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FOOD PRODUCTION TECHNOLOGY

THE SCIENTIFIC AND PRACTICAL PRINCIPLES OF CREATING PRODUCTS WITH INCREASED PROTEIN CONTENT

A. V. Bannikova[a,*], I. A. Evdokimov[b]

[a] Saratov State Agrarian University named after N.I. Vavilov,
Teatralnaia square 1, Saratov, 141012 Russian Federation

[b] North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355009 Russian Federation

* e-mail: annbannikova@gmail.com

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Abstract: The studies show that there is an increased interest in the market for the production of protein-fortified products due to the clearly observed trend of general decline in the protein intake by the population. The purpose of this work is to develop the new types of dairy products with increased protein content using the principle of products gradation for protein content, and to assess their functional and textural characteristics. The applied methods included an assessment of the rheological and textural characteristics, an optical microscopy, and the characteristic of color parameters of the products developed. It has been shown that the protein performs various functions in dairy products, such as changes in structural properties of the dairy products, reduction of fat level, and fortification of the products with the rich source of the branched-chain essential amino acids. The assessment of the textural properties of the developed products, which includes the measurements of the viscosity versus shear rate relationship, of the functional and technological properties of foam (fold and stability), and of the texture parameters (hardness and adhesion), has showed the acceptability of the developed technological solutions. The inclusion of the desired level of protein into the dairy products has not significantly affected their textural characteristics. An analysis of the viscosity-shear rate relationship has demonstrated the similar trends in the rheological properties for all the products studied. The texture of the new products was analyzed instrumentally pointing to their similarity with the commercial versions of the products containing half as much of the complete protein. The results of the study indicate the similar values of the color attributes of the developed products. The studies of the biological value of the new products has showed an increased content of the essential amino acids to an average of up to 76.9%, 80%, and 80.7% in cream, drinks, and desserts, respectively, as compared to their commercial counterparts. The amount of leucine, which is an amino acid that plays a fundamental role in the muscle protein synthesis, increased up to 61.9%, as compared to the commercial variants. This study can lay the foundation for the further development of a wide range of the structured food products with increased protein content.

Keywords: Dairy products, protein, viscosity, foam properties, texture, adhesion, biological value

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INTRODUCTION

The protein is one of the major cell components, and therefore plays an important role in functioning of a human organism. The protein, as a macronutrient, is built from the smaller units called amino acids that can be synthesized in a human organism or must be supplied from the external sources. In general, the increase in the protein intake up to 25–30 grams per meal is one of the requirements for obtaining the required amount of essential amino acids necessary to maintain or improve the health of the adult population [1, 2].

Thus, in early 2010, according to the Russian Federal State Statistics Service, nearly one in eight Russians, i.e. 12.9% of the country population, was aged 65 years or over. In the long run, the scale of aging of the Russian population will demonstrate increasing growth. Thus, according to the official demographic projection, in 2030, the share of the population aged 65 and over will increase up to 18% (according to the most optimistic scenario of growth of the total population of Russia), and up to 19.4% (according to the pessimistic scenario). This is a challenge for the changing demand for the access to the affordable services of good quality within the sphere of pension provision, health care, and social services,

as well as for the support of food industry. People aged over 65 experience decrease in the metabolism and changes in the physiology that significantly affects their nutritional needs. The wise choice of food products and a balanced diet are important for the people aged over 65 in order to maintain their health and quality of life.

Despite the growing worldwide consumer awareness about the importance of healthy eating, nearly fifty percent of people living in the developed countries suffer from a lack of essential nutrients [3]. With age and differences in the availability of nutrients, the changes in food consumption habits resulted in an imbalance between the essential nutritional ingredients contained in diet and the actual presence thereof in the consumable food products. Thus, it would be beneficial to review the criteria for food in order to prevent and treat the inadequate intake of protein in the diet of adults. Consequently, there is an increased interest in the market for the production of the protein-fortified products [4].

The quality and quantity of protein are the key considerations, since by consuming the "right" type of protein in the "right" time it is possible to achieve the consistency of the physiological responses and to optimize the protein intake. Among the amino acids that are important for the protein synthesis, the scientists distinguish the branched-chain amino acids (leucine, isoleucine, and valine), which possess the ability to stimulate protein synthesis. To improve the muscle protein synthesis, the scientists proposed to additionally include the diet with leucine, an amino acid that plays a core role in protein synthesis in cells [5].

The growing consumer interest in the easily accessible food products, which meet the nutritional needs required, led to the production of various products with specific nutritional purposes. In particular, the industry uses whey proteins (isolates and concentrates) and caseinates to produce the protein-fortified food products [6]. It is known that the texture and sensory properties of dairy products are largely dependent on the type of protein in the system. However, a market analysis has shown that the protein-fortified products are not always easily accessible for the consumers and, furthermore, do not completely satisfy the content of the desired amount of protein.

The casein is a conjugated protein, which is formed during milk fermentation. The coagulation of casein in milk occurs under the action of the proteolytic enzymes of rennet juice (cheese), the acids generated by lactic acid bacteria (cottage cheese), or by direct adding of acids (technical casein). The casein is one of the basic proteins of milk, cheese, yogurt, and other dairy products along with the whey proteins (albumin, etc.). The casein contains all the essential amino acids and is therefore an important food protein. The dried casein is a white powder with no taste and odor. In the human digestive tract, under the action of gastric enzymes (rennin, etc.), the caseinogen of milk is transformed into the casