDE ON THE ANTIOXIDANT ACTIVITY OF MEET

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Abstract: Meat technological processing has a significant effect on the dynamics and depth of the oxidative processes. The study on the antioxidative activity of the extracts, obtained from the salted meat samples before and after the thermal treatment, was carried out in order to establish a mechanism of action of the main technological processes (meat salting and thermal treatment) on the oxidative changes. The subjects of the research were the samples of pork *Longissimus dorsi* muscles, which were minced and salted with sodium chloride in the amount of 0.0, 2.0, 3.5 and 5.0%. The antioxidative activity was determined by the rate of oxidation of reduced by oxygen form of 2.6–Dichlorophenolindophenol. The catalase activity was measured spectrophotometrically by the rate of hydrogen peroxide decomposition; the superoxide dismutase activity was measured by the rate of inhibition of the pyrogallol autoxidation; the glutathione peroxidase activity was measured by the rate of NADPH decomposition. The research was carried out at V.M. Gorbatov Federal Research Center for Food Systems of RAS (Russia). According to the study results, meat salting initiated a decrease in the meat antioxidative activity by 9.2–18.0% depending on the sodium chloride concentration. Meat salting with sodium chloride in the amount of 5.0% led to a decrease in the activity of glutathione peroxidase by 24.6%, catalase by 60.1% and superoxide dismutase by 33.7%. The correlation dependence between the antioxidative activity and catalase activities, as well as between superoxide dismutase and glutathione peroxidase activity was revealed: the absolute values of the correlation coefficients were 0.97, 0.99 and 0.94 respectively. In the conducted research a decrease in the meat antioxidative activity by 22.9–28.3% ($p < 0.05$) was recorded under the action of high temperatures (72 ± 2°C) as a result of catalase inactivation and catalase partial inactivation of superoxide dismutase and glutathione peroxidase. The thermal treatment neutralized the sodium chloride negative effect on the antioxidative activity and activity of the meat antioxidative enzymes ($p > 0.05$). The obtained results on a decrease in the antioxidative activity in the meat salting process justify the necessity to develop approaches that allow reducing the sodium chloride content in meat products in order to retard the oxidative changes without deterioration of product consumer characteristics.

Keywords: Antioxidative activity, glutathione peroxidase, catalase, superoxide dismutase, sodium chloride

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INTRODUCTION

The fat oxidation processes have long been a subject of fundamental and applied research in terms of studying the mechanisms, process dynamics, methods of short-stop, effect on the product quality etc. A comprehensive interest in the problem of fat oxidation in meat products is due to significant influence of the lipids chemical modifications on the formation of the quality and safety of meat and meat products, including nutritional value and consumer characteristics (color, taste, smell, consistency) [1–3]. Prevention of fat oxidation is of paramount importance for meat industry, boosting production of quality products and increase in their shelf life.

Antioxidative system of meat is presented by non-enzymatic components (transferrin, vitamins A, E, K etc.) and by antioxidative enzymes (superoxide dismutase, catalase, glutathione peroxidase etc.) [4–6]. Fat-soluble bioantioxidants (phospholipids, tocopherols, vitamin A, carotenoids, ubiquinone, vitamin K complex, steroid hormones) carry out their protective function in biological membranes; water-soluble bioantioxidants (ascorbic, citric, nicotinic and benzoic acids; sulfur-containing compounds: cysteine, homocysteine, lipoic acid; phenolic compounds: polyphenols, flavonoids; ceruloplasmin, transferrin, lactoferrin, albumin, urea, uric acid) carry out their protective function in the cytosol of cells, inter-cellular fluid, blood plasma and lymph [7]. However, the total antioxidative activity of meat raw materials depends on the amount of antioxidants and their interaction, as well as on the presence of substances, which themselves do not have anti- or pro-oxidative effect, but can intensify or retard the effect of antioxidants.

A number of technological factors influence on the dynamics and depth of oxidative processes course:

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moisture mass fraction, heat treatment, light, type of packaging etc. [8–10]. Food ingredients, used in the production of meat products, also play a significant role in the formation of oxidation products [11, 12]. Sodium chloride, food phosphates, citrates, ascorbates *etc.* should be noted among such components. However, if salts of phosphoric, citric and ascorbic acids are known for their antioxidative properties, then the available data, concerning the nature of the sodium chloride action on the lipids hydrolysis and oxidation, as well as concerning the mechanisms of these changes, are quite controversial [13, 14].

It is known that oxidative processes are often due to the atmospheric oxygen action and can be suppressed or significantly retarded due to the natural complex of antioxidative defense of an organism, which includes a number of enzymes and native hydrophobic and hydrophilic biologically active compounds; in this context, the objective of this work was to establish the dynamics of impact of technological doses of sodium chloride on antioxidative activity of meat with the case study of *Longissimus dorsi.* This involved the study of the antioxidative activity and the activity of antioxidative enzymes (catalase, superoxide dismutase and glutathione peroxidase) in supernatant, extracted from meat samples.

STUDY OBJECTS AND METHODS

The objects of the study were the samples of pork *Longissimus dorsi* muscles of the big white female 2nd-category-pork of 2 years old. Taking into account the technological doses of sodium chloride in sausage and meat products recipes, the range of sodium chloride concentration was chosen from 2.0 to 5.0% for research. In order to distribute salt in meat raw materials regularly, meat was minced with a mincing machine through the lattice holes of 2–3 mm and was salted with sodium chloride according to State Standard R 51574 in the amount of 0.0; 2.0; 3.5 and 5.0%. Taking into account the small degree of meat mince, the prepared samples were kept during 24 h at a temperature of 4 ± 2°C, after which they were packed in vacuum and subject to thermal treatment until the temperature is 72 ± 2°C. Determination of antioxidative activity and the activity of antioxidative enzymes was carried out before and after thermal treatment.

The extraction was performed according to the method, described by Hernandez *et al.*, with some modification [15]. The minced pork samples were subject to extraction with laboratory dispersing equipment (LDE) (Labotex, Russia) with the use of 0.05 M phosphate buffer (pH 7) as an extractant during 3 minutes, at the volumes ratio of the extracted sample and extractant solution of 1 : 5, temperature of 4–5°C and the stirring speed of 5000 rpm. The extract was separated by centrifugation at a speed of 15.000 rpm during 15 min at a temperature of 4.0°C with the centrifuge Sigma 3K30 (Germany). The supernatant was filtered through the glass wool Sigma-Aldrich (Germany).

To obtain supernatant from the thermally treated raw material, it was minced with a mincing machine through the lattice holes of 2–3 mm, then it was subject to extraction with LDE (Labotex, Russia) with the use of 0.05 M phosphate buffer (pH 7) as an extractant during 3 minutes, at the volumes ratio of the extracted sample and extractant solution of 1 : 5, temperature of 4–5°C and the stirring speed of 5000 rpm. The extract was separated by centrifugation at a speed of 15.000 rpm during 15 min at a temperature of 4.0°C with the centrifuge Sigma 3K30 (Germany). The supernatant was filtered through the glass wool Sigma-Aldrich (Germany).

Determination of antioxidative activity was based on the registration of the oxidation rate of reduced 2.6-Dichlorophenolindophenol (2.6-DCPIP) by oxygen, dissolved in reaction medium; herewith, colorless leuco form of 2.6-DChPhIPh turned to colored form with maximum absorption at 600 nm [16]. The optical density was measured with the photometer BioChem SA (HTI, USA). Inhibition coefficient (IC) with supernatant of autoxidation 2.6-DCPIP was the indicator of antioxidative activity [16].

Determination of the catalase activity was performed according to the method, described by Jin G. *et al.* [17] with some modification. 0.1 ml of supernatant was mixed with 2.9 ml 11 Mm of hydrogen peroxide in phosphate buffer (pH 7.0) at room temperature (22 ± 2°C). The resultant mixture was stirred thoroughly, transferred into the cuvettes with the distance between the active faces of 1 cm, and the decrease in optical density of the test samples was measured immediately after the start of the reaction and after 3 min of incubation at a wavelength of 240 nm with the photometer KFK-3-01 "ZOMZ" (Zagorsk, Russia). The calculation of the decrease in the hydrogen peroxide concentration was produced taking into account the extinction coefficient of 39.4 $M^{-1}cm^{-1}$. As a catalase activity unit, the amount of hydrogen peroxide in mmol was taken, which is decomposed during 1 min while adding the supernatant, obtained by extraction of 1 g of meat; the results were expressed in U/g of meat.

Determination of the superoxide dismutase activity was performed by the method, described by Gatellier P. *et al.* [18] by measuring the pyrogallol autoxidation inhibition. 75 µl of supernatant were mixed with 75 µl 10 Mm of pyrogallol solution in 2850 µl 50 Mm of phosphate buffer (pH 8.2). The resultant mixture was stirred thoroughly, transferred into the cuvettes with the distance between the active faces of 1 cm, and the decrease in optical density of the test samples was measured immediately after the start of the reaction and after 2 min of incubation at a wavelength of 340 nm with the photometer KFK-3-01 "ZOMZ" (Zagorsk, Russia). The pyrogallol autoxidation was determined in the blank sample in the same reaction mixture, adding, instead of the supernatant, the same volume of distilled water (blank sample). As a superoxide dismutase activity unit, the ability of the sample to inhibit 50% of the reaction was taken; the results were expressed in U/g of meat.

Determination of the glutathione peroxidase activity was performed according to the method, described by Jin G. *et al*. [17] with some modification. The reaction mixture was prepared, containing 1 ml 75 mM of phosphate buffer (pH 7.0), 10 µL 150 mM

of reduced glutathione, 10 µL 46 E/mL of glutathione reductase, 30 µL 25 mM of EDTA, 30 µL 5 mM of NADPH, 200 µL of supernatant and 10 µL of 20%-TritonX-100. The volume of the ready mixture was 1.5 ml. Addition of 50 µL of 7.5 mM H_2O_2 initiated the reaction. Conversion of NADPH into NADP + was registered with the photometer KFK-3-01 "ZOMZ" (Zagorsk, Russia) at a wavelength of 340 nm during 3 min taking into account the extinction coefficient of 6220 $M^{-1}cm^{-1}$. As a glutathione peroxidase (E) activity unit, the amount of mol of NADPH was taken, which is decomposed during 1 min while adding the supernatant, obtained by extraction of 1 g of meat; the results were expressed in U/g of meat.

All experiments were carried out in triple replication. Statistical processing of the results, determination of the Pearson correlation coefficients and the approximation values of reliability were performed using the programme MS Excel. Assessment of the statistical significance of the differences between parameters was performed using the Student's t-test.

RESULTS AND DISCUSSION

Meat salting had a significant influence on the meat antioxidative activity. Statistical processing of the obtained data showed that the change in the antioxidative activity with increase in salt concentration occurred in a linear fashion.

According to the obtained results (Fig. 1), addition of sodium chloride in the amount of 2.0, 3.5 and 5.0% led to a decrease in the meat antioxidative activity by 9.2% ($p < 0.05$), 13.3% ($p < 0.05$) and 18.0% ($p < 0.05$) respectively.

Taking into account the significant role of antioxidative enzymes as a natural antioxidative protection, the identified regularity of changes in the antioxidative activity of the meat supernatant, depending on the sodium chloride concentration, required a more detailed study of the main components of the meat antioxidative system: of catalase, which catalyzes decomposition of hydrogen peroxide with formation of water and oxygen; of superoxide dismutase, which initiates the conversion of superoxide into oxygen and hydrogen peroxide, and of glutathione peroxidase, which catalyzes the recovery of peroxides due to tripeptide-glutathione [19].

It is known that the antioxidative activity indicates the total protection of meat system from peroxidation, which is toxic for the structures of cell membranes and for the functional activity of proteins-enzymes. The increase in the antioxidative activity indicates a high ability to withstand the effects of factors, which activate free radical oxidation of lipids; decrease, on the contrary, indicates a reduction of antioxidative defense. Thus, salting weakens the natural defense of the meat system from oxidation and thereby initiates the oxidative changes in meat.

Catalase is a heme-containing enzyme, which decomposes hydrogen peroxide in meat and meat products. As a result, decrease in the catalase activity in meat initiates lipids peroxidation. According to the research results, sodium chloride inhibited the catalase activity (Fig. 2). Addition of sodium chloride in the amount of 2.0, 3.5 and 5.0% led to a decrease in the catalase activity by 42.6% ($p < 0.05$), 52.2% ($p < 0.05$) and 60.1% ($p < 0.05$) respectively.

Increase in doses of sodium chloride led to a decrease in the catalase activity by the logarithmic law. The obtained data are consistent with the research results of Jin G. *et al.*, who identified the inhibitory influence of sodium chloride on the catalase activity in a dry-cured ham [17].

On the contrary, Lee *et al.* stated that there was no significant effect of sodium chloride in the amount of 2.0% on the pork catalase activity in the process of freezing, which is obviously due to the leveling effect of salt on the catalase activity as a result of its stabilization at low subzero temperatures [20].

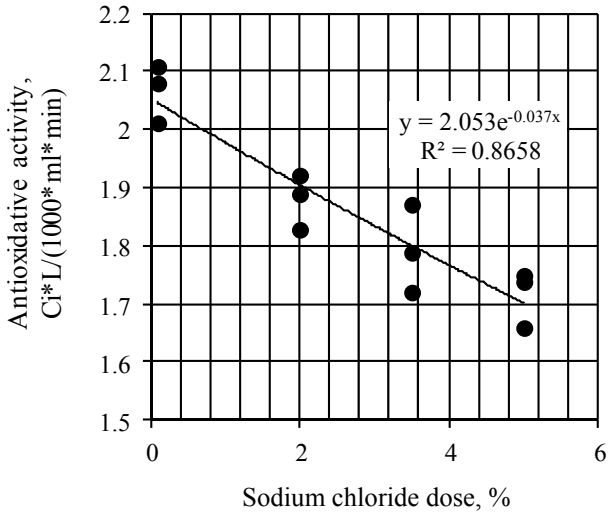

Fig. 1. Impact of sodium chloride on the total meat antioxidative activity.

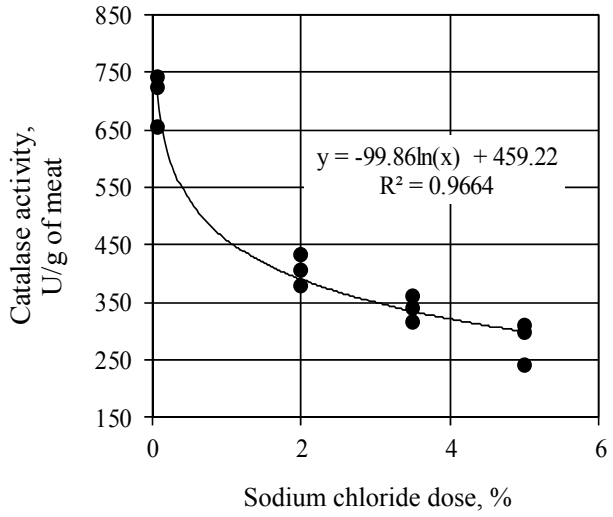

Fig. 2. Impact of sodium chloride on the catalase activity.

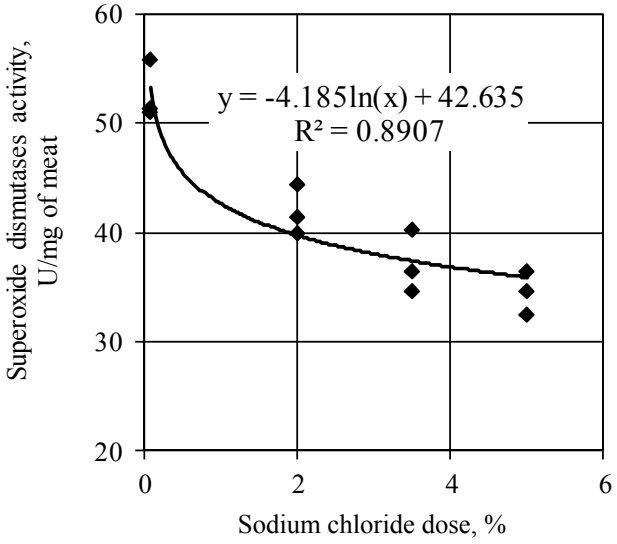

Fig. 3. Impact of sodium chloride on the superoxide dismutase activity.

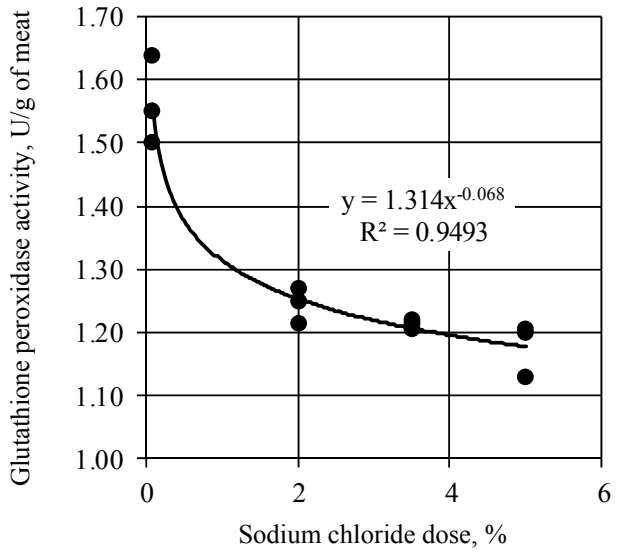

Fig. 4. Impact of sodium chloride on the glutathione peroxidase activity.

Similar tendency was observed while studying the effect of salt on the superoxide dismutase activity (Fig. 3). Increase in doses of salt decreased the superoxide dismutase activity by the logarithmic law. According to the obtained results, addition of sodium chloride in the amount of 2.0, 3.5 and 5.0% led to a decrease in the superoxide dismutase activity by 20.8% ($p < 0.05$), 28.6% ($p < 0.05$) and 33.7% ($p < 0.05$) respectively.

Similar results were obtained in the works of Jin G. *et al.*, according to the conclusions of which addition of sodium chloride in the amount of 5.0% led to a decrease in the superoxide dismutase activity in comparison with the samples of dry-cured ham, salted in 1.0% of sodium chloride. Lee *et al.* found out the inhibition of the superoxide dismutase activity under the action of salt in minced and frozen pork.

Sodium chloride had also an inhibitory effect on the glutathione peroxidase (Fig. 4). Decrease in the glutathione peroxidase activity, depending on the sodium chloride dose, occurred by the power law.

Addition of sodium chloride in the amount of 2.0, 3.5 and 5.0% led to a decrease in the glutathione peroxidase activity by 20.4% ($p < 0.05$), 22.4% ($p < 0.05$) and 24.6% ($p < 0.05$) respectively.

According to the results, obtained in the present experiment, sodium chloride had a less pronounced effect on the glutathione peroxidase activity in comparison with its effect on catalase and superoxide dismutase. Thus, meat salting with sodium chloride in the amount of 5.0% led to a decrease in the activity of glutathione peroxidase by 24.6% in comparison with the decrease in the catalase activity by 60.1% and in superoxide dismutase by 33.7%.

The obtained research results were partially consistent with the ones of Jin G. *et al.* [17] and of Lee *et al.* [20], who determined a negative correlation between the salt concentration and the activity of meat antioxidative enzymes. However, the research results of Jin G. *et al.* showed the smallest decrease in the superoxide dismutase activity at salting in comparison with the rest antioxidative enzymes, while Lee *et al.* determined the maximum decrease in the superoxide dismutase activity upon addition of 0.5–2.0% of sodium chloride in comparison with the catalase and glutathione peroxidase activity. According to the data, obtained by Gheisari H. R. and Eskandari M., the addition of sodium chloride slows down the inhibition of the glutathione peroxidase activity while keeping in salting in comparison with the unsalted meat [21].

Such contradictory data are obviously due to the methodological inconsistency of studies and the different selection of experiment objects. Thus, Jin G. *et al.*, for example, studied the impact of sodium chloride on the meat antioxidative activity in the process of production of dry-cured bacon. Lee *et al.* to determine the antioxidative enzymes activity used sodium chloride not in the process of meat salting, but added it in the previously prepared supernatant, isolated from unsalted meat.

It is known that thermal treatment causes denaturation of enzymes. Thus, the catalase activity decreases at a temperature above 35°C and is completely inhibited at incubation at 65°C during 5 min [22]. Glutathione peroxidase denaturation is observed after incubation at a temperature of 70°C during 40 min [23], while superoxide dismutase is inactivated under at a temperature of 80–100°C [24].

Taking into account the fact that thermal stability of the protein components can be influenced by ionic strength [25], the effect of sodium chloride on the antioxidative activity and the antioxidative enzymes activity in thermal-treated meat products was studied.

According to the obtained data, the antioxidative activity of the studied samples after thermal treatment decreased by 22.9–28.3% ($p < 0.05$) (Fig. 5).

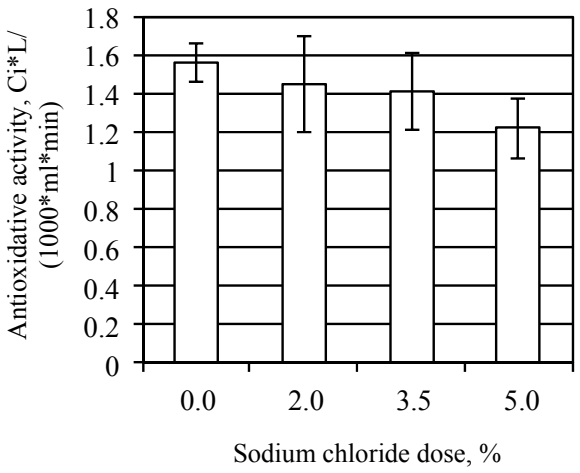

Fig. 5. Impact of sodium chloride on the total meat antioxidative activity after thermal treatment.

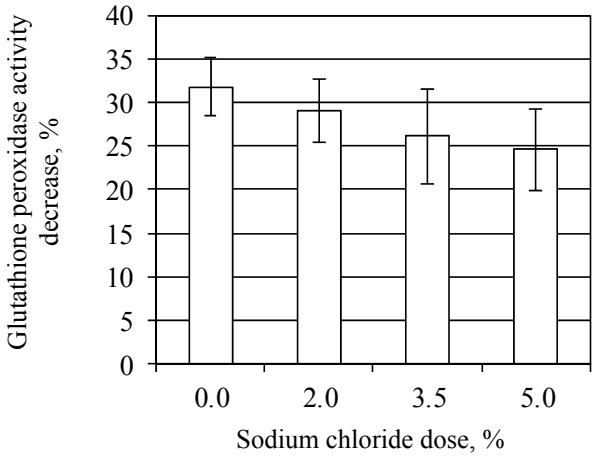

Fig. 6. Impact of sodium chloride on decrease in glutathione peroxidase activity after thermal treatment.

Nevertheless, the addition of salt had no significant effect on the value of the meat antioxidative activity ($p > 0.05$).

The research results showed that thermal treatment up to 72 ± 2°C did not lead to complete destruction of superoxide dismutase, the activity of which, however, decreased by 74.1–90.0% ($p < 0.05$) in comparison with the values before thermal treatment. The obtained research results were consistent with the ones of Sharapov M. G. *at al.*, according to which at a temperature of 75°C there was a decrease in the superoxide dismutase activity by ~ 80% in comparison with its activity at 25°C [26]. The superoxide dismutase high thermostability is associated with a large number of sulfhydryl groups in its structure [27].

The catalase activity in meat raw materials after thermal treatment was not detected.

Thermal treatment with a temperature of up to 72 ± 2°C influenced the glutathione peroxidase activity to a lesser extent, its decrease was 24.6–31.8% ($p < 0.05$) of the initial value before the thermal treatment (Fig. 6), which is obviously due to the manifestation of the maximum efficiency of glutathione peroxidase at 37–40°C [28] and its negligible activity at the determined during the experiment temperatures of the samples - at 4 ± 2°C (before thermal treatment) and after reaching 72 ± 2°C. The obtained research results were consistent with the ones of Hoac T. *et al.*, who stated, based on the study of temperature impact on the poultry antioxidative enzymes activity, that at a temperature of 60 C, 20% of glutathione peroxidase were destroyed, and at a temperature increase up to 80 C ~ 80% of enzyme were denatured [29].

It should be noted that the salt content had no significant effect on the activity of superoxide dismutase and glutathione peroxidase of the meat samples after thermal treatment ($p > 0.05$ in comparison with unsalted meat).

Partial preservation of the samples antioxidative activity after thermal treatment, apparently, is explained by the content of non-enzymatic components in the meat antioxidative system, which are involved in the compensation of oxidative processes and which also retain partial activity of superoxide dismutase and glutathione peroxidase. Besides, it is known that glutathione peroxidase has a greater affinity with hydrogen peroxide in comparison with catalase [27]. Therefore, this enzyme plays a significant role in the formation of antioxidative defense. In this regard, preservation of up to 68.2% of the glutathione peroxidase activity, resulted in thermal treatment, obviously contributed significantly to the formation of the antioxidative activity of thermally treated meat raw material.

It should be noted that still the researches have not reached a consensus on the interrelation of the antioxidative enzymes activity and the oxidative changes of meat and meat products. Thus, according to the results of the works of Hernandez *et al.* [15] and Jin G. *et al.* [17], a negative correlation between the antioxidative enzymes and the formation of secondary oxidation products was determined. However, Sarraga C. *et al.* found that a higher level of the glutathione peroxidase activity during the addition of sodium chloride was accompanied by an increase in the amount of malonic aldehyde in dry-cured pork products. Scientists explain the presented results by the sodium chloride ability to stabilize muscle tissue, which decreases the importance of antioxidative enzymes for inhibition of oxidative processes [30]. In addition, meat is a multi-component system, containing biologically active substances that can have an indirect impact on pro- and antioxidative effect of sodium chloride. Therefore, the mechanism of impact of meat salting on the dynamics of oxidative changes in animal raw materials is still the subject of further study.

The results of this research contribute significantly to the study of influence of sodium chloride on oxidative changes. It is stated that addition of sodium chloride decreases the meat antioxidative activity as a result of inhibition of the antioxidative enzymes activity: of catalase, superoxide dismutase and

glutathione peroxidase. The data analysis confirmed the existence of correlation dependence between the total antioxidative activity and the catalase, superoxide dismutase and glutathione peroxidase activity in meat raw materials: the absolute values of the Pearson correlation coefficients were 0.97, 0.99 and 0.94, respectively. The use of thermal treatment neutralized the negative impact of sodium chloride on the antioxidtive enzymes activity. Taking into account the fact that decrease in the amount of sodium chloride in meat products will result in deterioration of its taste and technological characteristics, it is necessary to use approaches allowing developing technological recommendations for varying doses of sodium chloride through the use of its substitutes in production of meat products.

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BIOTECHNOLOGY

ANALYSIS OF INDICATORS OF ENZYME HYDROLYSATES OF FEATHER-DOWN RAW MATERIALS OBTAINED WITH THE USE OF MULTI-ENZYME COMPOSITION

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Abstract: Feeds, feed preparations and high-protein feed supplements are complex multicomponent compositions that, when stored, used, processed and transported, can change their physicochemical properties, microbiological indicators and toxicological properties. In this regard, it is very important to study the above properties and quality indicators. The paper describes a study of the peptide profile, physico-chemical and toxicological properties of the enzymatic hydrolysates of feather-down raw materials obtained during a multifactorial experiment to optimize the conditions for enzymatic hydrolysis of feather-down raw materials. The raw materials were previously subjected to a short-term hydrothermal treatment to improve the course of enzymatic hydrolysis. The enzymes obtained from bacterial organisms were used for the enzymatic hydrolysis of raw materials: Protolade B and Protease 2630#2256. The following results were obtained for the physicochemical properties of enzymatic hydrolysates: the content of protein and solids in the enzymatic hydrolysates of feather-down raw materials varied in the range of 1.80–3.00% and 2.6–4.4%, respectively. In this case, the mass fraction of protein was 67.7–70.6% in terms of the mass fraction of moisture. The mass fraction of ash, in terms of the mass fraction of moisture, did not exceed 0.43% in all the samples of enzymatic hydrolysates, the mass fraction of fat was 0.74%, the mass fraction of crude fiber was 1.28%, the mass fraction of sodium chloride was 1.92% the mass fraction of the impurities insoluble in hydrochloric acid was 0.77%. It has been shown that enzymatic hydrolysates corresponded to TU 9219-094-23476484-09 “Hydrolyzed feed flour. Feather protein concentrate” for their microbiological properties (QMAFAnM, coliforms, pathogenic microorganisms, toxin-forming anaerobic bacteria and bacteria of the genus Proteus) and chemical safety parameters.

Keywords: Feather, enzymatic hydrolysis, biosafety, physical and chemical properties, feed supplement

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INTRODUCTION

The main condition at the present stage of society is the development of technological progress by introducing energy and resource-saving processing lines into production and industry that allow to supply to the production of high-quality products and reduce the negative environmental consequences of production. This trend can be referred to the production of feed supplements [3, 11, 15].

Feeds, feed preparations and high-protein feed supplements are complex multicomponent compositions that, when stored, used, processed and transported, can change their physicochemical properties, microbiological indicators and toxicological properties. In this regard, it is very important to study the above properties and quality indicators [6, 11, 12].

The production of poultry products is the leading branch of agriculture for today and has a significant effect on the production of food products of other industries. Much attention is paid to the safety of feeds for poultry [10, 11].

The creation of an extensive fodder base is one of the main factors in the production of animal products. An important task that scientists have in the development of new feed supplements or feeds is the development of maximum quality control and safety of feed supplements and feeds [2, 12, 13].

The present stage of the studies aims at studying the physico-chemical properties of the enzymatic hydrolysates of feather-down raw materials obtained during a multifactorial experiment on optimizing the conditions of the enzymatic hydrolysis of feather-down raw materials at a laboratory level.

Feather-down raw materials were used after a short-term hydrothermal treatment as feather raw materials for enzymatic hydrolysates. The use of a preliminary

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short-term hydrothermal treatment allows for grinding feather raw materials simultaneously with reducing the density of a keratin pack partially breaking hydrogen bonds and reducing hydrophobic interactions. The resulting substrate is more easily subjected to an enzymatic attack.

The sanitary quality of such products has an enormous effect on the reproductive ability, productivity and health of animals, as well as a direct effect on their biological value. The sanitary quality of feeds is determined by the amount of the pathogenic microorganisms contained therein [5, 16]. Most attention in the sanitary evaluation of feed supplements and feeds is paid to such indicators as the total bacterial content, the presence of pathogenic microorganisms and toxins. The high amount of such pathogenic microorganisms in feeds as salmonella, clostridia, etc., leads to an infection and the development of diseases in animals and birds, their early death, and, consequently, to high economic losses [7, 16, 14].

Microbiological studies of animal and vegetable feeds are carried out in the department of veterinary and sanitary examination for the presence of: the total bacterial content, Salmonella, Escherichia coli, Proteus and toxin-forming anaerobic bacteria [8, 19]. The presence of salmonella in feeds and feed supplements is the cause of infectious salmonellosis. Salmonellosis affects young stock, including poultry, animals and humans. Salmonellosis is followed by a fever and profuse diarrhea, and may also cause lung diseases. In pregnancy, salmonellosis can be a cause of spontaneous abortion [9, 14]. Intestinal bacillus (lat. Escherichia coli) is a species of gram-negative rod-shaped bacteria, widely distributed in the lower intestine of warm-blooded animals. Most strains of E. coli are harmless, but the serotype O157:H7 can be a cause of severe food poisoning in humans and animals [4, 15, 17].

The content of microorganisms in poultry feeds is the main cause of poisoning and the emergence of infectious agents.

Equally important is the observance of the conditions of storage of feeds and also their transportation. Mixed feeds must be transported in compliance with sanitary standards, in dry, clean, uninfected vehicles without foreign odors. When loading, transporting and unloading mixed feeds should be protected from atmospheric precipitation. Mixed feeds are stored in dry, clean, uninfected, well-ventilated warehouses. If mixed feed was stored incorrectly in an unprepared and unadapted room, it is highly likely to be very dangerous to the life and health of animals [1, 12, 19].

STUDY OBJECTS AND METHODS

The following was used as the study object: the enzymatic hydrolysates from the poultry feather-down raw materials of Kuzbass Broiler, LLC (Kemerovo region, Russia).

When analyzing the obtained enzymatic hydrolysates of feather-down raw materials using a multienzyme composition, the following methods were used:

The mass fraction of crude protein was determined by ashing with sulfuric acid in the presence of a catalyst, followed by the alkalinization of the reaction product, the distillation and titration of the released ammonia according to GOST 32044.1–2012 “Feeds, mixed feeds and raw material. Determination of mass fraction of nitrogen and calculation of mass fraction of crude protein”.

The mass fraction of the ash insoluble in hydrochloric acid was determined in accordance with GOST 13496.14–87 “Mixed fodder, raw mixed fodder, fodder. Method for determination of ash insoluble in hydrochloric acid”.

The mass fraction of moisture was determined according to GOST 17681–82 “Flour of animal origin. Test methods”.

The mass fraction of whole protein was studied using the Dumas method with the use of a RAPID N Cube protein nitrogen analyzer.

The mass fraction of fat was determined in accordance with GOST 32905–2014 “Feeds, mixed feeds and raw material. Method for determination of fat content”.

The mass fraction of crude fiber in enzymatic hydrolysates was determined in accordance with the requirements of GOST 13496.2–91 “Fodders, mixed fodders and mixed fodder raw material. Method for determination of raw cellular tissue”.

The mass fraction of sodium chloride in enzymatic hydrolysates was determined using an ionometric method in accordance with the requirements of GOST 13496.1–98 “Mixed fodder and raw mixed fodder. Methods for sodium and sodium chloride determination”.

The mass fraction of calcium in enzymatic hydrolysates was determined using a complexometric method in accordance with GOST 26570–85 “Fodder, mixed fodder and mixed fodder raw material. Methods for determination of calcium”.

The mass fraction of the mineral impurities insoluble in hydrochloric acid in enzymatic hydrolysates was determined using a water flotation method in accordance with GOST 25555.3–82 “Fruit and vegetable products. Methods for determination of mineral impurities”.

The mass fraction of phosphorus in enzymatic hydrolysates was determined in accordance with GOST 26657–97 “Fodders, mixed fodders, mixed fodder raw materials. Methods for determination of phosphorus content”.

The number of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) was determined in accordance with GOST 10444.15–94 "Food products. Methods for determination of quantity of mesophilic aerobes and facultative anaerobes" and GOST 25311–82 “Feeding flour of animal origin. Methods of bacteriological analysis”.

The number of coliforms (coliform bacteria) was determined in accordance with GOST 30518–97 “Food products. Methods for detection and quantity determination of coliforms” and GOST 26670–91 “Food products. Methods for cultivation of microorganisms”.

The presence of pathogenic and toxin-forming microorganisms was determined in accordance with GOST 25311–82 "Feeding flour of animal origin. Methods of bacteriological analysis", the presence of salmonella was determined in accordance with GOST 30519–97 "Food products. Method for detection of *Salmonella*".

The presence of bacteria of the genus *Proteus* was determined according to GOST 28560–90 "Food products. Method for detection of bacteria of *Proteus*, *Morganella*, *Providencia genera*".

The content of arsenic in the samples studied was determined in accordance with GOST 26930–86 "Raw material and food-stuffs. Method for determination of arsenic".

The content of toxic elements was determined:
– for lead - according to GOST 26932–86 "Raw material and food-stuffs. Methods for determination of lead", cadmium - in accordance with GOST 26933–86 "Raw material and food-stuffs. Methods for determination of cadmium",
– strontium-90 - in accordance with 32163-2013 "Foodstuffs. Method for strontium Sr-90 content determination",
– pesticides - according to GOST 32194–2013 "Feeds, compound feeds. Determination of organochlorine pesticides residues by gas chromatographic method",
mercury - in accordance with GOST R 53183–2008 "Foodstuffs. Determination of trace elements. Determination of mercury by cold-vapour atomic absorption spectrometry (CVAAS) method after pressure digestion".
– copper - in accordance with GOST 26931–86 "Raw material and food-stuffs. Methods for determination of copper",
– zinc - in accordance with GOST 26934–86 "Raw material and food-stuffs. Method for determination of zinc",
– aflatoxin B1 - according to GOST 31653–2012 "Feedstuffs. Method of immunoenzyme mycotoxin determination",
– cesium-137 in accordance with GOST 32161–2013 "Foodstuffs. Method for cesium Cs-137 content determination".

The molecular weight distribution of enzymatic hydrolysates of keratin-containing raw materials was estimated using the method of exclusion chromatography. The chromatographic system included a Varian ProStar HPLC chromatograph (USA), a PS210 SDM pump, a PS410 Autosampler and a BioSep-SEC-S 2000 (7.8 x 300 mm) column from Phenomenex (USA). This kind of column is used for the analytical separation of low molecular weight proteins and peptides by gel filtration. The column was calibrated for standard water-soluble proteins and peptides from GE Healthcare (USA), Serva (Germany) and Sigma (USA) in the range of molecular weights from 451 to 440,000 Da covering its operating range. The optical density was registered using a streaming current detector with a photodiode array (Varian 335 PDA) in the range of 190–330 nm with a base wavelength of 214 nm. A 50 mM Na-phosphate 0.15 M NaCl buffer, pH 6.8, was used as an aluent. The elution rate was 1 ml/min. The volume of the sample applied to the column was 20 µl. The sample preparation included double centrifugation at 60000 g for 40 min. The chromatograms obtained were integrated with the calculation of the relative content of the high molecular weight protein fraction (MW > 10 kDa), the mid-molecular oligopeptide fraction (MW is 3–10 kDa) and the low molecular weight fraction (MW < 3 kDa) containing peptides and free amino acids.

RESULTS AND DISCUSSION

Enzymatic treatment involves the hydrolysis of the protein that splits into smaller fragments, including peptides and free amino acids. The functional and technological characteristics of protein hydrolysates, such as the dissolubility capacity, the moisture-retaining capacity, emulsification and others, and the biological activity of hydrolysates are determined by the depth of the hydrolysis of the initial substrate.

To increase the degree of enzymatic conversion, the source feather raw materials were subjected to a short-term hydrothermal treatment. The samples of the hydrothermally treated keratin-containing raw materials were obtained in accordance with the following technological parameters of hydrothermal hydrolysis:
– the initial feather moisture - 55%;
– the heating temperature - 190–200ºC;
– the heating duration - 90 sec.

The obtained sample of the hydrothermally treated feather raw materials (feather) was characterized according to physicochemical parameters. The physicochemical properties include density, hygroscopicity, mass the fraction of moisture, the mass fraction of fat, the mass fraction of ash, the mass fraction of protein, the mass fraction of crude fiber, the mass fraction of sodium chloride, the mass fraction of calcium, the mass fraction of the mineral impurities insoluble in hydrochloric acid. Table 1 presents the results of a study of the physicochemical properties of the source feather raw materials after hydrothermal treatment.

Table 1. Physicochemical parameters of the hydrothermally treated feather raw materials

Parameter	Actual result	Method of analysis
Mass fraction of moisture, %	6.3	GOST 17681-82
Mass fraction of protein, %	84.1	GOST 32044.1-2012
Mass fraction of fat, %	2.7	GOST 32905-2014
Mass fraction of ash, %	1.0	GOST 32933-2014
Mass fraction of crude fiber, %	0.8	GOST 13496.2-91
Mass fraction of sodium chloride, %	1.6	GOST 13496.1-98
Mass fraction of calcium, %	1.2	GOST 26570-85
Mass fraction of phosphorus, %	1.5	GOST 26657-97
Mass fraction of the mineral impurities insoluble in hydrochloric acid, %	0.5	GOST 25555.3-82

Table 2. Parameters for obtaining samples of enzymatic hydrolysates

Sample	irrigation module[a]	Enzyme activity[b] per 1 g of substrate, unit	Enzyme	Sodium sulphite coenzyme, % of feather weight	Hydrolysis time, h
HF-3	4	15	Protease 2630#2256	0.5	2
HF-28	8	15	Protease 2630#2256	0.5	4
HF-37	6	20	Protolade B	0.5	2
HF-54	8	30	Protolade B	0.5	4
HF-58	4	30	Protolade B	1.25	2
HF-68	8	30	Protolade B	1.25	2

Note. [a]hydromodule is the weight ratio of water and raw materials; [b] the activity of EP was determined in accordance with GOST 20264.2-88.

Table 3. Physicochemical properties of the enzymatic hydrolysates of feather-down raw materials obtained during a multifactor experiment at a laboratory level

Parameter	Parameter value for a sample					
	HF-3	HF-28	HF-37	HF-54	HF-58	HF-68
Mass fraction of moisture, %	95.7	96.9	97.4	97.3	95.6	96.1
Mass fraction of protein, %	2.98/69.3	2.19/70.6	1.80/69.2	1.87/69.3	3.00/68.2	2.64/67.7
Mass fraction of ash, %	0.02/0.43	0.01/0.3	0.005/0.19	0.005/0.19	0.01/0.23	0.01/0.26
Mass fraction of fat, %	0.01/0.23	0.002/0.06	0.002/0.08	0.02/0.74	0.003/0.07	0.005/0.13
Mass fraction of crude fiber, %	0.05/1.16	0.03/0.98	0.03/1.15	0.02/0.74	0.03/0.68	0.05/1.28
Mass fraction of sodium chloride, %	0.06/1.39	0.05/1.61	0.05/1.92	0.04/1.48	0.08/1.82	0.05/1.28
Mass fraction of calcium, %	0.54/12.6	0.40/12.9	0.34/13.1	0.35/13.0	0.60/13.6	0.54/13.8
Mass fraction of phosphorus, %	0.62/14.4	0.40/12.9	0.36/13.8	0.38/14.1	0.65/14.8	0.58/14.9
Mass fraction of the mineral impurities insoluble in hydrochloric acid, %	0.02/0.46	0.02/0.65	0.02/0.77	0.01/0.37	0.03/0.68	0.02/0.51*

Note. The numerator - without recounting to the mass fraction of moisture required by the specifications; the denominator - in terms of the mass fraction of moisture required by the specifications.

As it can be seen from the data presented in Table 1, the feather-down raw materials subjected to hydrothermal treatment meet the requirements of TU 9219–094–23476484–09 “Hydrolyzed feed flour. Feather protein concentrate” for the physicochemical parameters.

The feather, pretreated hydrothermally, was subjected to enzymatic hydrolysis during a multifactor experiment to optimize the conditions of enzymatic hydrolysis of feather-down raw materials (HF) at a laboratory level. The samples of the hydrolysates shown in Table 2 showed the most satisfactory results (% of protein, the yield of solids, the anion-exchange capacity of feather hydrolyzates in relation to peroxyl hydrolyzate) and were selected for a further study.

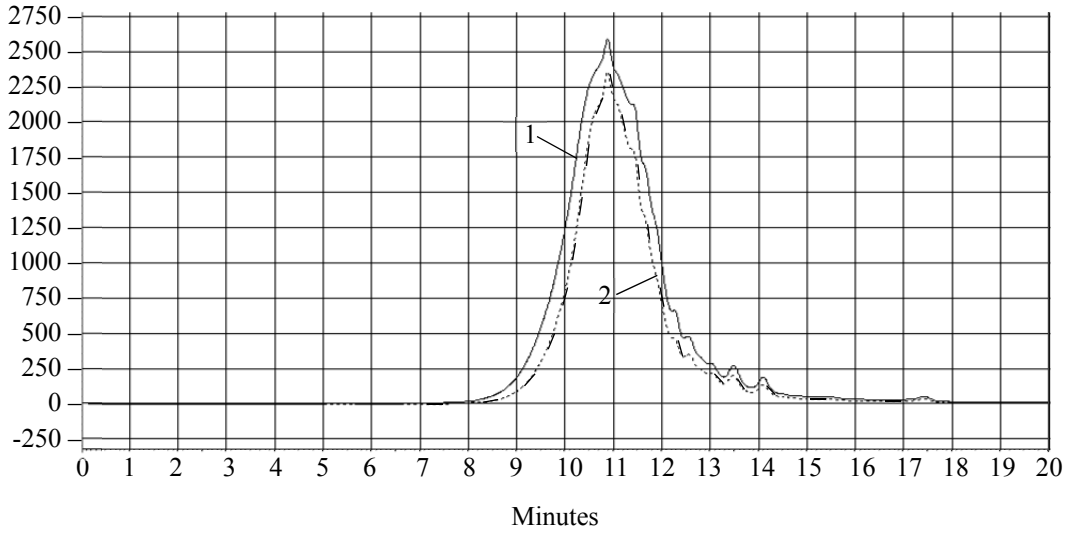
Further on, we studied the physico-chemical properties of six samples of enzymatic hydrolysates (Table 2) of the hydrothermally pre-treated feather-down raw materials obtained in the course of a multifactorial experiment to optimize the conditions for the enzymatic hydrolysis of feather-down raw materials at a laboratory level. Table 3 presents the results obtained.

The data presented in Table 3 show that the content of protein and solids in the enzymatic hydrolysates of the feather-down raw materials varies in the range of 1.80–3.00% and 2.6–4.4%, respectively. The mass fraction of protein in terms of the mass fraction of moisture is 67.7–70.6%. The mass fraction of calcium in terms of the mass fraction of moisture in the test samples varies in the range from 12.6 to 13.8%, the mass fraction of phosphorus ranges from 12.9 to 14.9%. The mass fraction of ash in terms of the mass fraction of moisture in all the samples of enzymatic hydrolysates does not exceed 0.43%, the mass fraction of fat - 0.74%, the mass fraction of crude fiber - 1.28%, the mass fraction of sodium chloride - 1.92% and the mass fraction of the impurities insoluble in hydrochloric acid - 0.77%. It also follows from Table 1 that the minimum protein content in terms of the mass fraction of moisture is in the hydrolysates with an irrigation module equal to 4. The content of solids and protein in the enzymatic hydrolysates of feather-down raw materials correlate with each other. This fact is apparently due to a high relative fraction of protein in the solids of enzymatic hydrolysates of feather-down raw materials. The content of moisture and ash of the enzymatic hydrolysates of feather-down raw materials correspond to the value range of these parameters for other animal hydrolysates.

The comparison of the data in Table 3, in terms of the mass fraction of moisture, testifies that the enzymatic hydrolyzates of the feather-down raw materials have satisfactory parameters for the mass fraction of ash, fat, crude fiber, sodium chloride, calcium, phosphorus and mineral impurities. As for the protein content, there is no such correspondence, due to which the further investigations within the framework of this study will be aimed at cleaning and degreasing the enzymatic hydrolysates of feather-down raw materials in order to increase the mass fraction of protein.

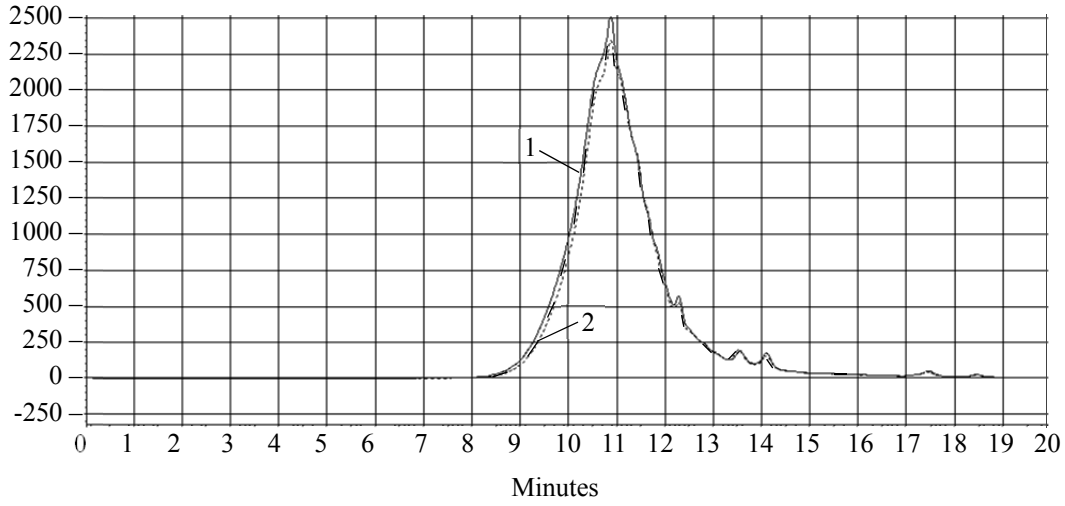
The analysis of the molecular mass distribution of enzymatic hydrolysates of keratin-containing raw materials of hydrolysates with the maximum yield of solids or the highest antioxidant capacity in relation to a peroxyl radical (HF-3, HF-28, HF-37, HF-54, HF-58, HF-68) was performed according to the procedure given. Table 4 presents the results obtained.

Figure 1 presents the profiles of hydrolysate elution.



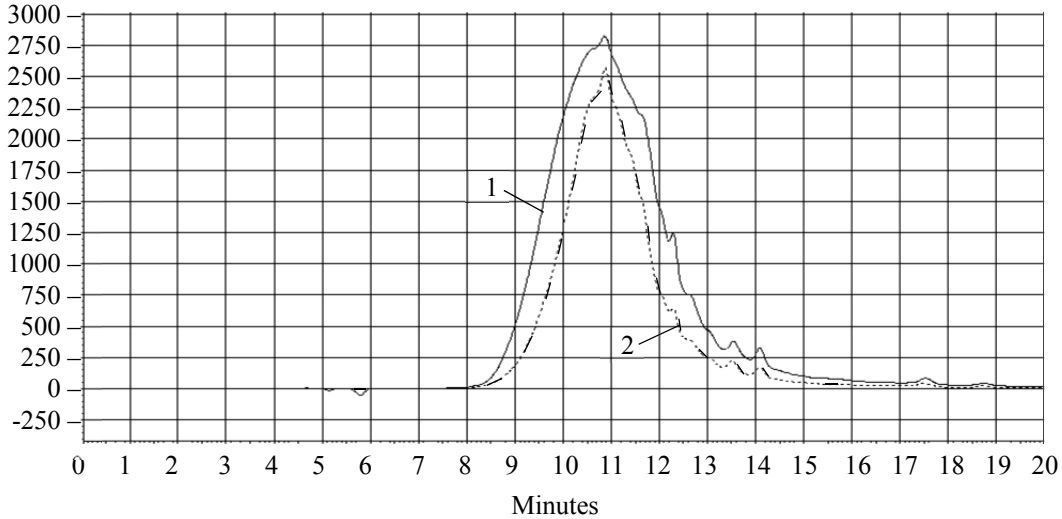
1 – Channel A-UV Spectral Photometer S-2500 N3.CDF; 2 – Channel A-UV Spectral Photometer S-2500 N28.CDF

(a)



1 – Channel A-UV Spectral Photometer S-2500 N37.CDF; 2 – Channel A-UV Spectral Photometer S-2500 N54.CDF

(b)



1 – Channel A-UV Spectral Photometer S-2500 N58.CDF; 2 – Channel A-UV Spectral Photometer S-2500 N68.CDF

(c)

Fig. 1. Elution profiles of hydrolysates (a) HF-3 and HF-8; (b) HF-37 and HF-54; (c) HF-58 and HF-68.

The elution profiles of the hydrolysates obtained when incubating the keratin-containing raw materials in a medium without the addition of an enzyme and sodium sulfite (Fig. 1) are characterized by the presence of well-marked peaks for 11 min of elution. In addition, there are peaks of low intensity that correspond to low-molecular compounds (13–14 min). The HF-58 sample, obtained when hydrolyzing the keratin-containing raw materials with a sodium sulfite content of 1.25% for 2 hours, with the hydromodule 4 and the activity of EP for Protolad B of 30 U/g, had the highest fraction (46.94%) of low-molecular compounds of the range < 3 kDa. The increase in the hydromodule to 8 (the HF-68 sample) resulted in a shift in the size of peptide molecules towards the range of molecular weights > 10 kDa. There is predominance in the content of peptides of an average molecular weight of 3-10 kDa (39–57%) in the samples HF-3, HF-28, HF-37 and HF-54.

Further on, the microbiological parameters of the hydrothermally treated feather-down raw materials (feather) were examined. Table 5 presents the results of these studies.

As it can be seen from the data presented in Table 4, the feather-down raw materials meets the requirements of TU 9219-094-23476484-09 “Hydrolyzed feed flour. Feather protein concentrate” for their microbiological properties (QMAFAnM, coliforms, pathogenic microorganisms, toxin-forming anaerobic bacteria and bacteria of the genus Proteus) and chemical safety parameters.

The microbiological properties of six samples of enzymatic hydrolysates were also studied. Table 6 presents the obtained results.

The data of Table 6 show that, according to microbiological properties, the samples of enzymatic hydrolysates of the pre-treated feather-down raw materials, obtained at a laboratory level, correspond to the current hygienic standards for the bacteriological safety of feeds and feed ingredients.

Within the framework of this study, the chemical safety indicators of the hydrothermally treated feather-down raw materials (feather) were studied. The study considered the content of lead, cadmium, arsenic and mercury, copper, zinc, aflatoxin B1, dichlorodiphenyltrichloromethylmethane, endosulfan, endrin, heptachlor and hexachlorocyclohexane, as well as the content of the ^{90}Sr and ^{137}Cs radionuclides, in the raw materials. Table 7 presents the study results.

The results of the studies presented in Table 7 show that the feather-down raw materials meet the requirements of TU 9219–094–23476484–09 “Hydrolyzed feed flour. Feather protein concentrate” for the chemical safety parameters.

Table 8 presents the results of the study of the physico-chemical indicators of six samples of enzymatic hydrolysates of the hydrothermally pre-treated feather-down raw materials obtained in the course of a multifactorial experiment to optimize the conditions for the enzymatic hydrolysis of feather-down raw materials at a laboratory level.

It follows from Table 8 that the content of toxic elements and radionuclides in the enzymatic hydrolysates of feather-down raw materials, obtained under optimal conditions of hydrolysis at a laboratory level, corresponds to the safety parameters specified in TU 9219–094–23476484–09 "Hydrolyzed feed flour. Feather protein concentrate".

Table 4. Molecular mass distribution of enzymatic hydrolysates of keratin-containing raw materials obtained during a multifactor experiment at a laboratory level

Example code	Share of components with the corresponding range of MW		
	> 10 kDa	3–10 kDa	< 3 kDa
HF-3	28.68	51.31	19.99
HF-28	27.13	57.83	15.02
HF-37	36.55	39.14	24.32
HF-54	25.94	53.88	17.41
HF-58	18.34	34.73	46.94
HF-68	41.15	35.21	23.62

Table 5. Microbiological parameters of the hydrothermally treated feather-down raw materials (feather)

Microbiological indicators	Norms	Test results	NTD for the test methods
The number of mesophilic aerobic and facultative anaerobic microorganisms, CFU per 1.0 g, no more than	$5.0 \cdot 10^5$	$1.0 \cdot 10^2$	GOST 10444.15-94
Product mass (g), in which coliform bacteria were not detected	50.0	50.0	GOST 25311-82, GOST 30518-97
Product mass (g), in which pathogenic microorganisms, including Salmonella, were not detected	50.0	50.0	GOST 25311-82, GOST 30519-97
Product mass (g), in which anaerobic bacteria (toxin-forming bacteria) were not detected	50.0	50.0	GOST 25311-82, GOST 29185-91
Product mass (g), in which *Proteus* were not detected	1.0	1.0	GOST 28560-90

Table 6. Microbiological indices of the enzymatic hydrolysates obtained under optimal hydrolysis conditions at a laboratory level

Microbiological indicators	Norms	Test results						NTD for the test methods
		HF-3	HF-28	HF-37	HF-54	HF-58	HF-68	
The number of mesophilic aerobic and facultative anaerobic microorganisms, CFU per 1.0 g, no more than	$5.0 \cdot 10^5$	$9.0 \cdot 10^1$	$1.8 \cdot 10^2$	$1.2 \cdot 10^4$	$2.2 \cdot 10^3$	$3.0 \cdot 10^1$	$1.1 \cdot 10^4$	GOST 25311-82, GOST 10444.15-94
Product mass (g), in which coliforms were not detected	50.0	50.0	50.0	50.0	50.0	50.0	50.0	GOST 25311-82, GOST 30518-97
Product mass (g), in which pathogenic microorganisms, including Salmonella, were not detected	50.0	50.0	50.0	50.0	50.0	50.0	50.0	GOST 25311-82, GOST 30519-97
Product mass (g), in which anaerobic bacteria (toxin-forming bacteria) were not detected	50.0	50.0	50.0	50.0	50.0	50.0	50.0	GOST 25311-82, GOST 29185-91
Product mass (g), in which Proteus were not detected	1.0	1.0	1.0	1.0	1.0	1.0	1.0	GOST 28560-90

Table 7. Indicators of chemical safety of the hydrothermally treated feather-down raw materials (feather)

Safety indicators	Actual value	Standard indicator	Methods
Lead, mg/kg	0.0020	Not more than 0.5	GOST 26932–86
Cadmium, mg/kg	0.0010	Not more than 0.3	GOST R 51301–99
Arsenic, mg/kg	not detected	Not more than 1.0	GOST 26930–86
Mercury, mg/kg	not detected	Not more than 0.2	GOST 53183–2008
Copper, mg/kg	10.0	Not more than 80.0	GOST 26931–86
Zinc, mg/kg	35.0	Not more than 250.0	GOST 26934–86
Content of aflatoxin B1, mg/kg	0.0050	Not more than 0.01	GOST 31653–2012
Content DDT (the sum of dichlorodiphenyltrichloromethylmethane, dichlorodiphenyl dichloromethylmethane and dichlorodiphenyldichlorethylene), mg/kg	0.0070	Not more than 0.05	GOST 32194–2013
Content of endosulfan (the sum of alpha and beta isomers and endosulfansulfate), mg/kg	0.020	Not more than 0.1	GOST 32194–2013
Content of endrin (the sum of endrin and delta-keto endrin), mg/kg	0.0050	Not more than 0.01	GOST 32194–2013
Content of heptachlor (the sum of heptachlor and heptachlorepoxide), mg/kg	0.0070	Not more than 0.01	GOST 32194–2013
Content of hexachlorocyclohexane (the sum of isomers), mg/kg	0.0060	Not more than 0.01	GOST 32194–2013
^{90}Sr, Bq/kg	not detected*	Not more than 200.0	GOST 32163–2013
^{137}Cs, Bq/kg	25.0	Not more than 600.0	GOST 32161–2013

Note. * not detected

Table 8. *Beginning*. Indicators of the chemical safety of the enzymatic hydrolysates obtained under optimal hydrolysis conditions at a laboratory level

Parameter	Parameter value for a sample					
	HF-3	HF-28	HF-37	HF-54	HF-58	HF-68
Lead, mg/kg	0.17	0.10	0.23	0.12	0.15	0.09
Cadmium, mg/kg	0.010	0.008	0.007	0.005	0.005	0.009
Arsenic, mg/kg	0.10	0.005	0.005	0.08	0.09	0.10
Mercury, mg/kg	0.005	0.0050	0.0080	0.004	0.011	0.0050
Copper, mg/kg	10.0	8.5	15.0	12.0	14.0	9.0

Table 8. *Ediing*. Indicators of the chemical safety of the enzymatic hydrolysates obtained under optimal hydrolysis conditions at a laboratory level

Parameter	Parameter value for a sample					
	HF-3		HF-3		HF-3	
Zinc, mg/kg	25.0	40.0	35.0	55.0	10.0	20.0
Content of aflatoxin B1, mg/kg	0.0050	0.005	0.0060	0.0025	0.0075	0.0035
Content of DDT (the sum of dichlorodiphenyltrichloromethylmethane, dichlorodiphenyl dichloromethylmethane and dichlorodiphenyldichlorethylene), mg/kg	0.010	0.010	0.009	0.005	0.008	0.010
Content of endosulfan (the sum of alpha and beta isomers and endosulfansulfate), mg/kg	0.050	0.030	0.020	not detected	0.050	0.010
Content of endrin (the sum of endrin and delta-keto endrin), mg/kg	0.0070	0.0070	0.0050	not detected	not detected	0.0060
Content of heptachlor (the sum of heptachlor and heptachlorepoxide), mg/kg	not detected	not detected	0.0060	not detected	not detected	0.0080
Content of hexachlorocyclohexane (the sum of isomers), mg/kg	0.0050	not detected	not detected	not detected	not detected	0.0050
^{90}Sr, Bq/kg	10.0	15.0	not detected	8.0	not detected	not detected*
^{137}Cs, Bq/kg	15.0	35.0	60.0	25.0	75.0	12.0

Note. * not detected.

Thus, the following results were obtained in the course of this study:

(1) The physico-chemical properties of the enzymatic hydrolysates of feather-down raw materials obtained during a multifactorial experiment on optimizing the conditions of the enzymatic hydrolysis of feather-down raw materials have been studied at a laboratory level. The content of protein and solids in the enzymatic hydrolysates of feather-down raw materials varies in the range of 1.80–3.00% and 2.6–4.4%, respectively. The mass fraction of protein, in this case, is 67.7–70.6% in terms of the mass fraction of moisture. The mass fraction of ash in terms of the mass fraction of moisture in all the samples of enzymatic hydrolysates does not exceed 0.43%, the mass fraction of fat - 0.74%, the mass fraction of crude fiber - 1.28%, the mass fraction of sodium chloride - 1.92% and the mass fraction of the impurities insoluble in hydrochloric acid - 0.77%.

(2) The molecular mass distribution of the enzymatic hydrolysates of keratin-containing raw materials of hydrolysates with the maximum yield of solids or the highest antioxidant capacity in relation to a peroxy radical has been analyzed. The elution profiles of the hydrolysates obtained when incubating the keratin-containing raw materials in a medium without the addition of an enzyme and sodium sulfite were characterized by the presence of well-marked peaks for 11 min of elution. In addition, there were peaks of low intensity that corresponded to low-molecular compounds (13–14 min). The HF-58 sample, obtained when hydrolyzing the keratin-containing raw materials with a sodium sulfite content of 1.25% for 2 hours, with the hydromodule 4 and the activity of EP for Protolad B of 30 U/g, had the highest fraction (46.94%) of low-molecular compounds of the range < 3 kDa. The increase in the hydromodule to 8 (the HF-68 sample) resulted in a shift in the size of peptide molecules towards the range of molecular weights > 10 kDa. There was predominance in the content of peptides of an average molecular weight of 3-10 kDa (39–57%) in the samples HF-3, HF-28, HF-37 and HF-54.

(3) The microbiological properties of the enzymatic hydrolysates of feather-down raw materials obtained during a multifactorial experiment on optimizing the conditions of the enzymatic hydrolysis of feather-down raw materials have been studied at a laboratory level. It has been shown that all the samples of enzymatic hydrolysates of the pretreated feather obtained at a laboratory level correspond to the parameters of microbiological safety specified in TU 9219–094–23476484–09 “Hydrolyzed feed flour. Feather protein concentrate” for their microbiological properties (QMAFAnM, coliforms, pathogenic microorganisms, toxin-forming anaerobic bacteria and bacteria of the genus Proteus).

(4) The chemical safety properties of the enzymatic hydrolysates of feather-down raw materials obtained during a multifactorial experiment on optimizing the conditions of the enzymatic hydrolysis of feather-down raw materials have been determined at a laboratory level. It has been shown that the experimental samples of enzymatic hydrolysates of feather-down raw materials completely correspond to the parameters of chemical safety in accordance with TU 9219–094–23476484–09 “Hydrolyzed feed flour. Feather protein concentrate” for the chemical and radiation safety parameters.

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DEVELOPMENT OF TEST PROCEDURES AND THE SEARCH FOR OPTIMAL POSITIONS OF THE PRIMERS PLANTING USING THE PROGRAM PRIMERQUEST FOR IDENTIFICATION OF PLANT OBJECTS

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Abstract: Identification has similarities and differences with other kinds of assessment activity: quality assessment, control management and certification. The final result of identification is verification of compliance or detection of falsification. Common features are tests for definition of actual values. This paper studies the design of universal primers for type identification of fruit raw material (strawberry, gooseberry, cherry, raspberry, banana, wild rose, kiwi). To further verify the specificity of primers, sequencing of fragments is produced, which are read by each from the primer pairs. For this purpose, 8 polymerase chain reactions (PCR-reactions) are initiated, one from each primer pair corresponding to one type of raw material. A single alignment matrix for each of the studied objects is created as a result. Re-verification of each matrix is conducted for the presence of read errors or other disputed single-nucleotide substitutions. It is stated that the alignment matrices of the nucleotide sequences of raspberry, strawberry (fragaria viridis), gooseberry, wild rose, cherry, banana and kiwi are aligned on all sides and the protruding "bases" do not disturb the future work of programmes for the primers design. Universal non-intersecting primers are chosen to identify the fruit raw material under studying. As a result of the use of various software packages and of the database GenBank NCBI, we managed to find a suitable DNA zone for each of the tested samples of fruit raw material at the level of generic differentiation for further development on its basis of the universal primers. It is zone 18S rDNA. All the found sequences have both the conservative part for planting a pair of primers, and the variable one for reliable identification of species or for phylogenetic analysis. As part of the study, all samples of fruit raw material have been identified.

Keywords: Fruit raw material, identification, PCR, matrix, primers

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INTRODUCTION

Identification as an activity has its own structure which includes objectives and tasks, objects and subjects, means and methods [1].

Identification has similarities and differences with other kinds of assessment activity: quality assessment, control management and certification. Common features are tests for definition of actual values and compliance test with the requirements of regulatory documents. The differences lie in the list of criteria; in the subjects which determine the assessment activity; in the final result. The final result of identification is verification of compliance or detection of falsification [2, 3].

The term "identification" is interpreted differently. The analysis of the regulatory documents showed that the term "identification" has the following definitions [13].

Identification is the procedure by which compliances of the products, submitted to certification, are established with the requirements for this type of products, set by the regulatory documents (Sertifikatsiya pischevykh produktov i prodovol'stvennogo syr'ya v RF [Certification of foodstuffs and food raw material in the Russian Federation], 1996).

As criteria of identification the indicators, meeting the following requirements, should be selected:
– typicalness for a particular type, name or homogeneous product group;
– objectiveness and comparability;
– ability to test;
– difficulty of falsification.

The greatest significance has the typicalness which can be characterized by complex or, less often, individual indicators that complement each other and have a varying degree of accuracy [4, 5].

The objective of this paper is the study of universal primers design for type identification of fruit raw material. The tasks of this paper include the selection of universal primers and the identification of such fruits and berries like cherry, strawberry, raspberry, gooseberry, wild rose, banana and kiwi.

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STUDY OBJECTS AND METHODS

Alignments were visualized in the programme GeneDoc.

Matrices were aligned from the two sides of alignments. Presence of protruding "bases" may disturb the future programme work [15].

In accordance with the objective and the tasks of the present paper, the study objects were: *Rubus idaeus* (raspberry, the grade “Nagrada”), *Fragaria vesca* (remontant wild strawberry, the grade “Berdskaya rannyaya”), *Ríbes úva-críspa* (garden gooseberry, the grade “Kooperator”), *Prunus fruticosa* (ground cherry, the grade “Altayskaya lastochka”), *Rosa majalis Herrm* (cinnamon rose), *Actinidia deliciosa* (kiwi delicatessen), *Músa paradisiaca* (banana of "extra" grade).

Primers were selected with the use of the programme PrimerQuest (http://eu.idtdna.com/Primerquest/Home/Index). Computer processing and sequences alignment were performed in the programmes ClustalW and GeneDoc, the construction of phylogenetic trees was performed in the programme Mega 6 [6, 7].

To further verify the specificity of primers, sequencing of fragments was produced, which are read by each from the primer pairs [14, 17]. For this purpose, 8 polymerase chain reactions (PCR-reactions) were initiated, one from each primer pair corresponding to one type of raw material [8, 9]. The obtained PCR-products were re-precipitated by ethanol in the presence of ammonium acetate, dried and then sequenced according to Sanger using the device ABI Prism 3500xl. The sequencer output data - chromatograms - were converted into nucleotide sequence and then, using the BLAST algorithm, were compared to the NCBI sequences, present in GenBank [10, 11].

RESULTS AND DISCUSSION

At this research stage previously conducted alignments were visualized and corrected in the program GeneDoc [12]. Thus, a single alignment matrix for each of the studied objects was created (Fig. 1–7). Re-verification of each matrix was conducted for the presence of read errors or other disputed single-nucleotide substitutions.

Matrices were aligned from the two sides of alignments. Presence of protruding "bases" may disturb the future programme work.

The analysis of the figure shows that the alignment matrices of the nucleotide sequences of raspberry, strawberry (fragaria viridis), gooseberry, wild rose, cherry, banana and kiwi are aligned on all sides and the protruding "bases" do not disturb the future work of programmes for the primers design.

Rectangular alignment matrices for each of the studied objects are presented in the figures.

Then, each matrix was loaded to the program PrimerQuest for sequences algorithmic analysis and search for optimal positions of the primers planting.

In the settings it was always stated that the maximum size of the amplicon, read by a pair of primers, should not exceed 300 b.p. An optimal pair of primers was selected from the ones, offered by the programme (Fig. 8). The following parameters were taken into consideration: primer length, annealing temperature, amplicon location.

Analyzing Fig. 8, optimal primers were selected.

Primers for the studied types of fruit raw material with the recommended parameters for PCR (visualization of the programme PrimerQuest) are indicated in Figures 9–15.

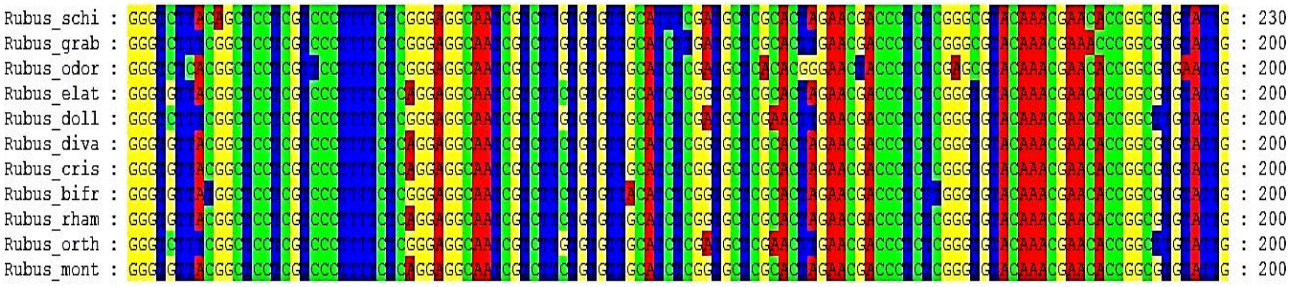

Fig. 1. Part of alignment matrix of *Rubus idaeus* nucleotide sequences.

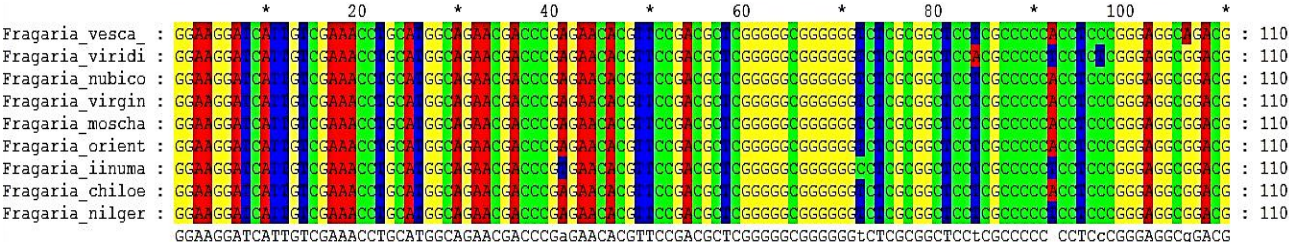

Fig. 2. Part of alignment matrix of *Fragaria vesca* nucleotide sequences.

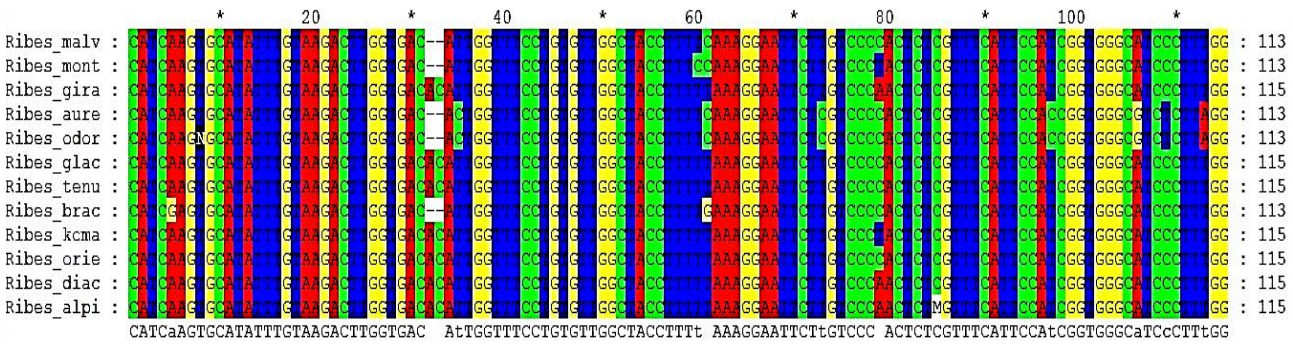

Fig. 3. Part of alignment matrix of *Ríbes úva-críspa* nucleotide sequences.

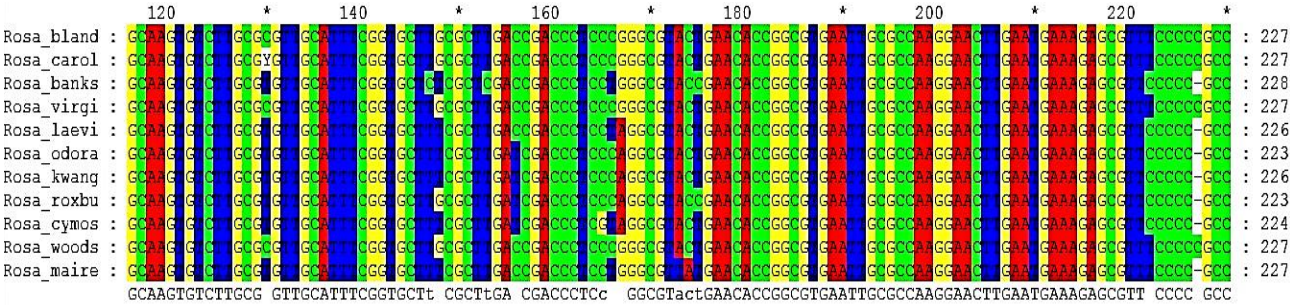

Fig. 4. Part of alignment matrix of *Rosa majalis Herrm* nucleotide sequences.

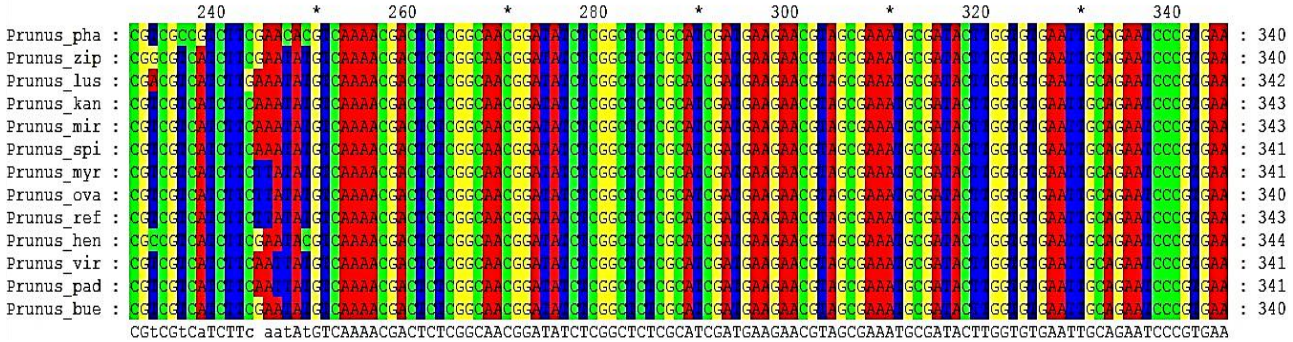

Fig. 5. Part of alignment matrix of *Prunus fruticosa* nucleotide sequences.

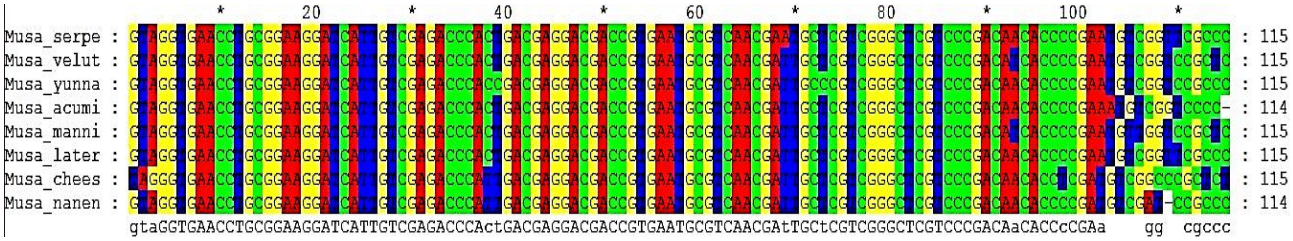

Fig. 6. Part of alignment matrix of *Músa paradisiaca* nucleotide sequences.

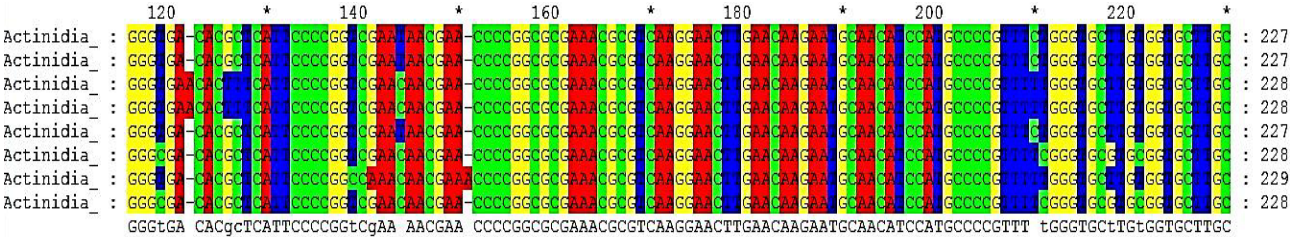

Fig. 7. Part of alignment matrix of *Actinidia deliciosa* nucleotide sequences.

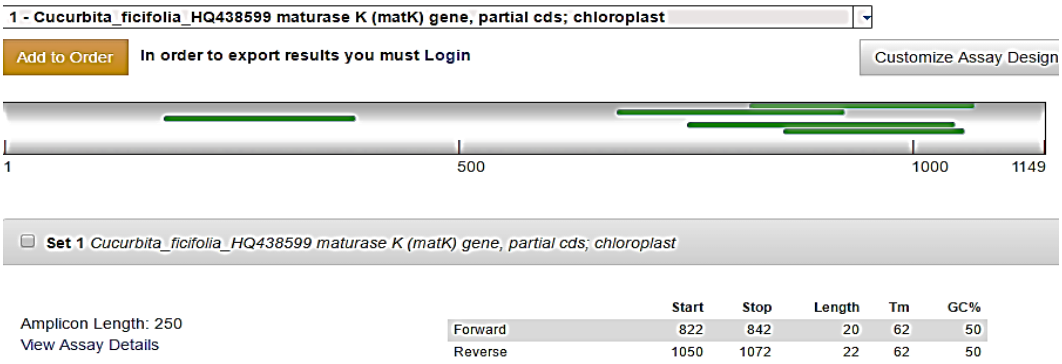

Fig. 8. Selection of an optimal primer pair in the programme PrimerQuest.

Parameter Set: General PCR (Primers only)
Sequence Name:
Amplicon Length: 271

		Start	Stop	Length	Tm	GC%
Forward	CGATGAAGAACGTAGCGAAATG (Sense)	354	376	22	62	45.5
Reverse	CGATAGGCAACAGAGGTTTGA (AntiSense)	604	625	21	62	47.6

Base	Sequence
1	TTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGAAACCTGCCCAGCAGAACGACCCGAGAACATGTTTCA
101	ACGCTTGGGGGCGAAGGGTCTTACAGCTCCTCGTCCCTTTTCTCGGGAGGCAATCGTCTTGTGTGTTGCATTTCGATGCTCGCACTAGAACGACCCTCTC
201	GGGCGTACAAACGAACACCGGCGTGTATTGCGCCAAGGAACTTGAATGAAAGAGCGTTTCCCCGTCGTCCCGGAAACGGTGTGCGTACGGTTGGTTACGT
301	CATCTTCAATATGTCTAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAAT
401	CCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGCCTGCCTGGGCGTCACACGTCGTTGCCCCCCCAACCCCC
501	TCGGGAGTTGGGCGGGACGGATGATGGCCTCCCGTGTGCTCCGTCATGCGGTTGGCATAAAAAACAAGTCCTCGGCGACTAACGCCACGACAATCGGTGG
601	TTGTCAAACCTCTGTTGCCTATCGTGTGCGCGTGTCGAACGAGGGCTCAATGAACCATGCTGCATTGATTCGTCGATGCTTTCAACGCGACCCCAGGTCA
701	GGCGGGGGTTACCC

Note. Hereinafter: Green - direct, Red - reverse

Fig. 9. Universal primers for *Rubus idaeus* identification.

Parameter Set: General PCR (Primers only)
Sequence Name:
Amplicon Length: 276

		Start	Stop	Length	Tm	GC%
Forward	CCGTGAACCATCGAGTCTTT (Sense)	351	371	20	62	50
Reverse	GCTTACCGACGCGCTTTA (AntiSense)	609	627	18	62	55.6

Base	Sequence
1	GGAAGGATCATTGTCGAAACCTGCATGGCAGAACGACCCGAGAACACGTTCCGACGCTCGGGGGCGGGGGGTCTCGCGGCTCCTCGCCCCCTCCTCCCGG
101	GAGGCGGACGTCTCGCGCGTCGCGCTCGGCGCTTCCGCCTGGCCGACCCTTCCGGGCGTACCGAACACCGGCGTGAATTGCGCCAAGGAACTTGAATGAA
201	AGAGCGTTCCCCCGCCGTCCCGGAGACGGAGACCGCGCGGGTGGTTCGTCGTCTTCAGTATGTCTAAACGACTCTCGGCAACGGATATCTCGGCTCTCGC
301	ATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCGTTAGGC
401	CGAGGGCACGTCTGCCTGGGCGTCACACGTCGTTGCCCCCCCGACCCCTTCGGGGGCCGGACGGGACGGATGATGGCCTTCCCGTGTGCCCCGTCACGCG
501	GTTGGCATAAATACCGAGTCCTCGGCGACCGGCGCCGCGACAATCGGTGGTTGTGAAACCTCGGTGCCTTGTCGCGTGCGTGAGTCGATCGCGGGACTTC
601	CTTAACCTTAAAGCGCGTCGGTAAGCCGACGCTTTCAACGCGACCCCAGGTCAGGCGGGTTACCCGCTGAATTTAA

Fig. 10. Universal primers for *Fragaria vesca* identification.

Parameter Set: General PCR (Primers only)
Sequence Name:
Amplicon Length: 269

		Start	Stop	Length	Tm	GC%
Forward	CGTCGTCTCATATGTCCATCAA (Sense)	14	36	22	62	45.5
Reverse	GGAGCAATAAAGCACCACATAC (AntiSense)	261	283	22	62	45.5

Base	Sequence
1	CTGTTGTCGCGTCCGTCGTCTCATATGTCCATCAAGTGCATATTTACAAGACTTGGTGACATTGGTTTCCTGTGTTGGCTACCTTTTCAAAGGAATTCTC
101	GTCCCAACTCTCGTTTCATTCCATCGGTGGGCATCCCTCTGTGGATTGTCTTGGTGGACCTTCAAGTGTTTCTCGTGTGCCATTCACGCTACATTTTWAT
201	GCGGCGATGACATGGTCCACGGGTTGCTACTCGTAATCTCGGATTCGGAACATGTTGTGGGTATGTGGTGCTTTATTGCTCCATCTGCCCAAGCAGAGCT
301	TCTGTTGCTCGGCAAGAACGACAGTCGTGCTCGTGTTGACCTCTCCGGCATGCATGTGCTCGGTTTGGCTCATGCGACGCCCGACTTCGCAAAGGAATGC
401	TACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTGT

Fig. 11. Universal primers for *Ríbes úva-críspa* identification.

Parameter Set: General PCR (Primers only)
Sequence Name:
Amplicon Length: 227

		Start	Stop	Length	Tm	GC%
Forward	GTTTCCTGTGTTGGCTACCT (Sense)	65	85	20	62	50
Reverse	TGGGCAGATGGAGCAATAAA (AntiSense)	272	292	20	62	45

Base	Sequence
1	CTGTTGTCGCGTGCGTCGTCTCATATGTCCATCAAGTGCATATTTGTAAGACTTGGTGACATTGGTTTCCTGTGTTGGCTACCTTTTCAAAGGAATTCTT
101	GTCCCCACTCTCGTTTCATTCCATCGGTGGGCATCCCTTTGGGGATTGTCTTGGTGGACCTTCAAGTGTTTCCTGTGTGCCATTCACGCTACATTTTAAT
201	GCGGCGATGACATGGTCCACGGGTTGCTACTCGTAATCTCGGATTCGGAATATGTTGTGGGTATGTGGTGCTTTATTGCTCCATCTGCCCAAGCAGAGCT
301	TCTGTTGCTCGGCAAGAACGACAGTCGTGCTCGTGTTGACCTCTCCGGCATGCATATGCTCGGTGTGGCTCATGCGACGCCCGACTTCGAAAAGGAATGC
401	TACCTGGTTGATCCTGCCAGTAGTCATA

Fig. 12. Universal primers for *Rosa majalis Herrm* identification.

Parameter Set: General PCR (Primers only)
Sequence Name:
Amplicon Length: 285

		Start	Stop	Length	Tm	GC%
Forward	CTTGGTGTGAATTGCAGAATCC (Sense)	362	384	22	62	45.5
Reverse	CATCTTTACTTCTAGCCCTCGAC (AntiSense)	624	647	23	62	47.8

Base	Sequence
1	ATTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCGAAACCTGCCCGGCAGAACGACCCGAGAACCAGTTTC
101	GGAACTGGGGGCGAGGGGTCTCGCGGCTCCTCCTCCCTTCGTCYCGGGAGGGTCGCGTCGCGTTCGCGCGGCCGGCCCTTCCGGGCGTACAAACGAACAC
201	CGGCGCGAATTGCGCCAAGGAACTTGAACGAGAGAGCGCGCCCCTGCGGCCCCGGAAACGGTGCGCGCGGGCGGCGTCGCCGTCTTCGAACACGTCAAAA
301	CGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCT
401	TTGAACGCAAGTTGCGCCCGAAGCCGTTAGGCCGAGGGCACGCCTGCCTGGGCGTCACGCGCCGTTGCCCCCCCGACCGATCCCTCGGGATCGCGGGGGG
501	CGGATGGTGGCCTCCCGTGCGCTCCGCCGCGCGGTTGGCATAAATACCAAGTCCCCGGCGACGCGCGCCACGACGATCGGTGGTTGCGAAACCTCGGTTG
601	CCCGTCGTGTGCGGCCGTCGCKCGTCGAGGGCTAGAAGTAAAGATGCTCGGCTCCGGCTCGGCTCTCAACGCGACCCCAGGTCAGGCGGGGTTACCCGCT
701	GAATTTA

Fig. 13. Universal primers for *Prunus fruticosa* identification.

Parameter Set: General PCR (Primers only)
Sequence Name:
Amplicon Length: 245

		Start	Stop	Length	Tm	GC%
Forward	GGAAGGATCATTGTCGAGACC (Sense)	15	36	21	62	52.4
Reverse	CGTTGCCGAGAGTCATACAA (AntiSense)	240	260	20	62	50

Base	Sequence
1	GTAGGTGAACCTGCGGAAGGATCATTGTCGAGACCCACTGACGAGGACGACCGTGAATGCGTCAACGAATGCTCGTCGGGCTCGTCCCGACAACACCCCG
101	AATGTCGGTTCGCCCTCGGGCGGGACGATCGAGGGGATGAACTACCAACCCCGGCGCGGATAGCGCCAAGGAACACGAACATCGAAGTCGGAGGGCCTCG
201	CTGCATGCAGGAGGCTACAATTCCGACGGTGACACCCCATTGTATGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATG
301	CGATACCTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAGGCCATCCGGCTAAGGGCACGCCTGCCTGGGCGTC
401	ACGCTTTCGACGCTTCGTCGTTGCCCCCTCGGGGGGTGGGGGCGAACGCGGAGGATGGCCCCCCGTGCCGGAAGGTGCGGTTGGCCGAAGATCGGGCCGT
501	CGGTGGTTGTCGAACACGACGCGTGGTGGATGCCTTGTGCGAGCCGTACGTCGTGCCTTCGGAACCCGGGCGAGGCCTCGAGGACCCAAGTCGTGGTGCG
601	AGTCGATGCCACGGACCGCGACCCCAGGTCAGGTGGGGCTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGA

Fig. 14. Universal primers for *Músa paradisiaca* identification.

Parameter Set: General PCR (Primers only)
Sequence Name:
Amplicon Length: 283

		Start	Stop	Length	Tm	GC%
Forward	GACCCGCGAACTTGTCTAATA (Sense)	18	39	21	62	47.6
Reverse	GCATTTCGCTACGTTCTTCATC (AntiSense)	279	301	22	62	45.5

Base	Sequence
1	AACCTGCCTAGCAGAATGACCCGCGAACTTGTCTAATACTCTCGGGGAAGCGAAAGGTTGGTTTTTATGGCCTCCTTTTTCTTCCCTTTGCCGGGTGTGC
101	TCGTGTTGCCCTATGGGTGACACGCTCATTCCCCGGTCGAATAACGAACCCCGGCGCGAAACGCGTCAAGGAACTTGAACAAGAATGCAACATCCATGCC
201	CCGTTTCTGGGTGCTTGTGGTGCTTGCTCTATCATAAACGAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGC
301	GATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCTGAAGCCATTAGGCCGAGGGCACGTCTGCCTGGGCGTCA
401	CGCATTGTGTCGCCCACCCGACTCAAGCCTTGCCAAGGCCTGCGTGTGGGTGGGCGGATATTGGCCCCCCGTGCACATTAGTGAACGGTCGGCCTAAAAA
501	TGAGTCCTTGGCAATGGACGTCACAACAAGTGGTGGTTGACAAACCGTTGCGTCCTGTTGTGCTTGCCCCCATTGCTAATGGTTTACTTTTGACCCTAGT
601	GTGCCGTTGCCACGGCTTCGATCGCGACCCCAGGTCAGGCGGGATTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGAAACTTACAAGGATT
701	CCCTTAGTAACGGCGAGCGAACCGGGAATAGCCCAGCTTGAAAATCGGGCGATCTCGTCGTCCGAATTGTAGTCTGGAGAAA

Fig. 15. Universal primers for *Actinidia deliciosa* identification.

2 **Table 1.** Universal primers for PCR test-systems

Name of food raw material	Nucleotide pair primer length	Nucleotide pair amplicon length	Primers
Strawberry (fragaria vesca)	20 18	276	CCGTGAACCATCGAGTCTTT GCTTACCGACGCGCTTTA
Gooseberry	22 23	269	CGTCGTCTCATATGTCCATCAA GGAGCAATAAAGCACCACATAC
Cherry	22 23	285	CTTGGTGTGAATTGCAGAATCC CATCTTTACTTCTAGCCCTCGAC
Raspberry	22 21	271	CGATGAAGAACGTAGCGAAATG CGATAGGCAACAGAGGTTTGA
Banana	21 20	245	GGAAGGATCATTGTCGAGACC CGTTGCCGAGAGTCATACAA
Wild rose	20 21	227	GTTTCCTGTGTTGGCTACCT TGGGCAGATGGAGCAATAAA
Kiwi	21 22	283	GACCCGCGAACTTGTCTAATA GCATTTCGCTACGTTCTTCATC
Pumpkin	24 20	297	AGATACGCCACTTCTGATGAATAA GGATGCCCTAACACGTTACA

On the basis of Figures 9–15, universal non-intersecting primers were selected for determination by PCR method of fruit raw material (strawberry, gooseberry, cherry, raspberry, banana, kiwi). These primers are represented in Table 1.

Analyzing the tabular data, with the use of various software packages and of the database GenBank NCBI, we managed to find a suitable DNA zone for each of the tested samples of fruit raw material at the level of differentiation for further development on its basis of the universal primers. It is zone 18S rDNA. All the found sequences have both the conservative part for planting a pair of primers, and the variable one for reliable identification of species or for phylogenetic analysis.

Thus, as a result of study, a single alignment matrix for each of the studied objects of fruit raw material was created with the use of the programme GeneDoc, re-verification of each matrix is conducted for the presence of read errors or other disputed single-nucleotide substitutions [12, 16].

Sequences algorithmic analysis and search for optimal positions of the primers planting are conducted with the use of the programme PrimerQuest with indication in the settings of maximum amplicon size, read by each primer pair, which does not exceed 300 b.p.

Optimal pairs for each type of fruit raw material are selected from the ones, offered by the programme, taking into consideration the following: primer length, annealing temperature, amplicon location.

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THE PROPERTIES OF BACTERIOCINS OBTAINED UNDER THE DIFFERENT CONDITIONS

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Abstract: Bacteriocines are substances of protein nature, which are produced by many strains of microorgamisms. Due to the fact that bacteriocins are effectively used as antimicrobial agents in medicine and food industry, isolation and study of the properties of new bacteriocins-producing strains is very important. It should be noted that production of bacteriocins by microbial cells directly depends on strain cultivation conditions and methods of bacteriocin isolation. Among other cultivation parameters such as temperature and the active acidity of the culture medium, the duration of cultivation directly influences the synthesis of bacteriocins. Thus, the selection of the optimum duration of strains cultivation and method of bacteriocin isolation would allow us to increase significantly the bacteriocin production and, hence the antimicrobial activity of the strains. Therefore, determination of the optimum duration of cultivation and method of bacteriocins isolation is a very important step for getting bacteriocins with maximum activity. In this study, the isolation of microbial cultures from the fresh onion surface and the identification of these strains by sequencing of the 16S RNA gene were carried out. Also their morphological and physiological-and-biochemical properties were studied. The optimum cultivation duration was performed by using the determination of the antimicrobial activity in different time intervals. The strain cultivation was carried out during 24 hours. Three methods were used to isolate bacteriocins. They were based on the using of activated carbon, ammonium sulfate, and chloroform. The antimicrobial activity of bacteriocins was studied by means of the disk diffusion method. The identification of the isolated strains has revealed that the strains belong to *Bacillus safensis* and *Bacillus pumilus* species. It was found that the bacteriocins had the greatest antimicrobial activity when being cultivated for 18 hours and using method based on ammonium sulfate precipitation.

Keywords: Bacteriocins, duration of cultivation, method of bacteriocin isolation, antimicrobial activity

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INTRODUCTION

Bacteria are able to synthesize metabolites being used in human life such as antibiotics, enzymes, amino acids and etc. [1, 2]. Thus, the isolation and study of the properties of new bacteriocin-producing strains are very important. Nowadays, special attention is paid to creating new antibiotics and antimicrobial agents due to wide spread of antibiotic resistant pathogenic strains [1, 3]. Some antimicrobial agents such as bacteriophages, probiotic bacteria [4], antimicrobial peptides [5, 6], and bacteriocins [7] have already been studied. They are produced by bacteria and are an alternative to antibiotics. Having a number of advantages, bacteriocins are one of the most promising components to create antibiotics; therefore, they are a subject of study nowadays.

Bacteriocins are anti-infection agents of protein nature produced by many bacterial strains which have a broad spectrum of antimicrobial activity and are safe for humans. Due to this fact, the use of bacteriocins in medical and food industry is future-oriented. Bacteriocins are a large group of heterogeneous bacterial antagonists differing considerably from one another in the molecular mass, biochemical properties, and the mechanism of action.

Bacillus strains have the ability to synthesize a wide spectrum of bacteriocins. The main bacteriocins-producing strains are *Bacillus licheniformis, Bacillus subtilis, Bacillus pumilus, Bacillus circulans, Bacillus cereus, Bacillus thuringiensis,* and *Bacillus amyloliquefaciens* [8, 9, 10].

Generally, *Bacilius* strains produce peptides and lipopeptide antibiotics [11] and have the ability to synthesize a wide spectrum of bacteriocins such as batsilin and fungumitsin, plipastatin and surfraktin [12], koagulin [13], tochiin [14], amilolichin [15]. A broad spectrum of antimicrobial activity of bacteriocins being produced by strains of *Bacillus* allows them not only to inhibit the growth of Gram-positive microorganisms but also to inhibit Gram-negative bacteria, yeast and fungi, which are pathogenic for human and animals.

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At present, a lot of information about bacteriocins has been studied: their genetic traits, mechanisms of action, molecular mass that allows us to classify bacteriocins, and strains being more sensitive to bacteriocins.

Mechanisms of bacteriocins actions are found to be different but generally bacteriocins exert their antimicrobial effect by breaking the cell wall or membrane of target organisms, either by inhibiting the biosynthesis of the cell wall or causing pore formation which leads to death [16].

Due to the ability of *Bacillus* strains to synthesize a large number of antimicrobial agents with a broad spectrum of the antimicrobial activity, the study of these strains is important for discovering new methods of their application [17]. Over the last five years new prospects for using bacteriocins have been offered, especially in pharmaceutical and food industry. Thus, the study of new bacteriocin types opens new promising advantages of their use.

Many researchers have concluded that bacteriocins have the ability to kill closely related species [18]. However, more detailed study showed that bacteriocins can take various forms and also exert the antimicrobial activity against other species. Practically, a range of antagonistic activity may be extended by regulating cultivation conditions of bacteriocins and by selecting the optimum isolation method. This would allow us to weaken cell barriers of target organisms and to increase the bacteriocin production [19, 20]. Therefore, the selection of cultivation duration of bacteriocins and method of their isolation is of great importance for producing bacteriocins with maximum antimicrobial activity [21].

In this study, we have given the results on the determination of the optimum cultivation duration of *Bacillus safensis* and *Bacillus pumilus* strains, and that of the optimum isolation method of bacteriocins which are produced by the strains.

OBJECTS AND METHODS OF STUDY

The subjects of research were *Bacillus safensis* B-12180 and *Bacillus pumilus* B-12182 from the Russian National Collection of Industrial Microorganisms (VKPM), having been isolated at the Kemerovo Institute of Food Science and Technology (University).

At various stages of the research the following materials were used: Bacto Peptone, beef extract, sodium chloride, yeast extract, dry bacterial agar, ammonium citrate, sodium acetate, disodium phosphate, magnesium sulfate heptahydrate, manganese sulfate pentahydrate, ethanol, activated carbon, ammonium sulfate, chloroform (Russia); glucose (Belarus); Tween, filters Millex-GV (USA); and antibiotics.

Isolation of microbial cultures. The cultures were isolated from the fresh onion surface. For this, a cut-up onion was put into a liquid culture medium and cultivated at 30±2, 37±2, and 45±2^{0}C from 1 to 5 hours. After growing biomass, the cultivation was carried out onto a solid culture medium which contains (in g/l) NaCl 12.0, Bacto Peptone 6.0, yeast extract 6.0, and dry bacterial agar 10.0. In order to obtain pure cultures, we performed inoculations every day until single colonies were formed. The identification of isolated colonies was carried out according to morphological properties, sporulation, and cell size by using Gram reaction method, oxidase, and catalase tests. For more accurate identification, a genetic analysis of the strains was carried out by sequencing of the 16S RNA gene [22].

Antibiotic resistance. Antimicrobial resistance of the isolated strains was studied by means of the disk diffusion method. The strains were cultivated on the solid culture medium containing (g/l) Bacto Peptone 10.0, beef extract 10.0, yeast extract 5.0, glucose 20.0, Tween 1.0, ammonium citrate 2.0, sodium acetate 5.0, disodium phosphate 2.0, magnesium sulfate heptahydrate 0.1, manganese sulfate pentahydrate 0.05, and dry bacterial agar 10.0. The samples were cultivated at the temperature of 30 ± 2°C and pH of 6.5 for 24 hours.

Determination of the optimum duration of cultivation. The strains were cultivated on the liquid medium containing (g/l) Bacto Peptone 10.0, beef extract 10.0, yeast extract 5.0, glucose 20.0, Tween 1.0, ammonium citrate 2.0, sodium acetate 5.0, disodium phosphate 2.0, magnesium sulfate heptahydrate 0.1, and manganese sulfate pentahydrate 0.05. The samples were cultivated at 30 ± 2°C for 24 hours (pH 6.5). The cultural liquid was subjected to centrifugation in order to separate the supernatant. Centrifugation was carried out at 7,000 g for 10 min. The supernatant was filtered using Millex-GV filters (0.22 μm). The antimicrobial activity against *Escherichia coli B-6954* strain was determined.

Determination of the medium composition. Available sources of carbon and nitrogen were chosen. During experiments we varied sources of nitrogen (peptone, tryptone, yeast extract), carbon (glucose, fructose, sucrose), and chloride (sodium chloride, calcium chloride), and also the ratio added components expressed in percent. Composition of the culture media is given in Table 1. The selection of a suitable medium was carried out by measuring the concentration of recombinant protein in the medium every 2 hours for 4 – 20 hours.

Determination of antimicrobial activity. The antimicrobial activity of bacteriocins was studied by means of the disk diffusion method [23]. Beef-extract agar was poured into Petri dish, dried under UV lamp for 1 hour after which the test microorganisms were inoculated onto the medium surface and dried again for 30 minutes with opened foil. Further, sterilized disks with 10 μl of bacteriocins were put onto the medium surface, dried for15 minutes, after which the dish was inverted, put into a thermostate for 15 minutes and inverted again. After aerobic incubation in the range from 30±2 up to 37±2^{0}C for 18 to 24 hours, the diameter of zones of inhabitation was measured.

The method of bacteriocin isolation based on using activated carbon. The strain cultivation was carried out in the liquid culture medium MRS. The cultural liquid was concentrated on hollow fibers then, sodium chloride was added and stirred in on a laboratory

rocker. The suspension then was centrifuged, pH of the supernatant was adjusted up to 3.0, and the obtained suspension was centrifuged. Water was added to the precipitate, after suspending the precipitate, ethanol was added, and the suspension was incubated at 0± 2°C for 30 minutes and centrifuged again. After removing ethanol, water and activated carbon were added into the solution and centrifuged in order to remove impurities adsorbed on carbon. Obtained aqueous solution was filtered though a membrane.

The method of bacteriocin isolation based on their precipitation with ammonium sulfate. The strain cultivation was carried out in the liquid culture medium MRS. The culture was centrifuged at 4,200 g for 30 minutes, after which ammonium sulfate was added up to 90 per cent saturation to sediment bacteriocins. After centrifugation at 4,200 g for 40 min, the precipitate was dissolved in 20 mM acetate buffer with pH 5.0, and centrifuged again at 4,200 g for 30 min to separate undissolved precipitate which was washed again in the buffer, and centrifuged. The obtained supernatant was used to determine the antimicrobial activity.

The method of bacteriocin isolation based on using chloroform. The strains were cultivated in the liquid culture medium MRS. Chloroform was added to the supernatant, the obtained suspension was stirred by using a magnetic shaker for 20 minutes and centrifuged at 10,400 g and 12 ± 2°C for 20 min. The top organic layer was carefully poured off and 5–10 ml of 0.1 M Tris-buffer with pH 7.0 was added for resuspending. The contents of the bottles (sediments, the surface layer, remainders of chloroform, and the cultural medium) and the mixture were mixed and centrifuged again at 12,100 g for 15 minutes. Then the precipitate was separated from the remaining chloroform and the medium.

RESULTS AND DISCUSSION

The strain isolation. Having obtained pure cultures, two strains were isolated and their morphological, physiological and biochemical properties were studied.

The first strain was Gram-positive, it had rod-shape cells, flat elevation, filiform edge, rough surface, beige color, and dense, uniform texture.

The second strain was Gram-positive, rod-shaped, scallop-edged, convex, smooth, white-colored, with soft uniform texture. The research of the isolated strains resistance to antibiotics showed that the first strain was less resistant to erythromycin, levofloxacin, ampicillin, cefotaxime, and vancomycin (inhibition zones were more than 25 mm). The strain showed higher resistance against levomycetin, tetracycline, and nitrofurantoin (inhibition zones were up to 20 mm).

The second strain demonstrated less resistance to amoxicillin, erythromycin, levofloxacin, doxycyclin, cefotaxime, and benzylpenicillin (inhibition zones were more than 25 mm). The strain turned out more resistant against levomycetin, ceftriaxone, amikacin, rifampicin, oxacillin, and nitrofurantoin (inhibition zones were up to 20 mm).

Identification of isolated strains. For the first strain, the results of the 16S rRNA gene sequence are given below:

CTAATACATGCAGTCGAGCGGACAGAAGGGAG
CTTGCTCCCGGATGTTAGCGGCGGACGGGTGA
GTAACACGTGGGTAACCTGCCTGTAAGACTGG
GATAACTCCGGGAAACCGGAGCTAATACCGGAT
AGTTCCTTGAACCGCATGGTTCAAGGATGAAA
GACGGTTTCGGCTGTCACTTACAGATGGACCCG
CGGCGCATTAGCTAGTTGGTGGGGTAATGGCTC
ACCAAGGCGACGATGCGTAGCCGACCTGAGAG
GGTGATCGGCCACACTGGGACTGAGACACGGC
CCAGACTCCTACGGGAGGCAGCAGTAGGGAAT
CTTCCGCAATGGACGAAAGTCTGACGGAGCAA
CGCCGCGTGAGTGATGAAGGTTTTCGGATCGTA
AAGCTCTGTTGTTAGGGAAGAACAAGTGCGAG
AGTAACTGCTCGCACCTTGACGGTACCTAACCA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCG
CGGTAATACGTAGGTGGCAAGCGTTGTCCGGAA
TTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCT
TAAGTCTGATGTGAAAGCCCCCGGCTCAACCG
GGGAGGGTCATTGGAAACTGGGAAACTTGAGT
GCAGAAGAGGAGAGTGGAATTCCACGTGTAGC
GGTGAAATGCGTAGAGATGTGGAGGAACACCA
GTGGCGAAGGCGACTCTCTGGTCTGTACTGAC
GCTGAGGAGCGAAAGCGTGGGGAGCGAACAG.

The phylogenic tree (Fig. 1) was constructed to determine homology of the strain.

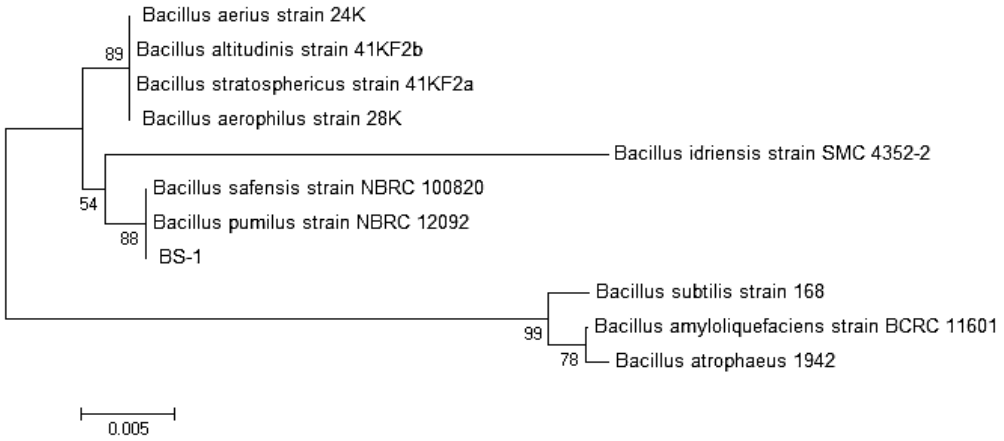

Fig. 1. Phylogenic analysis of the first strain. BS-1 is the notation of the strain.

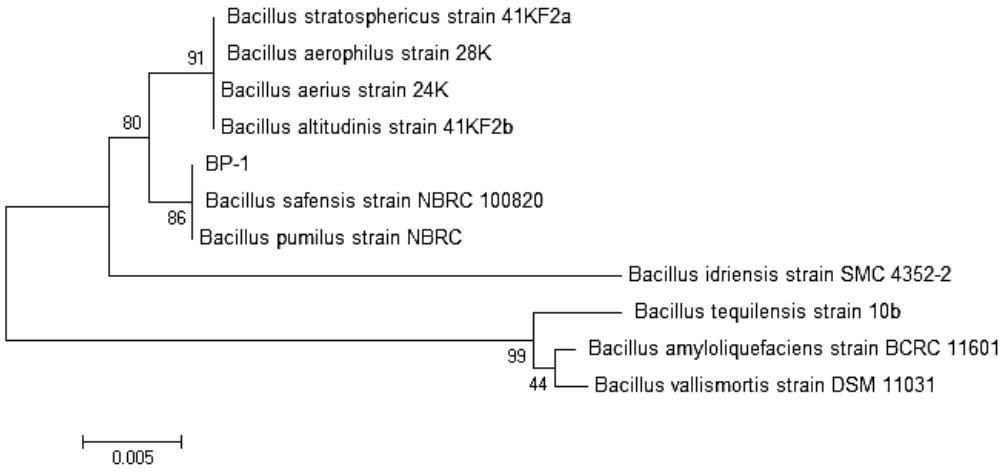

Fig. 2. The phylogenetic tree of the second strain. BP-1 is the notation of the strain.

Using nucleotide sequence of the isolated strain, its homology in relation to other strains of *Bacillus* was studied. The analysis was carried out by using NCBI data (national Center for Biotechnology Information). Ten nucleotide sequences with the highest homology to the studied strain were selected to build the phylogenetic tree for more accurate identification. The strains formed three main groups with the node stability 88, 89, and 99 per cent which indicates sufficiently high homology level of studied strains in formed groups. The strain of *Bacillus idriensis* SMC 4352-2 was isolated. From the results, the isolated strain was found to have the highest homology to *Bacillus safensis* and *Bacillus pumilus* strains. The analysis of morphological and physiological-and-biochemical properties revealed that the isolated strain belongs to *Bacillus safensis* specie.

For the first strain, the results of the 16S rRNA gene sequence are given below.

After the 16S rRNA gene sequencing, the following nucleotide sequence was obtained:

CTAATACATGCAGTCGAGCGGACAGAAGGGAGC
TTGCTCCCGGATGTTAGCGGCGGACGGGTGAGT
AACACGTGGGTAACCTGCCTGTAAGACTGGGAT
AACTCCGGGAAACCGGAGCTAATACCGGATAGT
TCCTTGAACCGCATGGTTCAAGGATGAAAGACG
GTTTCGGCTGTCACTTACAGATGGACCCGCGGC
GCATTAGCTAGTTGGTGGGGTAATGGCTCACCA
AGGCGACGATGCGTAGCCGACCTGAGAGGGTG
ATCGGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCC
GCAATGGACGAAAGTCTGACGGAGCAACGCCG
CGTGAGTGATGAAGGTTTTCGGATCGTAAAGCT
CTGTTGTTAGGGAAGAACAAGTGCGAGAGTAA
CTGCTCGCACCTTGACGGTACCTAACCAGAAAG
CCACGGCTAACTACGTGCCAGCAGCCGCGGTAA
TACGTAGGTGGCAAGCGTTGTCCGGAATTATTG
GGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTC
TGATGTGAAAGCCCCCGGCTCAACCGGGGAGG
TCATTGGAAACTGGGAAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAAT
GCGTAGAGATGTGGAGGAACACCAGTGGCGAA
GGCGACTCTCTGGTCTGTACTGACGCTGAGGAG
CGAAAGCGTGGGGAGCGAACAG.

Homology of the strain was determined by means of phylogenic analysis. The results are in Figure 2.

The analysis of the 16S RNA gene sequence of the isolated strain was performed to determine strains with the highest homology to the second isolated strain. In order to build the phylogenetic tree, strains with homology 97–99 per cent to the studied strain were chosen. The strains formed two main groups with the node stability 80 and 99 per cent. The homology level with bootstrap-value 70–100 per cent is considered to be high. That led to the conclusion that the homology level in the groups was high. The main groups were divided into three subgroups with the node stability 44–91 per cent. Strains of Bacillus idriensis SMC 4352–1 and Bacillus tequilensis BCRC 11601 were separated from the main groups. The phylogenic analysis showed that strains of Bacillus safensis and Bacillus pumilus are closely related to the studied strain. Taking into account the morphological and physiological-and-biochemical properties, the studied strain was designated as Bacillus pumilus.

Determination of the optimum duration of cultivation. The optimum cultivation duration was determined by measuring the antimicrobial activity in different time intervals. The cultivation was carried out at 30 ± 2^0C for 12, 18, and 24 hours (pH 6.5). The antimicrobial activity of bacteriocins against Escherichia coli B-6954 was studied by means of the disk diffusion method. The results are in Fig. 3 and Fig. 4.

It is seen from Fig. 3 that under the cultivation of *Bacillus safensis* strain for 12 hours, inhibition zones values varied from 7 up to 10 mm with a test culture. When cultivating for 18 hours, inhibition zones values were from 9 up to 11 mm, and for 24 hours the values were from 7 up to 10 mm. The strain showed the greatest activity against *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*.

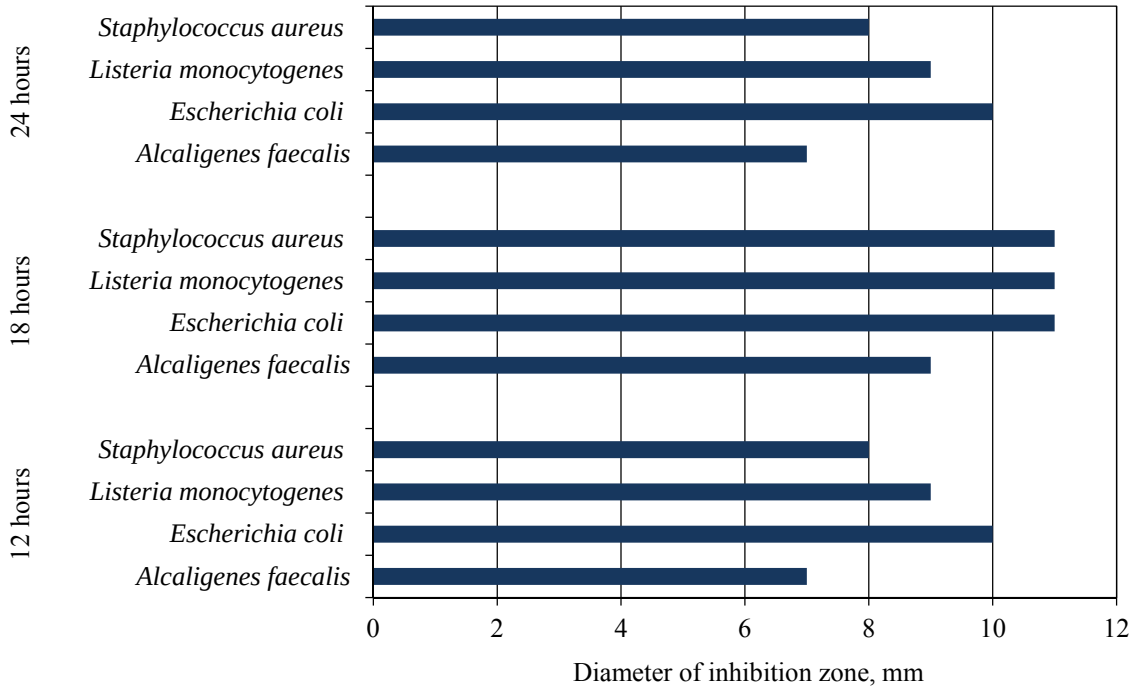

Fig. 3. The effect of the cultivation duration on the antimicrobial activity of *Bacillus safensis*.

Bacillus pumilus strain was cultivated for 12 hours and inhibition of pathogenic strains growth was observed. Inhibition zones were ranged from 7 up to 9 mm. With the increasing of time up to 18 hours, inhibition zones were ranged from 8 up to 10 mm. After cultivation of strain for 24 hours inhibition zones were from 7 up to 9 mm. It was found that *Bacillus pumilus* was more effective against *Staphylococcus aureus* strains.

Determination of the medium composition. Components of culture media and their concentrations that were used for strain cultivation are represented in Table 1.

The bacteriocin synthesis intensity was determined by measuring the concentration of recombinant protein in the medium. The results are given in Fig. 5 and Fig. 6.

It is seen that *Bacillus safensis* and *Bacillus pumilus* strains had the minimum protein concentration on the culture medium No. 1, while the maximum concentration was observed on the medium No. 6.

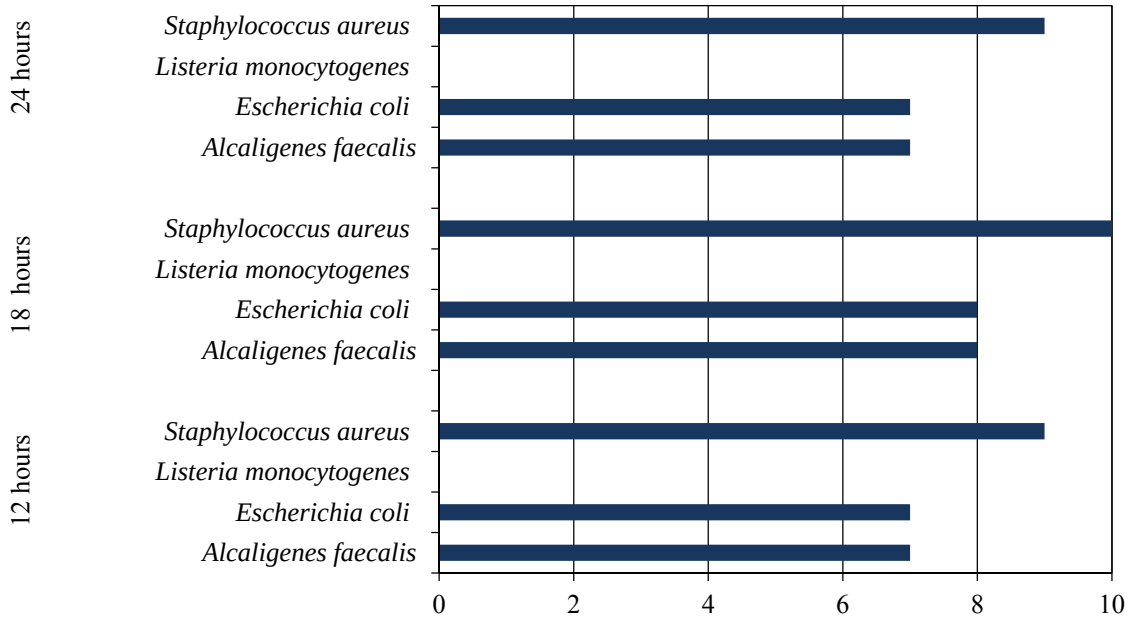

Fig. 4. The effect of the cultivation duration on the antimicrobial activity of *Bacillus pumilus*.

Table 1. Composition of culture media

Component	Mass content, g/dm^3								
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9
Peptone	12	12	12	–	–	–	–	–	–
Tryptone	–	–	–	12	12	12	–	–	–
Yeast extract	–	–	–	–	–	–	12	12	12
Pancreatic hydrolysate	12	12	12	–	–	–	12	12	12
Glucose	–	10	–	–	10	–	–	10	–
Fructose	10	–	–	10	–	–	10	–	–
Sucrose	–	–	12	–	–	12	–	–	12
Sodium acetate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Magnesium sulfate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ammonium sulfate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium chloride	6	6	5	5	5	6	6	6	5
Calcium chloride	2	2	3	3	3	2	2	2	3

Determination of the optimum method of bacteriocin isolation. Method of bacteriocin isolation considerably influences the activity and the number of isolated bacteriocins. Three methods of bacteriocin isolation were used. The first method is based on using activated carbon. The results are given in Table 2.

The results in Table 2 allow us to conclude that bacteriocins being produced by *Bacillus pumilus* strain had the inhibiting capacity against such pathogenic strains as *Leuconostoc mesenteroides, Escherichia coli, Enterobacter ludwigii, Salmonella enteric, Yersinia spp., Staphylococcus aureus,* and *Alcaligenes faecalis.* The diameter of inhibition zones was 9–10 mm.

The *Bacillus safensis* strain showed higher antimicrobial activity to pathogenic strains than *Bacillus pumilus*. Inhibition zones were 14–16 mm in diameter.

The second method of bacteriocin isolation was based on ammonium sulfate salting out with subsequent centrifugation and washing in the acetate buffer. The results of the antimicrobial activity of bacteriocins are represented in Table 3.

According to the results given in Table 3, the strain of *Bacillus safensis* was active against such strains as *Pseudomonas fluorescens, Candida albicans, Arthrobacter cumminsii, Alcaligenes faecalis, Escherichia coli, Enterobacter ludwigii, Erwinia aphidicola, Micrococcus luteus, Salmonella enterica, Listeria monocytogenes,* and *Staphylococcus aureus.* Inhibition zones were 14–18 mm.

The *Bacillus pumilus* strain has inhibited the growth of following test cultures: *Leuconostoc mesenteroides, Enterobacter ludwigii, Salmonella enterica, Yersinia spp., Staphylococcus aureus, Alcaligenes faecalis,* and *Escherichia coli.* The inhibition zone diameters were 10–11 mm.

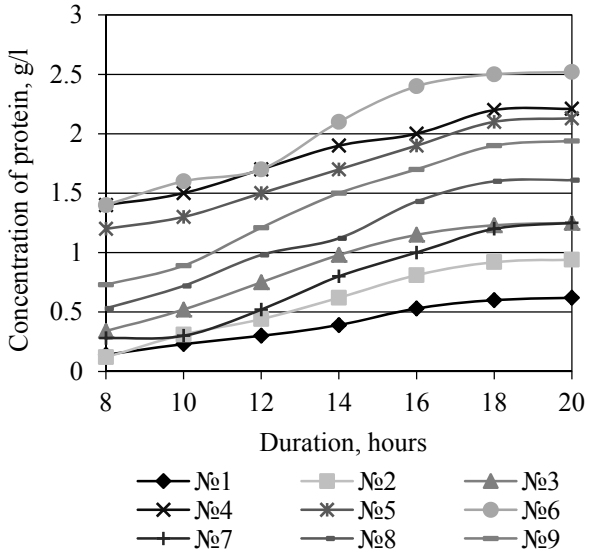

Fig. 5. Bacteriocin production of *Bacillus safensis* strain in a culture medium, depending on its composition.

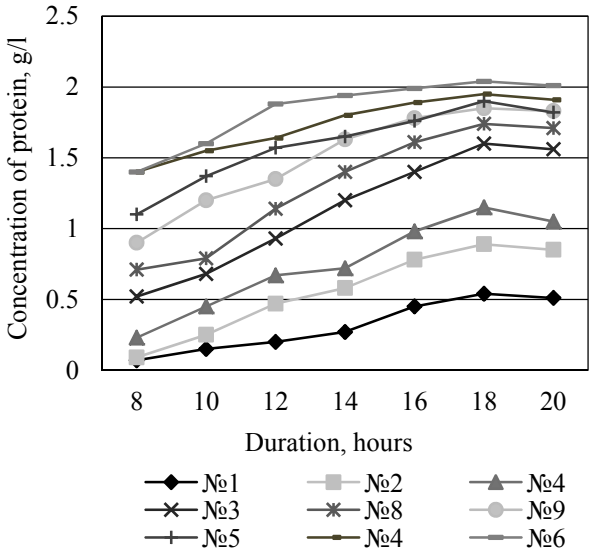

Fig. 6. Bacteriocin production of *Bacillus pumilus* strain in a culture medium, depending on its composition.

Table 2. Antimicrobial activity of bacteriocins isolated by using the first method

Test culture	Studied strain	
	Diameter of inhibition zones	
	Bacillus safensis	*Bacillus pumilus*
Pseudomonas fluorescens	14	0
Pseudomonas aeruginosa	0	0
Candida albicans	15	0
Leuconostoc mesenteroides	0	10
Arthrobacter cumminsii	14	0
Alcaligenes faecalis	15	8
Escherichia coli	16	9
Enterobacter ludwigii	15	9
Erwinia aphidicola	15	0
Micrococcus luteus	16	0
Salmonella enterica	16	9
Listeria monocytogenes	14	0
Yersinia spp.	0	10
Staphylococcus aureus	15	10

Table 3. Antimicrobial activity of bacteriocins isolated by using the second method

Test culture	Studied strain	
	Diameter of inhibition zones	
	Bacillus safensis	*Bacillus pumilus*
Pseudomonas fluorescens	14	0
Pseudomonas aeruginosa	0	0
Candida albicans	17	0
Leuconostoc mesenteroides	0	10
Arthrobacter cumminsii	15	0
Alcaligenes faecalis	16	9
Escherichia coli	18	10
Enterobacter ludwigii	18	11
Erwinia aphidicola	16	0
Micrococcus luteus	18	0
Salmonella enterica	18	10
Listeria monocytogenes	16	0
Yersinia spp.	0	11
Staphylococcus aureus	15	10

Table 4. Antimicrobial activity of bacteriocins isolated by using the third method

Test culture	Studied strain	
	Diameter of inhibition zones	
	Bacillus safensis	*Bacillus pumilus*
Pseudomonas fluorescens	14	0
Pseudomonas aeruginosa	0	0
Candida albicans	15	0
Leuconostoc mesenteroides	0	9
Arthrobacter cumminsii	13	0
Alcaligenes faecalis	0	8
Escherichia coli	15	8
Enterobacter ludwigii	15	0
Erwinia aphidicola	14	0
Micrococcus luteus	15	0
Salmonella enterica	15	8
Listeria monocytogenes	13	0
Yersinia spp.	0	0
Staphylococcus aureus	14	9

The third method allowed us to isolate bacteriocins by adding chloroform, isolating bacteriocins, centrifuging and resuspending in the Tris-buffer. The results are represented in Table 4.

It is seen from Table 4 that the *Bacillus safensis* strain has inhibited the growth of most pathogenic strains such as *Pseudomonas fluorescens, Candida albicans, Arthrobacter cumminsii, Escherichia coli, Enterobacter ludwigii, Erwinia aphidicola, Micrococcus luteus, Salmonella enterica, Listeria monocytogene,* and *Staphylococcus aureus.* The inhibition zones varied between 13 and 15 mm.

The *Bacillus pumilus* strain showed the antimicrobial activity to such pathogenic strains as *Listeria monocytogene, Salmonella enterica, Staphylococcus aureus, Alcaligenes faecalis,* and *Escherichia coli.* The inhibition zone diameters were 8–9 mm.

DISCUSSION

In this study, the isolation of two microbial cultures from the fresh onion surface was carried out and their morphological, genetic, physiological and biochemical properties were studied. The identification of the isolated strains has revealed that the strains belong to the species *Bacillus safensis* and *Bacillus pumilus.*

The optimum cultivation duration of *Bacillus safensis* and *Bacillus pumilus* strains was determined. The antimicrobial activity was found to be maximal after cultivation for 18 hours.

As the less antimicrobial activity was observed after cultivation duration of strains for 12 and 24 hours, 18 hours was considered as the optimum duration of cultivation.

It was found that the optimum culture medium for studied strains was the medium No. 6 where the maximum protein production was observed. The medium contained (in g/dm^3) tryptone 12.0, sucrose 12.0, sodium acetate 0.5, magnesium sulfate 0.2, ammonium sulfate 0.5, sodium chloride 6.0, and calcium chloride 5.0.

It was revealed that the bacteriocins produced by *Bacillus safensis* and *Bacillus pumilus* strains had the greatest antimicrobial activity when they were isolated using the method based on ammonium sulfate precipitation. This method was used for further work with bacteriocins.

The bacteriocins produced by *Bacillus pumilus* showed the antagonistic activity only against several pathogenic microorganisms, while the bacteriocins produced by *Bacillus safensis* were highly effective no matter which method of bacteriocin isolation was used.

Thus, the *Bacillus safensis* strain has been selected for further research, as the bacteriocins of this strain have high antagonistic activity and are one of the most promising components antibiotics creation.

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STANDARDIZATION, CERTIFICATION, QUALITY AND SAFETY

ESTIMATION OF QUALITY AND EFFICIENCY OF APPLICATION OF A POULTRY FEED SUPPLEMENT IN FEEDING HUBBARD BROILER CHICKENS

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Abstract: Poultry farming is one of the largest branches of agriculture with a high level of production of a variety of organic wastes that require special approaches to useful utilization. The article describes the problem of processing evisceration waste and keeping poultry in quality feed supplements and the estimation of their effectiveness in feeding broiler chickens. In accordance with the studies, it has been established that the studied feed supplements, with a share of feather-down waste of more than 80%, differ in a balanced amino acid composition, a high content of protein (89.9–90.2%) and sulfur-containing amino acids, that is cystine and tryptophan that have a high value in the process of fattening the bird. The ratio of 8 : 2 (feather-down waste : litter) is recognized rational and optimal for a further use - the feed supplement produced using this method contains glycine, the most important amino acid for chickens, and is characterized by a high content of calcium and phosphorus due to the components of litter. According to the safety indicators established by the regulatory documents, the feed supplement is recognized high-quality and safe. When estimating the toxicity of the feed supplement using the test organism *Tetrahymena pyriformis*, no adverse effect on the survival, mobility, growth and behavior of the cells was detected. When analyzing the relative biological value of the feed supplement using *Tetrahymena pyriformis*, it has been established that the average number of the cells grown with the use of the feed supplement is $(8.9 \pm 0.9) \times 10^4$ cells, which indicates its high nutritional value. Taking into account the results of the studies on the chemical and amino acid composition of the feed supplement, the data of microbiological and toxicological studies, and taking into account the toxicity estimation, it has been concluded that the studied supplement is safe and can be used in the birds diet.

Keywords: Feed supplement, poultry waste, effective microorganisms, waste bioconversion, microbial disposal, biopreparation, broiler chickens

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INTRODUCTION

Poultry farming is one of the key areas of business activity in the structure of agriculture. This is a science-intensive and dynamically developing industry with an annual world production growth rate of 6–9% [1, 2].

In the Russian Federation, along with the significant successes in poultry farming, there is an annual increase in the volume of waste generation of poultry enterprises that now make up more than 17 million tons per year [3, 4].

Waste of evisceration and poultry keeping is the most promising from the point of view of useful utilization and the creation of non-waste technologies. As for the chemical composition - the content of amino acids, the balance of micro and macro elements – feather-down raw materials, litter and other types of waste are a valuable source of nutrients in the production of feed supplements [5, 6]. Table 1 presents the chemical composition of feather-down raw materials. Table 2 presents the amino acid composition of feather-down raw materials in comparison with a human hair, a horse hair and sheep wool.

Table 1. Chemical composition of feather-down raw materials

Low-value keratin-containing raw materials	Mass fraction of			
	protein, %	mineral salts, %	fat, %	moisture, %
Chicken feather, turkey feather	76.4	1.5	3.3	18.8
Hen underwings	79.2–81.9	2.9	1.7	13.2–16.2
Broiler feather	82.5	2.2	1.8	13.5

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Table 2. Amino acid composition of feather-down raw materials in comparison with a human hair, a horse hair and sheep wool

Amino acids	Human hair	Horse hair	Sheep wool	Hen feather
Alanine	6.88	1.5	4.4	5.21
Valine	–	0.9	2.8	6.02
Leucine	12.12	7.1	11.5	10.83
Asparagine acid	–	0.3	2.3	7.04
Glutamic acid	8.0	3.7	12.9	14.03
Proline	–	3.4	4.4	–
Phenylalanine	0.62	–	–	4.74
Thirosine	3.3	3.2	2.9	5.06
Serine	–	0.6	0.9	5.28
Cystine	11.55	–	7.3	5.28

The existing technologies for processing poultry waste are based on the processes in which the bioavailability of useful components and their decontamination is achieved mainly through a high-temperature hydrothermal treatment or using acidic (alkaline) hydrolysis. Such methods often lead to the loss and racemization of essential amino acids, the formation of cyclopeptides and a significant decrease in the total biological value of the final feed products [7].

High-temperature drying or incineration is used in the technologies for useful litter disposal, which leads to a loss of more than 40% of nutrients. Sometimes formaldehyde is used to disinfect litter, but it also significantly reduces the nutritional and protein value of the feed produced [8, 9].

The analysis of the technologies used shows that the processing industry have some tasks of developing new modern environmentally friendly technologies for recycling waste from poultry enterprises, which allows, at high economic efficiency values, to produce feed, feed supplements and other technical products with improved characteristics.

A principally new approach of this trend is the creation of the bioconversion technologies based on the use of effective systems of destroying microorganisms [10, 11]. Such microorganisms are able to synthesize enzymes and enzyme complexes into the environment and provide the process of conversion of complex organic compounds that are part of waste into available forms. A unique feature of such strains is the ability to suppress the growth and development of pathogenic microflora of waste [11].

Thus, the study aimed at improving the biotechnological methods for recycling poultry wastes and developing a technology for producing a feed supplement from them is a relevant task, the solution of which is directed both at deep processing and the complete neutralization of waste.

STUDY OBJECTS AND METHODS

The object of the studies was a feed supplement from a mixture of feather-down raw materials and litter from Lohmann Brown chickens.

The paper aimed at estimating the quality and effectiveness of the developed feed supplement based on a mixture of poultry waste (chicken feather : litter) in feeding Hubbard broiler chickens.

Study of the feed supplement indicators. The process of utilization of mixtures of feather-down waste and litter was conducted under laboratory conditions as follows: the ground waste mixture was fed to a BioStat A plus bioreactor, 5 dm^3 in volume, and treated with an active culture of destroying microorganisms: *Bacillus pumilus SAFR-032*, *Microbacterium terregens AC1180, Bacillus fastidiosus B11090, Arthrobacter globiformis AC1529*, *Streptomyces olivocinereus AC1169, Acinetobacter sp. B3905*, which were in a nutrient medium (in the ratio of nutrient medium: microorganisms – 15 : 1). The bioconversion process begins immediately after the application of microorganisms and lasts for 72 hours. Upon completion, the microorganisms must be inactivated. The parameters of inactivation have been selected taking into account the cessation of the effect of extracellular enzymes of microorganisms on the substrate and the termination of the processes of division of the microorganisms themselves. After inactivation, the resulting enzymatic hydrolysis product is filtered using a filter with a mesh size from 1 to 0.5 mm to separate the coarse fraction.

The drying stage is feeding the spray dryer, the excess moisture is removed and the resulting feed supplement takes on a powdery form. The following stages include prepackaging and packaging the supplement.

For comparison, the samples of unprocessed waste mixtures are taken as the control samples:

– the chemical composition of the feed supplement was studied in accordance with GOST 17681-82 "Flour of animal origin. Test methods";

– the amino acid composition of the feed supplement was determined according to the requirements presented in GOST 32195–2013 "Feeds, compound feeds. Method for determination of amino acids" and GOST 32201–2013 "Feeds, compound feeds. Method for determination of tryptophan";

– the acid number was determined according to the requirements presented in accordance with GOST 13496.18-85 " Mixed fodder and fodder raw stuff. Methods for determination of fat acid value";

– the peroxide number was determined in accordance with the requirements presented in GOST 31485–2012 "Mixed feeds, protein-vitamin-mineral concentrates. Method for determination of peroxide number";

– the content of toxic elements was studied in accordance with GOST 30692–2000 "Fodders, mixed fodders and animal raw foodstuff. Atomic absorption method for determination of copper, lead, zinc and cadmium";

– the silver content was determined in accordance with GOST 18293–72 "Drinking water. Methods for determination of lead, zinc and silver content";

– the content of arsenic and mercury was determined according to the requirements of MBI FR.1.31.2002.00593 "The procedure for performing measurements of the mass concentration of arsenic

and mercury ions in feeds, feedstuff products and feed additives by the method of inversion voltammetry";
– the presence of pesticides was studied according to the "Guidelines for determination of organochlorine pesticides in water, food, feed and tobacco products by chromatography in a thin layer" (approved on 28.01.1980, No. 2142–80);
– to determine the content of radionuclides, a method based on the activity of gamma-emitting radionuclides in the sample using an SRP-68-01 radiometrometer was used. The studied sample of the feed supplement in a flat-bottomed chemical beaker was placed at the bottom of the well. A radiometer detector was placed in the sample of the supplement, which was covered with a plastic bag, so as to provide a position of the lower end of the device at a level of 2–3 cm from the bottom of the beaker.
– the microbiological studies were conducted in accordance with GOST 25311-82 "Feeding flour of animal origin. Methods of bacteriological analysis"; The selection for carrying out microbiological studies was carried out in accordance with the requirements of GOST 17536-82 "Feeding flour of animal origin. Specifications";
– the toxicity of the feed supplement was determined according to the "Methodological guidelines for the accelerated determination of toxicity of livestock products and 56 feeds" (approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 16.10.2000, No. 13-7-2/2156);
– the relative biological value (RBV) was determined according to the "Methodological recommendations for the use of the rapid method of biological assessment of products and feeds" (approved by the All-Union Academy of Agricultural Sciences named after Lenin, 1990);

Estimation of the effectiveness of the use of a feed supplement in feeding broiler chickens. In the course of the studies of zootechnical indices of the developed feed supplement for Hubbard broiler chickens, a supplement was added to the mixed feeds for the chickens of the control and experimental group (the number of birds is 45). The groups have been formed from day-old broiler chickens using a method of analogues. The stocking density, the light and temperature conditions are in accordance with the existing recommendations.

The groups were fed with crumbled mixed feeds with the application of a feed supplement in the premixes, in full. The same complete mixed feed was given to the chickens of all the groups (the control and the experimental one). The chickens of the experimental group consumed mixed feed with the addition of 100 g/100 kg of feed; the first group - 50 g/100 kg of feed. The chickens of the second group received dry crumbled mixed feed without a feed supplement (the negative control sample).

The following indicators were taken into account: feed consumption (daily accounting of feed residues); poultry livability (daily accounting of the dead bird); an increase in the live weight of the bird (alternate weighing using electronic scales); the daily average gain of live weight was calculated based on the results of the control weights; the conversion of feed was calculated as the feed costs required to increase 1 kg of weight gain over the growing period;
– the protein content was determined according to the requirements of GOST 25011-81 "Meat and meat products. Methods of protein determination";
– the moisture content was determined in accordance with GOST R 51479-99 "Meat and meat products. Methods for determination of moisture content";
– the fat content was determined according to GOST 23042-86 "Meat and meat products. Methods of fat determination";
– the ash content was determined in accordance with GOST 31727-2012 "Meat and meat products. Determination of total ash";
– the amino acid content was determined using an Aracus amino acid analyzer (PMAGmbH, Germany) according to standard procedures. The operation principle of the analyzer is based on the separation of a mixture of amino acids followed by postcolumn derivatization with ninhydrin.
– the content of oxyproline was determined according to GOST 23041-78 "Meat and meat products. Method for determination of oxyproline";
– the content of fat-soluble vitamins A and E was determined according to the regulations of GOST 32307-2013 "Meat and meat products. Determination of fat-soluble vitamins by high performance liquid chromatography";
– the content of water-soluble vitamins - in accordance with GOST R 55482-2013 "Meat and meat products. Method for determination of water-soluble vitamins";
– the content of minerals in meat was determined in accordance with the requirements of GOST R 55573-2013 "Meat and meat products. Determination of calcium by atomic absorption and titrimetric methods";
– the content of phosphorus was determined in accordance with GOST R 51482–99 "Meat and meat products. Spectrophotometric method for determination of total phosphorus content";
– the iron content was determined according to GOST 26928-86 "Food-stuffs. Method for determination of iron";
– the content of manganese was determined in accordance with GOST 55484-2013 "Meat and meat products. Determination of sodium, potassium, magnesium and manganese by flame atomic absorption";
– the silver content was determined in accordance with GOST 18293-72 "Drinking water. Methods for determination of lead, zinc and silver content";
– the concentration of hydrogen ions (pH) was determined according to the requirements of GOST R 51478–99 "Meat and meat products. Reference method for measurement of pH";
– the content of toxic elements such as lead and cadmium was determined in accordance with GOST 30178-96 "Raw materials and food-stuffs. Atomic absorption method for determination of toxic elements". Arsenic - according to GOST 26930-86 "Raw material and food-stuff. Method for determination of arsenic

content". The mercury content was determined according to GOST 26927–nation of mercury";
– the safety of meat of broiler chickens was determined in accordance with the requirements of the "Guidelines for the accelerated determination of the toxicity of animal and forage products" (approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 16.10.2000, No. 13-7-2/2156);
– the relative biological value (RBV) was determined according to the "Methodological recommendations for the use of the rapid method of biological assessment of products and feeds" (approved by the All-Union Academy of Agricultural Sciences named after Lenin, 1990);

All the studies were carried out in three replicates. The reliability of the values was determined using the Student's t-test.

RESULTS AND DISCUSSION

The quality of the feed supplement produced and the optimum ratio of substrate components (feather-down waste : litter) was estimated using the chemical and amino acid composition and biological value.

The safety of the feed supplement was estimated using the microbiological indicators, toxicity, pesticide and radionuclide content, acid and peroxide number.

Tables 3 and 4 present the results of the study of the chemical and amino acid composition of the feed supplement.

Table 4. Amino acid composition of the feed supplement*

1	Calcium, g/kg	0.2	0.5	1.3	1.8	1.7
2	Arginine	4.4	4.7	5.3	5.5	5.4
3	Valine	4.6	4.9	5.9	6.1	6.2
4	Histidine	0.3	0.4	0.5	0.7	0.8
5	Glycine	3.3	4.9	4.8	5.4	5.2
6	Isoleucine	3.2	5.8	5.2	5.5	5.4
7	Leucine	4.0	7.1	7.2	7.8	7.9
8	Lysine	0.3	1.3	1.9	2.2	2.1
9	Methionine	0.1	0.2	0.2	0.4	0.6
10	Threonine	2.1	3.8	3.9	4.9	4.2
11	Tryptophane	0.2	0.6	0.5	0.7	0.6
12	Phenylalanine	2.7	4.7	4.4	4.8	4.6
13	Cystine	1.1	2.1	2.2	2.6	2.6
14	Thirosine	1.0	1.4	1.5	2.1	2.0

Note. *the average value for three observations, $P < 0.05$.

The study used the ratios of the substrates of feather-down waste: litter of 0 : 1; 2 : 8; 5 : 5; 8 : 2 and 1 : 0 respectively.

Analyzing the data presented in Tables 3 and 4, it can be concluded that the studied feed supplements, with a share of feather-down waste of more than 80%, differ in a balanced amino acid composition, a high content of protein (89.9–90.2%) and sulfur-containing amino acids, that is cystine and tryptophan that have a high value in the process of fattening the bird. The highest content of these amino acids was noted in the cases of the experiment using the waste ratios in the shares of 8 : 2 and 1 : 0.

The ratio of 8 : 2 is recognized rational and optimal for a further use as the feed supplement produced using this method contains glycine, the most important amino acid for chickens, and is characterized by a high content of calcium and phosphorus due to the components of litter. Table 5 presents the results of the study of the safety indices of the feed supplement.

It can be seen from Table 5 that all the obtained indicators to determine do not exceed the established standards, which indicates the high quality and safety of the raw materials studied.

When estimating the toxicity of the feed supplement using the test organism *Tetrahymena pyriformis*, no adverse effect on the survival, mobility, growth and behavior of the cells was detected. The morphological indicators were also normal, which indicates the absence of toxicity.

When analyzing the relative biological value of the feed supplement using *Tetrahymena pyriformis*, it has been established that the average number of the cells of *Tetrahymena pyriformis* grown with the use of the feed supplement is $(8.9 \pm 0.9) \times 10^4$, which indicates its high nutritional value.

Taking into account the results of the studies on the chemical and amino acid composition of the feed supplement, the data of microbiological and toxicological studies, and taking into account the toxicity estimation, it can be concluded that the studied supplement is safe and can be used in the birds diet.

The genetic features, sex, age and physiological condition of poultry, keeping conditions, as well as taste and the structure of feeds and feed supplements determine the level of consumption for poultry.

When estimating the effectiveness of the feed supplement, an additive was applied to mixed feeds and feed mixtures at a dosage of 100 g/100 kg of feed for Hubbard chickens.

Table 5 presents the safety indices of the feed supplement.

Table 3. Chemical composition of the feed supplement*

Subitem No.	Designated parameter	Ratio of substrates (feather-down waste : litter)				
		0 : 1	2 : 8	5 : 5	8 : 2	1 : 0
1	Mass fraction of moisture, %	6.8	6.6	5.9	5.4	5.4
2	Mass fraction of fat, %	0.4	0.5	0.6	0.9	1.0
3	Mass fraction of protein, %	65.7	70.7	75.3	89.9	90.2
4	Mass fraction of ash, %	5.8	4.3	3.8	3.4	3.3
5	Mass fraction of phosphorus, %	0.1	0.1	0.1	0.3	0.2

Note. *the average value for three observations, $P < 0.05$.

2 **Table 5.** Safety indices of the feed supplement*

Subitem No.	Designated parameter	Result	MAL (maximum allowable limit)
Toxic elements, mg/kg, no more than			
1	Lead	0.17	5.0
2	Cadmium	0.014	1.0
3	Mercury	0.01	0.2
4	Arsenic	0.01	4.0
5	Argentum	0.8	50.0
Pesticides, mg/kg, no more than			
1	HCH (total of α, β, γ - isomers)	not detected	0.1
2	Dichlorodiphenyltrichloroethane and its metabolites	not detected	0.1
Radionuclides, Bq/kg, no more than			
1	Caesium -137	12	600
2	Strontium - 90	8	200
Microbiological indicators			
1	QMAFAnM, CFU/g, no more than	1.5×10^5	5×10^5
2	Salmonella, in 25 grams	not detected	not permitted
3	Coliforms, per 1 g	not detected	not permitted
4	Anaerobia in 50 g	not detected	not permitted
Physico-chemical parameters			
1	Acid number, mg KOH/g	0.5	20
2	Peroxide number, moles of active oxygen/kg	2.9	23.6

Note. *the average value for three observations, $P < 0.05$

Table 6. Feed consumption by broiler chickens

Period, days	Experimental group		Control 1		Control 2	
	Fact, g/day	For the period, g	Fact, g/day	For the period,g	Fact, g/day	For the period, g
Starting period (0–10)	28.7	287.0	27.9	279.0	28.8	288.0
Growing period (11–25)	88.4	1326.0	86.5	1297.5	87.5	1312.5
Finishing period (26–41)	170.8	2732.8	165.5	2648.0	166.5	2664.0
Total, g	–	4351.8	–	3947.7	–	3979.4

When estimating the effectiveness of the feed supplement, the supplied was added to the mixed feed of Hubbard chickens.

Table 6 presents the studies of consumption of the feed supplement by broiler chickens.

According to the data presented in Table 6, it can be concluded that the feed consumption at the first stage is at the same level in all the groups. This is explained by the identity of mixed feed during the starting period. At the second stage, the difference was 29 g for the first control sample and 14 g for the second. During the finishing period, the difference increased to 84.8 and 68.8 g respectively. Thus, we can talk about an increase in feed consumption by broiler chickens when the developed supplement is included in mixed feeds.

The livability of the bird is one of the most important indicators, which is characterized by the ratio of the final number of birds to the initial. Table 7 provides the results of the analysis of poultry livability.

Analyzing the data it can be said that the livability of broiler chickens was high and amounted to 100% for the experimental sample, and this indicator was 97.7% at the end of the finishing period for the control ones. However, the analysis of cases of a decrease in livability showed that it was not due to the effect of diets in the control groups. The use of the feed supplement does not adversely affect the livability of broiler chickens.

Table 8 presents the results of determining the live weight of broiler chickens.

Table 7. Livability of broiler chickens

Group	Number of birds at the beginning of the experiment	Starting period (0–10)		Growing period (11–25)		Finishing period (26–41)	
		Number of birds	%	Number of birds	%	Number of birds	%
Experimental group	45	45	100	45	100	45	100
Control 1	45	44	97.7	44	100	43	97.7
Control 2	45	45	100	45	100	44	97.7

Table 8. Live weight of broiler chickens

Period, days	Experimental group		Control 1		Control 2	
	Live weight, g	Average daily weight gain, g	Live weight, g	Average daily weight gain, g	Live weight, g	Average daily weight gain, g
11	290 ± 5.0	25.0	285 ± 4.3	24.7	289 ± 4.9	24.9
26	1230 ± 20.0	61.2	1190 ± 15.0	56.9	1213 ± 17.1	59.5
42	2600 ± 35.5	87.9	2570 ± 32.5	85.6	2597 ± 31.0	87.3

Table 9. Costs of mixed feed per unit of output

Parameters	Groups		
	Experimental	Control 1	Control 2
Total consumption, kg	220.5	208.0	217.5
Gain per group, kg	130.9	122.0	127.3
Feed consumption per kg of gain, kg	1.68	1.70	1.71

It follows from the data presented in Table 6 that starting from the second period, the growing one, there were deviations in the three groups. In the experimental group, the live weight was 1230 g, which is almost 3% higher than that in the control groups. In the finishing period, the difference will increase to 4.5%.

Thus, the use of feed supplements has a beneficial effect on the live weight of broiler chickens and contributes to its increase.

Feed conversion is an important indicator of its quality and nutrient utilization efficiency. This is a coefficient that shows the ratio of the amount of feed expended to the unit of output.

Table 9 presents the costs of mixed feed per unit of output.

Based on the obtained data, it was found that the feed conversion rate in the experimental group was 1.66%, which is lower than the values for the control groups. The feed costs in the experimental group correspond to the zootechnical norms. The best digestibility of nutrients contributed to an increase in the live weight of broiler chickens.

The indicators of meat of broiler chickens were estimated by such parameters as the chemical and amino acid composition, the content of minerals and vitamins and a biological value.

Table 10 presents the chemical composition of meat of broiler chickens, in particular the pectoral and femoral muscles.

The analysis of the chemical composition of the muscular tissue of broiler chickens has shown that the addition of mixed feed with a developed feed supplement to the diet has a positive effect on the composition of the muscle tissue - a high protein content, a decrease in the percentage of fat, and hence the improved quality of meat.

It should be noted that the total protein and fat content in meat is not sufficient for a full description of its nutritional value. Together with the essential amino acids that are part of complete proteins, there are incomplete proteins in the meat of broiler chickens. Thus, for the most complete estimation of nutritional value, the amino acid composition of the pectoral muscles was analyzed, the results of the analysis are presented in Table 11.

Table 10. Chemical composition of meat of broiler chickens*

Designated parameter	Groups		
	Experimental	Control 1	Control 2
Pectoral muscles			
Water	73.5	74.02	74.34
Solids	26.8	26.09	25.3
Protein	21.01	20.65	19.8
Fat	2.55	2.56	2.58
Ash	1.15	1.10	1.09
Nutritional value, kcal	117.89	115.83	114.92
Femoral muscles			
Water	72.45	73.09	72.98
Solids	27.67	27.09	26.87
Protein	17.65	17.12	16.74
Fat	9.02	8.94	8.54
Ash	1.13	1.12	1.12
Nutritional value, kcal	155.55	149.03	149.34

Note. *the average value for three observations, $P < 0.05$.

Table 11. *Beginning.* Amino acid content in the meat of broiler chickens

Amino acid	Groups		
	Experimental	Control 1	Control 2
Pectoral muscles			
Lysine	1.834 ± 0.001	1.820 ± 0.001	1.790 ± 0.004
Methionine	0.567 ± 0.003	0.528 ± 0.003	0.463 ± 0.003
Tryptophane	1.213 ± 0.001	1.214 ± 0.002	1.209 ± 0.001
Leucine	1.556 ± 0.003	1.534 ± 0.005	1.542 ± 0.004
Isoleucine	1.009 ± 0.006	1.001 ± 0.004	1.002 ± 0.006
Threonine	0.899 ± 0.005	0.846 ± 0.005	0.853 ± 0.003
Valine	0.947 ± 0.002	0.933 ± 0.004	0.949 ± 0.004
Histidine	0.618 ± 0.002	0.578 ± 0.001	0.564 ± 0.001
Arginine	1.401 ± 0.001	1.376 ± 0.003	1.381 ± 0.003
Phenylalanine	0.817 ± 0.004	0.805 ± 0.004	0.806 ± 0.001
Total of essential amino acids	10.989 ± 0.004	10.827 ± 0.004	10.745 ± 0.004
Alanine	1.390 ± 0.002	1.385 ± 0.004	1.367 ± 0.004
Glycine	1.439 ± 0.001	1.421 ± 0.001	1.428 ± 0.001
Cystine	0.210 ± 0.005	0.205 ± 0.006	0.194 ± 0.007
Glutamic acid	3.480 ± 0.003	3.473 ± 0.002	3.476 ± 0.002
Asparagine acid	0.218 ± 0.001	0.176 ± 0.002	0.200 ± 0.001
Proline	1.031 ± 0.002	1.026 ± 0.002	1.034 ± 0.004
Serine	0.884 ± 0.007	0.867 ± 0.006	0.871 ± 0.006
Oxyproline	0.249 ± 0.008	0.228 ± 0.005	0.236 ± 0.013
Total of nonessential amino acids	8.896 ± 0.005	8.856 ± 0.004	8.869 ± 0.005
Total	19.885 ± 0.005	19.683 ± 0.003	19.614 ± 0.002

Table 11. *Ediing.* Amino acid content in the meat of broiler chickens

Amino acid	Groups	Amino acid	Groups
	Experimental		Experimental
Femoral muscles			
Lysine	1.414 ± 0.001	1.400 ± 0.001	1.391 ± 0.004
Methionine	0.447 ± 0.001	0.421 ± 0.001	0.393 ± 0.001
Tryptophane	1.202 ± 0.001	1.189 ± 0.002	1.191 ± 0.001
Leucine	0.956 ± 0.001	0.894 ± 0.003	0.942 ± 0.004
Isoleucine	0.799 ± 0.003	0.781 ± 0.003	0.724 ± 0.005
Threonine	0.779 ± 0.005	0.736 ± 0.005	0.743 ± 0.003
Valine	0.835 ± 0.002	0.823 ± 0.004	0.802 ± 0.003
Histidine	0.523 ± 0.002	0.508 ± 0.001	0.504 ± 0.001
Arginine	1.675 ± 0.001	1.666 ± 0.003	1.590 ± 0.003
Phenylalanine	0.890 ± 0.005	0.815 ± 0.005	0.845 ± 0.003
Total of essential amino acids	9.456 ± 0.004	9.365 ± 0.003	9.412 ± 0.003
Alanine	1.380 ± 0.002	1.364 ± 0.004	1.343 ± 0.004
Glycine	1.215 ± 0.001	1.209 ± 0.001	1.234 ± 0.001
Cystine	0.169 ± 0.004	0.156 ± 0.004	0.144 ± 0.004
Glutamic acid	0.280 ± 0.002	0.243 ± 0.001	0.286 ± 0.001
Asparagine acid	0.183 ± 0.001	0.171 ± 0.002	0.154 ± 0.001
Proline	0.926 ± 0.001	0.902 ± 0.002	0.925 ± 0.004
Serine	0.743 ± 0.005	0.734 ± 0.005	0.671 ± 0.004
Oxyproline	0.367 ± 0.003	0.333 ± 0.003	0.341 ± 0.014
Total of nonessential amino acids	5.302 ± 0.002	5.235 ± 0.002	5.245 ± 0.002
Total	14.758 ± 0.005	14.600 ± 0.003	14.657 ± 0.002

Note. *the average value for three observations, $P < 0.05$.

When analyzing the composition of amino acids of broiler chickens meat, it was found that there are more amino acids in the pectoral muscles than in the femoral muscles. It was noted that the difference in total of all amino acids in the pectoral muscles in the experimental group was 0.202% and 0.271% in comparison with the control groups. The highest amino acid content was noted in the femoral muscle protein of the experimental group, it exceeded the value of the first control group by 0.158% and the second group by 0.101%. Such an excess was mainly due to the essential amino acids of femoral muscles.

In the human body, vitamins are not synthesized and must be supplied with food. Table 12 presents the content of vitamins in meat.

In accordance with the analysis of the data on the determination of the content of vitamins in the meat of broiler chickens, it has been established that the maximum amount of vitamins was in the meat of the experimental group that consumed mixed feeds with a feed supplement. The content of vitamin A and B12 in the pectoral muscles exceeded their content in the femoral muscles. The femoral muscles had a high content of vitamin B1.

Another important indicator of meat quality is the content of minerals. Along with proteins, fats and carbohydrates, they are a vital component of human nutrition (Table 13).

The data presented in Table 13 show that the accumulation of micro elements was the most complete in the femoral muscles. In general, a higher accumulation of microelements is noted in the experimental group.

The accumulation of calcium is on average higher by 20% in the experimental group than that in the control groups. The difference in phosphorus accumulation was more than 5% in the pectoral and more than 10% in the femoral muscles. The accumulation of iron and manganese have not shown a significant difference between the groups.

Table 12. Content of vitamins in the meat of broiler chickens

Vitamins	Groups		
	Experimental	Control 1	Control 2
Pectoral muscles, mg/100 g			
A(retinol)	0.40 ± 0.009	0.38 ± 0.011	0.34 ± 0.012
E(tocopherol)	0.17 ± 0.005	0.13 ± 0.005	0.15 ± 0.007
B1(thiamine)	0.071 ± 0.001	0.062 ± 0.0001	0.049 ± 0.001
B2(riboflavin)	0.18 ± 0.002	0.18 ± 0.002	0.15 ± 0.002
B3(pantothenic acid)	0.47 ± 0.001	0.36 ± 0.002	0.33 ± 0.001
B6(pyridoxin)	0.37 ± 0.007	0.33 ± 0.002	0.36 ± 0.002
B12(cyancobalamin)	0.41 ± 0.001	0.40 ± 0.001	0.39 ± 0.001
Femoral muscles, mg/100 g			
A(retinol)	0.33 ± 0.005	0.32 ± 0.001	0.29 ± 0.004
E(tocopherol)	0.014 ± 0.004	0.14 ± 0.009	0.011 ± 0.01
B1(thiamine)	0.087 ± 0.001	0.071 ± 0.002	0.079 ± 0.002
B2(riboflavin)	0.15 ± 0.002	0.12 ± 0.001	0.14 ± 0.003
B3(pantothenic acid)	0.54 ± 0.002	0.51 ± 0.005	0.50 ± 0.001
B6(pyridoxin)	0.32 ± 0.003	0.31 ± 0.002	0.31 ± 0.001
B12(cyancobalamin)	0.30 ± 0.004	0.30 ± 0.002	0.27 ± 0.001

Note. *the average value for three observations, $P < 0.05$.

Table 13. Content of minerals in the meat of broiler chickens*

Mineral substances	Groups		
	Experimental	Control 1	Control 2
Pectoral muscles, g/kg			
Calcium	0.57 ± 0.22	0.46 ± 0.28	0.29 ± 0.13
Phosphorus	4.23 ± 0.13	4.03 ± 0.16	3.89 ± 0.21
Iron	7.54 ± 0.31	7.51 ± 0.22	6.94 ± 0.24
Manganese	0.23 ± 0.091	0.13 ± 0.02	0.21 ± 0.032
Femoral muscles, g/kg			
Calcium	0.66+ ± 0.35	0.53 ± 0.31	0.45 ± 0.25
Phosphorus	5.08 ± 0.13	4.99 ± 0.16	4.56 ± 0.16
Iron	8.5 ± 0.31	8.40 ± 0.19	8.01 ± 0.24
Manganese	0.22 ± 0.011	0.20 ± 0.015	0.22 ± 0.015

Note. *the average value for three observations, $P < 0.05$.

Analyzing the data obtained during the study of the chemical composition of broiler chicken meat, it can be concluded that the use of mixed feeds with the addition of the developed feed supplement had a positive effect on the chemical and amino acid composition of the chicken meat of the experimental group, and also contributed to an increase in its food and energy values.

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PROVISION OF MICROBIOLOGICAL SAFETY OF OAT SEED GERMINATION

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Abstract: This paper describes methods to ensure microbiological safety of sprout seeds with no loss of functional and organoleptic properties, since germination of cereal crop seeds promotes intensive growth of microorganisms. The impact of the short-wave ultraviolet irradiation for 1.0, 1.5, 2.0, 2.5 hours, the ultrasound for 5, 10, 15, 20 minutes, the antimicrobial drug solution of 3, 5, 10% in concentration for 2 and 4 minutes, solution of potassium permanganate of 0.005, 0.01, 0.05% in concentration for 2 and 4 minutes, succinic acid solution of 5% in concentration and chitosan solution of 0.1 and 0.5% in concentration in succinic acid for 20, 40, 60 minutes was identified on such microbiological properties of the germinated oat seeds, as QMAFAnM, Coliform bacteria, the amount of yeast and molds. The study results revealed that sprout oat seeds treated with ultraviolet, ultrasound, solution of antimicrobial drug of 3% and 5% concentration, succinic acid solution is not effective enough. Upon the organoleptic analysis of the resulting product, it is noted that organoleptic properties of the test product change when sprout seeds are treated with antimicrobial solution of more than 5% in concentration and with the potassium permanganate solution of more than 0.01% in concentration. The microbiological safety of sprout oat seeds is ensured without their organoleptic property changed when treated with 0.005% solution of potassium permanganate for four minutes or 0.1–0.5% solution of chitosan in succinic acid for 60 minutes. The sprout seeds thus treated may be used as the functional additive to food products.

Keywords: Sprout seeds, oat, microbiological safety, UV irradiation, ultrasound, antimicrobial agent, potassium permanganate, succinic acid, chitosan

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INTRODUCTION

The dieting pattern of the Russian population is known for inadequate consumption of biologically valuable food. Under these conditions, it is more practicable to focus on food production rich in biologically active substances, functional ingredients that beneficially impact the health and metabolism of human body [1].

Currently, more attention is paid to the use of sprout seeds of cereals and legumes as such functional ingredients. It is advisable to introduce sprout seeds to mass consumption food products.

The oat is ranked special among cereals due to the higher protein content in seeds (9.0–19.5%), and its amino acid balance that is quite successful in terms of biological value. Its composition fully complies with the standard for proteins as specified by the Food and Agricultural Organization - the UN Food and Agriculture Organization [2]. In addition, unlike wheat, rye, barley and a range of other crops, the oat does not contain gluten. Absence of allergenic properties in oat products helps to expand the range of products that do not have contraindications in case of gluten-free diet [3].

Sprouting is used to increase the biological value of seeds. As compared with the original, the sprout grain contains considerably more highly digestible vitamins and minerals and, therefore, it has a high biological value [1, 4, 5]. The content of vitamins, macro- and micro-elements such as B1, B5, B6, B9, E, silicon in sprout oat, unlike non-sprout oat, increases by 1.5 times; the content of calcium, sodium, copper, iron, zinc and tin increases twice as much; the content of vitamins B2 – 5.5 times; and the content of Vitamin C – 15.7 times [6, 7]. When germinated, the concentration of natural antibiotics and growth stimulators sharply increase in the seed [8].

In addition, when seeds are germinated, proteins decompose to amino acids due to an increase in the protease enzyme activity. The part of amino acids is digested, and the retaining volume is further decomposed to nucleotides. Thus, the protein in the germinated seed becomes easily digestible where the content of essential amino acids increases [7, 9]. During germination, the total amino acid analysis showed an increase in essential amino acids, such as lysine and tryptophan, which is responsible for the biological value promotion of germinated oat seeds [10].

When germinated, the content of antioxidants sharply increases in seeds. It is identified that the total content of water-soluble antioxidants in initial (dry) oat seeds is 34 mg per 100 g, and in sprouts - 65 and 334 mg per 100 g, respectively, on the 2nd and 5th days upon germination [11].

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Thanks to a wide range of useful properties, germinated seeds of grain and legumen crops gain more popularity among many nations. Germinated seeds of various crops, food and dishes with seed sprouts, as well as seeds and devices for self-germination are on sale.

In November 2014, the US Centers for Disease Control and Prevention reported on hospitalization of 63 people from 10 states for food poisoning due to salmonella. According to the facility, those people got poisoned with dangerous bacteria as they consumed sprout beans by the manufacturer based in New York. The US Centers for Disease Control and Prevention noted that since 1990, sprout beans have been reported to be the cause of public poisoning in 30 cases. The reason is that this popular national product is grown under conditions favorable for harmful microorganism development: namely, in warm and humid environment [https://russian.rt.com/article/61123].

The water content in grain crops is relatively low (about 14%) preventing from the growth of microorganisms though they are found on grains in large quantities. The bulk of microorganisms appear on the grain during harvesting along with dust and soil particles [12]. The low moisture content of the grain is responsible for the inactive state of those microorganisms. However, during the grain germination and about 50% moisture content, microorganisms become active increasing in number: yeast – 5–10 times, fungi - 2.5–5 times, bacteria – 50–100 times. Therefore, microbiological surety of germinated seeds is the essential and urgent task [13] complicated by the most methods of seed treatment resulting in destruction of biologically active substances, such as vitamins, enzymes, antioxidants.

There are lots of methods known to reduce the microbiological seed contamination. Among them, there are physical (thermal and radiation) and chemical (oxidants, fumigants, inhibitors of enzymes and mycotoxins).

With high-temperature treatment regimes, the grain organoleptic properties and nutritional value unavoidably change.

One of the effective ways to solve the problem of the grain decontamination is the ultraviolet (UV) radiation use. UV radiation with the wavelengths of 205 nm to 315 nm has the bactericidal effect and efficiently inactivates various microorganisms, namely bacteria, spores, micro-fungi, etc. Unlike ionization, UV beam has the greater penetrating power and is absorbed by almost all solids. As a rule, UV photons penetrate for a few microns only; so, when solids are exposed to UV, only the thinnest layer is treated while the bulk of the substance is not affected. Accordingly, it does not change its biochemical properties. This is the advantage of UV treatment compared to other decontamination methods [14].

To date, the ultrasonic treatment of food media is under quite active discussion. UV radiation is the mechanical oscillation of over 20 kHz in frequency, which is above frequencies perceived by the human ear. The ultrasound can result in high-molecular compound breakdown, protein coagulation, enzyme inactivation, and destruction of microorganisms [15]. The most hazardous for microorganisms is the ultrasound at 20 kHz to 100 kHz at the intensity of 0.5 - 1.0 W/cm^2. Where, the treatment efficacy depends on the length of exposure, chemical composition of the medium, its viscosity, temperature, pH and initial bacterial count [16].

The advantage of food sterilization by ultrasound is that the product is not heated up to high temperatures and its taste properties remain the same. However, when using the ultrasound treatment, the low exposure intensity promotes the growth of colonies of microorganisms. The ultrasound differently affects vitamins in food products. Ascorbic acid can oxidize; B-group vitamins remain intact when exposed to low-frequency ultrasound, and A2 and D2 vitamins - when exposed at higher frequencies [17].

Chemical antiseptics have been widely used since the second half of the XIX century, when the chemical industry proceeded to synthesize a variety of organic substances with the preserving effect. However, the health legislation of most countries sets forth strict requirements in terms of antiseptics for food preserving by limiting the types of antiseptics, their doses and a variety of food products they are allowed for [18].

It was found that the amber acid at 0.05 g/ml has the non-selective antimicrobial effect against gram-positive, gram-negative bacteria and yeast-like fungi [19].

Recently, the option to use chitosan in agriculture, ecology, industry and microbiology is of high concern [20]. Chitosan is the linear polysaccharide (Figure 1) that may be produced by chitin deacetylation, the long-chain polymer of N-acetylglucosamine that the natural part of cell wall of fungi and bacteria and is found in the exoskeleton of arthropods and a range of other invertebrates [21]. Antibacterial activity of chitosan is well known as the study was published in 1990 where 298 strains were utilized [20].

This work aims to study the efficiency of methods to ensure microbiological safety of germinated oat seeds.

OBJECTS AND METHODS OF RESEARCH

The target of the research are the hulless oats for germination (Obraz zhizni LLC (Lifestyle), Altai Territory).

The seed to germinate should be mellow, and comply with GOST 28673-90 "Oats. Requirements for procurement and supplies". The selected grain was washed, soaked in clean drinking water at 15–20°C for 10–12 hours, germinated at the ambient temperature of 18–23°C for 4 days.

The moisture of the germinated seeds was identified by drying in drying ovens at the temperature of (140 ± 2)°C for 40 minutes upon pre-drying at (105 ± 2)°C for 30 minutes and grinding at the laboratory mill.

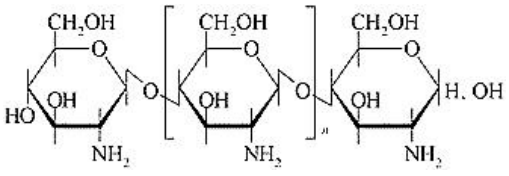

Fig. 1. Chitosan structural formula.

The final moisture content of germinated oat seeds was about 55%. To increase the length of safe storage, preserve the quality and improve processing properties, the germinated oat seeds were dried in drying ovens to reach the moisture content of 14%.

When drying the germinated grain, the temperature of the drying agent should not exceed 45°C. An increase in the temperature of the drying agent to intensify the process may result in enzymatic hydrolysis of proteins and starch, and the considerable reduction in the content of antioxidants. Drying at low temperatures is also undesirable since it slows the process and the resulting intensive breathing causes an increase in the loss of solids and in development of microbiological processes.

The dried germinated oat seeds were crushed at the laboratory mill to particles size 1 mm, not larger.

The prepared product was exposed to the following.

(1) Ultraviolet radiation. A thin layer of the test product was placed in the laminar box with TUV (Philips) disinfection lamps and exposed to short-wave UV radiation at the wavelength of maximum 253.7 nm at 20–25°C and the relative humidity of 80% for 1, 1.5, 2 and 2.5 hours.

(2) Ultrasound. Prior to drying, the germinated oat seeds were sonicated at 35 kHz in the ultrasonic bath at 20–25°C for 5, 10, 15 and 20 minutes.

(3) The antimicrobial drug «Unicons» (Alternativa NGO) that is known for a wide range of bactericidal, fungicidal and virucidal activity. Composition: polyazolidine ammonium, 1,2,3-trihydroxypropane, water. The manufacturer recommends the external treatment by soaking or sprinkling with the solution prepared at the rate of 1 part of additive for 10–20 parts of water. Seeds were immersed in the preparation solutions concentrated at 3, 5 and 10% for 2 and 4 minutes.

(4) Potassium permanganate solution. Oat seeds were immersed in the potassium permanganate solutions concentrated at 0.005, 0.01 and 0.05% for 2 and 4 minutes.

(5) Amber acid solution. The germinated seeds were immersed in 5% acid solution at the room temperature regularly stirred for 20, 40 and 60 minutes.

(6) Food chitosan (Bioprogress LLC). It is known that water-soluble chitosans are able to very quickly suppress the bacterial growth. However, this effect is less remarkable than for acid-soluble chitosans: minimal values of the inhibitory concentration of acid-soluble chitosans are much less (0.03–0.1%) than water-soluble (0.05–0.8%) [20]. The solution of 0.1 and 0.5% alimentary acid-soluble chitosan in 5% solution of amber acid was used in the work. Oat seed were treated by being immersed into 100 ml of chitosan solution at the room temperature and regular stirring for 20, 40 and 60 minutes.

To reduce the negative impact of chemicals, disinfected seeds were three times with washed up with the sterile distilled water.

Germinated oats seeds were sonicated, treated with antimicrobial solutions, potassium permanganate, amber acid and chitosan solutions in amber acid immediately as they germinated. Then, the seeds were dried and ground by the technology above.

Table 1. Microbiological characteristics of non-treated germinated oat seeds

Microbiological properties	QMAFAnM, CFU/g	CGB, in 0.01 g	Yeasts, CFU/g	Fungi, CFU/g
Rated parameters as per TR TS 021/2013	$5.0•10^3$	not permitted	not rated	$5.0•10^1$
Control sample	$4.6•10^6$	found	$6.4•10^2$	$5.5•10^1$

Table 2. Microbiological properties of UV treated germinated oat seeds

Processing time, h	Microbiological properties			
	QMAFAnM, CFU/g	CGB, in 0.01 g	Yeasts, CFU/g	Fungi, CFU/g
1.0	$2.4•10^5$	found	$4.3•10^2$	$2.2•10^2$
1.5	$8.0•10^4$	found	$5.0•10^2$	$5.5•10^1$
2.0	$1.0•10^5$	found	$4.4•10^2$	$2.0•10^1$
2.5	$3.5•10^5$	found	$7.8•10^1$	$3.6•10^1$

All seed samples were tested to identify the Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM), Escherichia coli group bacteria (CGB), yeasts and fungi as per standard practices.

Microbiological characteristics of germinated oat seeds should comply with requirements of TR TS 021/2011 on *Food Safety*.

After the germinated seeds were treated, the changes in their organoleptic properties were reported as they complied with regulatory document requirements.

RESULTS AND DISCUSSION

Microbiological characteristics of non-treated germinated oat seeds were determined (Table 1). As expected, samples showed *Escherichia coli* group bacteria and a significant number of mesophilic aerobic and optionally anaerobic microorganisms that does not meet requirements of regulatory documents; thus, the test product is not applicable for human consumption without adequate treatment.

The results of microbiological studies of UV treated germinated oat seeds are given in Table 2.

As it is seen in Table 2, when exposed to UV, the total microbial contamination decreases by an average of one order; there is no dependence on the length of treatment. The UV treated test product does not reduce the level of contamination to the acceptable level.

Changes occurring in bacteria and lower organisms exposed to UV radiation are reported to be in three stages as follow: motion excitation and enhancement, destructive change occurrence, cell death as a result of photochemical processes. The germicidal efficiency curve of UV radiation matches the absorption spectrum of nucleic acids. This means that the UV target is DNA molecules [22]. However, UV radiation of same wavelengths and same intensity is reported to cause the selective bactericidal action, that is, the dose that kills one type of bacteria only inhibits others. First of all, this is due to the unique DNA structure of each living organism. Division is the cell function most sensitive to

UV rays. Certain dose of exposure causes a stop in the division of about 90% of bacterial cells. UV rays cause changes in nucleic acids that adversely affect the growth, division, heredity of cells, that is, their major manifestations of life activity [22].

Table 3 presents the results of microbiological studies of germinated oat upon after sonication.

As it is seen in Table 3, the compliance with the regulatory documentation is not reached even with ultrasonic treatment for 20 minutes. The effect of ultrasound on microorganisms is associated with the cavitation phenomenon. Cavitation is the process of cavity formation in the liquid medium filled with vapors of the liquid itself that immediately shut. The resulting pressure pulses are capable of destroying lots of biological objects, including microorganisms. To note, the phenomenon of hydrodynamic cavitation, despite the long-term studies, does not seem to be fully studied. Most researchers who studied cavitation processes agree that this phenomenon remains unpredictable in some of its manifestations [15].

Compliance of microbiological properties with regulatory documentation is reached by treating germinated oat seeds with 10% antimicrobial solution. Upon the organoleptic analysis of the resulting product it was noted that organoleptic properties of the product vary when treating germinated seeds with the antimicrobial solution "Unicons" concentrated at over 5%: unpleasant smell and flavor develop. Solutions of potassium permanganate are widely used in medicine and in food industries to inactivate microorganisms. In brewing, potassium permanganate is used to disinfect malt in the amounts of 10–15 g per 1 m^3 of water.

As it is seen in Table 5, the treatment of germinated oat seeds with the potassium permanganate solution significantly reduces the level of microbiological contamination of the product. When seeds are soaked in 0.005% solution of potassium permanganate for 4 minutes, the microbiological properties of the product comply with requirements of regulatory documentation. Upon the organoleptic analysis it was noted that when the use of the potassium permanganate solution concentrated at over 0.01% results in variations of appearance of germinated oat seeds: they get darker. The results of organoleptic analysis of the test product after treatment with antimicrobial and potassium permanganate solutions are shown in Fig. 2. The results of microbiological studies of germinated oat seeds treated with solutions of amber acid and chitosan are presented in Table 6.

The treatment of oat seeds with the amber acid solution reduces the level of their microbiological contamination by an average of 2 orders. Antibacterial action of amber acid is most likely due to that organic acids are lipophilic in undissociated form, and can easily penetrate into the cytoplasm through the bacterial cell membrane. Once inside the cell, where the pH is approximately neutral, these acids dissociate, releasing protons. This results in propagation of the proton driving force that suppresses the enzyme system, transfer of nutrients, amino acids, energy metabolism and DNA synthesis. The bactericidal action of organic acids may also occur resulting from anion accumulation inside the cell. The pH lowering inside the cell causes the microbial cell to use its energy to release protons resulting in the cell depletion [19].

Table 3. Microbiological properties of germinated oat seeds upon ultrasound treatment

Processing time, min	Microbiological properties			
	QMAFAnM, CFU/g	CGB, in 0.01 g	Yeasts, CFU/g	Fungi, CFU/g
5	$2.4•10^5$	found	$4.3•10^2$	$1.4•10^1$
10	$9.2•10^4$	found	$5.0•10^2$	3.0
15	$8.2•10^4$	found	$3.5•10^2$	not found
20	$4.6•10^4$	found	$7.8•10^1$	not found

Table 4. Microbiological properties of germinated oat seeds as treated with antimicrobial agent

Concentration of solution,%	Length of treatment, min	Microbiological properties			
		QMAFAn, CFU/g	CGB, in 0.01 g	Yeasts, CFU/g	Fungi, CFU/g
3	2	$2.1•10^5$	found	$1.8•10^2$	$5.0•10^1$
3	4	$1.8•10^5$	found	$2.1•10^2$	$4.8•10^1$
5	2	$3.6•10^4$	found	$6.4•10^1$	$1.0•10^1$
5	4	$2.6•10^4$	found	$4.0•10^1$	$1.1•10^1$
10	2	$8.8•10^2$	not found	$1.0•10^1$	not found
10	4	$5.8•10^2$	not found	not found	3.0

Table 5. Microbiological properties of germinated oat seeds as treated with the potassium permanganate solution

Concentration of solution,%	Length of treatment, min	Microbiological properties			
		QMAFAn, CFU/g	CGB, in 0.01 g	Yeasts, CFU/g	Fungi, CFU/g
0.005	2	$6.4•10^3$	found	$3.5•10^1$	$1.2•10^1$
0.005	4	$4.8•10^3$	not found	$2.0•10^1$	5.0
0.01	2	$1.5•10^3$	not found	$1.3•10^1$	6.3
0.01	4	$1.2•10^3$	not found	$1.0•10^1$	5.6
0.05	2	$7.5•10^2$	not found	5.0	5.0
0.05	4	$2.0•10^2$	not found	not found	3.4

Table 6. Microbiological properties of germinated oat seeds after treatment with chitosan solution in 5% amber acid solution

Concentration of chitosan solution,%	Length of treatment, min	Microbiological properties			
		QMAFAn, CFU/g	CGB, in 0.01 g	Yeasts, CFU/g	Fungi, CFU/g
0	20	$1.9•10^4$	found	$2.2•10^2$	$5.0•10^1$
0	40	$2.6•10^4$	found	$1.0•10^2$	$1.5•10^1$
0	60	$1.6•10^4$	found	$1.1•10^2$	$1.5•10^1$
0.1	20	$9.1•10^3$	found	$5.8•10^2$	$2.0•10^1$
0.1	40	$9.1•10^3$	found	$3.0•10^2$	$4.0•10^1$
0.1	60	$5.0•10^3$	not found	$1.5•10^2$	$1.5•10^1$
0.5	20	$8.3•10^3$	found	$8.5•10^1$	$3.0•10^1$
0.5	40	$7.5•10^3$	found	$6.4•10^1$	$2.5•10^1$
0.5	60	$4.0•10^3$	not found	$7.0•10^1$	$1.5•10^1$

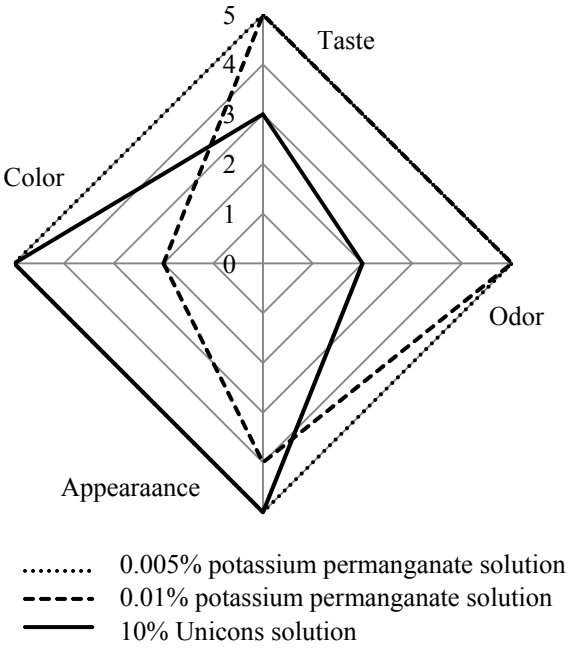

Fig. 2. Organoleptic properties of germinated oat seeds treated differently.

Nevertheless, the antimicrobial effect of amber acid is not sufficient. When chitosan concentrated at 0.1% and 0.5% is added to the amber acid solution, microbiological properties of the test product considerably improve. Moreover, the compliance with the regulatory documentation is reported when germinated seeds are treated with 0.1–0.5% chitosan solution for at least 60 minutes. In fact, chitosan directly affects the morphology of the treated microorganism. The scientific literature specifies that at pH <6.0, low-molecular chitosans are under efficient conditions for antimicrobial, antioxidant and preservative actions to occur in food products, as required. Other studies indicate that, besides its direct antimicrobial action, chitosan causes a series of plant protection reactions aimed at increasing the production of glucan hydrolases, phenolic compounds and synthesis of specific phytoalexins with antifungal action [20, 23].

No changes in organoleptic properties of germinated oat seeds treated by amber acid and chitosan are reported.

CONCLUSION

By the outcome of researches conducted we may conclude that the compliance of microbiological properties of germinated oat seeds with regulatory documentation with no change in their organoleptic properties is reached by treating seeds with the potassium permanganate solution concentrated at 0.005% for 4 minutes and chitosan solution at 0.1–0.5% in 5% solution of amber acid for 60 minutes.

The chitosan solution is more preferable for germinated seed disinfection due to its properties like biodegradability, biological compatibility, nontoxicity [21, 23]. In addition, chitosan and its derivatives have a wide range of biological effect: antibacterial, fungicidal, adsorption action, antioxidant, antineoplastic, anticholesterolemic, immunoadjuvant properties [24, 25].

Thus, germinated oat seeds treated with 0.1–0.5% chitosan solution in 5% amber acid are proved to be functional product with microbiologic safety with a wide range of wholesome properties. They can be consumed directly as ready-to-use product, or as the functional food additive.

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INDICATORS OF QUALITY OF CANNED MILK: RUSSIAN AND INTERNATIONAL PRIORITIES

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Abstract: The main indicators of the quality and safety of dairy canned food in the range of low, intermediate and high humidity related to their distribution in Russia and abroad are considered. A comparative analysis of the quality parameters of traditional canned milk produced by the interstate standards in force in Russia, including obligatory conditions of compliance with technological requirements and sanitary and hygienic norms for production, when compared to similar products manufactured according to international standards, demonstrates competitive indicators of quality and safety. The basic technological approaches are investigated and a number of additional evaluation criteria for the utilization of various technologies and assessments of the quality of finished products are considered. Data on alternative raw ingredients, food additives and technological aspects that contribute to improving the quality of products, including storage stability, are reviewed. Separately presented are the integral criteria which, excludes the presence of falsified products. The principles of creating technologies for canned dairy products of functional purpose, including gero-dietetics, are described. Thus, based on a modern regulatory and technical base and using existing production capacities for the production of high-quality dairy canned food, the only necessary element for solving the problem of complete import substitution of canned dairy products in Russia is to increase the volume of raw material production.

Keywords: Canned milk, normative documents, product quality, evaluation criteria, storage stability, falsification

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INTRODUCTION

The importance of milk and dairy products, including canned, in the structure of human nutrition cannot be overestimated. High nutritional value, the balance of macro- and microcomponents, the presence of biologically active substances, and good digestibility are all necessary elements for the normal functioning of the human body. Due to the production and use of canned milk, problems such as the seasonality of providing the population with dairy products, supplying them to remote regions of the Russian Federation, where the development of dairy cattle breeding is difficult. In addition, due to its long shelf life, canned milk is included in the nomenclature of food reserves to ensure food independence, which in turn is a component of Russia's economic security.

According to the market survey of dairy products compiled by the Federal State Institution «Special Center-Aid in the Agro-Industrial Complex» of the Ministry of Agriculture based on Federal State Statistics Service of Russian Federation, Federal Customs Service of Russia, National Statistical Committee of Belarus and Eurasian Economic Commission data over the past five years, the annual production of canned milk in the Russian Federation was 330–370 thousand tons, with import of 200–240 thousand tons, and export of 40–70 thousand tons. The main share of production, import and export of canned milk (more than 70%) is in the form of four traditional types: milk powder, dry cream, condensed milk with sugar, and condensed cream with sugar. Great attention is given to the issues related to the

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product quality, security, falsification, both in our country and abroad [1, 2, 3].

Due to the significant transformations in the field of technical regulation that are a logical consequence of the establishment of the Eurasian Economic Union (EAEU) and the Customs Union (CU) in recent years, the following interstate standards have been developed, and are currently in effect in the case of traditional dairy preserves in Russia [4, 5, 6]:

– GOST 31688-2012 with the change № 1 "Canned milk. Milk and cream sweetened condensed. Specifications", the date of introduction of the standard – 01.07.2013, the date of introduction of the change - 01.05.2016;

– GOST 33629-2015 "Canned milk. Dry milk. Specifications", the date of introduction of the standard - 01.07.2016;

– GOST 33922-2016 "Canned milk. Dry cream. Specifications", the date of introduction of the standard - 01.09.2017.

In the territories of the EAEC and the CU, the main producing countries of these types of canned food are Russia and Belarus. Therefore, the basis of interstate standards was the content of national documents of these states:

– GOST R 53436-2009 "Canned milk. Milk and sweetened condensed cream. Specifications";

– GOST R 52791-2007 "Canned milk. Dry milk. Specifications";

– STB 1858-2009 "Dry milk. General specifications";

– GOST R 54661-2011 "Canned milk. Dry cream. Specifications".

In addition, international scientific requirements, regulated by the Codex Alimentarius, were taken into account when developing interstate standards.

Codex Alimentarius is a collection of food international standards, guidelines, and instructions adopted by the FAO/WHO International Commission covering the main types of food, regulating product safety and quality when entering the world market. The Codex Alimentarius Commission was formed in 1963 and currently has 188 members (187 countries and 1 organization – the European Union) and 219 participants (56 international government organizations, 147 private organizations, and 16 United Nations organizations) which includes 99% of the world population. The Russian Federation has been a member of the Code since 1993. The Codex standards are recommended for voluntary application by its members, forming the basis for the formation of national policy in the field of technical regulation [7].

The Codex Alimentarius Committee on Milk and Dairy Products has developed the following international standards for these types of canned milk:

– CODEX STAN 282-1971 "Standard for Sweetened Condensed Milks", adopted in 1971;

– CODEX STAN 207-1999 "Standard for Milk Powders and Cream Powder", adopted in 1999.

It should be stressed that when creating interstate standards for canned milk, the goal was to maximally harmonize the relevant Codex standards. As a result, a new classification group (partly defatted canned food) was introduced into interstate standards, new quality indicators were established and current norms were readjusted.

Since the industrial development of society, great attention has been constantly given to the issues of improvement of food quality. The complexity and multifaceted nature of the problem are obvious even now, as they affect various technical, technological, economic, and social aspects of human activities. In the accepted sense of the term, the quality of food products is a complex of product characteristics, including its safety, consumer properties, nutritional and energy value, that is meant to satisfy human need for food and health preservation.

The quality indicators for all of Russia's standardized canned milk products can be divided into three groups:

– organoleptic - product characteristics, determined by means of sensory systems, such as visual, tactile, olfactory and taste;

– physico-chemical - standardized physicochemical characteristics of specific types of products;

– safety indicators - microbiological indicators and identification of potentially dangerous substances in the quantities regulated by law.

Due to the different approaches of food quality appreciation in Russia and abroad related to the content of regulatory acts, the control, testing, monitoring and allocation of responsibility between state bodies and industry, it is difficult to make a direct comparison "one to one" of the above indicators. Therefore, it is best to focus attention on the fundamental criteria of the assessments of quality.

ASSESSMENT OF QUALITY

Organoleptic indicators are a special category of comparison. The Technical Regulations of the Customs Union and the Alimentarius Codes have significantly different approaches to their narration. In TR specific quality indicators are used, established in correspondence with interstate or national standards and regulations. By contrast, the Alimentarius Codes requirements to the organoleptic indicators are not standardized. According to its concept, food quality characteristics like appearance, consistency, color, smell and taste are not a part of product controlling system. On the contrary, the market decides whether these characteristics are satisfactory, and the producers establish them in relation to the consumers' demand.

Organoleptic characteristics of condensed and dried dairy canned products in correspondence with TR CU 033/2013 "On Food Safety", GOST 31688-2012, GOST 33629-2015, GOST 33922-2016 are given below in the unificated form in Table 1.

Qualitative assessment of Russian condensed with sugar and dried milk canned food throughout the shelf life of organoleptic indicators should meet the requirements as specified in Table 1.

Tables 2 and 3 show the physicochemical parameters of dairy canned food, as regulated by inter-governmental and international standards.

2 **Table 1.** Organoleptic parameters of condensed with sugar and dried milk canned food

Name of indicator	Organoleptic characteristic	
	Milk and cream, condensed with sugar	Dry milk and cream
Appearance and consistency	Homogeneous, viscous throughout the mass without the presence of sensible organoleptic lactose crystals. It is permissible to have a powdery consistency and a slight lactose deposit on the bottom of the container during storage.	Homogeneous fine dry powder. A small number of co-lobules scattered by light mechanical impact
Color	Uniform throughout the mass. For milk partially defatted or whole condensed with sugar and cream condensed with sugar - white with a cream shade. For skim milk condensed with sugar - from white to white with a slightly blueish tint Uniform throughout the mass.	Uniform throughout the mass. For milk powder - white or white with a light cream shade. For dry cream - white with a light cream color.
Taste and smell	The taste is sweet, pure with a distinct taste and smell of pasteurized milk (for skimmed milk, partially defatted, whole condensed with sugar) or pasteurized cream (for cream condensed with sugar), without foreign flavors and smells. It is permissible for milk which is fat-free condensed with sugar to not have enough expression of the taste of milk. It is allowed to have a light forage taste.	Pure, characteristic of pasteurized milk (for dry milk) or characteristic pasteurized cream (for dry cream)

Table 2. Physicochemical parameters of condensed milk with sugar and condensed cream with sugar

The name of the indicator CODEX STAN 282-1971	The norm in accordance with							
	GOST 31688-2012				CODEX STAN 282-1971			
	SCSM	PSCSM	SCM	SCHM	SCSM	PSCSM	SCM	SCHM
Mass fraction of fat,%	≤ 1	1–8.5	≥ 8.5	≥ 19	≤ 1	1-8	≥ 8	≥ 16
Mass fraction of moisture, %, not more than	30	28.5	26.5	26	–	–	–	–
Mass fraction of dry milk residue, %, not less than	26	26	28.5	37	24	24	28	–
Mass fraction of dry defatted milk residue,%, not less than	–	–	–	–	–	20	–	14
Mass fraction of protein in dry non-fat milk residue,%, not less	34	34	34	34	34	34	34	34
Mass fraction of sucrose,%	44–46	43.5–46	43.5–45.5	37–39	–	–	–	–
Acidity, °T, not more than	60	55	48	40	–	–	–	–
Viscosity, Pa·s	–	–	3–15	–	–	–	–	–
Group of purity, not less than	I	I	I	I	–	–	–	–
Size of lactose crystals, μm, not more than	15	15	15	15	–	–	–	–

Note. SCSM – Sweetened Condensed Skimmed Milk; PSCSM – Sweetened Condensed Partly Skimmed Milk; SCM – whole condensed milk with sugar (according to CODEX STAN 282-1971 this type of product has the name "Sweetened Condensed Milk"); SCHM – cream condensed with sugar (according to CODEX STAN 282-1971 this type of product has the name "Sweetened Condensed high-fat Milk"); «–» – the value of the indicator by the standard is not standardized.

Table 3. Physical and chemical parameters of milk powder and dry cream

The name of the indicator	The norm in accordance with							
	GOST 33629-2015, GOST 33922-2016				CODEX STAN 207-1999			
	SMP	PSM	WMP	CP	SMP	PSM	WMP	CP
Mass fraction of fat,%	≤1.5	1.5–26	26–41.9	≥42	≤1.5	1.5-26	26–42	≥42
Moisture content,%, not more than	5	4	4	4	5	5	5	5
Moisture content,%, not more than	34	34	34	34	34	34	34	34
Mass fraction of lactose,%	47–54	39–52	31.5–40	–	–	–	–	–
Acidity *	14–21	14–21	14–21	14–20	≤18	≤18	≤18	–
Solubility index, not more than **	0.2	0.2	0.2	0.4	1.0	1.0	1.0	–
Group of purity, not less than	I	I	I	–	–	–	–	–
Burnt particles, not higher	–	–	–	–	Disk B	Disk B	Disk B	–

Note. SMP – Skimmed Milk Powder; PSM – partly skimmed milk powder; WMP – whole milk powder; CP – cream powder; * – the dimension of the indicator in accordance with GOST 33629-2015 and GOST 33922-2016 – «°T», according to CODEX STAN 207-1999 – «ml 0.1N NaOH / 10g dry defatted milk residue»; ** – the dimension of the indicator in accordance with GOST 33629-2015 and GOST 33922-2016 – «cm^3 of a wet sediment», according to CODEX STAN 207-1999 – «ml».

Analyzing the data of Table 2 and 3, it can be stated that the physico-chemical indicators of conserved dairy products produced by interstate standards are not inferior, with some standards exceeding similar indicators of the Codex Alimentarius standards.

Indicators of condensed milk canned with sugar.

In GOST 31688-2012 mass fractions of fat for whole condensed milk with sugar (not less than 8.5%) and cream condensed with sugar (at least 19%) are left unchanged with respect to the national standard that was in effect in the Russian Federation; their values do not contradict Norms CODEX STAN 282-1971 (not less than 8% and 16% respectively). This is due to the fact that since the second half of the last century and up to the present, Russia's dairy-canning industry produces products with the aforementioned mass proportions of fat on a scientifically based, experimentally tested, practically tested basis used when implementing technologies and recipes.

The mass fraction of the dry milk residue in Russian condensed canned food with sugar (26–28.5%) is 0.5–2% higher than in the corresponding types of products under the Code. Despite the fact that differences in values at first glance can be called small, they are due to a fundamentally different technological approach related to formation of the quality of finished products intended for long-term storage. In addition, the increased content of dry milk residue contributes to the increase in the food value of canned food, including consumer appeal due to the saturation of the taste and flavor of the dairy product.

Of particular importance is the indicator «the mass fraction of protein in the skimmed milk powder residue - not less than 34%», which was included in interstate standards for canned milk by analogy with the Codes. This indicator indirectly describes the naturalness of the milk material used, which is produced from cow's milk. In the production of canned goods, concentration of all its constituent parts, except for water which evaporates, takes place simultaneously with the preservation of the initial ratio between them, including between the protein and the dry, skimmed milk residue. Using the data from the identification of raw milk from different types of agricultural animals, given in Annex 6 of TR TC 033/2013, you can calculate this indicator, which will be different for other animals, or much lower (milk of the donkey – 19.5%, horse – 23.6%, goats – 26.4%, and camels – 31.7%), or significantly higher (sheep milk – 41.5%, and buffalo – 42.9%). This indicator, both in the world and in the national standardization, is included not only in documents for dairy, dairy components, but also for milk-based canned food and canned milk, namely:

– CODEX STAN 250-2006 “Standard for a Blend of Evaporated Skimmed Milk and Vegetable Fat”;
– CODEX STAN 251-2006 “Standard for a Blend of Skimmed Milk and Vegetable Fat in Powdered Form”;
– CODEX STAN 252-2006 “Standard for a Blend of Sweetened Condensed Skimmed Milk and Vegetable Fat”;
– GOST R 54649-2011 “Milk-containing dry canned foods. Specifications”;
– GOST R 54666-2011 “Canned milk. Sterilized condensed milk. Specifications”;
– GOST 31703-2012 “Canned milk-containing sweetened condensed. General specifications”;
– GOST 33921-2016 “Canned milk. Condensed sweetened cooked milk. Specifications”;
– GOST 33923-2016 “Canned compound sweetened condensed milk. Specifications”.

What is especially important, is the ratio of protein to dry skim milk remains one of three (two others - the mass fraction of fat and milk solids) included in the basic definitions of all types of dairy canned food TR CU 033/2013, such as Condensed milk, milk and cream condensed with sugar, milk and cream are dry.

GOST 31688-2012 obligatorily normalizes the requirements for the mass fraction of moisture, sucrose, acidity, viscosity, purity group and the size of lactose crystals (milk sugar), as important for quality control of the product during storage. In CODEX STAN 282-1971, these indicators are not standardized, with the exception of recommendations on the amount of sugar, the minimum value of which is limited by good manufacturing practice, allowing preservation of the quality of the product during storage, while the maximum quantities do not allow for crystallization of the sugar. However, long-term scientific and practical studies of condensed milk canned with sugar demonstrated that with long-term storage it is important to standardize the content of sucrose and moisture in the regulatory documentation. Due to the use of osmotically active substances, a «preserving» effect (osmoanabiosis) occurs, which significantly increases the storage capacity of the products. Osmoanabiosis - increased osmotic pressure (16 MPa or more) occurs at the border of the solution/microbial cell. Due to the difference in the concentration of substances in the solution and inside the cell, dehydration of the protoplasm of the cell occurs, accompanied by compression (plasmolysis), separation from the cell wall and disruption of vital functions. By the degree of exposure to bacteria, the most common sugars can be arranged in the following order: fructose > glucose > sucrose > lactose. In both Russia and abroad, as applied to traditional condensed milk preserves, sucrose was introduced as an osmotically active substance into the condensed milk in the form of a water solution of beet or cane sugar. Lactose in condensed milk crystallizes without having a significant effect on osmotic pressure, and therefore does not have an individual preservative effect. Recently developed technologies of canned food for functional purposes, wherein osmotically active substances, not only various sugar mono-substances but also their compositions (isomaltulose, fructose, and lactulose) are used. In connection with the foregoing, the quantity and quality of sugar used in the production of canned dairy products is of great importance. In addition, to ensure the storage of products for a long period, it is necessary to regulate the ratio between sucrose and moisture in the product, with the concentration of sucrose, not less than 60.0% for condensed milk with sugar, not less than 58.7% for condensed cream and sugar. The concentration is understood as the ratio of the mass fraction of sugar to the sum of the mass fractions of sugar and water in the

finished product («sugar number», «sugar ratio»). However, some microorganisms (*Catenularia fuliginea*, and *Aspergillus glaucum*) contaminating the product can adapt to increased osmotic pressure, so condensed milk or cream with sugar must be protected against the entry of secondary microflora with observation of the recommended temperature storage regimes [8–11].

A role in the evaluation of the quality of canned dairy products belongs to the dimensions of the crystals of milk sugar. The consistency of the product, including consumer properties, depends on their magnitude. The process of crystallization of milk sugar, including the transition from a molecular solution to a solid crystalline state, is very specific in the production of canned food. When the mixture of milk with sugar is thickened, the concentration of all dry substances, including lactose, increases by 2.5 fold (in proportion to the decrease in the water content). At the end of the condensation (not less than 69.0% of dry matter), the lactose content is 12.5%, and the water content is no more than 31.0%. Thus, the concentration of lactose in the aqueous part of the finished product («lactose number») is about 30.0% and, since the solubility of lactose is low (at 18°C in 100g of water only 15.5 g of lactose dissolves), under these conditions there is a saturated state and, under cooling, uncontrolled crystallization will inevitably occur. The temperature of the condensed milk mixture with sugar when discharged from the vacuum apparatus is 50–60°C, and the storage temperature of the finished product in accordance with the recommendations of the current regulatory documents should not exceed 10–20°C. Therefore, cooling the condensed milk mixture with sugar is a prerequisite. In this case, lactose passes from a saturated solution to a supersaturated state, and then to a crystalline state. Formation of the consistency of the product is achieved only by performing the necessary technological methods for cooling the product by introducing crystallization centers (embryos) in the form of fine crystalline lactose (seed) with a crystal size of not more than 4 μm. Other factors (raw material quality, primary milk processing, pasteurization, thickening) do not affect the crystallization of lactose. It is necessary to properly prepare the seed, carefully observe the cooling regimes, so as to maximize the amount of lactose. Otherwise, when the product is stored under low temperature conditions, then the system becomes supersaturated again, with further crystallization occurring in the remaining portion of the dissolved sugar. The crystals that have fallen as a result of the spontaneous event are large, which can reach a size of 20–25 microns instead of the standard of «no more than 15 microns». In this case, the product will have a flaw of consistency (mealyness, or sandiness) and it can be used only for industrial processing [12–15].

Indicators of dry milk canned food. The ranges of the fat content of Russian dry canned milk are practically the same as the Codex Alimentarius, in addition to dry whole milk, for which the upper limit of the range is limited to 41,9%, which corresponds to the identification indices regulated by TR CU 033/2013. Dry milk product with a fat content of 42% in the Russian Federation is a dry cream that has been standardized more than 60 years ago and is produced in this fat content since then to the present. In addition, the term «dry cream» is included in TR 033/2013, which is defined as a dry milk product with a mass fraction of milk solids – not less than 95%, fat – not less than 42% and mass fraction of milk protein in dry non-fatty substances Milk – not less than 34%.

The production of dry dairy products is based on the principle of xeroanabiosis, that is, from normalized milk, moisture is removed by drying, bringing it to the minimum amount at which microbiological and enzymatic processes cannot proceed intensively. For dry partially defatted and whole milk, as well as for dry cream, the mass fraction of moisture in interstate standards is represented by a more «rigid» value – 4% in relation to the standard in the Code. Moisture content in the range 2.5–4.0% is optimal for the listed species and corresponds to a monomolecular layer of adsorption-bound moisture. The mass fraction of moisture for skimmed milk powder (not more than 5%) is due to the increased content of protein and lactose it has. Moisture content of more than 7% in dry milk leads to destabilization of the fatty phase of milk powder and the crystallization of lactose, which contributes to the appearance of various defects worsening its quality. Excessively low mass fraction of moisture (less than 2.4%) lead to the appearance of a salty aftertaste in the product during storage [12].

In dry milk products, as well as in condensed canned foods, the protein content of the skimmed milk powder is normalized (at least 34%). The significance of this indicator was noted above.

The standards for milk powder and cream, which are in force in the Russian Federation, stipulate mandatory requirements for acidity and solubility index, while the GOST for milk powder also regulates the mandatory standards for the mass fraction of lactose and the purity group. In the Code, acidity, solubility index and burnt particles are additional indicators of quality that are intended for voluntary application by commercial organizations but do not presume their use at the state level.

Comparing the norms on acidity and solubility index in GOSTs and CODEX STAN, as well as taking into account the nuances of the methods of their determination in different countries, it can be concluded that they are consistent. Thus, the determination of acidity in the Russian Federation is carried out in a sample of a certain mass calculated by a special computer program, depending on the type of product [16]. In the Codex, however, a sample of the product containing 10 g of a skimmed milk powder is used to determine the titrated acidity. Differences in the norms of the solubility index in accordance with GOST (cm^3 of wet cake) and CODEX STAN (ml) are related to the instruments and equipment used, as well as the mass of the sample. To assess the correlation of the norm specified in the Code, the value of the solubility index according to GOST is multiplied by 5.

The determination of the lactose content in Russian dry canned milk is needed due to the widespread

industrial use of this product as a raw material component for the production of various food products, and also to detect cases of falsification by unscrupulous producers of dried milk [4, 17].

The value of the indicator «burnt particles» by interstate standards for dry dairy products is not currently standardized, which is due to the lack of a standard for the method for determining this parameter. The method of determining the purity group burnt particles does not refer to mechanical contamination. The probability of the appearance of burnt particles in dry milk is unpredictable, since in the course of the technological process, there may be cases of molten milk in places where hot air is supplied to the drying tower with a temperature of more than 140°C. As a result, deposits may appear on the internal surfaces of the equipment, where the product is exposed to prolonged thermal effects. Burnt particles are detected visually in the form of small brown or black spots in the mass of milk powder or when it is restored. In accordance with the interstate standardization plan for 2016–2018, the Russian Federation is developing an interstate standard for milk powder for the production of baby food products, which is intended to include this indicator with the words of the method of its determination, as well as additional indicators compared with «regular Heat treatment class» and «bulk density».

In international practice, the indicator «heat treatment class» or «heat class» is used and normalized quite some time ago. It is defined as the ratio of the difference between the total protein and the sum of whey proteins to the total protein at a certain value of the active acidity. In the process of production of milk powder, raw milk is heat treated (pasteurization, thickening, and drying), causing its chemical and technological properties to undergo changes. The lower the overall temperature effect on the initial milk, the less transformations and technological changes occur in it, and therefore more physiologically significant (useful) components will remain in the dried milk, allowing it to have a wider range of uses. Therefore, it is important for both the manufacturer and the consumer of dry milk to estimate the degree of the overall heat treatment in order to solve the problem of its specific application in order to obtain high-quality products, for example, to direct milk powder for the production of dairy products, in the production of which a good clot Cottage cheese, cheese), or produce dairy products, canned milk, cereals, etc. Currently, the following classification is used, which is based on the fact that as the degree of heat treatment increases, an increasing proportion of whey protein in milk is denatured and becomes associated with casein:

– low-temperature heat treatment (not less than 6.0 mg UNWON per gram);
– moderate heat treatment (from 1.51 to 5.99 mg UNWON per gram);
– high-temperature heat treatment (not more than 1.5 mg UNWON per gram), where UNWON is the concentration of un-denatured whey protein nitrogen.

Proceeding from the foregoing, it will be logical and expedient to introduce into the draft standard for dry milk, which will be used in the production of various dairy products for children's nutrition, the indicator «heat treatment class» with the norm corrected on the basis of numerous studies of at least 4.5 mg UNWON per gram, indicating a moderate or low-temperature heat treatment.

The indicator «bulk density» is of great practical importance. It is used both for technological and marketing purposes to manage the process of drying milk; Evaluation of the quality of construction of drying plants and diagnostics of their condition in the process of work; In forecasting the suitability of products for long-term storage; When assessing the state of production after long-term storage; To calculate the required volumes of storage tanks and storage facilities; and When dosing dry milk into various types of consumer and shipping packaging. In addition to the above, this indicator can serve as additional indirect evidence in detecting the falsification of the product. The density of dry milk depends on the density of its constituent particles, their size, surface appearance, shape, compactness, the chemical composition of the raw milk raw materials, etc. In GOST 31977-2012 “Dried milk products. Method for determination of bulk density” defines three types of indicator “bulk density”:

– volumetric bulk density – the ratio of the mass of the product to its volume in a graduated cylinder without sealing the product;
– loose bulk density – the ratio of the mass of the product to its volume after 100 strokes;
– Bulk density – the ratio of the mass of the product to its volume after 625 strokes.

Based on numerous scientific and practical studies, the following values of the "bulk density" indicator for dry milk will be included in the draft standard:

– fat-free – from 0.560 to 0.680 g/cm^3;
– partially defatted – from 0.520 to 0.640 g/cm^3 (for dry milk with a mass fraction of fat from 25 to 26% – from 0.440 to 0.550 g/cm^3);
– solid – from 0.330 to 0.420 g/cm^3.

In the near future, introduction of such physical and chemical indicators as burnt particles, heat treatment class and bulk density into the interstate standard for milk powder will significantly improve the quality of Russian products.

The main characteristic of any food products entering the domestic or foreign markets is its security. Presence of such dangerous factors as biological, chemical, physical substances, present in food products or coming in contact with them, can become a potential cause of negative impact or even constitute a serious threat to human health.

In CU, the relevant documents are accepted: TR CU (Technical Regulations of Custom Union) 021/2011 «On Food Safety» and TR CU 033/2013, establishing the maximum permissible standards for microbiological indicators and potentially dangerous substances of specific types of canned food. In the Codex Alimentarius, the requirements for microbiological criteria for condensed with sugar and dry canned milk are regulated by CAC/GL 21-1997 «Principles and guidelines for the establishment and application of microbiological criteria related to foods», and to the

permissible levels of potentially dangerous substances (contaminants) – CODEX STAN 193-1995 «General standard for contaminants and toxins in food and feed» and CAC/RCP 57-2004, «Code of hygienic practice for milk and milk products», which formulate general principles and requirements for food safety. Comparative analysis of the features of the requirements of TR CU and Codex with respect to the permissible levels of microbiological indicators, chemicals, veterinary drugs, pesticide residues is a complex of complex issues from different scientific and technical subject areas.

Microbiological safety standards are noted in Annex 1 of TR CU 021/2011, as applied to milk preserves, wherein only the indicator «pathogenic microorganisms, incl. Salmonella». The remaining permissible levels of microorganisms are established in TR CU 033/2013 (Annex 8). The summary requirements for the permissible microbiological standards in canned milk are presented in Table 4.

As can be seen in Table 4, microbiological TR requirements are a combination of indicators for pathogenic and indicator microorganisms that are evaluated in the final product. In CAC/GL 21-1997, the requirements for microbiological criteria are aimed to identify microorganisms that are directly related to food-dependent diseases throughout the food chain from farm to consumer. Control of microorganisms is performed during the production process. The results are confirmed again, when the products are already in circulation in the market. From the point of view of health threats, when comparing the micro-biological safety of canned milk in our country and abroad the most important group of micro-organisms are pathogenic microorganisms, the permissible standard of which is the same. In fact, only these microorganisms are the only group on which it is possible to make a comparison. Thus, in the countries of the European Union (EU), according to Commission Regulation (EC) 2073/2005 «On microbiological criteria for foodstuffs», which is built on the principles of CAC/GL 21-1997, milk powder except for the absence in 25g of pathogenic microorganisms of the genus Salmonella, in 25g not allowed staphylococcal entero toxins, and during the process determine the Enterobacteriaceae (less than 10 CFU/g) and coagulase-positive staphylococci (maximum 10 CFU/G). The last two criteria do not apply to milk powder, intended for further processing in the food industry. In addition, provisions of Commission Regulation 2073/2005 entitle the inspection agency, if there is reasonable threat to security, to conduct research on the production organisms are not identified in the Regulations, and also require market participants to identify other food organisms (other than those listed in Regulations) as microbiologically significant risks in the framework of self-control programs based on HACCP principles. Thus, this makes it possible, as necessary, to expand the list of controlled microorganisms.

The index «a potentially dangerous substance», used in the TR CU or «contaminants» up, use, being operated in the Codex Alimentarius, means any substance inadvertently trapped in the food pro-induction during the manufacturing process, the packaging, transportation or storage of such products, or by Pollution from the environment. Since it is generally believed that the pollution has a negative impact on food safety and may pose a risk to human health, domestic and international public authorities have taken measures to control the content of the hard exponents in foods.

In Table 5 shows allowable levels of potentially hazardous substances (toxic elements, mycotoxins, antibiotics, pesticide, radionuclide, dioxins, melamine) in canned milk under Annex 3 and 4 TR CU 021/2011.

Codes, guidelines and guidelines set maximum levels of contaminants in food products and apply to a wide range of foods. However, it is significantly inferior in coverage to the norms of the CU. In the case of milk, these documents set the following maximum permissible levels:

– lead – 0.02 mg/kg (if the milk is partially or completely dehydrated, then the appropriate concentration ratio is used);
– tin – 250 mg/kg;
– aflatoxin M_1 – 0.5 mg/kg (if the milk is partially or completely dehydrated, then the appropriate concentration ratio is applied);
– levomycetin – in the EU countries this antibiotic is prohibited in the treatment of animals; EU MRLs are not
– tetracycline group – 0.1 mg/kg; In the treatment of animals
– penicillin and its derivatives – the norms are established for each specific derivative;
– cesium-137 – 1000 Bq/kg;
– strontium-90 – 100 Bq/kg;
– melamine – 2.5 mg/kg.

Table 4. Acceptable levels of microorganisms in canned milk

Name of the type of milk preserves	MAFAM*, CFU**/cm^3 (g), not more	Volume (mass) of the product, cm^3 (g), In which are not allowed		
		CGBF*** (Coliforms)	Pathogenic, incl. Salmonella	Staphylococcus aureus
Milk / cream, condensed with sugar, in:				
- consumer packaging;	2×10^4	1	25	–
- transport packaging	4×10^4	1	25	–
Cow milk powder for:				
- direct use;	5×10^4	0.1	25	1
- industrial processing	1×10^5	0.1	25	1

Note. * The amount of mesophilic aerobic and facultative anaerobic microorganisms; ** Colony forming units; *** Bacteria of the group of *Escherichia coli.*

Table 5. Acceptable levels of potentially dangerous substances in canned milk

Name of indicator	Permissible level in canned milk	
	Condensed with sugar	Dry
Toxic elements, mg / kg, not more than: - lead - arsenic - cadmium - mercury - tin (for products in a tin container) - chrome (for products in chrome tare)	 0.3 0.15 0.1 0.015 200.0 0.5	(in terms of the recovered product) 0.1 0.05 0.03 0.005 200.0 0.5
Mycotoxins, mg/kg, not more than: - aflatoxin M_1	 0.0005	 0.0005
Antibiotics, mg/kg: - levomycetin - tetracycline group - streptomycin - penicillin	 Not allowed (<0.0003) Not allowed (<0.01) Not allowed (<0.2) Not allowed (<0.004)	 Not allowed (<0.0003) Not allowed (<0.01) Not allowed (<0.2) Not allowed (<0.004)
Pesticides, mg/kg, not more than (in terms of fat): - hexachlorcyclohexane (α,β,γ - isomers) - DDT ant its metabolites	 1.25 1.0	(in terms of the recovered product) 1.25 1.0
Radionuclides, Bq/kg, not more than: - specific activity of cesium-137 - specific activity of strontium-90	 300 100	 500 200
Dioxins, mg/kg, not more (in terms of fat)	0.000003	0.000003
Melamine, mg/kg	Not allowed (<1.0)	Not allowed (<1.0) (in terms of recovered product)

With regard to pesticides, for example, in the EU their list and maximum allowable doses represent a constantly updated database in the form of an Internet portal that includes both authorized and unauthorized substances.

When comparing the Russian and international security indicators, it is clearly possible to establish compliance of the requirements with the permissible levels of Aflatoxin M_1. The remaining parameters have different values, in one case in the TP more rigid (the content of tin, antibiotic tetracycline, cesium-137, melamine), in the other – in the Codes (lead content, strontium-90, prohibition on the use of levomycitin).

For the production of condensed with sugar and dried milk canned food, a variety of dairy raw materials are used, the list of which is presented in Table 6.

All dairy raw materials used in the Russian Federation for the development of traditional types of canned milk products are standardized in the form of national and interstate documents. In international practice from the listed in Table 6 raw materials. These codes only regulate the requirements for plums, dried milk and dried cream.

The quality of raw milk and other dairy raw materials depends on the quality of the finished product. If we consider the problem «from the field», it is necessary to carry out measures to increase the number of cattle and carry out selection work aimed at creating breeding stock and the formation of certain quality indicators of milk, respectively [18, 19]. In recent years, the requirements to the quality and safety of dairy raw materials on the territory of the EAEU and CU member countries have been tightened, and almost all its types are subject to either national or interstate standards.

Traditional canned milk can be attributed to products of multi-purpose. In addition to their direct consumption in food, their other purpose is to use a huge amount of various food products in production, the quality of which, of course, is affected by the species and the component amount of its raw materials.

Table 6. The list of dairy raw materials used for the production of traditional canned milk

Condensed milk canned with sugar		Dry canned milk		
GOST 31688-2012	CODEX STAN 282-1971	GOST 33629-2015	GOST 33922-2016	CODEX STAN 207-1999
Milk cow raw, Milk cow pasteurized - raw materials, Milk degreased - raw materials, condensed milk - raw materials, Cream - raw Milk	Milk, Cream, Dry milk, Cream dry, Milk concentrate, Permeate milk	Milk cow raw, Milk cow pasteurized - raw materials, Milk degreased - raw materials, condensed milk - raw materials, Cream - raw milk	Milk cow raw, Milk cow pasteurized - raw materials, Milk degreased - raw materials, condensed milk - raw materials, Cream - raw milk	Milk, cream, Milk concentrate, Permeate milk

Table 7. List of food additives used for the production of traditional canned milk

Condensed milk canned with sugar		Dry milk canned		
GOST 31688-2012	CODEX STAN 282-1971	GOST 33629-2015	GOST 33922-2016	CODEX STAN 207-1999
Stabilizing agents				
Sodium phosphate E339 Sodium citrate E331 Potassium phosphate E340 Potassium citrate E332	Sodium citrate E331 Potassium citrate E332 Calcium citrate E333	–	–	Sodium citrate E331 Potassium citrate E332
Viscosity regulators				
–	Potassium chloride E508 Calcium chloride E509	–	–	Potassium chloride E508 Calcium chloride E509
Acidity regulators				
–	Calcium carbonate E170 Sodium phosphate E339 Potassium phosphate E340 Calcium phosphate E341 Pyrophosphate E450 Triphosphates E451 Pyrophosphate E452 Sodium carbonate E500 Potassium carbonate E501	–	–	Sodium phosphate E339 Potassium phosphate E340 Pyrophosphate E450 Triphosphates E451 Pyrophosphate E452 Sodium carbonate E500 Potassium carbonate E501
Emulsifiers				
–	Lecitin E322	–	–	Lecitin E322 Mono and diglycerides of fatty acids E471
Thickener				
–	Carraginan E407	–	–	–
Additives to prevent caking and clumping				
–	–	–	–	Calcium carbonate E170 (i) Ortho-phosphate calcium 3-substituted E341 (iii) Ortho-phosphate magnesium 3-substituted E343 (iii) Magnesium carbonate E504 (i) Magnesium oxide E530 Silicon dioxide amorphous E551 Calcium silicate E552 Silicates of magnesium E553 Aluminosilicate sodium E554
Antioxidants				
Ascorbic acid E300 Sodium ascorbate E301 Potassium ascorbate E303 Dihydroquercetin	–	Dihydroquercetin	Dihydroquercetin	L-Ascorbic acid E300 Sodium ascorbate E301 Ascorbyl palmitate E304 Butylhydroxyanisole E320
"–" the use of standard additives not regulated				

In this regard, attention should be paid to allowing the Codex Alimentarius to use in the production of dry and condensed milk canned foods an extremely large list of different food additives: acidity regulators and viscosities, hardeners, stabilizers, thickeners, emulsifiers, caking and clumping inhibitors, synthetic antioxidants (Table 7). Although these food additives in Russia are also included in the list for use in the production of food products TR CU 029/2012 «Safety requirements for food additives, flavors and technological aids», but not all of their use in the development of canned dairy products is technologically justified and eco-friendly and economically feasible. Therefore, the use of only four salt-stabilizers (citrates and phosphates of sodium and potassium) and antioxidants (dihydroquercetin, ascorbic acid and its salts), and for dry canned food - only dihydroquercetin, the introduction of which is scientifically justified, is normalized for the production of Russian condensed milk canned food with sugar standards, which has been experimentally confirmed by many years of research and numerous studies with positive results.

In CODEX STAN 207-1999 as an antioxidant recommended is ascorbic acid, its salts and butylhydroxyanisole. Long-term research by the All-Russian Research Institute of Dairy Industry,

since the 70th of the last century, as there are numerous publications and scientific reports, the introduction of the above-mentioned antioxidants is ineffective with regard to whole milk powder, in addition, they are synthetic antioxidants. In the last decade, both in Russia and abroad, natural antioxidants are preferred. Such an antioxidant is currently recognized as dihydroquercetin (DHQ).

DHQ is a biologically active substance of plant origin of domestic production and belongs to the group of bioflavonoids - flavananol. As a result of many years of research, the organic part of Siberian and Dahurian larch wood, which grows in Western and Eastern Siberia, and also in the Far East, is recognized as an environmentally friendly plant raw material for industrial production in Russia. Currently, only the Russian Federation is the only producer of DHQ in the world on an industrial scale from larch wood. The capacities of its production, which do not depend on seasonality, make it possible to meet the needs of chemical, pharmaceutical and food enterprises, both in Russia and for export . DHQ is non-toxic, does not have allergic, embryotoxic, immunotoxic, or mutagenic properties, being characterized by high antioxidant activity, superior to quercetin, rutin, β-carotene and a number of synthetic antioxidants. In addition, dihydroquercetin is characterized by a wide spectrum of biological activity, has a capillary-strengthening, lipid-lowering (anti-sclerotic), radioprotective action, helps normalize microcirculation and improves the rheological properties of the blood. DHQ was investigated for all the necessary parameters required for the production of medicines and food additives in the 90s of the 20th century. Technical specifications were developed for it, a pharmacopoeia article providing for its use in medicines and dietary supplements was developed. The results obtained allowed to consider it not only as a pharmaceutical preparation, but also as a food additive for use in various branches of the food industry, both as an antioxidant and as a functional ingredient. DHQ was introduced in SanPiN 2.3.2.1293-03 «Hygienic requirements for the use of food additives» in the list of food additives and is classified as a class of antioxidants that do not have a harmful effect on human health when used for food production, and subsequently, in the same quality – in TR CU 029/2012. At the present time GOST 33504-2015 «Food additives. Dihydroquercetin. Specifications», which regulates the requirements for DHQ for its use in the food industry as an antioxidant. The use of DHQ in the technology of dairy canned food provides an opportunity to significantly increase their shelf life while preserving the original properties of the products [20].

CONCLUSION

Comparative analysis of quality indicators of traditional canned milk produced by the interstate standards in force in Russia, with the obligatory condition of compliance with technological requirements and sanitary and hygienic norms for their production, in relation to similar products manufactured according to international standards, has shown that they have a competitive indicator Quality and safety.

Using the existing production facilities for the development of high-quality dairy products due to the increase in the volume of processing of dairy raw materials and the current regulatory framework, in the near future in Russia it will be possible to implement a complete import substitution of these types of products.

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USE OF THE METHOD OF ELECTRON PARAMAGNETIC RESONANCE FOR DETERMINATION OF ABSORBED DOSES OF IONIZING RADIATION OF DIFFERENT TYPES OF MEAT AND FISH RAW MATERIALS

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Abstract: To determine the absorbed doses of ionizing radiation of food, it is recommended to use the method of electron paramagnetic resonance (EPR), but, at the same time, determination method improvement is necessary. The objective of the study is improvement of the technique of sample preparation and analysis of the obtained results by means of quantitative estimation of the absorbed doses of ionizing radiation on the basis of EPR spectrum parameters, case study is irradiated meat and fish. Bone tissue samples (BTS) of meat and fish were subject to irradiation processing with ionizing radiation in the following doses: 3, 9, 10, 12 kGy with UELR-10-10C2 linear electron accelerator with energy up to 10 MeV. Study of the irradiated samples was performed with the automated portable X-band EPR spectrometer of the brand Labrador Expert. The improved technique of preparation of bone tissue samples for analysis differs with the increased duration of drying - up to 24 hours, which allows to obtain the stable EPR spectra in multiple replications. Correlation dependence of EPR signal area increase and irradiation dose is determined: for beef it is 0.98, for pork it is 0.98, for poultry it is 0.996 and for fish it is 0.99. Strong statistical relationship between the irradiation dose and the absorbed dose is determined: for beef it is 0.94, for pork it is 0.94, for poultry it is 0.96 and for fish it is 0.94. Processing of EPR spectra of meat and fish BTS by means of amplitude calculation and EPR peak width and area allows to determine the absorbed dose with a high degree of confidence ($p \leq 0.05$). For bone tissue of pork, beef, poultry and fish, irradiated with the dose of 12 kGy, amplitude is $4.8 \cdot 10^{-4}$, $5.08 \cdot 10^{-4}$, $1.11 \cdot 10^{-4}$ and $3.44 \cdot 10^{-5}$ p.u., peak area is – $6.73 \cdot 10^{-3}$, $6.94 \cdot 10^{-3}$, $3.68 \cdot 10^{-3}$ and $3.07 \cdot 10^{-3}$.

Keywords: Ionizing radiation, bone tissue samples, meat, fish, irradiation dose

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INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO), during the entire supply chain from agricultural raw materials processing and food production to retail outlets, quantitative and qualitative losses of food are significant, and their reduction is in priority for enterprises of food and processing industry [1].

According to the data of 2016, in Russia cooled products have a lead in the total amount of production: beef has a percentage of 80.3% in the total amount of production, pork - 94.2%, poultry has 64.1% and fish - 22.3%. The mentioned food products are perishable [2, 3], and their losses during storage are 0.1–0.2% in the total amount of production. In this regard, improvement of traditional methods and development of new ones of meat and fish preservation is a relevant issue of scientific researches and practical recommendations.

Herewith, processing with ionizing radiation of agricultural raw materials and food may be one of the most modern and promising methods of preservation. The ionizing radiation sources are: γ-emitting radionuclides ^{60}Co or ^{137}Cs, X-rays with radiation energy of or below 5 MeV and electron beams with radiation energy of or below 10 MeV.

The analysis of literature suggests that low doses (0.25–0.5 kGy) of ionizing radiation inhibit the growth of mesophilic aerobic, facultative aerobic microorganisms, coliform bacteria, fungi and yeast [4]; the dose of 3 kGy of meat and meat products prolongs their shelf life to 14 days at a storage temperature of 0–3°C; the dose of ≥ 4.5 kGy reduces microbiological content [5–7], but leads to formation of radiolysis products, reduction of fatty acids and thiamine (B_1) concentration and pH displacement to acid medium, which leads to excessive oxidation and appearance of undesirable flavour in fish [8].

The most important advantages of food irradiation processing over the traditional methods are significant increase in their shelf life, as well as low energy and money costs [9, 10].

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At the same time, there is no consensus among scientists over the recommended doses of meat and fish irradiation, as well as over justification for large-scale use of irradiation processing of food products and food raw materials.

Despite this fact, since 1992 more than 60 countries allowed processing with ionizing radiation of meat and fish with a maximum dose of absorbed radiation of 10 kGy - it is a safe dose, determined by the decision of the joint Committee of Experts of FAO/International Atomic Energy Agency (IAEA)/World Health Organization (WHO). Irradiated food products are identified by marking with the Radura symbol.

Scientists found that under irradiation free radicals and radiotoxins may be formed in the meat bone [11, 12]. To identify and establish the fact of irradiation of food raw materials and food products, the method of electron paramagnetic resonance is used, which allows to establish only the fact of irradiation/non-irradiation of meat and meat products, containing bone tissue, but not the irradiation dose. In this regard, improvement of the technique of sample preparation and analysis of the obtained results for determination of the absorbed dose is opportune and relevant. In practice the method of electron paramagnetic resonance is used. The authors of the sources [13, 14] state that the establishment of the fact of irradiation with EPR-method is possible due to the presence of long-lasting free radicals, especially of the radical anions CO_2^-, CO_3^{3-}, SO_2^- and SO_3^-. The authors of the source [15] used EPR for detection of irradiation in the bones of agricultural animals and poultry, fish and mollusc shell, and found that the EPR signals are stable in mammal bones and mollusc shells.

Carrying out the research on irradiated seafood and poultry in different ways (thermoluminescence, radioluminescence and electron paramagnetic resonance methods), the author of the source [16] argues that these methods are comparable, but the use of the EPR method is more effective for solids.

The experimental studies, mentioned in the source [15], show that in bone tissue of irradiated fish the intensity of the radiation-induced EPR signal increases at the absorbed dose, as well as non-linear relationship between radiation dose and the height of the EPR signal is determined. The same data were obtained by the authors of this source.

EPR spectroscopy is as follows: under the influence of ionizing radiation there is a chain reaction of excitation of molecules with the appearance of highly active free radicals, and the analytic signal appears - EPR spectrum, which is recorded with spectrometer.

It is stated that the electron paramagnetic resonance method allows to compare the spectra before and after irradiation of poultry bone tissue samples. The irradiated bone tissue sample has a pronounced spectrum [17].

A number of researchers found that the intensity of the EPR signal is maintained over time. The authors of the source [18] note that the radiation-induced EPR signal can be detected after 60 days. The authors of the sources [10, 19] found that after 2 years since irradiation, 44–75% of the bone tissue samples retain the original EPR spectrum.

The objective of the study is improvement of the technique of sample preparation and analysis of the obtained results by means of quantitative estimation of the absorbed doses of ionizing radiation on the basis of EPR spectrum parameters, case study is irradiated meat and fish.

OBJECTS AND METHODS OF STUDY

Cooled (0- (+2)°C) samples are taken for irradiation (primary sample):

– of beef and pork with a bone from the carcase shoulder after 48 hours since slaughter, with a mass of 2 kg;

– of chicken meat with a bone with a mass of 1.4 kg;

– of fish (Cyprinus carpio) with a mass of 1.5 kg.

To determine the irradiation absorbed dose, fish and meat samples were subject to irradiation processing with the following doses: 3, 9, 10, 12 kGy in the Centre of Radiation Sterilization of the Ural Federal University named after the first President of Russia B.N. Yeltsin with UELR-10-10C2 linear electron accelerator (Russia) with energy up to 10 MeV, designed for irradiation of food products and medical instruments. The main characteristics of the electron beam are indicated in Table 1. To control the dose after irradiation, the method of photospectroscopy was used by means of measuring optical density of the irradiated polymer film on the spectrophotometer at a wavelength of 512 nm.

We have improved the method of sample preparation of bone tissue samples (BTS) to determine the absorbed dose of ionizing radiation: fish tubular bones and spine are cleaned from muscle tissue, the middle part of the bone with a length of 3–10 cm is cut out, and then they are fully cleaned with a scalpel from the remnants of meat, tendons, membranes and bone marrow. The bone is washed with distilled water and dried with filter paper. Then it is dried in a dryer at a temperature of 39–40°C during 24–30 hours until the residual moisture of 3–4 %, cooled, incubated at a room temperature during 30 40 minutes and crushed to the size of separate fragments up to 0.5x0.5x0.5 mm with a total mass of at least 0.05 g.

Table 1. Parameters of the beam of accelerated electrons of UELR-10-10C2 linear electron accelerator

Parameter	Characteristic
Maximum energy of accelerated electrons, MeV	10
Maximum average current of dumped electron beam, mA	1
Adjustment range of electron energy, MeV	8–10
Frequency of electronic current impulses sequence, 1/s	50–240
Maximum size of irradiation field at a distance of 100 mm from exhaust foil, mm	600×20
Uniformity of irradiation field along the reamer length on the surface of the irradiated cases, %	± 5
Frequency of electron beam scanning, Hz	1–3

Table 2. Main technical characteristics of EPR spectrometer

Technical characteristics	Value
Sensitivity, spin/0.1 mT, up to	1×10^{11}
Signal channel frequency of microwave frequency, GHz	9.2
Maximum capacity of microwave frequency, mW	50
Constant magnetic field induction, T	0.328 ÷ 0.03
Magnetic field modulation frequency, Hz	2–12200
Magnetic field modulation amplitude, mT	4.8–0.001
Magnetic field absolute error, mT, up to	0.05

The mentioned technique of BTS preparation for research differs from the existing one with an increase in drying duration up to 24–30 hours, which allows to obtain more stable EPR spectra and, consequently, the higher reliability of the results at a lower error of the received data.

BTS are weighed in grams with an accuracy of the third decimal digits and are placed in marked glass phials. EPR spectrum is determined immediately after the preparation of the samples under research.

The choice of the dosimetry system (equipment) for determination of the absorbed dose is associated with the fact that one of the most effective and practicable methods of investigation and identification of radicals, induced by radiation, is electron paramagnetic resonance spectroscopy. Bone tissue density, structure and composition are heterogeneous, the variations of the absorbed dose differ on the surface, inside and in different parts of the sample, therefore the use of precisely the EPR method of the average sample allows to obtain reliable and repeatable results. EPR spectrometry was performed with the automated portable X-band EPR spectrometer of the brand Labrador Expert (Russia), which is the working dosimeter.

The main technical characteristics of EPR spectrometer are indicated in Table 2.

Quartz vessel with a height of 10.0 ± 0.5 mm was filled with BTS; then BTS were placed in the resonator operating area at a set fixed depth. BTS irradiation was performed at a temperature of 18–22°C indoors, atmospheric pressure of 746–748 mm Hg and air humidity of 45–59%. Studies were performed in ten-fold replication at the irradiation frequency of 9200 MHz, in the range of magnetic field from 3000 to 3500 Gs with selection of optimum values of conversion time, modulation amplitude and gain. To normalize the signal-to-noise index, microwave frequency capacity was set between 4 and 6 dBm. Reference sample (high-stability reference) was used for comparison of signals: the number of paramagnetic centres (PC) on the basis of manganese oxide (with concentration of paramagnetic centres of $5.9\cdot10^{14}$ spin/MT). As control samples, BTS of non-irradiated products were used, in which no EPR spectra were fixed.

Measurement and processing of EPR spectra was carried out using special computer programs to the EPR spectrometer, which allowed to set the parameters of the EPR spectrum for each sample of bone tissue. EPR spectrum parameters were shown in the automatic mode on the computer screen. The following parameters of radiation signal were determined: g-factor; amplitude; signal width and area. The same parameters with the same units of measurement are determined on the German spectrometers Bruker, which allows to compare the results of measurements.

In the experiment the value, used for assessment of the degree of ionizing radiation impact on the BTS under study (the value is assigned in radiation process), was considered as radiation dose; energy of ionizing radiation, absorbed by irradiated BTS (was determined according to formula (1)), was considered as absorbed dose.

Formula (1) was used for determination of the absorbed dose of bone tissue samples.

$$D = [NPC \cdot L_O\, I\, (M \cdot L_M)]\, 10^{-15}, \qquad (1)$$

where NPC is the number of paramagnetic centres, which corresponds to the 3rd component of the EPR spectrum of the reference sample; L_O is the arithmetic mean of the EPR signal intensity of the bone tissue sample, p.u.; M is the mass of the bone tissue sample, g; L_M is the EPR signal intensity of the 3rd component of the reference sample, p.u.

The research results are analysed with the method of analysis of variance with the use of Student coefficient.

RESULTS AND DISCUSSION

After irradiation of pork samples with the dose of 3 kGy (g-factor 2.0047 ± 0.0002) in the field range of 3260–3285 Gs, peak amplitude is $(3.06 \pm 0.03)\cdot10^{-5}$ p.u., signal width is 11.66 ± 0.63 Gs and peak area is $(5.75 \pm 0.04)\cdot10^{-4}$ p.u. ($p \leq 0.05$); with the dose of 9 kGy (g-factor 2.0035 ± 0.0001) at width signal decrease up to 9.81 ± 0.15 Gs or by 15.8%, there is an increase in peak amplitude either up to $(2.15 \pm 0.032)\cdot10^{-4}$ p.u. or 7.0-fold-increase, in signal area there is an increase either up to $(3.84 \pm 0.21)\cdot10^{-3}$ p.u. or 6.7-fold-increase ($p \leq 0{,}05$); with the dose of 10 kGy (g-factor 2.0025 ± 0.0001) signal amplitude increased by 24.2% up to $(2.67 \pm 0.02)\cdot10^{-4}$ p.u. in comparison with the pork samples, irradiated with the dose of 9 kGy, and peak area increased by 11.1% up to $(4.27 \pm 0.01)\cdot10^{-3}$ p.u.; peak width decrease by 9.8% up to 8.85 ± 0.02 Gs ($p \leq 0.05$) was indicated.

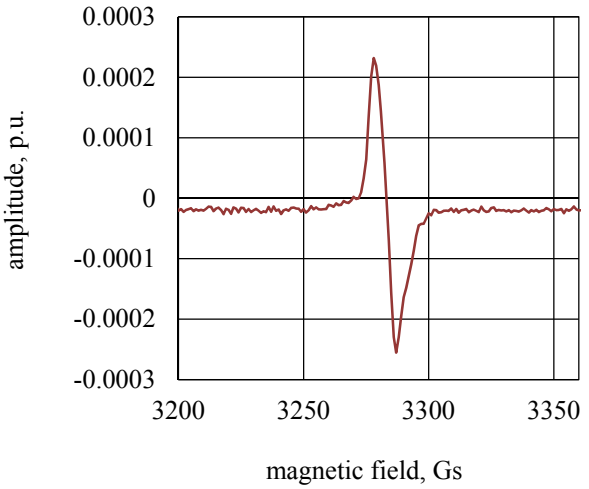

Fig. 1. BTS spectrum (pork), irradiated with the dose of 12 kGy (g-factor 2.0002 ± 0.0001).

Pork samples irradiation with the dose of 12 kGy (Fig. 1) led to amplitude increase by 79.8% up to $(4.8 \pm 0.01)\cdot10^{-4}$ p.u. and peak area increase by 57.6% up to $(6.73 \pm 0.29)\cdot10^{-3}$ p.u. at signal width decrease by 5.3% up to 8.38 ± 0.02 Gs ($p \leq 0.05$) in comparison with pork, irradiated with the dose of 10 kGy.

Beef samples, irradiated with the dose of 3 kGy (g-factor 2.0028 ± 0.0001) in the field range of 3260–3300 Gs, had peak amplitude of $(2.78 \pm 0.04)\cdot10^{-5}$ p.u. and signal width of 10.51 ± 0.01 Gs. Peak area is $(5.14 \pm 0.05)\cdot10^{-4}$ p.u. ($p \leq 0.05$).

After beef samples irradiation with the dose of 9 kGy (g-factor 2.0027 ± 0.0001), there is a 8.1-fold-increase in peak amplitude up to $(2.24 \pm 0.03)\cdot10^{-4}$ p.u. at width decrease by 18.7% up to 8.54 ± 0.18 Gs and 7.7-fold-increase of peak area up to $(3.95 \pm 0.03)\cdot10^{-3}$ p.u. ($p \leq 0.05$).

Analysis of beef samples, irradiated with the dose of 10 kGy (g-factor 2.0027 ± 0.0001), showed that at signal amplitude increase by 22.3% up to $(2.74 \pm 0.08)\cdot10^{-4}$ p.u. and width by 0.8% up to 8.47 ± 0.08 Gs, signal area increase is indicated by 8.9% up to $(4.3 \pm 0.01)\cdot10^{-3}$ p.u. ($p \leq 0.05$) in comparison with the beef samples, irradiated with the dose of 9 kGy.

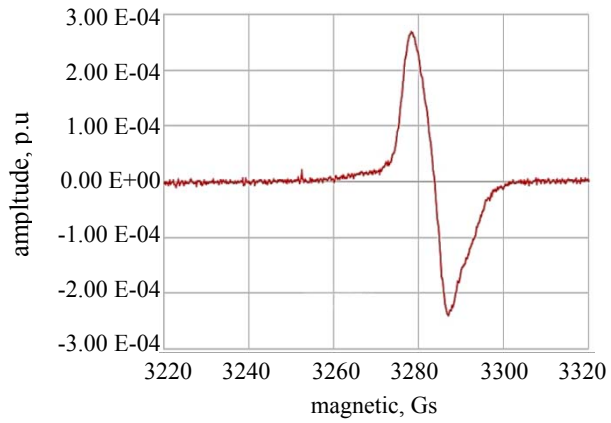

Fig. 2. BTS spectrum (beef), irradiated with the dose of 12 kGy (g-factor 2.0026 ± 0.0001).

Beef BTS irradiation with the dose of 12 kGy (Fig. 2) leads to 1.9-fold-increase in EPR signal peak amplitude up to $(5.08 \pm 0.01)\cdot10^{-4}$ p.u. and peak area increase by 61.4% up to $(6.94 \pm 0.02)\cdot10^{-3}$ p.u. at signal width decrease by 1.8% up to (8.32 ± 0.12) Gs ($p \leq 0.05$) in comparison with beef, irradiated with the dose of 10 kGy.

After poultry bone tissue samples irradiation with the dose of 3 kGy (g-factor 2.0046 ± 0.0001) in the field range of 3260–3290 Gs, peak amplitude is $(1.67 \pm 0.07)\cdot10^{-4}$ p.u., signal width is 10.54 ± 0.40 Gs and peak area is $(4.30 \pm 0.04)\cdot10^{-3}$ p.u. ($p \leq 0.05$); with the dose of 9 kGy (g-factor 2.0048 ± 0.0001) there is a change in EPR signals parameters in comparison with the poultry bone tissue samples, irradiated with the dose of 3 kGy: 5.9-fold-increase in peak amplitude up to $(9.92 \pm 0.01)\cdot10^{-5}$ p.u. at signal width increase by 14.9% up to 12.01 ± 0.03 Gs and 6.7-fold-increase in peak area up to $(2.88 \pm 0.11)\cdot10^{-3}$ p.u. ($p \leq 0.05$); with the dose of 10 kGy (g-factor 2.0051 ± 0.0001) signal peak amplitude increases by 0.6% up to $(9.98 \pm 0.11)\cdot10^{-5}$ p.u. and peak area increases by 6.9% up to $(3.08 \pm 0.01)\cdot10^{-3}$ p.u. in comparison with the poultry BTS, irradiated with the dose of 9 kGy; peak width increase is indicated by 6.7% up to 12.09 ± 0.05 Gs ($p \leq 0.05$).

Poultry BTS irradiation with the dose of 12 kGy (Fig. 3) leads to 1.1-fold-increase in peak amplitude up to $(1.11 \pm 0.01)\cdot10^{-4}$ p.u. and peak area increase by 20% up to $(3.68 \pm 0.04)\cdot10^{-3}$ p.u. at signal width increase by 1.7% up to 12.29 ± 0.01 Gs ($p \leq 0.05$) in comparison with the poultry BTS, irradiated with the dose of 10 kGy.

After fish samples irradiation with the dose of 3 kGy (g-factor 2.0047±0.0001) in the field range of 3260–3290 Gs, peak amplitude was $(6.29 \pm 0.01)\cdot10^{-6}$ p.u., signal width was 9.81 ± 0.02 Gs and peak area was $(4.07 \pm 0.04)\cdot10^{-4}$ p.u. ($p \leq 0.05$).

Fish samples irradiation with the dose of 9 kGy (g-factor 2.0032 ± 0.0001) leads to change in EPR spectrum parameters: 5.1-fold-increase in peak amplitude is indicated up to $(3.19 \pm 0.01)\cdot10^{-5}$ p.u., as well as increase in width by 38.1% up to 13.55 ± 0.01 Gs and 6.26-fold-increase in peak area up to $(2.55 \pm 0.01)\cdot10^{-3}$ p.u. ($p \leq 0.05$) in comparison with the fish BTS, irradiated with the dose of 3 kGy.

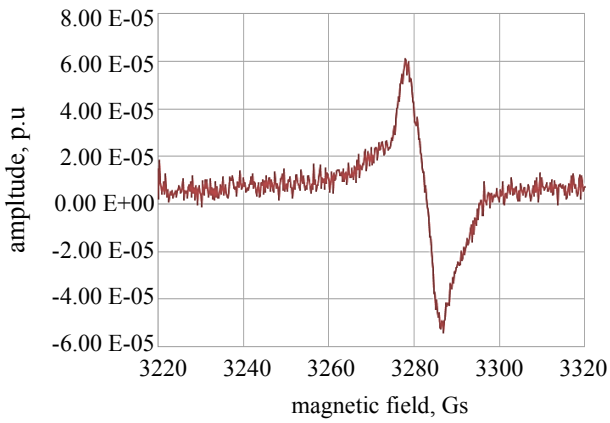

Fig. 3. Poultry BTS spectrum, irradiated with the dose of 12 kGy (g-factor 2.0029 ± 0.0001)

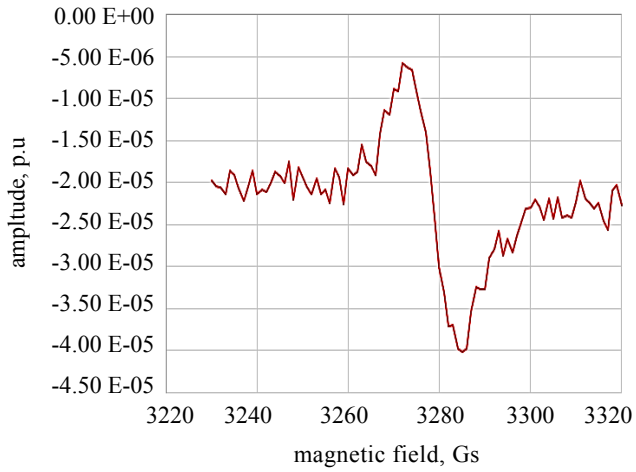

Fig. 4. Fish BTS spectrum, irradiated with the dose of 12 kGy (g-factor 2.0047 ± 0.0001).

As a result of irradiation of fish BTS with the dose of 10 kGy (g-factor 2.0047 ± 0.0001), peak amplitude increase is indicated by 1.9% up to $(3.25 \pm 0.01)\cdot10^{-5}$ p.u., as well as increase in signal width by 1.7% up to 13.78 ± 0.01 Gs and in peak area by 2.7% up to $(2.62 \pm 0.01)\cdot10^{-3}$ p.u. ($p \leq 0.05$) in comparison with fish BTS, irradiated with the dose of 9 kGy.

Increase in all parameters is indicated in fish BTS, irradiated with the dose of 12 kGy (Fig. 4) in comparison with fish BTS, irradiated with the dose of 10 kGy: of peak amplitude by 5.8% up to $(3.44 \pm 0.07)\cdot10^{-5}$ p.u., of signal width by 7.0% up to 14.74 ± 0.05 Gs, of peak area by 17.2% up to $(3.07 \pm 0.01)\cdot10^{-3}$ p.u. ($p \leq 0.05$).

The results of research of samples of bone tissue of different animals, poultry and fish after processing with ionizing radiation with different doses (3, 9, 10, and 12 kGy) showed different ability to absorb ionizing radiation: cattle and pork bone tissue is the most susceptible of absorption.

It is stated that the change in EPR signals amplitude of bone tissue samples does not depend linearly on irradiation dose (Fig. 5). EPR signals width has a clear dependence on irradiation dose and decreases with its increase in pork and beef BTS. EPR signals width increases in poultry and fish BTS (Fig. 6): a high degree is identified of correlation dependence of EPR signals width change and irradiation dose: for beef it is 0.97, for pork - 0.99, for poultry - 0.98 and for fish - 0.99.

EPR signals area of pork and beef BTS increases significantly with the increase in irradiation dose of 9 kGy. A high correlation degree is identified for all samples: for beef and pork it is 0.98, for fish and poultry - 0.99 and 0.996 respectively (Fig. 7).

At increase in the irradiation dose the absorbed dose has a clear tendency towards an increase in all BTS (Fig. 8). For pork the multiple correlation coefficient (absorbed dose-irradiation dose-signal area) is 0.98; for beef - 0.99; for poultry - 0.94; for fish - 0.96. Dependence of change in the absorbed dose and irradiation dose, as well as signal area, for pork, beef, poultry and fish BTS, is indicated in Figures 9–12.

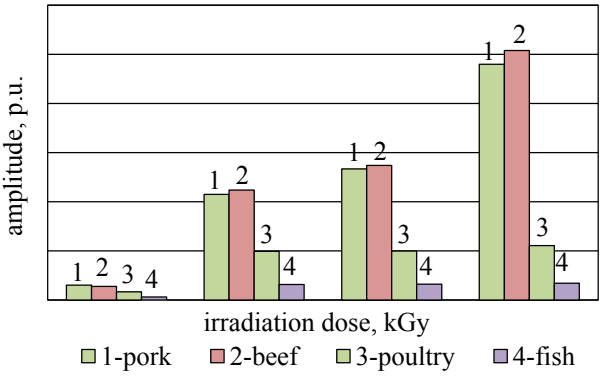

Fig. 5. EPR signals amplitude of pork, beef, poultry and fish BTS, irradiated with different doses (3, 9, 10, 12 kGy).

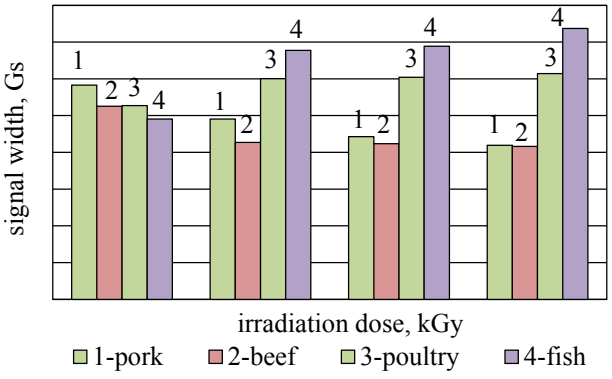

Fig. 6. EPR signals width of pork, beef, poultry and fish BTS, irradiated with different doses (3, 9, 10, 12 kGy).

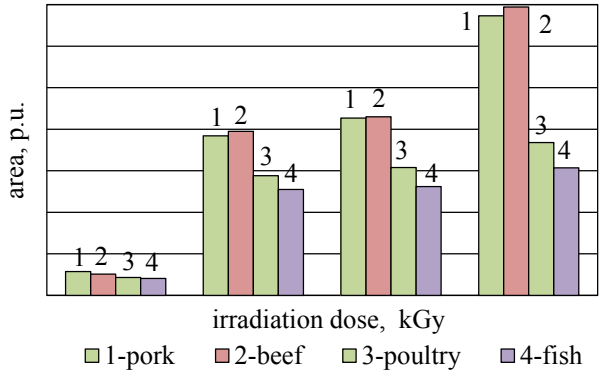

Fig. 7. EPR signals area of pork, beef, poultry and fish BTS, irradiated with different doses (3, 9, 10, 12 kGy).

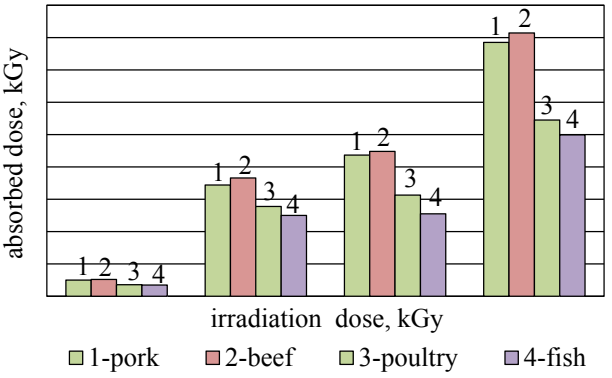

Fig. 8. Absorbed dose of pork, beef, poultry and fish BTS, irradiated with different doses (3, 9, 10, 12 kGy).

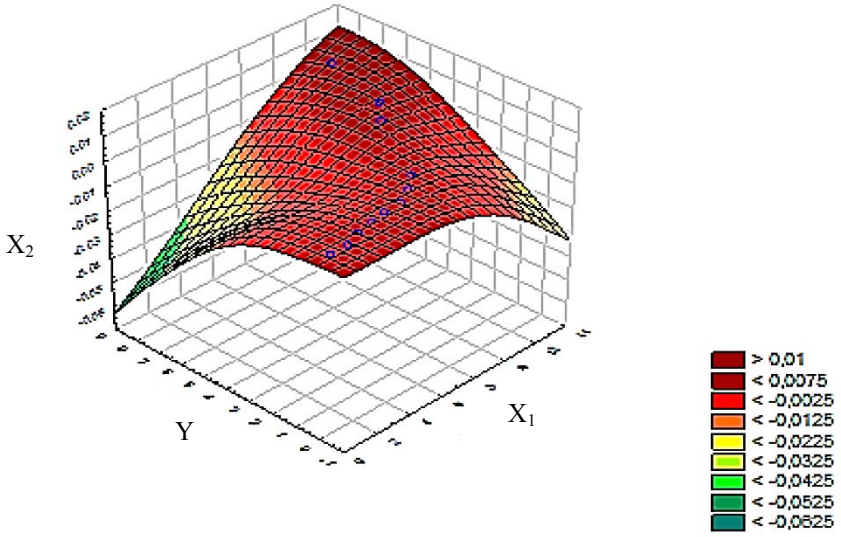

Fig. 9. Dependence of change in the absorbed dose (Y) and irradiation dose ($X_{1)}$, as well as signal area (X_2) for pork BTS ($Y = -0.7877+0.24079X_1+745.6338X_2$).

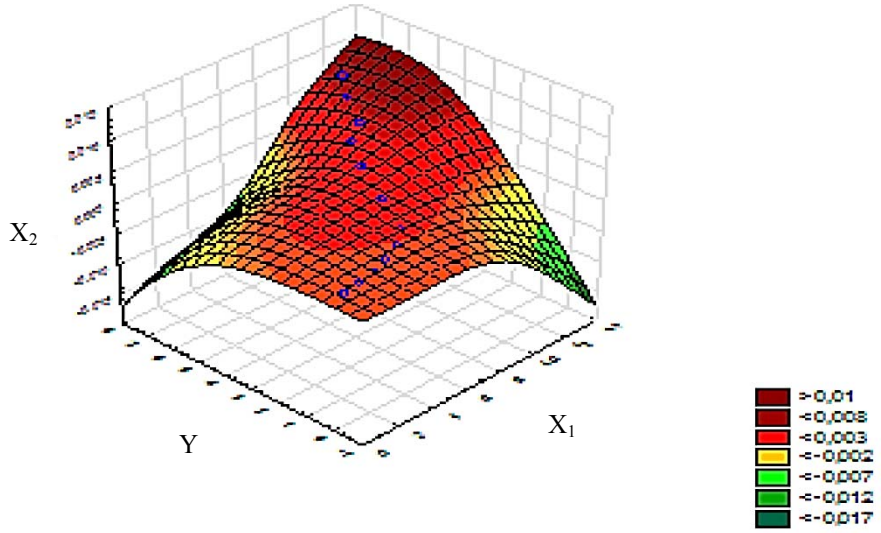

Fig. 10. Dependence of change in the absorbed dose (Y) and irradiation dose ($X_{1)}$, as well as signal area (X_2) for beef BTS ($Y = -0.33131+0.152599X_1+847.1903X_2$).

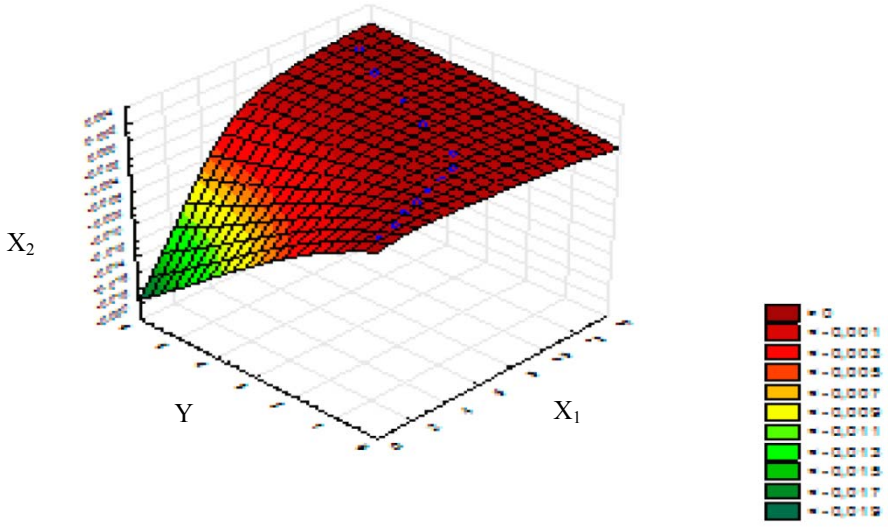

Fig. 11. Dependence of change in the absorbed dose (Y) and irradiation dose ($X_{1)}$, as well as signal area (X_2) for poultry BTS ($Y=-1.81791+0.222756X_1+3551.512X_2$).

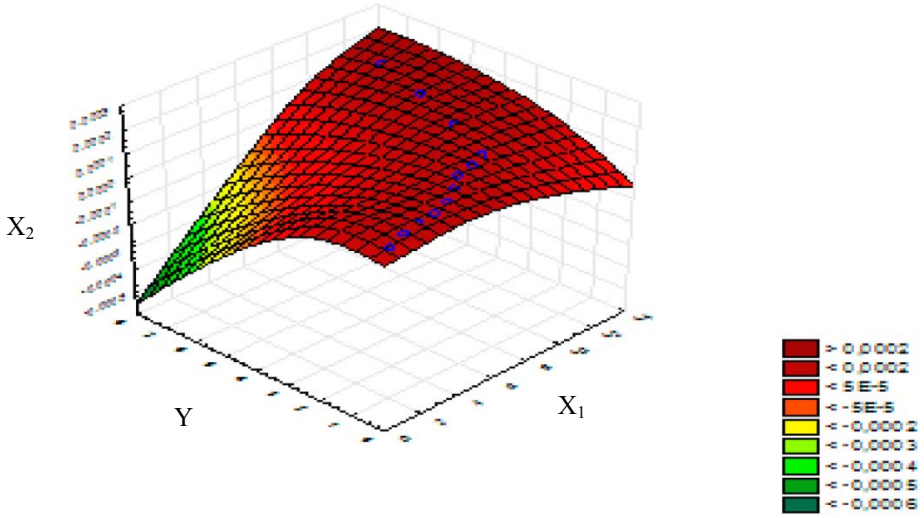

Fig. 12. Dependence of change in the absorbed dose (Y) and irradiation dose ($X_{1)}$, as well as signal area (X_2) for fish BTS ($Y = -13.7797+0.121923X_1+105588.3X_2$).

As a result of the conducted researches it is stated that at increase in irradiation dose of pork BTS from 3 kGy to 12 kGy, there is a 15.7-fold-increase in amplitude and 11.7-fold-increase in area, EPR signal width reduces up to 28.1% ($p \leq 0.05$); in beef BTS the parameters change in the following way: amplitude increases 18.3 times, area - 13.5 times, peak width by 20.8% ($p \leq 0.05$). In poultry BTS there was an increase in all EPR signal parameters: amplitude increases 6.7 times, area - 8.6 times, peak width by 16.7% ($p \leq 0.05$). Similarly there was a change in fish BTS parameters: respectively amplitude increased 5.5 times, peak area - 7.5 times, peak width by 50.3% ($p \leq 0.05$).

Experiments showed that despite processing bone tissue samples with identical doses of ionizing radiation, the absorbed dose depends on the type of vertebrates, the tissue structure of the sample, water-holding capacity and on other factors. Pork and beef samples most reacted to the change in irradiation dose by all studied parameters. Thus, at increase in irradiation dose amplitude increases, peak width decreases and, consequently, area increases.

Stable correlation dependence of EPR signal area and irradiation dose is fixed: for beef and pork it is 0.99, for poultry and fish - 0.996 and 0.99 respectively (the strength degree of statistical relationship according to Cheddock is very high). The absorbed dose at increase in irradiation dose increases substantially, which is proved by the area under the EPR spectrum signal line. Correlation coefficient by the absorbed dose is high. It is: 0.94 for beef, pork and fish and 0.96 for poultry.

As a result of the conducted research, the technique of sample preparation is improved, which differs with the increase in duration of bone tissue samples drying up to 24 hours, which allows to obtain the stable EPR spectra in multiple replications. The technique is offered of processing of the obtained results of the irradiated meat and fish EPR spectrum, which allows to determine the absorbed dose of ionizing radiation (permissible variation of signal amplitude, width and area at the level of ± 4% at D›1):

– for pork bone tissue, irradiated with the dose of 3 kGy, amplitude is $3.06 \cdot 10^{-5}$ p.u., width is 11.66 Gs, peak area is $5.75 \cdot 10^{-4}$ p.u.; at the dose of 9 kGy amplitude is $2.15 \cdot 10^{-4}$ p.u., width is 9.81 Gs, peak area is $3.84 \cdot 10^{-3}$ p.u.; at the dose of 10 kGy amplitude is $2.67 \cdot 10^{-4}$ p.u., width is 8.85 Gs, peak area is $4.27 \cdot 10^{-3}$ p.u.; at the dose of 12 kGy amplitude is $4.8 \cdot 10^{-4}$ p.u., width is 8.38 Gs, peak area is $6.73 \cdot 10^{-3}$ p.u.;

– for beef bone tissue, irradiated with the dose of 3 kGy, amplitude is $2.78 \cdot 10^{-5}$ p.u., width is 10.51 Gs, peak area is $5.14 \cdot 10^{-4}$ p.u.; at the dose of 9 kGy amplitude is $2.24 \cdot 10^{-4}$ p.u., width is 8.54 Gs, peak area is $3.95 \cdot 10^{-3}$ p.u.; at the dose of 10 kGy amplitude is $2.74 \cdot 10^{-4}$ p.u., width is 8.47 Gs, peak area is $4.3 \cdot 10^{-3}$ p.u.; at the dose of 12 kGy amplitude is $5.08 \cdot 10^{-4}$ p.u., width is 8.32 Gs, peak area is $6.94 \cdot 10^{-3}$ p.u.;

– for poultry bone tissue, irradiated with the dose of 3 kGy, amplitude is $1.67 \cdot 10^{-5}$ p.u., width is 10.54 Gs, peak area is $4.3 \cdot 10^{-4}$ p.u.; at the dose of 9 kGy amplitude is $9.92 \cdot 10^{-5}$ p.u., width is 12.01 Gs, peak area is $2.88 \cdot 10^{-3}$ p.u.; at the dose of 10 kGy amplitude is $9.98 \cdot 10^{-5}$ p.u., width is 12.09 Gs, peak area is $3.08 \cdot 10^{-3}$ p.u.; at the dose of 12 kGy amplitude is $1.11 \cdot 10^{-4}$ p.u., width is 12.29 Gs, peak area is $3.68 \cdot 10^{-3}$ p.u.;

– for fish bone tissue, irradiated with the dose of 3 kGy, amplitude is $6.29 \cdot 10^{-6}$ p.u., width is 9.81 Gs, peak area is $4.07 \cdot 10^{-4}$ p.u.; at the dose of 9 kGy amplitude is $3.19 \cdot 10^{-5}$ p.u., width is 13.55 Gs, peak area is $2.55 \cdot 10^{-3}$ p.u.; at the dose of 10 kGy amplitude is $3.25 \cdot 10^{-5}$ p.u., width is 13.78 Gs, peak area is $2.62 \cdot 10^{-3}$ p.u.; at the dose of 12 kGy amplitude is $3.44 \cdot 10^{-5}$ p.u., width is 14.74 Gs, peak area is $3.07 \cdot 10^{-3}$ p.u.

The obtained results have an important significance for arrangement of the regulatory system in the framework of updating the requirements of the European and International standards for safety and quality assurance of food.

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FOOD HYGIENE

THE POTENTIAL OF PINE NUT AS A COMPONENT OF SPORT NUTRITION

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Abstract: The development of personified medicine, aimed at prevention, makes relevant any development of foodstuffs with improved quality characteristics, including by addition of natural plant ingredients. Nuts are a high-calorie food with a high protein and fat content, including pine nuts. They have a positive impact on human health and attract the attention of researchers due to their anti-inflammatory and antioxidant characteristics. The study objects were samples of a nut kernel of *Pinus sibirica*, growing in the Kemerovo Oblast. In the *Pinus sibirica* nut samples the protein composition (15–16%) is not lower by content than in many other kinds of nuts; as for the fat content (62–67%), the greatest one belongs to linolenic acid; oleinic and linolenic acids are the next by content. Palmitic acid dominates among the saturated fatty acids. The studied nut samples exceeded the ones of the Tuva Republic, the Far East region and China by many indicators of nutrition value. By the protein and fat content of the studied nut samples are comparable with the ones of the Far East region. By the protein content they exceed the nut samples of China (15%); by the fat content - the ones of Tuva (40%). It is stated that by chemical and microbiological parameters the *Pinus sibirica* nuts, growing in the Kemerovo Oblast, satisfy the requirements of the current normative documents, they do not have any toxic effect on a human, and their nutrition value can be considered as a promising ingredient for various food products, including sport nutrition and special food.

Keywords: *Pinus sibirica*, nut kernel, chemical compound, nutrition value, sport nutrition

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INTRODUCTION

On the domestic market of functional food, sport nutrition and dietary supplements of plant origin mainly products are represented, based on ingredients, supplied by foreign producers (India, South-East Asia and southern Europe). At the same time, the Russian Federation, with its potentially large raw material base for the production of functional food and dietary supplements of plant origin, occupies a small volume of this market segment. At the moment, the requirements of the domestic market in food additives and functional food ingredients are satisfied through import by 75–80%.

A promising source of biologically active substances is wild-growing raw materials. In the Siberian Federal District 6.5% of wild-growing products are implemented, in other Russian regions - 18.5%. The share of export in total sales of wild-growing products of Tomsk companies is 36.5%, the main export (80%) goes to China.

In the Siberian Federal District on the territory of 5114800 km², covered with forests and marshes, considerable biological resources are located. These include first of all wild plants (berries, mushrooms and nuts). Nuts are a high-calorie food with a high content of fat, most of which is represented by unsaturated fatty acids [1]. Nuts also contain a significant amount of fiber, folic acid, minerals and antioxidative substances [2, 3, 4]. The interest of researchers to nuts is due to their nutrition content. Influence of nuts on health, cardiovascular diseases risk mitigation [5], high cholesterol [6, 7] and diabetes [8, 9] was studied. Nuts are also often considered as a source of selenium [10–14].

The most studied nuts are almonds, hazelnuts, walnuts, pistachios and cashews, however, only few researchers have examined the characteristics of pine nuts. Pine nuts differ with a high content of protein, unsaturated fatty acids and dietary fiber, low-molecular carbohydrates, vitamins (folic acid, niacin, tocopherol, B6 and B2), minerals, phytoesterols and polyphenols [15–18]. There are few works, in which characteristics of *Pinus sibirica du tour* nuts of the Kemerovo Oblast have been studied. The objective of this paper is to study the potential of seeds of *Pinus sibirica* of the

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Kemerovo Oblast as a component of functional food for people with a high physical activity.

OBJECTS AND METHODS OF STUDY

Objects of study were the samples of Pinus sibirica nut kernel, growing in the Kemerovo Oblast (Tashtagol region, crop of 2016 and 2017). The shell was preliminarily separated from the pine nut kernel, sample No. 1 is from the crop of 2016, sample No. 2 is from the crop of 2017.

Moisture mass fraction was determined according to GOST 31852-2012 (ISO 6756:1984) "Peeled pine nuts".

A sample was placed in the distillation apparatus flask; a sufficient amount of distillate (toluene, xylene) was added to it, so that to fully cover the sample, taken for analysis. The flask content was mixed with slewing. The apparatus was assembled, the receiver was filled with a solvent by pouring it through the cooler until it started to overflow into the distillation flask. Cold water was turned on.

The flask was heated until the distillation speed reached approximately four drops per second. Heating was going on until water began to collect in the graduated part of the receiver.

Condensation was removed from the cooler from time to time during distillation. 5 cm^3 of the solvent were used to clean the moisture, which deposited on the cooler or receiver walls. To separate water from the solvent, a copper spiral was put in the receiver and cooler, which was periodically moved up and down, thus causing deposition of water on the receiver bottom.

Distillation process was going on until the water level in the graduated receiver was permanent during 15 min, then heating was stopped. The receiver was immersed in water at room temperature at least for 15 min or until the solvent became transparent, then the water volume was measured with accuracy rate of 0.1 cm^3.

Moisture mass fraction in % was measured according to the formula:

$$X = V \cdot \rho \cdot 100 / M, \qquad (1)$$

where V is the volume of water, collected in the receiver with a graduated test tube, cm^3; ρ is the water density, ρ=1 g/cm^3; M is the mass of the sample, taken for analysis, g.

Protein mass fraction was measured by express-method of combustion according to Dumas using the express analyser Rapid N Cube (Elementar, Germany).

Protein fractional content was measured by ion-exchange chromatography using a chromatograph ARACUS.

Fat mass fraction was measured according to GOST [State Standard] 10857–64 Interstate Standard oily seeds. Oil content measuring method.

Kernel weighed amount, taken with consideration for oil content, was thoroughly minced and put in the weighed holder, prepared in advance. The holder was closed and put in the Soxhlet apparatus for extraction. To the extractor a clean flask was connected, which was preliminarily dried during 1 h at 100–105°C and weighed after cooling. Diethyl ether was poured to the extractor and connected with the cooler, after which extraction began.

After extraction ether was distilled and oil was dried in a dryer at a temperature of 100–105°C until the constant mass. The first weighting was carried out after 1–1.5 h, the next was in 30 minutes. In case of twice increase in mass, drying was stopped and the minimal mass was taken for measuring.

The fat content in % in kernels, freed from dirt, and dried, was measured according to the formula:

$$X = (m - m_1) \cdot 100 / m_2, \qquad (2)$$

where m is the flask mass with oil, g; m_1 is the mass of the empty flask, g; m_2 is the weighed amount of the dried seeds, g.

The fatty acid content was analyzed with gas-chromatography method using a gas-liquid mass-spectrometer GCMS-QP2010 Ultra (Shimadzu, Japan).

The ash mass fraction was measured according to GOST 26226–95 "Fodder, compound fodder, compound fodder raw materials. Crude ash measuring methods".

In a crucible, dried to a constant weight, the tested sample was placed with a mass of approximately 0.5–2.0 g (the amount of the tested ash should be at least 50 mg). The sample was put in the crucible without compression so that atmospheric oxygen flew to its lower layers. Up to half of the crucible was filled with the sample.

The crucible with the sample was weighed with the accuracy rate of 0.001 g; then it was placed in a cold furnace and temperature was increased up to 200–250°C (until smoke appeared, it is allowed to carry out preliminary combustion at the open door of a muffle, heated to dark red heat (525 ± 25)°C on an electric heater or gas burner, in a fume hood, avoiding ignition of the sample).

After the smoke exhalation stopped, the furnace temperature was adjusted to (525 ± 25)°C and the crucible with the sample was being annealed during 4–5 h. The absence of the coal particles and the ash uniform grey color indicated the complete ashing of the material.

The mass fraction of crude ash (X) in % in the test sample was measured according to the formula:

$$X = (m_2 - m_0) \cdot 100 / (m_1 - m_0), \qquad (3)$$

where m_0 is the crucible mass, g; m_1 is the mass of the crucible with the sample before ashing, g; m_2 is the mass of the crucible with ash, g;

The vitamin content was measured with a capillary electrophoresis method with the use of the system of capillary electrophoresis Kapel'-105 (Lumex, Russia); the method is based on the migration and separation of ionic forms of the analyzed components under the influence of electric field due to their different electrophoretic mobility, with the following registration at a wavelength of 200 nm.

The vitamin PP content was measured according to GOST R 50479–93. "Products of fruit and vegetable processing. The vitamin PP content measuring method".

The sample weighed amount with a mass of 1.0–10.0 g was ground in a porcelain mortar with 1.5 g of calcium hydroxide. Then the mortar content was transferred in portions in a conical flask with a capacity of 100 cm^3, washing away 50–60 cm^3 of water in small portions. The flask with the sample

was heated during 90 min on a boiling water bath, having closed preliminary the flask neck with a small funnel or a special glass insert stopper, and shaken periodically. After heating, the flask was cooled to room temperature. Then the hydrolysate volume was adjusted with water to 75 cm^3, stirred, cooled during 2 hours on an ice bath or placed in a refrigerator for the whole night. The cooled hydrolysate was filtered or centrifuged.

The filtrate volume of 25 cm^3 was placed in a cylinder with a capacity of 50 cm^3, 1–2 drops of phenolphthalein solution and sulfuric acid solution 2.5 mol/dm^3 were added by drops until discoloration.

8 test tubes or flasks with ground stoppers were used for carrying out the color reaction. In three tubes 5 cm^3 of the working standard solution of vitamin PP were added with a pipette. In 4 tubes 5 cm^3 of the obtained filtrate were added with a pipette, in the control tube 5 cm^3 of water instead of the filtrate were added. All tubes were closed with stoppers and heated on a water bath at a temperature of 48–52°C during 5–10 min. Then, in the tubes with a standard vitamin solution, in the tube with water and in two tubes with the test filtrate, 2 cm^3 of rodenberger were added from the burette in a fume hood.

All tubes were closed with stoppers, shaken and left on a water bath at a temperature of (50 ± 2)°C during 10 min. After 10 min all the tubes were cooled with water to room temperature and left for 10 min in a dark place, then 3 cm^3 of metol solution were added to each of them, they were shaken and left for 1 h in a dark place. Then the optical density of the solutions was measured with a spectrophotometer. Distilled water was a control solution.

Vitamin E content was measured according to GOST R 54634–2011 "Functional food. Vitamin E measuring method".

For carrying out the alkaline hydrolysis, 5–20 g of the analyzed sample were placed in a flat-bottomed flask with a capacity of 100–500 cm^3. 5–20 cm^3 of water were added to the dry material and heated on a water bath at a temperature of 60–70°C, stirring during 5 min. Then 50–150 cm^3 of ethyl rectified alcohol were added, 0.2–2.0 g of antioxidant (ascorbic acid, hydroquinone, butylhydroxytoluene) and 3–40 cm^3 of 50%-potassium hydroxide-solution, all these were then heated during 15–40 min on a water bath with a reflux condenser at a temperature of 80–100°C.

After hydrolysis, the flask content was rapidly cooled to (20 ± 5)°C and transferred in portions to the separatory funnel. The flask was rinsed with water, the volume of which is equal to the volume of the added ethyl alcohol, and water was poured into the same funnel. Tocopherols were extracted with diethyl ether, ethyl acetate, n-hexane and n-hexane with the addition of diethyl ether in a volume ratio of 1 : 1 during 2 min.

The extraction was repeated three or four times with the extractant portions of 50–100 cm^3. The combined extract was washed from alkali three or four times with water portions of 50–150 cm^3 until the alkaline wash water disappears (according to universal detector paper). To remove water, the extract was filtered through a filter with 2–5 g of anhydrous sodium sulfate. Then, the extract was evaporated to dryness with the use of a rotary evaporator and re-suspended in n-hexane.

The obtained solution was analyzed with a method of normal-phase high performance liquid chromatography (NP HPLC).

The macronutrient content (phosphorus, potassium, magnesium, manganese, iron, iodine) was measured with atomic absorption spectrophotometry. The analysed samples were transferred to the atomic state and the optical density of the atomic vapour of the determined element was measured in a certain spectral range. The element concentration was measured by the intensity of the light absorption by the atomic vapour of the determined element with a specific wavelength. To obtain the atomic vapour, a gas burner with spray was used. The light source was a lamp with a hollow cathode.

The micronutrient content was measured with a capillary electrophoresis method with the use of the system of capillary electrophoresis Kapel'-105 (Lumex, Russia), which is based on the separation of cations due to the differences in their electrophoretic mobility during migration in quartz capillary in the electrolyte under the action of electric field with the following registration of the difference of optical absorption by electrolyte and cations in the UV-area of the spectrum (wavelength is 254 nm).

Chemical and microbiological safety indicators. Measurement of mercury is according to GOST 26927–86 "Raw materials and foodstuffs. Mercury measuring method (with Amendment No. 1)".

Measurement of arsenic is according to GOST 26930–86 "Raw materials and foodstuffs. Arsenic measuring method (with Amendment No. 1)".

Measurement of lead is according to GOST 26932–86 "Raw materials and foodstuffs. Lead measuring method (with Amendment No. 1)".

Measurement of cadmium is according to GOST 26933–86 "Raw materials and foodstuffs. Cadmium measuring method (with Amendment No. 1)".

Measurement of pesticides is according to GOST 30349–96 "Fruits, vegetables and products of their processing. Methods of measuring of residual quantities of organochlorine pesticides".

Measurement of mycotoxin is according to GOST 30711–2001 "Foodstuffs. Methods of detection and determination of aflatoxins B(1) and M(1)".

The total number of yeast and mold fungi is in accordance with GOST 10444.12–2013 "Microbiology of foodstuffs and fodder. Methods of identification and calculation of the number of yeast and mold fungi (with Amendment)".

The number of coliform bacteria is according to GOST 31747–2012 "Foodstuffs. Methods of detection and determination of coliform bacteria".

The number of pathogenic microorganisms is according to GOST ISO 22118–2013 "Microbiology of foodstuffs and fodder. Polymerase chain reaction (PCR) for detection and quantitative accounting of pathogenic microorganisms in foodstuffs. Technical characteristics".

Statistical analysis. All repeated experiments were performed triply. Data processing was carried out with standard methods of mathematical statistics. The test of homogeneity of the obtained value selection was performed with the use of the Student criterion. Differences between means are considered significant when the confidence interval is smaller than 5% ($P \leq 0.05$).

Table 1. Chemical compound of pine nut samples

Parameter	Mass fraction, %	
	sample No. 1	sample No. 2
Moisture	5.20 ± 0.52	4.87 ± 0.49
Protein	15.15 ± 0.76	16.04 ± 0.80
Fats	62.10 ± 3.11	66.72 ± 3.34
Ash	2.51 ± 0.25	2.24 ± 0.22

RESULTS AND DISCUSSION

All nuts, including pine nuts, have a high fat and protein content, which determines their high energy value. A decreased risk of disorders of metabolic exchange and morbidity of diabetes of the 2nd type [19] are associated with the intake of protein of plant origin, such as nuts, legumes and grains. The results of determination of chemical compound of the pine nut kernel samples are presented in Table 1. Lipids are a predominant component of the pine nut kernel (62.10–66.72%). Also pine nuts, including our samples, differ with a high protein content and are second only to peanuts [20]. The moisture content in the nuts is up to 8.0%, which meets the requirements of GOST 31852-2012 (ISO 6756:1984).

The study results of the qualitative composition of the protein component in pine nuts in the Kemerovo Oblast are indicated in Fig. 1 and Table 2. In water-soluble protein fraction of the samples No. 1 and No. 2, fractions with a molecular weight of 19 kDa (line B2, C2) and 11 kDa (line B5, C5) dominate.

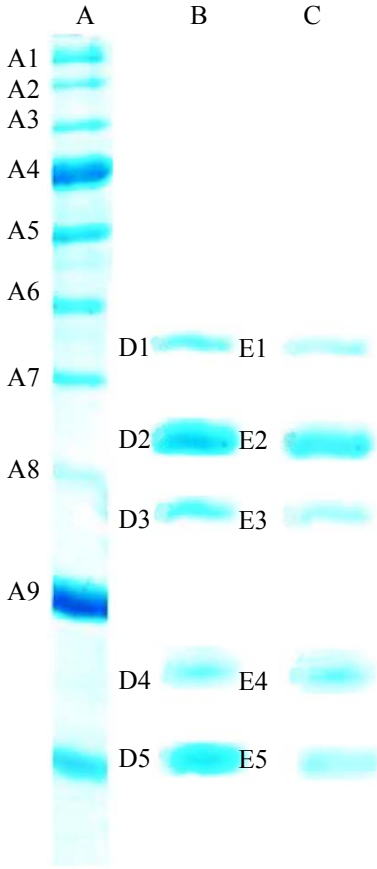

Fig. 1. Profile of water-soluble protein fraction of pine nut kernel: track A - marker, track B - sample No. 1, track C - sample No. 2

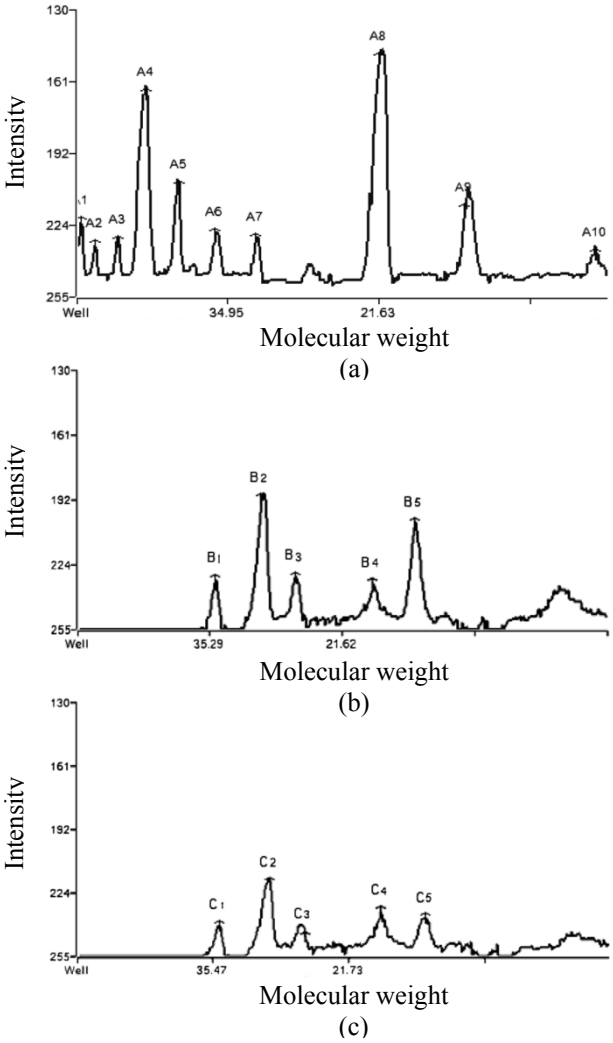

Fig. 2. Protein profile (a) in the marker, (b) sample No. 1, (c) sample No. 2.

Table 2. Protein profile of water-soluble fraction in the marker and in the pine nut samples

Track name	Line number	Molecular mass, kDa	Mobility coefficient, R_f
A	A1	227.17	0.0223
	A2	115.89	0.0487
	A3	67.83	0.0911
	A4	45.55	0.1449
	A5	33.96	0.2056
	A6	25.81	0.2812
	A7	21.19	0.3557
	A8	17.09	0.4611
	A9	13.88	0.5928
B	B 1	23.39	0.3167
	B 2	19.17	0.4007
	B 3	17.09	0.4611
	B 4	12.64	0.6770
	B 5	11.31	0.7572
C	C 1	23.39	0.3167
	C 2	18.94	0.4070
	C 3	17.09	0.4611
	C 4	12.73	0.6622
	C 5	11.10	0.7572

Note. A - in the marker, B - sample No. 1, C - sample No. 2.

Table 3. Amino acid content of the pine nut kernel

Amino acid name	Content, g/100 g of protein	
	sample No. 1	sample No. 2
Alanine	5.44 ± 0.27	5.33 ± 0.27
Arginine	15.41 ± 0.77	15.44 ± 0.77
Asparagine acid	5.89 ± 0.29	6.12 ± 0.31
Valine	3.37 ± 0.17	3.52 ± 0.18
Histidine	2.84 ± 0.14	2.79 ± 0.14
Glycine	4.58 ± 0.23	4.65 ± 0.23
Glutamic acid	11.84 ± 0.59	11.75 ± 0.59
Leucine+Isoleucine	15.73 ± 0.79	15.70± 0.79
Lysine	6.04 ± 0.30	5.84 ±0.29
Methionine	1.66 ± 0.08	1.62 ± 0.08
Proline	5.47 ± 0.27	5.52 ± 0.28
Serine	6.72 ± 0.34	6.81 ± 0.34
Thirosine	2.86 ± 0.14	2.80 ± 0.14
Threonine	3.15 ± 0.16	3.10 ± 0.15
Triptophane	1.18 ± 0.06	1.24 ± 0.06
Phenylalanine	6.49 ± 0.32	6.52 ± 0.33
Cystine	1.33 ± 0.07	1.25 ± 0.06

Amino acid content of the pine nut sample is indicated in Table 3. The analyzed samples differ with a high content of such essential amino acids as leucine and isoleucine, phenylalanine and lysine: 15.72 g, 6.50 g and 5.94 g per 100 g of protein respectively. Also, there is a high content of such non-essential amino acids as arginine, glutamic acid, serine, asparagine acid, proline and alanine: 15.43 g, 11.80 g, 6.77 g, 6.00 g, 5.50 g and 5.39 g per 100 g of protein respectively.

Nuts are a good source of fat and are considered good for health due to the high content of unsaturated fatty acids [21, 22]. The fatty acid content of the pine nut kernel is indicated in Table 4. The total content of saturated fatty acids in the pine nut kernel is 9.53%, of unsaturated fatty acid – 90.47%. In the analyzed samples linolenic acid (omega-3 fatty acids) has the highest content - approximately 43% from the total fat content, oleinic and linolenic acids are the next by content - approximately 24% and 21% respectively. Palmitic (5.23%) and stearic (2.82%) acids dominate among the saturated fatty acids.

Table 5. Vitamin content of the pine nut kernel

Vitamin name	Vitamin content, mg/100 g of product	
	sample No. 1	sample No. 2
Water-soluble vitamins		
B1 (thiamine)	0.540 ± 0.180	0.470 ± 0.160
B2 (riboflavin)	0.270 ± 0.090	0.240 ± 0.080
B3 (niacin, PP)	3.780 ± 1.280	3.900 ± 1.330
B5 (nicotinamide, nicotinic acid)	0.450 ± 0.150	0.520 ± 0.180
B6 (pyridoxin)	0.120 ± 0.040	0.100 ± 0.030
C (ascorbic acid)	0.670 ± 0.230	0.740 ± 0.250
Fat-soluble vitamins		
E (alpha-tocopherol)	8.330 ± 0.830	8.120 ± 0.810
K (phylloquinone)	0.050 ± 0.005	0.050 ± 0.005

Table 6. Macro- and micronutrient content of the pine nut kernel

Element name	Element content, mg/100 g of product	
	sample No. 1	sample No. 2
Macronutrients		
Potassium	602.30 ± 30.10	595.60 ± 29.80
Calcium	15.90 ± 0.80	17.60 ± 0.90
Magnesium	246.00 ± 12.30	255.80 ± 12.80
Sodium	7.10 ± 0.40	7.90 ± 0.40
Phosphorus	789.00 ± 39.50	795.20 ± 39.80
Micronutrients		
Iron	5.67 ± 0.28	5.59 ± 0.28
Manganese	8.73 ± 0.44	8.88 ± 0.44
Copper	1.27 ± 0.06	1.39 ± 0.07
Zinc	4.41 ± 0.22	4.35 ± 0.22
Iodine	0.15 ± 0.01	0.12 ± 0.01

Table 4. The fatty acid content of the pine nut kernel

Fatty acid name	Fatty acid index	Fatty acid content, g/100 g of fat	
		sample No. 1	sample No. 2
Saturated			
Myristinic acid	$C_{14:0}$	0.44 ± 0.02	0.52 ± 0.03
Palmitic acid	$C_{16:0}$	5.18 ± 0.26	5.27 ± 0.26
Stearinic acid	$C_{18:0}$	2.89 ± 0.14	2.75 ± 0.14
Arachic acid	$C_{20:0}$	0.95 ± 0.05	1.06 ± 0.05
The amount of unsaturated fatty acids		9.46 ± 0.47	9.60 ± 0.48
Unsaturated			
Palmitoleic acid	$C_{16:1}$	0.35 ± 0.02	0.46 ± 0.02
Oleic acid	$C_{18:1}$	24.05 ± 1.20	24.27 ± 1.21
Linoleic acid	$C_{18:2}$	42.20 ± 2.11	42.77 ± 2.14
Linoleic acid	$C_{18:3}$	20.98 ± 10.49	20.43 ± 1.02
Gondoic acid	$C_{20:1}$	0.87 ± 0.04	0.93 ± 0.05
Eicosadienoic acid	$C_{20:2}$	0.65 ± 0.03	0.57 ± 0.03
Eicosatrienoic acid	$C_{20:3}$	1.44 ± 0.07	0.97 ± 0.05
The amount of unsaturated fatty acids		90.54 ± 4.53	90.40 ± 4.52

Table 7. Chemical and microbiological pine nut safety indicators

Parameters	Content, mg/kg	
	sample No. 1	sample No. 2
Mercury,	not detected	not detected
Arsenic	0.0500 ± 0.0030	0.0350 ± 0.002
Lead	0.1000 ± 0.0050	0.0600 ± 0.0003
Cadmium	0.0100 ± 0.0005	0.0070 ± 0.0004
Aflatoxin B1	0.0050 ± 0.0003	0.0030 ± 0.0002
Hexachlorocyclohexane (the sum of isomers)	0.0060 ± 0.0003	0.0050 ± 0.0003
Dichlorodiphenyltrichloroethane and its metabolites	0.0100 ± 0.0005	0.0150 ± 0.0008
Product mass (g), in which coliform bacteria were not detected	0.01	0.01
Product mass (g), in which pathogenic microorganisms, including Salmonella, were not detected	25.00	25.00
Fungi, CFU/g	$1.00 \cdot 10^1$	$1.10 \cdot 10^1$

Nuts have a wide range of micro-, macronutrients and vitamins in sufficient quantities [23, 24], which can have a positive impact on health and contribute to the prevention of nutritional deficiency (Tables 5–6). Pine nut kernel samples are rich in a fat-soluble vitamin alpha-tocopherol and water-soluble vitamins PP, C, B1 and B5: 8.23 mg and 3.84 mg, 0.71, 0.51 mg, 0.49 mg per 100 g of the product respectively. The main macronutrients in the samples content are phosphorus (792.1 mg/100 g of the product), potassium (600.0 mg/100 g of the product) and magnesium (250.9 mg/100 g of the product); as for micronutrients, the main ones are manganese (8.81 mg/100 g of the product), iron (5.63 mg/100 g of the product) and zinc (4.38 mg/100 g of the product).

Chemical compound and content of micro - and macronutrients and vitamins may vary depending on climatic and soil conditions [25]. The protein content in the kernels of Kuzbass *Pinus sibirica* is comparable with the protein content of the nuts of cedar of Tuva and the Far East region [26, 27] and exceeds by approximately 15% the samples, obtained in China [28]. By content the nut kernels of the Far Eastern cedar are less full-valued than the ones of *Pinus sibirica* [27]. The fat content in our samples is comparable with the content of nut samples of China and the Far East region and exceeds by more than 40% the samples of Tuva.

The difference in content of micro - and macronutrients between our nut samples and the ones of China and Tuva was as follows: potassium is +20% and -15%; calcium is 400% and -2%; phosphorus is +40% and +4% respectively [26, 28]. The zinc and vitamin B2 content in Kuzbass and China nuts is comparable, by 25% less by sodium and by 400% by vitamin E, exceeds 3000 times by the vitamin B1, by other micro- and macronutrients, except iodine, exceeds by 40%-220% [28].

While growing and ripening, nuts, including pine nuts, can accumulate in their fruits toxic chemicals (mercury, arsenic, lead, etc.), as well as during storage and transportation they can accumulate toxins (waste products of fungi, pathogens, etc.). Pine nut samples (Table 7) satisfy the requirements of the current regulations (CU TR [Technical Regulations of the Customs Union] 021/2011, SanPiN [Sanitary Regulations and Norms] 2.3.2.1078–01) by chemical and microbiological parameters and do not have a toxic effect on a human.

CONCLUSIONS

The desire to prevent cancer, cardiovascular, gastrointestinal and other diseases preserves the relevance of developments of foodstuffs of high biological value with addition of natural ingredients. To achieve this goal, a full-valued protein, individual amino acids, probiotics, prebiotics, vitamins, micro- and macronutrients and plant raw material [28–36] are considered as supplements. Traditionally, nuts are considered as a source of nutrients to improve the food quality. Those, who eat nuts, usually do not feel the deficiency of vitamins A and C, folate, calcium, iron, magnesium and zinc [37, 38]. Pine nuts of *Pinus sibirica* differ with a high content of proteins, in some samples the content is equal to the one in a peanut. Nuts have a positive impact on health due to their anti-inflammatory and antioxidative characteristics [39, 40], which reduce the impact on cholesterol level. Nutritional value of *Pinus sibirica* nuts allows to using them as ingredients in different food, such as: cereals, bread and cakes, cheese and functional products, including sport nutrition.

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DEVELOPMENT OF A FUNCTIONAL BASIS OF PHYTO-BEVERAGES WITH AN INCREASED ANTIOXIDANT ACTIVITY FOR THE CORRECTION OF NUTRITION OF PATIENTS WITH DIABETES MELLITUS

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Abstract: Diabetes mellitus is the most threatening form of human metabolic disorder and of malnutrution as one of the main causes of its development. The purpose of the studies is the development of a functional basis of phyto-beverages based on herbal medicinal raw materials with a high antioxidant activity for the correction of nutrition for diabetic patients. The object of the studies was plant medicinal raw materials that grow mainly in the territory of the Kemerovo region, various vegetative parts of plants were used: leaves (of cranberry, knotgrass, common St. John's wort, blindweed, common plantain and common horsetail); roots (of elecampane, common burdock, common dandelion); valves (common beans); shoots (of blueberry). The antioxidant activity of water extracts from plant raw materials mixtures was determined using a spectrophotometric method based on the determination of concentration of malonic dialdehyde in biological material. The following compositions of mixtures have been experimentally determined: 1) common St. John's wort, great nettle, common dandelion, blueberry, common horsetail; 2) knotgrass, great nettle, blueberry, common horsetail; as well as the following extraction parameters: the degree of grinding of raw material is 5–8 mm, the ratio of solid (medicinal plant raw material) and liquid (water) phases is 1 : 7, the temperature is 50 ± 1°C, the extraction time is 6 hours. A possibility of preserving finished extracts with alcohol up to 8% of the volume fraction has been determined, which allows to keep them for 30 days providing the alcohol content in the beverage on the basis of the extract of not more than 0.5%, according to the applicable requirements. It has been shown that the created extracts have an antioxidant activity.

Keywords: Antioxidant activity, phyto-beverages, diabetes mellitus, nutrition correction

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INTRODUCTION

The modern way of life of the majority of population of the developed countries often leads to disturbances in the metabolic processes of the human body. Carbohydrate metabolism disorders, in particular diabetes mellitus (DM), have become one of the most serious forms of metabolic disorders in recent decades, both in terms of the scale and the level of disability. Since 1980, the global incidence of diabetes has nearly doubled, having risen from 4.7% to 8.5% among the adult population. According to the predictions of the World Health Organization (WHO), diabetes will have ranked seventh among the causes of death by 2030 [1]. Over the past 10 years, the number of people with diabetes mellitus (DM) in the world has grown more than twice, having reached 415 million by the end of 2015. According to the predictions of the International Diabetes Federation, 642 million people will have suffered from diabetes by 2040 [2]. Figure 1 presents a comparative rating of the countries that are leaders in terms of the number of people aged 20–79 with diagnosed diabetes mellitus, compiled from the data for 2011 and 2015. [12]. At the same time, in most cases there is a significant increase in the number of the diseased for the period from 2011 to 2015: in particular, China is in the lead not only in terms of the number of people suffering from DM, which is explained by the largest population, but also in terms of the maximum rate of an increase in incidence - + 21% in 2015 compared to 2011.

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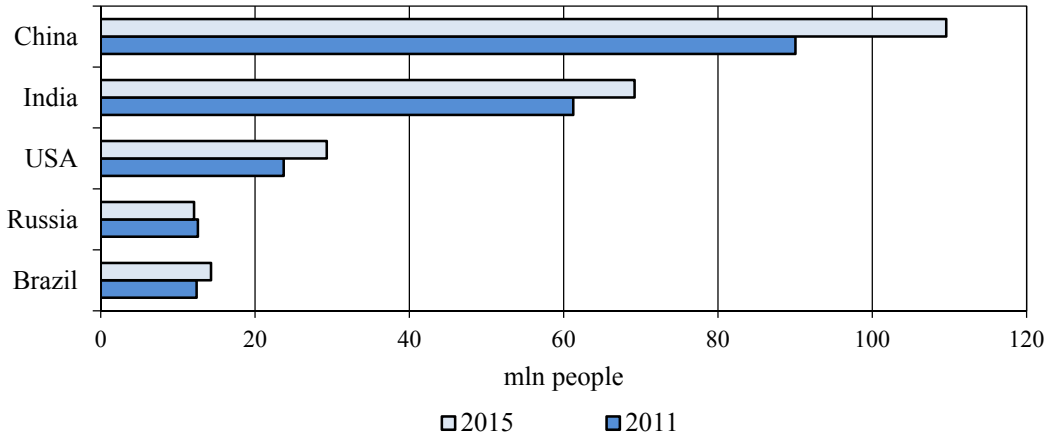

Fig. 1. Number of the people aged 20–79 with diagnosed diabetes mellitus [1, 2].

Despite the fact that the situation with the incidence of diabetes in Russia looks more stable against the background of other countries, the data of the studies show that among the adult population of Russia aged 20–79 years, 19.3% had prediabetes and 5.4% of the population had type 2 diabetes (which is the most common) [3]. At the same time, there is an opinion that the true number of patients with diabetes is approximately 3–4 times higher than the officially registered number, and reaches about 7% of the population.

The main cause of the development of type 2 diabetes is often nutrition disorders, i.e. this disease is alimentary-dependent. One of the most effective measures to prevent alimentary-dependent diseases is the use of the functional foods that can correct the diet. As a rule, the diseases that have a high level of spread are prevented by the production and sales of functional everyday food products consumed by all the population groups. Traditionally, in Russia, such products include grain products, bread and bakery products. There is an experience of enriching them with the micronutrients deficient in the diet of most Russians [4, 5]. In diabetes, the consumption of products of this group is recommended to be limited, therefore it is necessary to seek other ways of solving the problem.

The results of domestic and foreign studies indicate the advisability of supplementing the diet of patients with products with a high content of biologically active substances (BAS), including antioxidants, to increase the efficiency of therapy in patients with diabetes. The additional intake of antioxidants contributes to the prevention of complications, normalizes oxidation-reduction processes and increases the adaptive capacity of the body [6, 7, 8]. An effective source of natural antioxidants is plant raw materials, including medicinal plant raw materials. Some significant positive experience in its use in the complex therapy of diabetes mellitus has been accumulated [9, 10, 11, 12, 13, 14, 15]. Currently, there are more than 150 species of antidiabetic plants belonging to 50 families.

There are several opinions on the mode of hypoglycemic action of medicinal plants in modern preventive medicine:

(1) Plant raw materials supply alkaline radicals to the body. In a weakly alkaline solution in the presence of calcium hydroxide ($Ca(OH)_2$), glucose is spontaneously converted to fructose and mannose, resulting in an increase in the body's alkaline reserve, which can improve the uptake of glucose by tissue [7].

(2) Flavonoids contained in a lot of plants have a hypoglycemic effect, the mechanism of which is to increase the concentration of calcium that stimulates the secretion of insulin by pancreatic cells using flavonoids. Favonoids also act as vitamin P - they strengthen the walls of capillaries and contribute to an increase in the body resistance [8].

(3) Some plants (mainly that of aster family) contain inulin which is called "plant insulin." Inulin affects normal glycemia, reduces a high blood sugar level; regulates carbohydrate and lipid metabolism; improves the availability of minerals (zinc and copper), which have a hypoglycemic effect; in general, it positively affects the human immune system [7].

The use of medicinal plant raw materials as a source of antioxidants in the production of foodstuffs places a number of limitations in the choice of a product which is a functional carrier. This is due to both the flavor profile of source raw material and the need for preliminary extraction of the functional basis. This fact determines the priority of such a homogeneous group of products as non-alcoholic beverages in terms of the formation of organoleptic indicators and technological characteristics.

Non-alcoholic beverages are a popular and mass consumer product, however, the traditional formulation implies in most cases the use of sugar in quite large quantities. A relation between the use of a number of non-alcoholic beverages and different raw materials and the increased risk of development of type 2 diabetes mellitus has been revealed [16, 17]. In this regard, the studies aimed at producing functional beverages for people with carbohydrate metabolism disorders are relevant. The studies in this area are being performed [18, 19], but the assortment of these beverages in the market is insufficient.

The paper aims at developing the functional basis of phyto-beverages based on plant medicinal raw materials with a high antioxidant activity for the correction of diabetic patients.

OBJECTS AND METHODS OF STUDY

The plant medicinal raw materials that grow on the territory of the Kemerovo region (except for cranberry and blueberry) have been used as the study objects, gathering and harvesting were performed when the corresponding vegetative part of the plant, which is a functional carrier, grew ripe:

– cranberry (leaf), from Latin *Vaccínium vítis–idaéa*;
– knotgrass (leaf), from Latin *Polýgonum aviculáre*;
– elecampane (root), from Latin *Ínula helénium*;
– common St. John's wort (leaf), from Latin *Hypericum*;
– great nettle (leaf), from Latin *Urtíca dióica*;
– common burdock (root), from Latin *Arctium láppa*;
– common dandelion (root), from Latin *Taráxacum officinále*;
– blindweed (leaf), from Latin *Capsélla búrsa–pastóris*;
– common plantain (leaf), from Latin *Plantágo májor*;
– kidney bean (valves), from Latin *Phaséolus*;
– blueberry (shoots), from Latin *Vaccínium myrtíllus*;
– common horsetail (leaf), from Latin *Equisetum arvense*.

All the raw materials were used in the dry state. In addition, phytomixtures and their aqueous extracts were the study objects at different stages of the study (see Table 1 for the composition).

Research methods. The actual nutrition of people suffering from carbohydrate metabolism disorders (type 2 diabetes mellitus) was studied by means of a survey using specially developed questionnaires in accordance with the methodological guidelines "Rationalization of nutrition of the population in the sanitary and epidemiological service" on the basis of Kemerovo Municipal Clinical Hospital No. 3. The questionnaires were processed using a computer program developed by Kemerovo Institute of Food Science and Technology (University), developed using a database of the chemical composition of foods and prepared meals and taking into account nutrient losses during cooking.

The organoleptic evaluation of plant extracts was carried out in accordance with GOST 6687.5 "Non-alcoholic industry products. Methods for determination of organoleptic indices and products volume". In the finished extracts, an appearance, transparency, color, flavor and taste were determined in terms of the possibility of developing beverages with high consumer properties on their basis (an attractive appearance, a harmonious taste and flavor).

The content of solids in plant extracts was determined using a standard refractometric method.

The content of ascorbic acid in plant extracts and non-alcoholic beverages was determined by means of the titration of ascorbic acid with Tillmans paint (2,6-dichlorophenolindophenol), the principle of which is based on the ability of ascorbic acid to quantitatively reduce 2,6-dichlorphenolindophenol.

The content of polyphenolic compounds (in terms of catechine) in dry plant raw materials and plant extracts was determined according to the pharmacopoeial Leventhal method based on the light oxidability of polyphenolic compounds with a solution of 0.1 mole/dm^3 of potassium permanganate in an acid medium in the presence of an indicator and an indigosulfonic acid catalyst.

Currently, due to the relevance of the studies aimed at determining the antioxidant activity in food systems, various electrochemical [17, 18, 19], fluorescent methods of analysis are used [20]. In this paper, the antioxidant activity of water extracts from the mixtures of plant raw materials was determined using a spectrophotometric method based on the determination of the concentration of malonic dialdehyde using thiobarbituric acid in the biological material, which is more often used in medical practice. The method is based on the reaction between malonic dialdehyde and thiobarbituric acid, which proceeds, at a high temperature and a low value of pH, with a change in the colored trimethine complex that contains one molecule of malonic dialdehyde and two molecules of thiobarbituric acid. The maximum absorption of the complex accrued to 532 nm. Therefor, a test standard serum of a healthy person (taken from one healthy person as a standard test object) was used and, adding the test substances to the selected aliquot from the serum volume, malonic dialdehyde was determined after incubation. The dissolution of thiobarbituric acid and the incubation of the sample was carried out in the presence of Triton X-100, the mixture was stirred at a constant oscillation frequency, the reaction was arrested by the addition of the extract under study. Before the determination of the optical density of the sample, Trilon B and a mixture of ethanol and chloroform were added. The concentration of malonic dialdehyde in serum was calculated using Formula 1.

$$C_{mda} = \frac{(D1 - D2) \times U2}{E \times L \times U2}, \quad (1)$$

where C_{mda} is the concentration of malonic dialdehyde, mmol/dm^3; D_1 is the optical density of the sample with serum; D_2 is the optical density of the control sample; U_1 is the volume of serum taken for calculation, ml; U_2 is the final volume of the mixture, ml; L is the length of the cuvette, cm; E is the extinction coefficient (156 mM^{-1}cm^{-1}).

Consequently, the higher the concentration of malonic dialdehyde in blood serum, the lower the antioxidant activity of the extracts studied.

RESULTS AND DISCUSSION

Any preventive measures to reduce alimentary-dependent diseases are associated with two main activities: the development / introduction of educational programs for groups of people with certain diseases and the development / saturation of the market with functional food products. As a rule, functional food products are designed to optimize the diets of all groups of the population and, in particular, for people who are predisposed to and / or have an alimentary-dependent disease. The first step in the optimization of diets is the study of actual nutrition, the results of which allow us to identify deviations from the norm and to propose the target directions of the studies within the general prevention task.

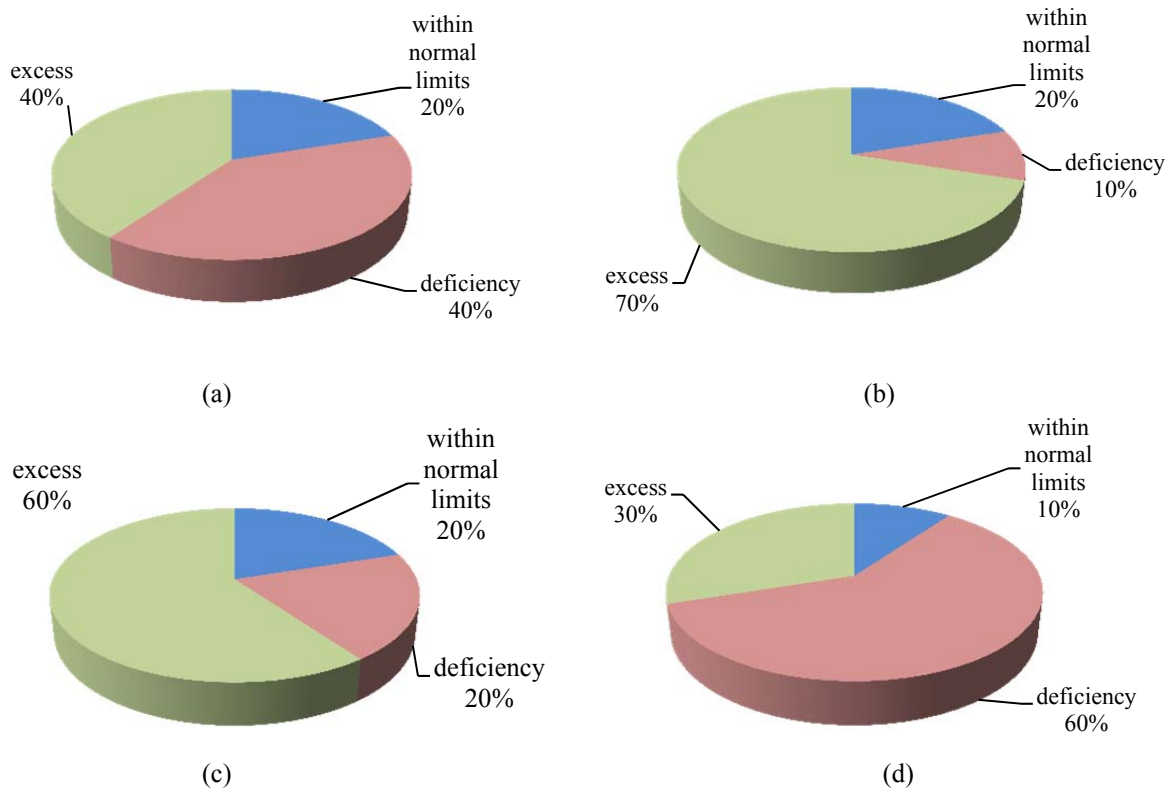

Fig. 2. Compliance of the caloric value and the content of macronutrients to the norms in the diets of the studied group of people with type 2 diabetes mellitus: (a) the compliance of the caloric value of diets, (b) the compliance of protein content in the diet, (c) the compliance of fats content in the diet, (d) the compliance of carbohydrates content in the diet

In the group of respondents in the number of 130 people (52 men and 78 women) with diagnosed diabetes mellitus (DM), diets were analyzed to determine the degree of their compliance with the principles of balanced nutrition and the recommendations of nutritionists.

Figure 2 provides the results of compliance with the norms of the caloric value of diets, as well as the content of the main nutrients.

The results showed that the caloric value of the diets in general for the whole group of the observed people was on average 1905 calories per day (at the rate of 1800–1975). The caloric value was within normal limits in 20% of patients; the caloric value of the diets was reduced on average by 10–17% in 40% of the surveyed people, and the caloric value of the diets of 40% of the men and women surveyed had a caloric value of 2250–2300 kcal.

The consumption of nutrients by respondents is significantly differentiated, which is caused by a number of things: a social status, the economic accessibility of foods, the specific knowledge about nutrition in DM and food culture in general. Thus, the average daily protein intake averaged 75.4 g in the group. The protein intake within normal limits was in 20% of men and women; protein deficiency was in 10% of the observed people, and excess protein - in 70% (up to 50%) of them. Animal protein the content of which should be at least 55% of the total, is important, in fact it was 45.1 g (59.8%) per day. The diets of 55% of respondents showed a significant content of animal protein, mainly caused by a high level of consumption of meat and poultry, sausages, canned meat and eggs. There are almost no meat products in the diets of people with low incomes, the protein intake is mainly due to the consumption of dairy products, herewith, there is lack of protein in the diet.

The average daily fats intake in the observed patients averaged 74.2 g in the group, which, at first glance, corresponds to the physiological needs. However, the use of fats within the norm is only observed in 20%, for the majority of respondents (60%) there is an excess of fat in the diet reaching 30%, and there is lack of lipids in the diet in 20% of respondents. According to medical records, 22% of respondents suffer from atherosclerotic vascular disease, which may be a result of a high content of saturated animal fats in the diet.

Despite the fact that polyunsaturated fatty acids contained in vegetable oils help to lower the cholesterol level in blood, have an anti-sclerotic effect, the percentage of vegetable fats consumption in 70% of the surveyed is reduced and is 5.2 g per day. Only 10% of respondents consume vegetable fats within normal limits, 30% of them had a slight excess of the norm of vegetable fats content in their diets (up to 12.3 g).

There was a decrease on average by 15–30% in the consumption of total of carbohydrates in 60% of respondents. Probably, this fact is due to the willful restriction of carbohydrate-containing foods within the prescribed diet. However, the nutrition of 30% of people is characterized by an excessive consumption of

carbohydrates due to an excessive use of bread. For the whole of the group, the consumption of carbohydrates was 61.8% of the total caloric intake (with the recommended 50–56%), there is an exclusion of refined carbohydrates from the diet (sugar, confectionery). The respondents use sugar substitutes to give a sweet taste to products.

The total content of cellulose and pectin in the diets corresponds to the physiological norms: the average dietary fiber intake was 13.7 g, including fiber - 10.3 g and pectin - 3.4 g. However, these amounts are not enough to prevent complications and correct the behavior of DM.

On average, the level of intake of ascorbic acid with food corresponds to the recommendations. At the same time, 20% of respondents get vitamin C within normal limits, 40% feel lack of it, and 40% have a slight excess of it. The content of vitamin A in the diets has on average been reduced by almost 50% compared with the norm, and the content of vitamin PP - by 15–25%. There is lack of vitamin A in 90% of the respondents, and lack of vitamin PP - in 70%. The content of vitamin B_1 in the diet exceeds the norm by 0.5–12% in 60% of people with carbohydrate metabolism disorders, 20% feel lack of this vitamin. The average daily intake of vitamin B_2 corresponds to nutritional standards and averages 1.44 mg only in 40% of respondents. It should be noted that the imbalance in the intake of vitamins can reduce the efficiency of prevention and treatment of DM.

The study of the mineral composition of diets showed that the average daily calcium and magnesium content in diets of men and women is in most cases below the recommended level, there is only a small excess of calcium in 20% of respondents and a small excess of magnesium - in 10%. There is a high iron intake - to 27.1 mg per day - in 40% of the observed people, a low iron intake - to 12.4 mg - in 30%, and iron intake was only normal in 30%. However, it should be noted that the main part is iron from plant raw materials with a low digestibility. The level of phosphorus intake corresponds to the recommendations on average in 30%, 40% of people feel lack of phosphorus, and 44% (up to 1510 mg) - an excess of it. The ratio of Ca and P is upset in favor of phosphorus in the studied diets as well, and the ratio of Ca and Mg is upset in favor of Mg.

Thus, the analysis of diets of people with diagnosed type 2 diabetes mellitus indicates an imbalance in the consumption of nutrients and energy. In general, there is an increased energy contribution of the fat component (mainly due to animal fats), a low consumption of vegetable fats and a number of vitamins and minerals. These factors reduce the efficiency of prevention and treatment of DM and contribute to various aggravating violations.

As noted above, non-alcoholic beverages are one of the most advanced groups of food products for manufacturing functional products, which is explained by its popularity with the population, all-season consumption, quite a simple production process and a high digestibility of biologically active substances (BAS) of raw materials. In this regard, the products of this group should be used in the diet of people with diabetes mellitus as well - with the aim of optimizing it. The substantiated choice of the functional basis will allow:

– firstly, to exclude food additives from the formulation (dyes and flavors),

– secondly, to make the product therapeutic and preventive, in the context of this paper - with a high antioxidant activity.

Thus, the joint use of traditional and proven methods used in the treatment of diabetes mellitus - phytotherapy and diet therapy - was used during the study, which is justified from both the medical and preventive and the socio-economic points of view. An important aspect was a steady tendency observed in Russia to increase consumer interest in a healthy lifestyle, including interest in food products that contain natural biologically active substances.

The expediency of using medicinal plants in the production of beverages for DM is due to the fact that the considerable list of them has a hypoglycemic effect. In addition, these plants have a positive effect on the body in general, since there are a large number of complications of different organs of the body in DM.

At the first stage of the study, an analysis of scientific and technical literature containing recommendations of practical medicine for collecting medicinal plants that have a hypoglycemic effect was made. When choosing mixtures for the studies, we followed the data on the chemical composition (the absence of potent substances, the high content of vitamins, antioxidants), the proven pharmacological properties, the abundance and availability of raw materials for procurement on an industrial scale, and therefore we followed the raw material base of the Kemerovo region. It is necessary to note the experience of a number of small enterprises for processing plant raw materials and producing functional beverages with a scientific support of scientists from KemIFST and KSMU.

An important factor in the choice of medicinal raw materials was also the presence and diversity of taste and flavor characteristics, as well as coloring agents, since the use of plants that contain bitters and a lot of tannins to create beverages is undesirable.

Taking into account the above, of all the variety of the mixtures used in medical practice 10 collections that have a hypoglycemic effect in diabetes mellitus and have the organoleptic characteristics adequate for the preparation of the beverage were preferred (Table 1).

As can be seen from Table 1, various vegetative parts of plants (leaves, flowers, fruits, etc.) were used:

– leaves (of cranberry, knotgrass, common St. John's wort, blindweed, common plantain and common horsetail);

– roots (elecampane, common burdock, common dandelion);

– valves (common beans);

– shoots (of blueberry).

Table 1. Composition of the antidiabetic mixtures selected for the studies

No. of mixture	Medicinal plant raw materials											
	Cranberry (leaves) from Latin *Vaccínium vítis-idaéa*	Knotgrass (leaves) from Latin *Polýgonum aviculáre*	Elecampane (root) from Latin *Ínula helénium*	St. John's wort (leaves) from Latin *Hypericum*	Great nettle (leaves) from Latin *Urtica dióica*	Common burdock (root) from Latin *Arctium láppa*	Common dandelion (roots) from Latin *Taráxacum officinále*	Blindweed (leaves), *from Latin Capsélla búrsa-pastóris*	Common plantain (leaves) from Latin *Plantágo májor*	Kidney beans (valves) from Latin *Phaséolus*	Blueberry (shoots), *from* Latin *Vaccínium myrtíllus*	Common horsetail (leaves) from Latin *Equisetum arvense*
1					+		+				+	
2				+	+						+	
3				+	+		+				+	+
4					+		+		+		+	
5	+				+						+	
6		+	+		+		+				+	
7						+				+	+	
8		+			+		+				+	
9		+			+						+	+
10		+			+			+				+

All the selected plants contain a large number of flavonoids, tannins, organic acids, vitamins and microelements. Elecampane, common burdock and dandelion contain inulin, have a choleretic, diuretic, antiseptic, general tonic and antioxidant effect, strengthen the walls of capillaries and regulate metabolism, in particular, have a positive effect on carbohydrate and lipid metabolism. Knotgrass, common St. John's wort, common dandelion, blueberry and common burdock are also recommended by Methodical recommendations MR 2.3.1.1915–04 "Recommended levels of consumption of food and biologically active substances" as alternative sources of flavonoids intaken by the body. Most of these plants are also widely used in other branches of the food industry.

It should be noted that the total content of plants in mixtures does not exceed five, which is optimal from the point of view of the possibility of predicting the projected functional properties of beverages. In addition, it is this number of plants that is recommended for the production of phyto-beverages. The purpose of experimental studies was to confirm the hypothesis of a hypoglycemic effect in mixtures and to rank mixtures by the quantitative content of BAS-antioxidants.

Determination of regimes of extraction of biologically active substances. The biologically active substances (BAS), which cause the antioxidant and hypoglycemic effect, transit to a solution while being extracted from plants. In this regard, the next step was to select the parameters of extraction of biologically active substances from the antidiabetic mixtures. The following criteria were set to select an extraction method:

– the harmonious organoleptic properties of the extract,
– the output of biologically active substances that determines the phyto-effect;
– the high antioxidant activity of the extract (AOA);
– the availability of the technology of the selected method of extraction of biologically active substances.

The most common is the method of BAS extraction in both the food industry and in medicine. The efficiency of the extraction process is effected by a number of factors: the nature of the extractant, the temperature and duration of extraction, the concentration of substances in the system and hydrodynamic conditions, the anatomical structure and the degree of grinding of plant material, the water absorption coefficient and the raw material-extractant ratio.

The following requirements were imposed on the extractant: the used extractant must be safe and accessible, and also provide the maximum yield of the necessary compounds. Based on the analysis of the existing experience and recommendations, preference is given to the aqueous extraction of plant raw materials, which fully meets the above requirements: it is available and completely safe, and makes possible the transition to a solution not only for antioxidants, but also for the compounds that provide a taste and flavor composition of the beverage (polysaccharides, tannins, organic acids, etc.). Purified water is pharmacologically indifferent, has a large diffuse capacity and high desorption properties.

The optimal raw materials- extractant ratio was selected taking into account the water absorption (Wa) coefficient. Providing that the common coefficient is 1.5 for roots; 2.0 for flowers and herbs, and there are both of them in mixtures, the calculated coefficient was 1.75.

The ratios of the liquid and solid phases were experimentally determined. To determine the ratio of the liquid and solid phases, the weighed quantity of the studied raw materials was filled with water, which is 1 cm higher than the upper layer of the raw materials. When using excessively large amounts of water, extracts have a low content of solids; when the

volume of the liquid phase is insufficient, the process of diffusion of substances into an extractant deteriorates and the extraction of extractive substances will be incomplete. To optimize the extraction process in production, it is recommended to carry out the process of extracting BAS from the gathered raw materials, not from the individual components.

It has been experimentally determined that the optimum ratio of the solid (medicinal plant raw materials) and liquid (water) phases is 1 : 7.

An extraction temperature regime has been determined, which is (50 ± 1) °C - this temperature provides a high yield of taste and flavor substances of raw materials and the maintenance of activity of the most valuable biologically active compounds. This temperature provides an increase in the solubility of tannins, starch, pectin and other substances, and an increase in diffusion.

It is known that the phase contact area, the efficiency of release of substances from the plant cell due to tissue rupture, the rate of penetration of the extractant and of dissolution of substances depend on the degree of grinding of plant raw materials. When determining the necessary degree of grinding of the raw materials used, we followed the fact that coarse grinding (particles of more than 10 mm in size) leads to an insufficient extraction of biologically active substances - antioxidants. Grinding raw material to particles of less than 1 mm contributes to the appearance of a large number of deteriorated cells, from which nonnutritive fibers, insoluble particles and colloids transit into the extract, which impairs the organoleptic characteristics of the extract, and the resulting liquid is subsequently difficult to purify. Thus, the degree of grinding of raw materials was 5–8 mm in the studied mixtures.

To determine the duration of extraction with a certain periodicity, a yield of solids was measured. The measurements were carried out every hour using the refractometric method. The optimum extraction time was determined based on the maximum content of substances in the extract, which was 5 hours for mixtures No. 1, 4, 7 and 10 (the maximum solids content was within 2.7–3.2%), and 6 hours for mixtures from No. 2, 3, 5, 6, 8 and 9 (3.0–3.2%) (Fig. 3 and 4). The obtained values of the content of solids can be considered rational in accordance with the technology of extraction of raw materials and the recommendations of All-Union Research Institute of Brewing, Non-Alcoholic and Wine Industry (Moscow). Extracts of plant raw materials with the content of solids of 1.5% and lower are considered ineffective.

The recommended time of extraction of the plant raw materials under study for production was determined using the maximum yield of solids and the organoleptic characteristics of the extracts. It was 6 hours for all the types of the studied plant raw materials. The process cycle of extraction with duration of 6 hours is caused not only by the output of the maximum amount of extract, but also by the "ripening" of the extract, which is expressed in providing fullness and harmony for taste.

After extraction, the liquid phase was separated and the yield of the extract was determined. The yield of extracts was from 65.0 to 78.6% for various mixtures, the minimum yield of extracts being 8, 9, 2 and 10, the maximum - 3, 4, 5, 6 and 1. Valid correlation dependences of the effect of individual plants on the yield of the extract were not revealed. Different yields of extracts are explained by a different qualitative and quantitative composition of mixtures, but in general it is effective for industrial production.

In the non-alcoholic industry, to produce beverages from plant raw materials in order to increase the shelf life, alcoholic extracts preserved to a volume fraction of alcohol of 16–20 are widely used. When adding the extracts preserved in alcohol to a volume fraction of 16% or more to the beverage, in the amounts necessary to have a preventive effect, the alcohol content in the finished beverage will exceed the norm, which should not be more than 0.5% (according to GOST 28188–2014 "Non-alcoholic drinks. General specifications"), by several times. Based on the literature data and the results of our own studies on the detection of bacteriostatic properties of certain types of plant raw materials obtained earlier, a hypothesis has been put forward on the possibility of reducing the alcohol content in an alcohol extract.

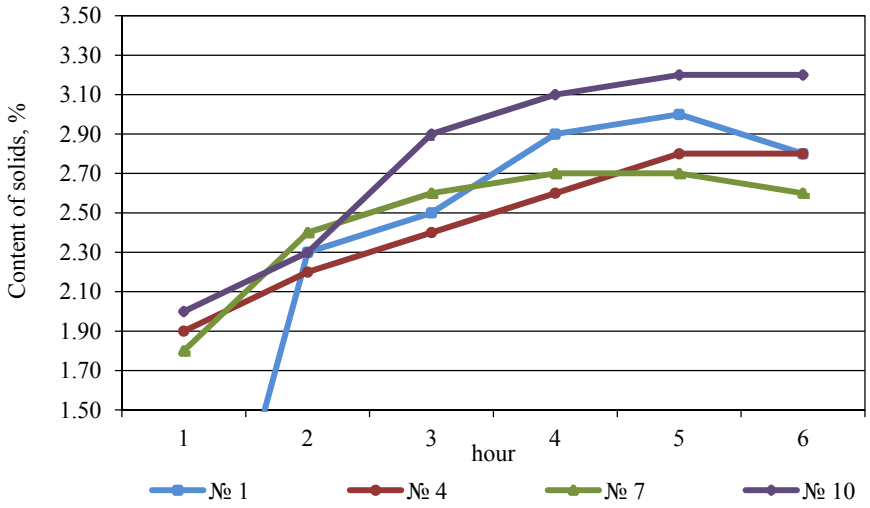

Fig. 3. Dynamics of accumulation of solids in the extracts from mixtures No. 1, 4, 7 and 10.

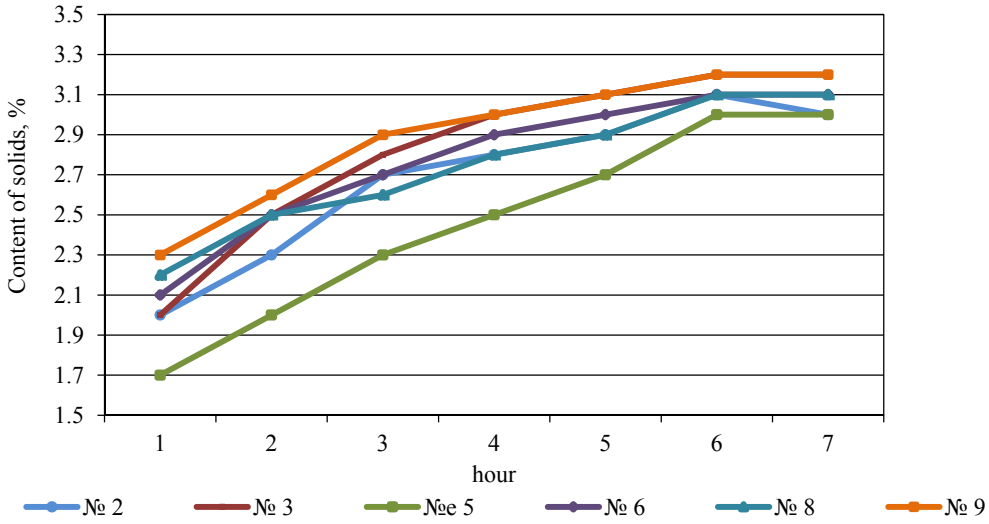

Fig. 4. Dynamics of accumulation of solids during the extraction of mixtures No. 2, 3, 5, 6, 8 and 9.

The theoretical calculation showed that when an alcoholic extract is added in the amount of 8 cm^3 to 100 cm^3 of the finished beverage, with the alcohol content of not more than 0.5% in it, the extract can only be fortified to a volume fraction of alcohol of 8%. Samples of extract from Mixture No. 3 containing 8% of volume fraction of alcohol were prepared and subjected to microbiological testing (Table 2).

The results obtained show that coliforms, pathogenic microorganisms, yeast and molds are not detected in the extract. However, the content of mesophilic aerobic and facultative anaerobic microorganisms increases as the extract is stored and it exceeds the norm on the 39th day of storage - 5.6×10^4 CFU / g at a rate of 5.0×10^4 CFU/g, which, taking into account the safety coefficient, suggests a possible shelf life of 30 days for the extract. According to GOST 28188-2014 "Non-alcoholic drinks. General specifications", the shelf life of non-alcoholic beverages of specific names, the storage and transportation conditions are established by the manufacturer in technological instructions or formulations based on the results of the studies of a pilot batch of beverages, allowing to correct the quantitative indicators of products obtained in laboratory conditions. In this regard, the results of laboratory studies of obtaining extracts and the recommendations are basic for the development of a project of technological documentation.

Study of antioxidant activity of the extracts from plant raw materials. The value of antioxidant activity (AOA) of the studied mixtures is a complex indicator that characterizes the total content of a number of biologically active substances. The analysis of reference data showed that the studied samples are rich in flavonoids, among which quercetin, avicularin, luteolin, kaempferol, hyperoside, rutin and other substances are the most common, contain ascorbic acid, catechins, tannin, sterols, free organic acids, etc.

The bioflavonoids, classified as vitamin P, form a single oxidation-reduction system together with vitamin C. Within the framework of this paper, it is proposed to use the aqueous extraction of plant raw materials and it can be stated that of the whole range of the bioflavonoids present in the analyzed raw materials water-soluble polyphenols (catechin, epicatechingallate, epicatechin, etc.) will mainly transit to an extract, and, in this regard, the antioxidant activity of the extracts will mainly be determined by the content of phenolic compounds and ascorbic acid.

According to the above, a content of phenolic compounds (in terms of catechine) was determined in the extracts. The studied mixtures can be ranked by the content of phenolic substances in the following order (Fig. 5): No. 3, 9, 2, 8, 10, 5, 6, 7, 4, 1. By the content of ascorbic acid, respectively: No. 9, 8, 3, 5, 6, 10, 4, 2, 7, 1.

Based on the results of the organoleptic evaluation of the extracts obtained, we predicted their effect on the taste and flavor of the phytonoproteins based on them. Table 3 presents total of the obtained results, five most promising extracts for the production of treatment and prophylactic beverages have been selected based on the analysis of the data presented - No. 2, 3, 8, 9 and 10.

Table 2. Microbiological indicators of the herbal extract preserved to the volume fraction of alcohol of 8%

Microbiological indicators	Admissible levels	Content of microorganisms						
		Day 7	Day 14	Day 21	Day 28	Day 30	Day 35	Day 39
Coliforms per 1 g	not permitted	not detected	not detected	not detected	not detected	not detected	not detected	not detected
Pathogenic, including salmonella, per 25 g	not permitted	not detected	not detected	not detected	not detected	not detected	not detected	not detected
QMAFAnM, CFU/g,	not more than 5.0×10^4	1.8×10^3	2.8×10^3	3.0×10^4	1.8×10^4	3.8×10^4	4.6×10^4	5.6×10^4
Yeasts and molds, CFU/cm^3, not more than	10	not detected	not detected	not detected	not detected	not detected	not detected	not detected

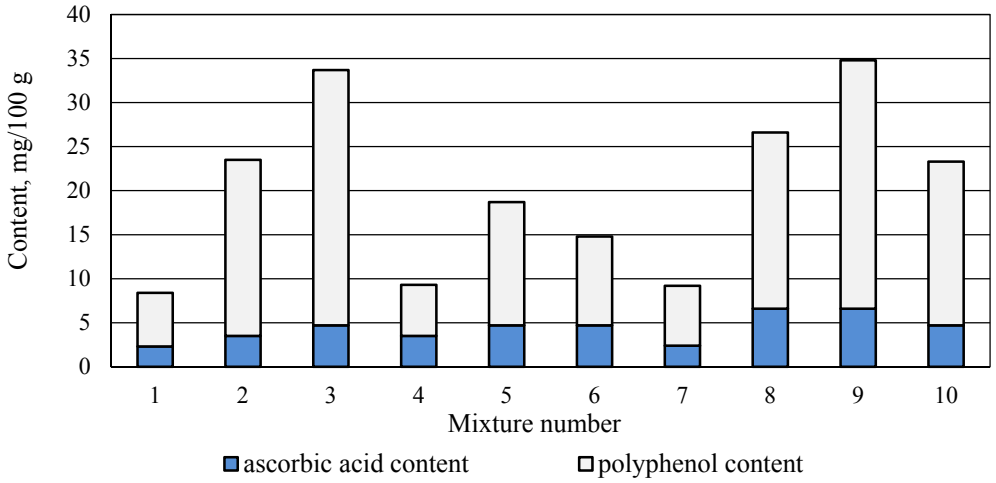

Fig. 5. Content of antioxidants in the extracts from plant raw materials.

Table 3. Organoleptic and physicochemical indicators of the water extracts prepared from phytomixtures

No. of mixture	Content of solids,%	Content of ascorbic acid, mg/100g	Transparency, color and appearance	Flavor and taste
1	3.0 ± 0.2	2.4 ± 0.3	Opaque; dark brown	Slight taste, characteristic of the grass
2	3.1 ± 0.3	3.5 ± 0.4	Opaque; greenish brown	The taste is harmonious, characteristic of the grass
3	3.2 ± 0.2	4.7 ± 0.2	Opaque; brown	The taste is full, characteristic of the grass, with a pleasant note
4	2.8 ± 0.2	3.5 ± 0.4	Opaque; light brown	The taste is blank, the flavor is weak, characteristic of the grass
5	3.0 ± 0.3	4.7 ± 0.4	Opaque; dark brown	The taste is pleasant and poor
6	3.1 ± 0.3	4.7 ± 0.4	Opaque; greenish brown	Needlessly bitter, characteristic of the grass
7	2.7 ± 0.3	2.4 ± 0.3	Opaque; light brown	The taste is imperfect and inharmonious
8	3.1 ± 0.3	6.6 ± 0.3	Opaque; greenish brown	Light, sour-bitter, pleasant
9	3.2 ± 0.2	6.6 ± 0.3	Opaque; dark brown	Slightly astringent, full, with a bit of sourness
10	3.2 ± 0.3	4.7 ± 0.2	Opaque; brownish light green	A light flavor, a pleasant taste

Due to the fact that the used method for determining polyphenol compounds allows us to determine the amount of phenolic substances, not all of which belong to bioflavonoids, the final stage of the studies was the determination of the total antioxidant activity of the extracts of the selected mixtures.

The level of antioxidant activity of extracts was judged by the content of malonic dialdehyde in blood serum, as there is an inverse relation between these indicators (the higher AOA, the lower the content of malonic dialdehyde). Table 4 presents the study results.

As shown by the data presented in Table 4, the administration of the obtained extracts into blood serum makes the lipid peroxidation processes slower, and, consequently, leads to a decrease in the concentration of malonic dialdehyde. According to antioxidant activity, the studied extracts can be ranked as follows: No. 3 > No. 9 > No. 2 > No. 8 > No. 10. The extracts prepared from mixtures of plant raw materials No. 3 and No. 9 have the highest value of antioxidant activity, they are the most promising for use as a functional basis of beverages for the correction of diabetic patients. It should be noted that these extracts previously showed the highest content of ascorbic acid and polyphenols (see Fig. 5), which indicates a direct dependence of total of AOA on them.

Based on the results obtained, formulations of the functional basis (of extracts) of phyto-beverages with a high antioxidant activity have been developed, Table 5 presents the consumption of components per 100 dal.

Table 4. Content of malonic dialdehyde in blood serum

No. of mixture	List of inward raw materials	Concentration of malonic dialdehyde, C_{mda}, mmol/l
No. 2	Common St. John's wort, great nettle, blueberry	0.0188
No. 3	Common St. John's wort, great nettle, common dandelion, blueberry, common horsetail	0.0135
No. 8	Knotgrass, great nettle, common dandelion, blueberry	0.0283
No. 9	Knotgrass, great nettle, blueberry, common horsetail	0.0169
No. 10	Knotgrass, great nettle, blindweed, common horsetail	0.0291

Table 5. Consumption of components per 100 dal (without regard to losses)

Name of food raw material	Units of measurement	Amount of raw material
Extract from Mixture No. 3		
Common St. John's wort (leaves)	kg	33.4
Great nettle (leaves)	kg	33.4
Common dandelion (roots)	kg	33.4
Blueberry (shoots)	kg	33.4
Common horsetail (leaves)	kg	33.4
Drinking water (extractant)	dm^3	1670
Ethyl alcohol, 96% vol (preservation agent)	dm^3	80
Yield	dm^3	1000.0
Extract from Mixture No. 9		
Knotgrass (leaves)	kg	35.8
Great nettle (leaves)	kg	35.8
Blueberry (shoots)	kg	35.8
Common horsetail (leaves)	kg	35.8
Drinking water (extractant)	dm^3	1430
Ethyl alcohol, 96% vol (preservation agent)	dm^3	80
Yield	dm^3	1000.0

Thus, the extracts developed on the basis of medicinal plant raw materials (mainly local raw materials) contain biologically active substances and have an antioxidant activity to varying degrees. The analysis of the functional orientation of the recommended mixtures of medicinal herbs for patients with diabetes mellitus on the basis of the results of the studies has shown the expediency of using mixtures No. 3 and No. 9 for the industrial production of phyto-beverages. A possibility of fortification of the finished extracts to 8% of the volume fraction of alcohol has been determined, due, on the one hand, to their 30-day storage capacity, which is important for industrial production, on the other hand, to the reduction of the quantitative content of the extract in the beverage to the preventive one at a given level of quality, namely, with the alcohol content of not more than 0.5% according to the requirements of regulatory documentation.

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CHEMISTRY AND ECOLOGY

THE CHOICE OF SORBENT FOR ADSORPTION EXTRACTION OF CHLOROFORM FROM DRINKING WATER

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Abstract: At present, providing the population with clean drinking water is one of the most important urgent problems of our time. Due to seasonal changes in water composition and violation of water treatment technology, the conventional process is not always effective to ensure water purification of organic compounds. Moreover, more hazardous contaminants may form unlike the previous ones. Humic substances act as the main source of chloroform formation for water decontamination during the water treatment. Adsorption chloroform extraction from water was studied under static conditions using KAU, SKD-515, BAU, AG-3, AG-OV-1 carbon, ABG semi-coke, PFS polymer sorbents and Porolas T and active nonwoven fabric that differ in the production method, structure and specific surface. Main regularities, features and mechanism for adsorption extraction of chloroform from water are identified for test sorbents. The Freundlich and Langmuir equations (theories of monomolecular adsorption), the Dubinin-Radushkevich equation, modified for adsorption from aquatic solution (theory of micro-pore bulk filling) and the BET equation (generalized theory of polymolecular adsorption of Brunauer, Emmett, and Teller) are used to define sorbents and calculate adsorption parameters. To study the potential to increase the sorbent adsorption capacity due variations in the surface structure and chemistry, sorbents were modified by hydrochloric acid solutions and sodium hydroxide. In absence of experimental studies, the technique is developed to define the limiting value of organic compound adsorption not interacting with surface functional groups of carbon sorbents with the developed system of micropores. The sorbent with the best adsorption properties regarding chloroform was recommended.

Keywords: Adsorption, active carbons, polymeric sorbents, chloroform

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INTRODUCTION

For the past decades, the biosphere has been considerably contaminated, including aquatic ecosystems. The health of population depends on the quality of water resources, the rational integrated use thereof and protection [1]. Being the main source for utility and drinking water system, the natural water contains the considerable amount of pollutants of natural and anthropogenic origin, including organic contaminants. In addition, the water treatment process poses the risk of possibility for more dangerous secondary toxic contaminants to generate. The water from natural water bodies is mainly decontaminated by chlorine-containing reagents. In water, chlorine interacts with natural organic substances, including humic substances, producing halogen-organic compounds. The nature of organic contaminants in the source water, the dose of chlorinating agent, temperature, the length of chlorination and other factors influence the generation of these products and relative content thereof.

For the last decade, people changed their mind as to address the issue of water with the content of halogen-containing compounds in water. Due to the likelihood of adverse effects on health, including metabolic disturbances in case of chronic ingestion intake, the chloroform poses a hazard as the priority and rather popular decontamination by-product available in all water supply systems with chlorinated reagents used. Chloroform accounts for 80–90% of all halocarbons generated in water during chlorination. This compound may be regarded as the indicator of the content of chlorinating products in water [2]. Containing in water in concentrations in excess of the maximum permissible

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value, the chloroform has the general toxic and carcinogenic effect on the human body [3]. As classified by the International Agency for Research on Cancer (IARC, Lyon, France) being the part of WHO, chloroform is referred to 2B Group, i.e., to potentially carcinogenic substances (potentially hazardous) to humans. Many authors [4–9] mention the possibility of cancer development in humans, most often pancreatic, bladder, colon and rectal cancer associated with the consumption of chlorinated chloroform-containing water.

The danger of chlorinated water consumption is the ability of chloroform to cause the most severe delayed action. The direct cytotoxic effect of chloroform is identified on liver cells that are responsible for higher permeability of cell membranes [10]. The development of hematopoiesis process disorder is the consequence of changes in oxidative and antioxidant processes proved by the identified dependence of erythrocytes reduction likelihood in blood due to chloroform content.

The data [10] is compiled on the adverse effect of chloroform on children's health to indicate that at the chloroform content of less than 0.3 MPC, the risk assessment for critical organs and systems chronically exposed to chloroform conducted in line with P2.1.10.1920-04 "Guidelines for Risk Assessment for Public Health Exposed to Chemicals Contaminating the Environment" was adequate to define the unacceptable level of risk for blood circulatory system, liver, kidneys, and central nervous system. The more profound examination of children assisted in getting a package of laboratory parameters to identify the development of adverse effects with negative effects of chloroform, supplied with the drinking water.

As per the genetic testing in children [11] who have been long exposed to organochlorine compounds orally and consume the chloroform-containing drinking water, an the unacceptable chronic (non-carcinogenic) pathology risk is formed with the endocrine system. Non-carcinogenic risk is shown as the probability to develop chronic intoxication symptoms over the certain period which is quantitatively associated with the increase in the overall incidence with no any specific forms of disease.

The effect of halocarbons on the female reproductive function was revealed. The consumption of water disinfected with chlorine during pregnancy may result to the birth of children with severe congenital defects, in particular, with heart and brain defects, as considered by the research team headed by Uni Yaakkola from the University of Birmingham (UK). The investigator assessments revealed that the higher content of chlorinated by-products significantly increases the risk of occurrence of three congenital malformations: septum defect between two ventricles (the septum opening results in the mix of arterial and venous blood and insufficient supply of oxygen to blood), "wolf mouth" (cleft in the palate) and anencephaly (partial or even full absence of the cranial vault bones and brain tissue) [12] In addition, a range of compilations indicate on pregnancy disturbance caused by chloroform: delay in fetal development, weight loss of newborns, premature birth [13,14].

Observations in the USA (Iowa State) made it possible to establish that the consumption of chloroform-containing drinking water by the pregnant at concentrations of more than 0.009 mg/dm^3 results in the higher risk of the fetal development delay [15]. The maximum permissible concentration of chloroform as per international and national standards for drinking water quality is as follows: in the EEC standards - up to 0.001 mg/dm^3, WHO - up to 0.030 mg/dm^3, RF - up to 0.060 mg/dm^3 (hygienic standards 2.1.5.2280-07), USA - up to 0.100 mg/dm^3. In this view, it is necessary to exclude chloroform in drinking water.

One of ways to reduce the chloroform concentration in water is its adsorption by various materials of the developed surface or specific properties. Most often adsorbents are active carbons (AC) and polymeric sorbents with the selectivity of adsorption of organic substances. In this view, this paper aims at the study of adsorption properties of the large group of domestic carbon sorbents with respect to chloroform.

OBJECTS AND METHODS OF STUDY

The research objects were the commercial active carbons based on SKD-515, AG-3, AG-OB-1 make black coal, BAU charcoal, KAU carbon obtained from apricot kernels (produced by the Sorbent OJSC, Perm), ABG semi-coke (produced by Carbonika-F CJSC, Krasnoyarsk), PFS polymer sorbent (PFR), Porolas T polymer sorbent (produced by Sorbent JSC, Perm), activated nonwoven fabric (ANF) that differ in nature, production method, porosity of structure and specific surface area. Brief description of physical and chemical properties of active carbons and polymeric sorbents is presented in Table 1.

The AG-OV-1 carbon is produced from the crozzling coal of coal-bearing base. The main production stages include: grinding, granulation, carbonization at 500–600°C, activation at 900–950°C, sieving and packing. The AG-3 carbon is produced from the Kuznetsk candle coal. Main production stages cover: semi-coking, grinding, granulation, carbonization, steam activation. The SKD-515 carbon is the sorbent on the coal-bearing basis known for the developed porous structure and high internal surface. Main stages of production include two-stage heat treatment, activation and granulation. The coal of KAU grade is made of apricot bone putament by crushing it followed by sieving. A kernel-based sorbent may undergo multiple regeneration as it is known for the large microporous structure and higher strength. The BAU carbon is the wood-based sorbent made from the hardwood. Main stages of production include the following: sieving, activation at 850°C, crushing and grinding. However, this sorbent is recommended for small-sized sorption columns with fixed bed due to low mechanical characteristics (strength, micro- and mesoporous structure). ABG is the brown coal gasified carbon sorbent obtained from the coal-tar raw material (semi-coke). The semi-coke is produced from coal mined in the Kansko-Achinsky Basin section. This unique technology for semi-coke production is low-cost. Polymer phenolic-formaldehyde resin-based sorbent (PFS) [16].

Table 1. Physical and chemical properties of sorbents

Parameter	Sorbent brand							
	KAU	AG-3	AG-OV-1	SKD-515	BAU	ABG semi-coke	PFS	Porolas T
Bulk density, g/dm^3	417	465	531	526	240	490	374	550
Strength, %	90	88	70	75	60	70	86	95
Weight content of total ash,%	5.4	8.0	15.0	31.0	7.0	12.0	1.3	–
pH of water extract	9.25	6.45	6.85	7.65	7.85	7.45	7.85	–
Total pore volume, cm^3/g	0.97	0.88	0.44	0.7–1.0	0.47	0.5–0.57	1.1	2.52
Pore volume, cm^3/g								
Micro-	0.31	0.26	0.32	0.28	0.25	0.09	0.34	0
Meso-	0.66	0.09	0.14	0.11	0.10	0.38	0.76	2.52
Macro-	–	0.53	0.44	0.33	–	0.12	–	0
Adsorption activity by iodine, %	78	60	65	56	60	60	226	–
Pellet shape	irregular	cylindrical	irregular	cylindrical	irregular	crushed	globe-shaped	spherical

Table 2. Physical and chemical characteristics of the activated nonwoven fabric

Parameter	Dimension	Value
Width of fabric	mm	400–500
Fabric thickness	mm	3–5
Surface density	g/m^2	50–250
Specific surface area	mg/g	over 1000
Total pore volume	cm^3/g	over 0.8
Moisture of fabric	%	less than 5
Adsorption capacity for benzene	mg/g	over 280
Adsorption capacity for iodine	%	over 80
Adsorption capacity for phenol	mg/g	over 200

Porolas sorbent, as the highly porous non-ionic polymer, is the polystyrene obtained by granular inert solvent copolymerization of divinylbenzene and ethylstyrene iwhere nert solvents are easily removed from the finished product. The change in the ratio of initial monomers of divinylbenzene and ethylstyrene, the solvent volume and nature (isooctane, benzene, toluene, cyclohexane, isobutanol, etc.) results in the wide range changes in the porous structure of such adsorbents of the similar chemical structure. Highly porous non-ionic polymers are predominantly classified as mesoporous sorbents, with few micro-pores that may be accessed by organic molecules.

The activated non-woven fabric (ANF) is predominantly produced from viscose production waste. Main production stages include: carbonization in the inert gas (CO_2) and the activation of textile (cellulose, viscose, and synthetic) materials of both woven and non-woven fabric at 900°C. Carbonization and activation of textile materials commit to micropore formation. The activated non-woven fabric and activated carbon differ in the surface condition by the crystallinity degree (crystallized sections alternate with random sections (amorphous) and degree of carbon surface homogeneity. The activated non-woven fabric surface is more homogeneous as compared with the active coal surface. Sorption carbon fabrics differ with the large structure of the thread porous space forming these fabrics, as well as with high mechanical strength, low air resistance and unique kinetic parameters. The activated non-woven fabric is produced as the fabric with physical and chemical characteristics shown in Table 2.

To clarify peculiarities of chloroform adsorption, the adsorption equilibrium was studied on active coal and polymer sorbent solutions within the range 0.0042 to 25.13 mmol/dm^3 in concentration. The chloroform in aqueous solutions was determined as per the common gas chromatography procedure with the electron capture detector [17]. The time to reach the adsorption equilibrium was determined under static conditions by the additional series of tests to be within 24 hours, not longer. The adsorption value (a, mmol/g) for test sorbents of the certain weight (m, g) was determined by the difference of the initial (C_0, mmol/dm^3) and equilibrium (Cp, mmol/dm^3) chloroform concentrations in the aqueous solution of certain volume (V, dm^3):

$$a = \frac{C_0 - C_p}{m} * V .$$

The test sample adsorption with the exception of polymer sorbents PFS and Porolas T was studied

without any pre-arrangement. In view that PFS and Porolas T have the hydrophobic shell that makes it difficult to study the chloroform adsorption equilibrium from aqueous solutions, it was removed by treating sorbents with ethyl alcohol or acetone for 8 hours followed by washing polymer sorbents with distilled water until the excess solvent was completely removed.

The effect of sorbents treatment with HCl and NaOH solutions on adsorption properties was evaluated using active coals of AG-3, SKD-515, and AG-OV-1 brands. They were placed in acid (alkali) solution for 24 hours followed by washing with distilled water.

Porous properties of active coals (total surface area, microporous area, volumes) were studied by the method of low-temperature adsorption of nitrogen at 77°C using the Sorbtometr M (manufactured by the Boreskov Institute of Catalysis, Siberian Branch of the RAS, Novosibirsk).

RESULTS AND DISCUSSIONS

Adsorption isoterms refer to one of major evaluation criteria for the sorbent adsorption properties that allow determination of the sorbent activity dependence (sorption capacity) on adsorbate concentration under equilibrium conditions. The chloroform adsorption isotherms are plotted from aqueous solutions presented in Fig. 1 based on experimental data. It was established that the adsorption capacity of test adsorbents decreases in terms of chloroform in the row as follows: ANF>KAU>SKD-515>BAU>AG-3> AG-OV-1>PFS>ABG>Porolas T.

In the region of low chloroform concentrations (initial section), the adsorption isotherm for all the tested sorbents has a linear pattern, and is, consequently, described by the Henry equation. In the high-concentration region, the adsorption isotherm is L-shaped as per the Hils's classification. The obtained results indicate that the most effective sorbent for chloroform extraction from aqueous solutions is the activated nonwoven material due to the large specific adsorption surface and the AU KAU. However, since they are ore expensive as compared with other sorbents, SKD-515, BAU, AG-3 and AG-OV-1 are the most effective activated carbons for chloroform extraction. Despite the lower adsorption capacity, the ABG semi-coke is the local inexpensive stock that does not require regeneration and, as processed, it may be provided to combustion plant as the solid fuel. The chloroform adsorption volume on PFS polymer sorbents and Porolas T is lower than that on activated carbon. One of reasons for lower chloroform adsorption on polysorbates as compared with activated carbon adsorption is the screening by hydrogen atoms of carbon atoms of polysorbate frame polymer chains that prevents the chloroform adsorbed molecules from approaching the polymer carbon atoms at the distance reached when adsorbed on the surface of carbon materials.

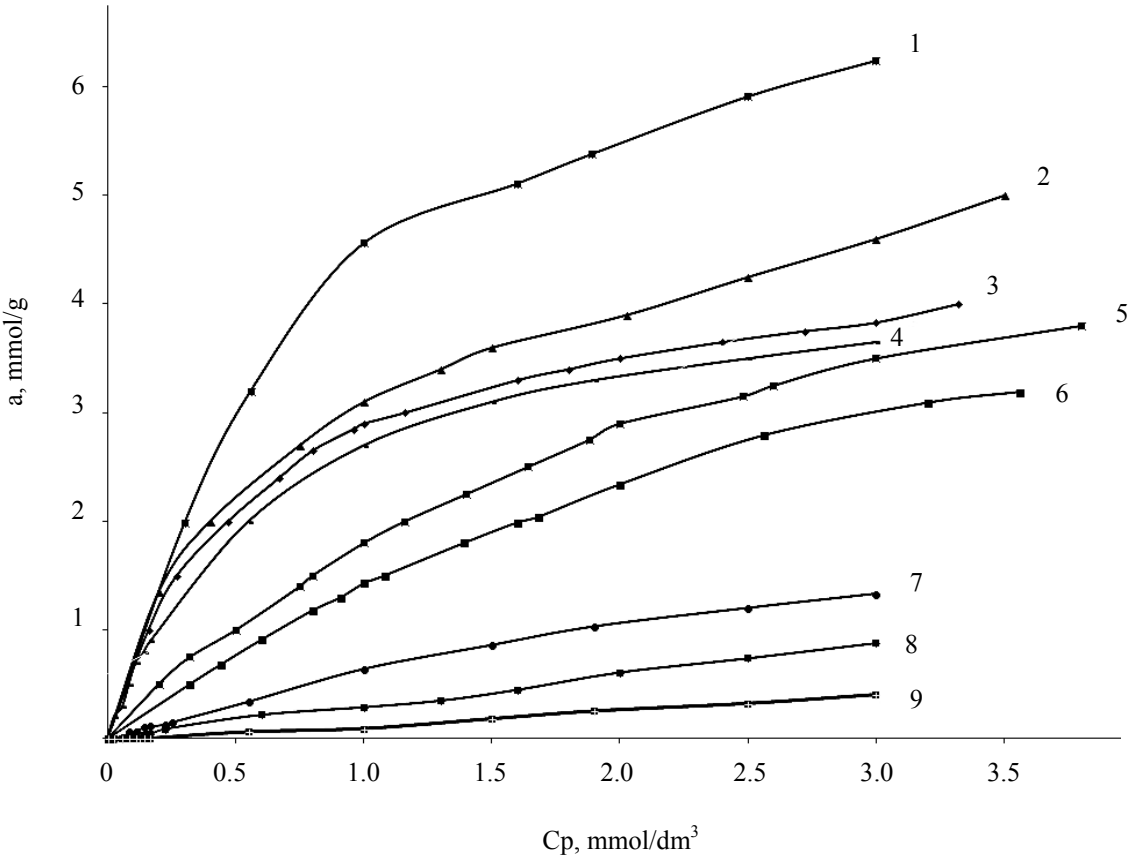

Fig. 1. Isotherms of chloroform adsorption from aqueous solutions on sorbents as follow: (1) ANF, (2) KAU, (3) SKD-515, (4) BAU, (5) AG-3, (6) AG-OV-1, (7) PFS, (8) ABG, (9) Porolas T.

For more detailed characterization of test sorbents and to calculate main adsorption parameters, monomolecular adsorption theories were used (Freundlich and Langmuir equation), the theory of micropore bulk filling (Dubinin-Radushkevich equation modified for aqueous solution adsorption) and the generalized theory of polymolecular adsorption of Brunauer, Emmett and Teller (BET). Theoretical isotherms of chloroform adsorption are calculated by parameters obtained shown in Fig. 2 using the example of AU AG-3. All adsorption isotherms are reviewed in appropriate linearization coordinates of these equations. The activated carbon chloroform adsorption isotherms in coordinates of equations of Langmuir, Freindlich, Dubinin-Radushkevich, and BET are shown in Fig. 3 using the example of AU SKD-515. A comparative analysis of experimental and theoretical adsorption isotherms indicated that all the equations used well describe adsorption isotherms experimentally obtained on AU. Consequently, all adsorption equations may be used to describe the adsorption process on these sorbents. The calculated values of adsorption parameters for chloroform adsorption are given in Table 3.

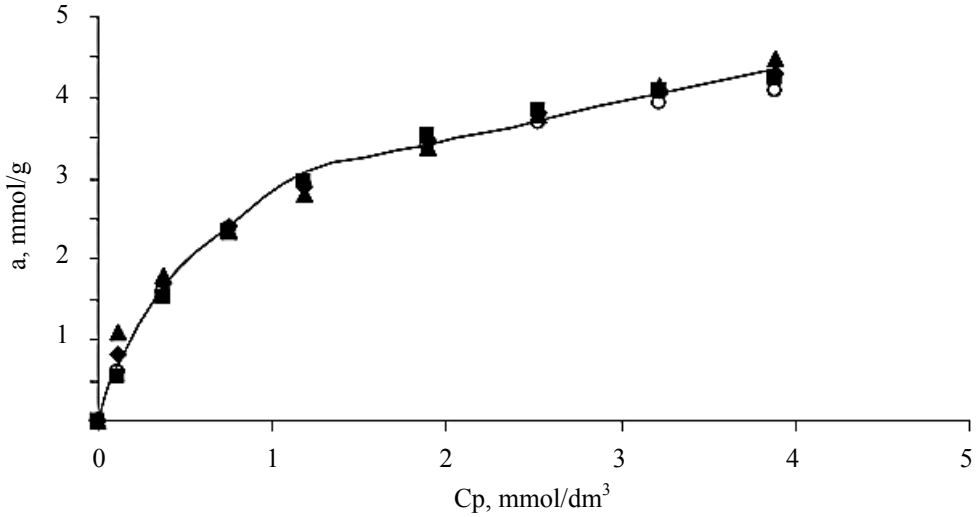

Fig. 2. Experimental (–) and calculated chloroform adsorption isotherms by equations of Langmuir (■), Freundlich (▲), Dubinin-Radushkevich (♦), BET (○) from aqueous solution on AU AG-3.

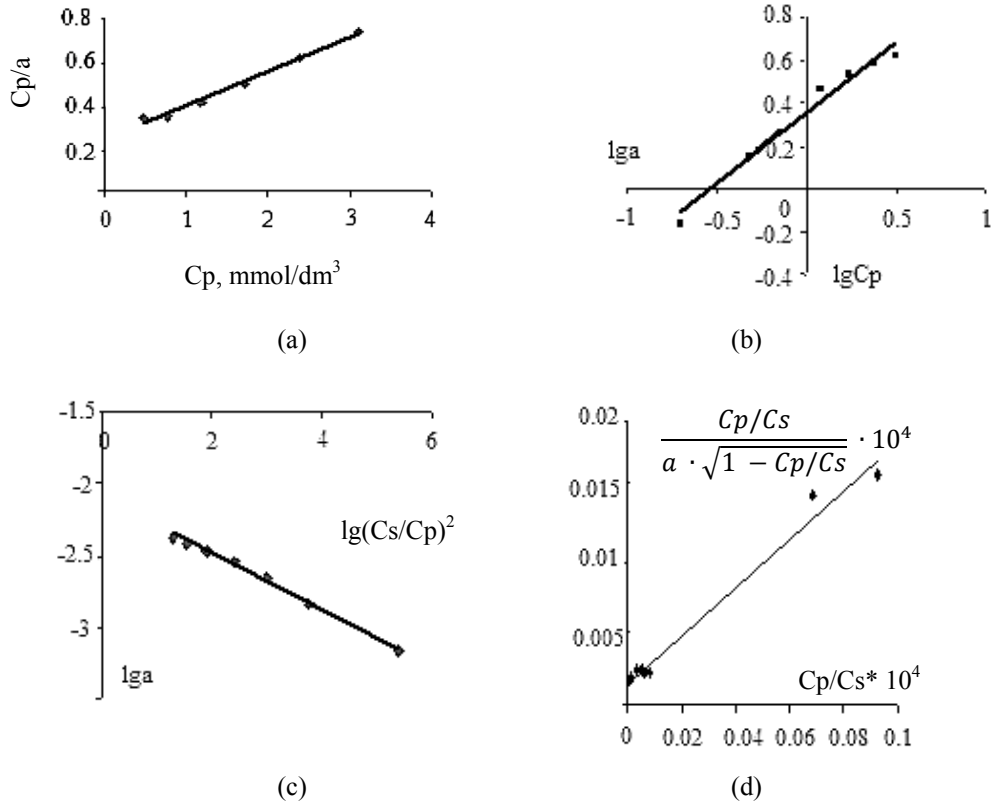

Fig. 3. Isotherms of chloroform adsorption on AU SKD-515 in the linearization coordinates of equations Langmuir (a), Freindlich (b), Dubinin-Radushkevich (in), BET (g).

Table 3. Parameters of chloroform adsorption from aqueous solutions by sorbents under static conditions

Sorbent make	Adsorption parameters calculated by the equation of									
	Langmuir		Freindlich		Dubinin-Radushkevich			BET		
	a_{max}, mmol/g	K	β, mmol/g	1/n	a_{max}, mmol/g	E_0, kJ/ mmol	W, dm^3/kg	a_{max}, mmol/g	K	Q, kJ/mol
AG-3	5.27	1.07	2.63	0.82	6.85	10.49	0.64	4.6	58.75	10.13
AG-OV-1	–	–	2.27	0.81	6.54	10.10	0.1892	–	–	8.23
SKD-515	6.26	0.67	0.52	0.85	7.22	8.50	0.86	5.53	33.49	8.77
KAU	–	–	–	–	7.96	10.40	0.85	–	–	–
PFS	–	–	–	–	4.56	11.70	0.49	–	–	–
ABG	4.99	0.14	0.70	0.35	3.54	7.03	0.38	3.22	9.86	5.91
BAU	–	–	0.62	0.82	5.63	10.00	0.3218	–	–	14.15
Porolas T	–	–	1.6975	0.38	–	–	–	–	–	–
ANF	–	–	0.5537	0.09	5.30	14.12	0.49	–	–	14.55

The values of the limit adsorption volume of tested sorbents (0.49–0.86 cm^3/g) indicate that the chloroform adsorption is subject to the volume mechanism of microporous filling. The values of characteristic energy (7.03–14.12 kJ/mmol) allow assuming the larger microporous structure of activated carbons.

Isotherm forms and values of adsorption heat allow preliminary conclusion on the mechanism of chemical compound interaction with the carbon sorbent surface. Isotherms of L type show that the interaction between adsorbed molecules of chemical compounds is insignificant, and the activation energy does not depend on the extent of sorbent surface filling. Isoterms of L class and calculated adsorption heats (5.91–14.55 kJ/mol) indicate that the chloroform adsorption from aqueous solutions is of physical pattern and is due to van der Waals forces.

To clarify the mechanism of chloroform adsorption, the potentiometric titration as per Böhm was performed as well as characteristics of the sorbent porous structure (porometry). The porosimetry data allow identification of the non-specific interaction share (dispersion interaction), and titration data define the fraction of specific interaction due to hydrogen bond of the surface oxygen-bearing function groups of AU with the adsorbate, that is, the amount of OBFG on the sorbent surface. Main characteristics of the porous structure of tested AU and the potentiometric titration data are shown in Tables 4 and 5.

The porosimetry data indicated (Table 4) that the volume of micropores in AU is pro rata their adsorption capacity in terms of chloroform, the adsorption of which is of physical nature and is predominantly shown in micropores. There is the strong dependence of the adsorption capacity on the number of micropores: KAU>SKD-515>BAU>AG-3>AG-OV-1>ABG. This is what explains the high adsorption capacity of sorbents with a large number of micro-pores.

The chemical condition of the surface may affect the adsorption behavior of sorbents, either. Yet, all carbons possess adequate function groups that interact with active sorbate groups, increasing the adsorption capacity (Table 5). Data comparison of adsorption capacity, structure and surface chemistry confirm that non-specific interaction is the only mechanism for chloroform adsorption. Chloroform with no groups capable of interacting with function groups on AU surface is adsorbed only in pores with the clear structure-specific dependence of adsorption reported.

Based on experimental data review, the technique to calculate the coefficient b is offered that describes the relationship between the micropore volume ($V_{ми}$, cm^3/g) and the value of the limiting adsorption (a_{max}, mmol/g), which allows without additional studies to determine the adsorption limit of any porous material by with respect to chloroform. The values of the coefficient b are shown in Table 5.

Table 4. Main characteristics of the test sorbent porous structure

sample	S_{micro}, m^2/g	V_S, cm^3/g	V_{meso}, cm^3/g	V_{micro}, cm^3/g
KAU	1419	0,730	0.110	0.620
BAU	586	0,455	0.103	0.352
AG-3	490	0,340	0.060	0.270
SKD-515	404	0,561	0.202	0.359
AG-OV-1	369	0,459	0.241	0.218
ABG	–	0,260	0.240	0.020

Table 5. Surface condition of activated carbons

AU	The content of active oxygen in mmol-eq/g of carbon (n_{kfg}, μmol-eq/m^2)		
	-OH phenolic	$-COOH_{heav}$ carboxylic	-COO- lactone
SKD-515	0.181	–	0.157
AG-OV-1	0.213	0.032	0.078
AG-3	0.321	0.035	0.039
ABG	0.130	0.020	0.040
KAU	0.194	0.090	0.060

Table 6. Relationship between the volume of micropores and the value of limiting sorbent adsorption

Carbon grade	KAU	AG-3	SKD-515	BAU
a_∞, mmol/g	7.96	6.85	7.22	5.63
V_{mi}, cm^3/g	0.31	0.26	0.28	0.25
$b = a_\infty / V_{mi}$	25 ± 7	26.4	25.8	22.5
b_{cp}	25.10 ± 2.04			

Table 7. Henry's Constants for Treated and Technical Sorbents

AU grade	Black AU	AU treated with HCl	AU treated with NaOH
SKD-515	7304	7389	7293
AG-3	6328	6337	6326
AG-OV-1	3813	3798	3798

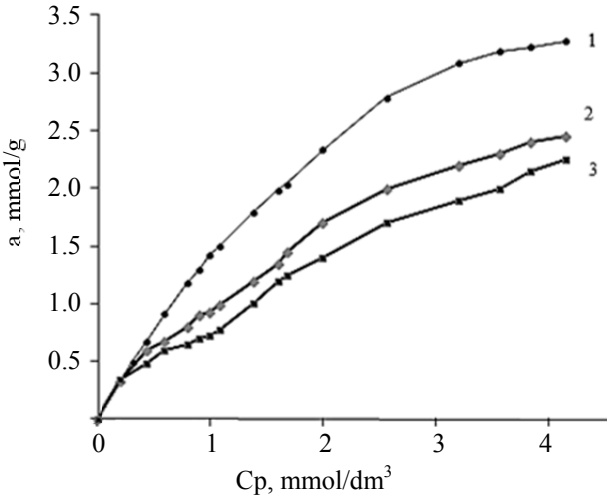

Fig. 4. Isotherms of chloroform adsorption from aqueous solutions on AU AG-OV-1: (1) technical; (2) treated with HCl; (3) treated with NaOH.

The applicability of the proposed method is verified by comparing the value of limiting adsorption calculated by the proposed method and the value obtained during the test on the example of halocarbon adsorption (chloroform, carbon tetrachloride, difluorochloromethane, trifluoromethane) with activated carbon AG-OV-1. The discrepancy between experimental data and calculated values of the limiting adsorption does not the error of chloroform concentration determination in the solution (10–12.5%). This technique to find the limiting adsorption of sorbents in terms of chloroform can be recommended for other class substances not interacting with surface function groups of sorbents with the large system of micropores.

To increase the adsorption capacity of AU, the effect of sorbent pretreatment with hydrochloric acid and sodium hydroxide sokutions was investigated. It is found that the isotherm pattern of chloroform adsorption within the initial section with industrial activated carbons treated with acid (alkali) is similar, that is, in the region of low chloroform concentrations, the amount of chloroform adsorption by carbon sorbents does not almost depend on the method of preparation thereof. The Table 7 presents similar values of Henry's constants rated for treated and commercial carbons that, in turn, indicate on zero influence of the sorbent preparation technique on the chloroform adsorption in the low-concentration region.

The data in Fig. 4 show based on the example of AU AG-OB-1 that regions of high chloroform concentrations report on the decrease in the adsorption value for all sorbents treated with HCl or NaOH solutions. Such effect is probably due to the influence of acid or alkali where a portion of micropores transformed to mesopores with the sorption to be considerably lower than in micropores.

A decrease in the ash content verifies a change in the carbon structure caused by the treatment. The content is determined by gravimetry (on average, the ash content of carbon reduced by 18%). Yet, to note that the partial sorption of reagents (HCl or NaOH) may occur during the treatment resulting in the reduction of the sorption space, and, consequently, to the decrease in the value of ultimate adsorption of carbon to chloroform. Therefore, to purify the drinking water of chloroform, it is practicable to recommend the use of technical sorbents (without pretreatment).

CONCLUSIONS

Thus, the study of adsorption properties of test sorbents under static conditions indicates on their high adsorption capacity in terms of chloroform and allows their arrangement as per extraction efficiency in a series: ANF>KAU>SKD-515>BAU>AG-3> AG-OV-1>PFS>ABG>Porolas T that is related to the different structure of test sorbents. Studies show that the chloroform adsorption predominantly takes place in micropores due to non-specific interaction. The activated carbon treatment with HCl or NaOH solutions results in the decrease in adsorption capacity of sorbents due to changes in their structure by the treating reagent. AU SKD-515 can be recommended for adsorption extraction by the results of studies.

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BRIEF COMMUNICATION

SOME PECULIARITIES RELATED TO FORMATION OF DRIED MILK PRODUCTS PROPERTIES

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Abstract: The current development of drying technologies makes it possible to manufacture dried milk products which, when recovered, practically do not differ from the original ones. At the same time, the choice of various auxiliary dried milk conditioning processes impacts decisively on its physical-chemical, organoleptic and reconstitution (instant) properties. This article presents the results of research covering the impact of individual methods of vibration treatment and transportation of dried milk products manufactured by two-stage spray drying on their characteristics. The properties of dried milk products were evaluated using both traditional and specially developed methods. To measure the instant properties, a specially developed index – relative dissolution rate – was used. Analysis of changes in properties of samples of dried milk products selected at different stages of the drying process indicates a significant decrease in the average particle size, an increase in the free fat mass fraction and consequently a decrease in the relative dissolution rate after passing through instantizer, vibrating sieve and aerosol transport system. And the most significant changes are observed during milk powder passing through aerosol transport system. In general, the results obtained indicate the significant impact of the drying method, systems of treatment, transportation and intermediate storage of dried milk on its properties that should be taken into account when improving the relevant processes and equipment.

Keywords: Dried milk products, powder milk, dried instant milk, spray drying, two-stage drying, disk spraying, nozzle spraying, instant properties, relative dissolution rate, drying chamber

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INTRODUCTION

The properties of dried milk products produced by spray drying method are very diverse due to differences in their composition, pre-treatment regimes, instrument and process peculiarities of the main and auxiliary drying processes. In this case differences in the drying process conditions executed by traditional and two-stage methods consisting in the fact that the particles dehydration conditions in the process of two-stage drying promote acquiring of an agglomerated structure are of great importance.

As a consequence, the products obtained by these methods differ from each other by quite a number of characteristics including granulometric composition [1, 2], solubility indices (instant properties) [3, 4], free fat mass fraction [3, 4], and bulk weight [5, 6].

The present studies are devoted to studying impact of some auxiliary processes of dried milk treatment using traditional and two-stage drying process on the mentioned indices.

OBJECTS AND METHODS OF STUDY

The dried milk products properties were evaluated using both conventional and specially developed methods. In particular, the mass fractions of moisture, fat, free fat and bulk weight were measured by conventional methods. The particle size distribution was evaluated by sieving the product through a set of sieves with a mesh size of 2; 0.5; 0.25 mm, followed by weighing the four resulting fractions and determining the average weighted nominal particle diameter [3]. To characterize the instant properties, a specially developed indicator – the relative dissolution rate – was used [1, 3].

To accelerate and improve the accuracy of this analysis, we used a device that worked as follows.

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A sample of usual or instant whole milk powder in an amount of 5 g is poured into a mixing bowl, into which 35 ml of water with a temperature of 20 ± 0.5°C is then added. Then, the electric motor driving the agitator is turned on. After the end of stirring, which lasts 5 s, a vacuum pump starts and a filtration process begins. The filter, which is a porous glass plate at the bottom of the mixing bowl, passes only the dissolved product. At the end of the filtration process, which usually lasts no longer than 2 s, the vacuum pump is stopped. Then the mass fraction of solids in the dissolved product is refractometrically determined.

The relative dissolution rate was estimated by the percentage content of the dry milk sample transferred to the solution on the assumption that the current relative dissolution rate is proportional to the maximum one that corresponds to the dry matter content of the fully reconstituted dry milk. In the case of estimating the relative dissolution rate of dry skim milk or milk powder with a fat mass fraction of 15%, the amount of water in which the dissolution process is carried out using the device varies proportionally.

Using the above figures, the properties of samples of five kinds of dried milk products, varied by different fat content and selected at different stages of the technological process were analyzed:

– Sample 1 is dried whole instant milk added with surfactants (DWM-instant);

– Samples 2 and 7 are dried instant skim milk (DSM-instant), sample 3 is dried whole milk added with surfactants (DWM with SAA);

– Sample 4 is dried milk with 15% mass fat fraction (DM 15%);

– Samples 5 and 6 are dried whole milk with 25% mass fat fraction (DWM).

In the course of the research, spray dryers with a disk (Fig. 1) and a nozzle (Fig. 2) spraying equipped with twin-shell shakers 2 were used (hereinafter the position numbers correspond to both drying schemes shown in Fig. 1 and 2 for drying. A drying unit intended for obtaining dry whole milk, an instant, with surfactant additives, was provided with an additional powder wetting system at the outlet of the drying chamber 22 and a surfactant application unit after the first drying stage of the powder 24.

The operating principle of the dryer (Fig. 1) is described below. The first stage of drying the product takes place in a vertical cylindrical drying chamber 1. The condensed product from a condensed milk tank 3 is fed to the drying chamber 1 by means of a food pump 1, where it is sprayed with a spray atomizer and dried in a hot air stream, after the latter has been prepared in a system consisting of a purification filter 5, a blowing fan 6 and a main air heater 7. The product dried to a humidity of 6–8% due to the conical shape of the lower part of the drying chamber enters the fluidized bed of an agglomeration chamber 23. The fluidization mode in the agglomeration chamber is created by supplying through the perforated bottom of the additional air prepared with the air filter 5, a fan 8 and an air heater 9.

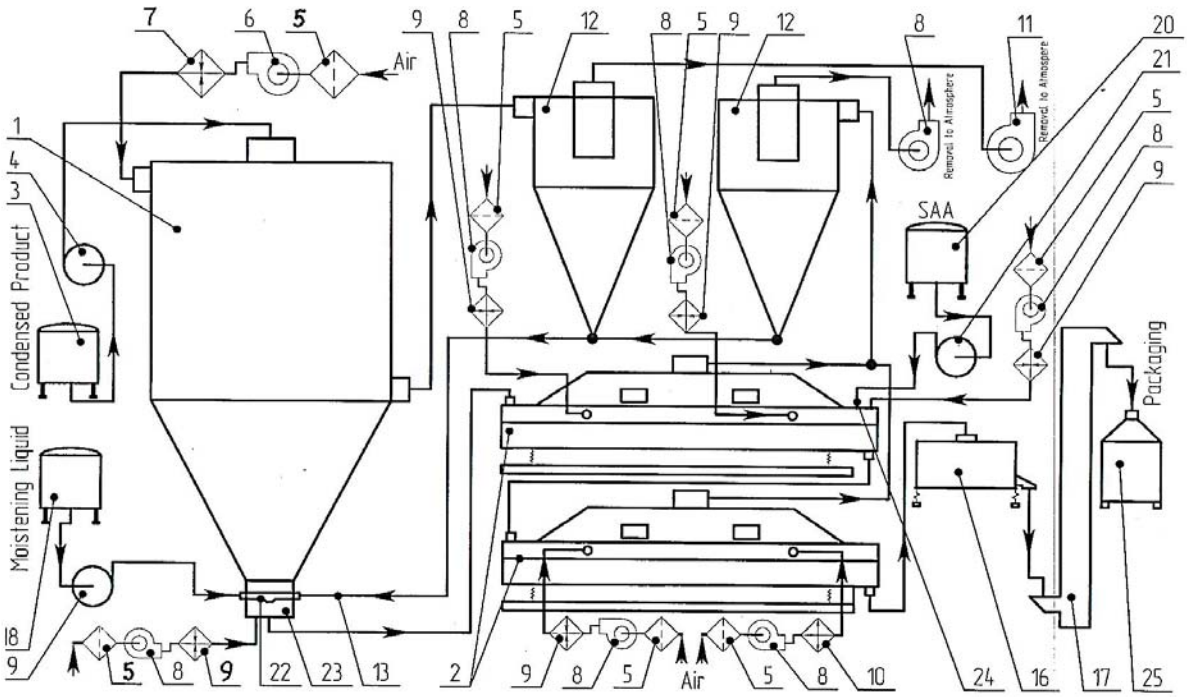

Fig. 1. Diagram of installation for dried instant milk production: 1 – drying chamber; 2 – vibratory convective drier; 3 – tank for condensed milk; 4 – feed (product) pump; 5 – outside air cleaner filter; 6 – mechanical blower; 7 – main heater; 8 – fan; 9 – heater; 10 – air cooler; 11 – main fan; 12 – cyclone; 13 – cyclone fraction recovery system; 16 – vibrating sieve; 17 – bucket elevator (belt elevator); 18 – tank for moistening liquid; 19 – pump for moistening liquid supply; 20 – tank for surfactants; 21 – pump for surfactants supply; 22 – block for moistening liquid spraying; 23 – agglomeration chamber; 24 – block for surface active agents spraying; 25 – container.

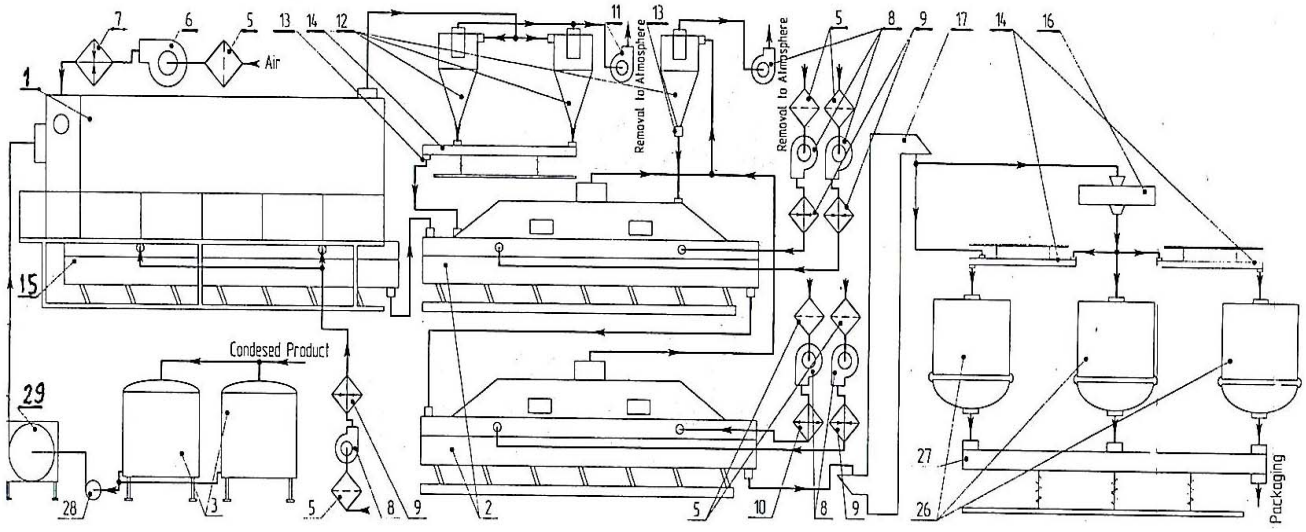

Fig. 2. Diagram of the drying chamber (OSV): 1 – drying chamber; 2 – vibratory convective drier; 3 – tank for condensed milk; 5 – outer air cleaner filter; 6 – forced draft blower; 7 – main heater; 8 – fan; 9 – heater; 10 – air cooler; 11 – main fan; 12 – cyclone; 13 – cyclone fraction recovery system; 14 – vibrating conveyor; 15 – granulator; 16 – vibrating sieve; 17 – bucket elevator (belt elevator); 26 – cooling fan for drying chamber end wall; 27 – hopper; 28 – discharging vibrating conveyor; 29 – line pump; 30 – high pressure pump.

The guaranteed formation of agglomerates of the product particles in the agglomeration chamber 23 feds a moisturizing liquid (e.g., skimmed milk) to the surface of the layer, for which purpose a special humidifying liquid spraying unit 22, a pump 19 for supplying it, and a storage tank 18 serve. Small particles of the cyclone fraction of the product entering the agglomeration chamber 23 from the cyclones are also involved in the agglomeration process using a cyclone fraction recovery system 13.

Agglomerated product from the sintering chamber enters the first shaker (instantizer) 2, where it is dried in a fluidized bed to standard moisture content. At the interface of the first and second shakers, there is a spraying unit for surfactants 24 used for the production of fat-containing products. The surfactant enters this unit from a reservoir 20 by means of a pump 21. In the same area, the fan 8 supplies hot air through the filter 5 and the air heater 9 for preliminary distribution of surfactants in the product volume. Further uniform distribution of surfactants along the surface of the agglomerates is ensured by blowing hot air through the product layer of the first section of the second shaker 2. In the second section of this shaker, the particles are cooled and their final structure is formed. Particles are cooled by air prepared in a cooler 10. The finished product passes through a vibrating screen 16 after the second shaker, where the lumps formed accidentally during drying and agglomeration are separated, after which the product is reloaded, with a bucket elevator (belt elevator) 17, into separate containers 25 with a capacity of about 1 m3 each, in which the product is transported to the packaging station. The use of the bucket elevator 17 and containers 25 is caused by the need to ensure minimal mechanical impact on the formed agglomerated particles of the product. Cleaning of waste air in the drying chamber and shaker from the trapped small particles of the product takes place in cyclones 12. The fan 8 and an exhaust (main) fan 11 are used to remove air from the cyclones.

The production of dry milk products without surfactants was carried out without the use of an agglomeration chamber 23, a surfactant spray assembly 24, a belt elevator 17 and containers 25. At the same time, the transportation of dry milk to the packaging station was carried out by aerosol transport system.

The operating principle of the nozzle horizontal dryer (Fig. 2) is described below. The first stage of drying the product takes place in a horizontal rectangular drying chamber 1. The condensed product from a condensed milk tank 3 is fed via an intermediate pump 28 and a high-pressure pump 29 to the drying chamber 1, where it is sprayed with mechanical injectors and dried in a stream of hot air after the preparation of the latter in a system consisting of a purification filter 5, a blower 6 and a main air heater 7. The product dried to a moisture content of 6-8% enters the fluidized bed of a granulator 15 located under the drying chamber. In the fluidized bed of the product of the granulator 15, due to residual moisture, partial agglomeration of the particles takes place. The fluidization mode in the granulator 15 is created by applying vibration and feeding through the perforated bottom of the additional air prepared with the air filter 5, a fan 8 and an air heater 9. The product from the granulator 15 enters the first and second shakers (instantizers) 2, where it is dried in the fluidized bed to standard moisture content and cooled. The fine particles of the product cyclone fraction are returned to the first shaker with the aid of a vibrating conveyor 14 and the return systems of the cyclone fraction 13. Particles are cooled by air prepared in a cooler 10. The finished cooled product from the second shaker, using bucket elevator (belt elevator) 17, flows through a vibrating screen 16 into vibrating conveyors 14 and is distributed to hoppers 26. From the hoppers 26 the product is fed to the packaging station with the use of an unloading

conveyor 27. The vibrating screen 16 serves to separate out randomly formed lumps during the drying process. Cleaning of waste air in the drying chamber and shaker from the trapped small particles of the product takes place in cyclones 12. The fan 8 and an exhaust (main) fan 11 are used to remove air from the cyclones.

The following basic equipment was used for dried milk production after the instantizer discharge:

– Sample 1 – vibrating sieve (Pos. 16), bucket elevator (Pos.17), intermediate small-sized containers 0.5 m^3 (Pos. 25);

– Samples 2, 3, 4 and 5 – vibrating sieves (Pos.16), aerosol transport lines (Pos. 13), hoppers for buffering (Pos. 27);

– Samples 6 and 7 – vibrating sieves (Pos.16), bucket elevator (Pos. 17), horizontal vibrating conveyors (Pos. 14).

The capacity of driers equipped with disc atomizer and instantizer was 1500 kg/h of evaporated moisture, the drier with spray-type atomizers – 1000 kg/h. The drier inlet air temperature was maintained at the level of 175–180°C. Outlet air temperature was maintained at the level ensuring the specified product moisture content at the drying chamber outlet. Air temperature in the first and second sections of the instantizer was maintained at the level of 70–115°C, in the cooling section – at the level of 6–13°C. The air supply parameters in all cases ensured the production of the finished product with the standard moisture content (2.5–4%).

RESULTS AND DISCUSSION

The results of the systems impact providing the additional processing and dried milk transport within the different variants of its production are presented in Fig. 3. In all cases mass moisture fraction at the drying chamber outlet was 6–8%. DWM with SAA samples were the exception. Mass moisture fraction in milk powder at the dying chamber outlet was 5.4–5.5%.

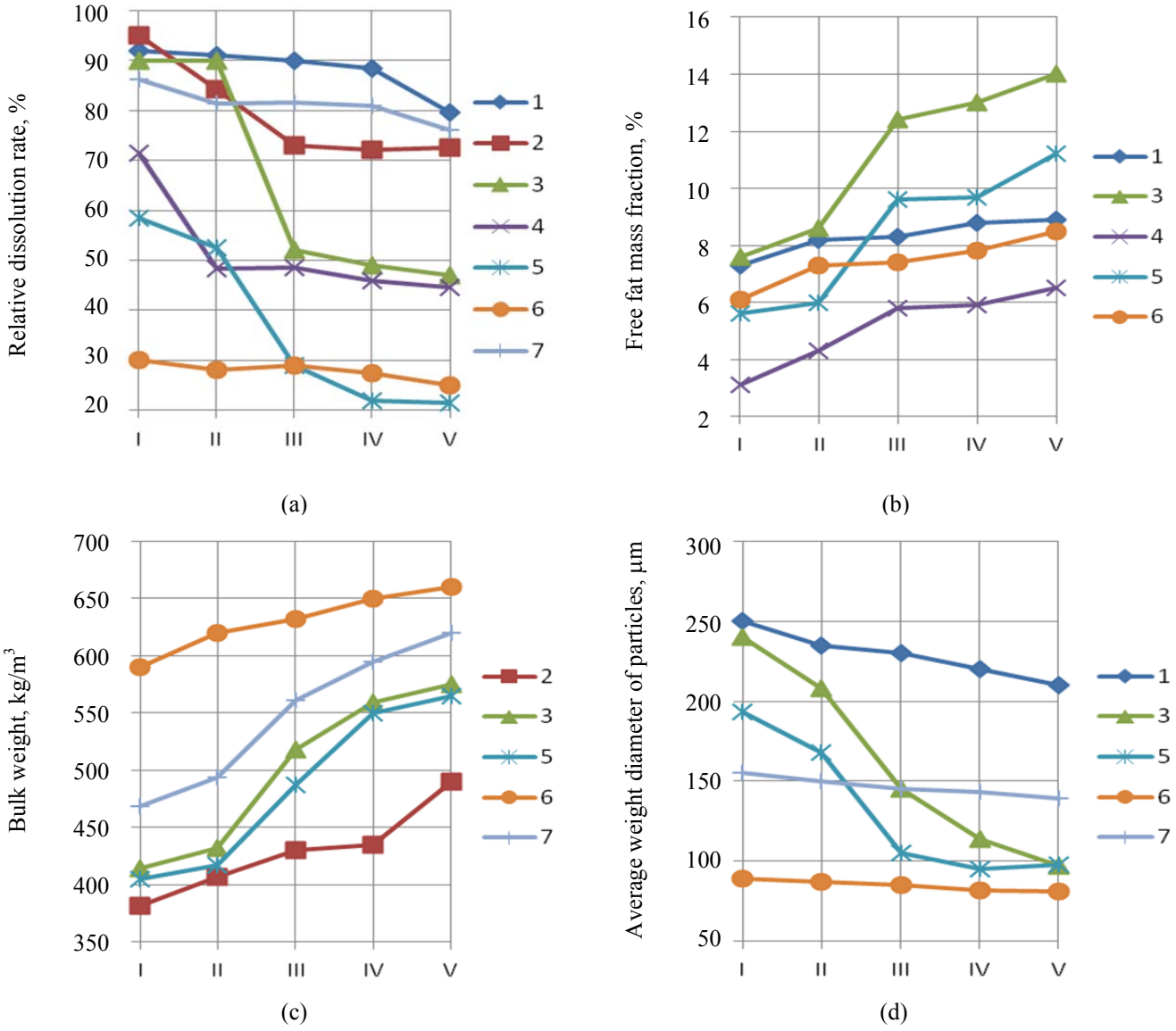

Fig. 3. Changes of dried milk properties as a result of the additional treatment system impact and conveyance at different stages of the process: 1 – at the drying chamber outlet; II – after instantizer and vibrosieve; III – at the conveyance system outlet; IV – after intermediate storage in hoppers and containers; V – after packaging; 1 – instant DWM with SAA additives; 2,7 – instant DSM; 3 – DWM with SAA additives; 4 – DM 15%; 5,6 – DWM (samples 6 and 7 are produced with nozzle spraying and the rest – at the driers with disc spraying).

The analysis of the obtained results indicates the significant impact of dried milk processing systems after drying on its fat phase stability, average size of particles, bulk weight and instant characteristics. One can state that the traditionally utilized systems and particularly vibrating sieves and conventional aerosol transport lines have a negative effect on these properties (samples 3, 4 and 5). Thus, dried milk particle size distribution is changed to the reduction of the largest particles portion (agglomerates) in the product. Herewith the most significant changes of the granulometric composition are observed when the milk powder passes through the aerosol transport system.

The supplementary tests of the manufactured product showed that immediately after leaving the drying chamber the milk powder contains relatively small number of single particles. The mechanical effect of conventional transportation systems leads to an increase in the proportion of single particles by more than 50%, as well as to a drop in the average particle size below 120 μm. These changes are properly agreed with bulk weight amount change in the dried milk production process.

Thus, the passage of dry milk products through instantizer, vibrating sieve and aerosol transport leads to a significant decrease in the average particle size and an increase in the free fat mass fraction. As a consequence, this contributes to a decrease in the relative dissolution rate, which is particularly important for fat-containing dried milk products.

The results of the studies also indicate a positive effect of dried milk transportation method by belt elevator, which provides slight influence on the grain size distribution, free fat content and instant properties.

It should be mentioned that the regimes of the product processing in the instantizer effect significantly the change of dried milk instant properties. As in the previous case, these changes have more apparent negative character for fat-containing dried milk products. The mechanical impact in the aerosol transport systems results in the reduction of the particles average size and increase of single particles amount, which also leads to a deterioration of the instant properties even in the case of surfactants feeding to the first section of the instantizer (sample 3). At the same time, the use of additional agglomeration prior the first section of the instantizer, the process of SAA application and gentle transport systems as well as intermediate storage of the product impart the product high instant properties (sample 1).

As for skim milk, a two-stage process, combined with the use of gentle ways of dried milk transportation are sufficient in order to impart the product high instant properties (sample 7).

In general, the results obtained indicate a significant effect of the processing systems, transportation and intermediate storage of dried milk, obtained by various methods.

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There should be no references to publications that are not readily available. These include institutional regulations, state standards, technical requirements, unpublished works, proceedings of conferences, extended abstracts of dissertation, and dissertations.

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The following papers are sent to the editorial board.

1. A soft version of the article typewritten in MS Word. The file of the article should be entitled by the first author's surname, e.g., PetrovGP.doc. The data file must only contain a single document.

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4. A cover letter to the editor-in-chief from the responsible organization with the conclusion about the work urgency and recommendations for its publication, carrying the date, reference number, and the head's signature.

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For the article file use the format *.doc. Do not use Russian letters and spaces in the file's name.

The article file is to be identical to the printed original submitted to the editorial board.

The article's textual file is to include the title of the article, an abstract, a structured text, references, a separate sheet of captions for the diagrams, and tables (each on a separate sheet). Structural chemical formulae are placed in the body of text.

The articles are accepted in the text editor Microsoft Word using the font Times New Roman for texts, Symbol for Greek letters, and MathematicalPi2 for handwritten and gothic symbols. The standard font size is 14.

For tables, use Word (Table – Add Table) or MS Excel.

Recommendations for typewriting formulae

Mathematical equations forming a separate line should be typed in the MathType formula editor as a whole.

It is not allowed to combine a table, a text, and an inserted frame within the same unit. For the equations printed in the MathType, it is necessary to keep to the standard style of symbols and indices, their size and placing. Forced manual changing of individual symbols and formula elements is not allowable!

Dimensions

They are separated from the figure by a space (100 kPa, 77 K, 10.34(2) A), except for degrees, percent, and permille: 90°, 20°C, 50%, 10‰. Fraction dimensions: 58 J/mol, 50 m/c^2.

For complex dimensions, it is allowed to use both negative degrees and parentheses (J/ mol-1 K-1), {J/ (mol K) or J(mol K)-1}, if it makes reading easier. The main requirement is the unified manner of writing dimensions.

While enumerating and giving numerical spaces, the dimension is set for the last number (18–20 J/mol), with the exception of angular degrees.

For Celsius degrees: 5°C, not 5°. Angular degrees are not omitted: 5°–10°, not 5–10°.

Dimensions for variables are written with a comma (E, kJ/mol); for logarithms, in square brackets without a comma: ln t [min].

Spaces between words

References to diagrams and tables are typed with spaces (Fig. 1, Table 2).

Inverted commas and brackets are not separated by spaces from the included words: (at 300 K), (a); not (at 300 K), (a).

There should be a space between the № or § sign: (№ 1; § 5.65), but no space in numbers with letters: (IVd; 1.3.14a; Fig. 1d).

In geographical names, there is a space after the period: р. Енисей, г. Новосибирск.

References to cited works

Initials are put after authors' and editors' names and are not separated by spaces: Иванов, А.А., Petrov, B.B.

The year, volume, number, etc. are separated from one another and from the figures by spaces: 1992, vol. 29, no. 2, pp. 213.

To refer to the issue number of both Russian and foreign journal, use the № sign. In the titles, the word *journal* is shortened for Journ.

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The journal publishes black-and-white illustrative material.

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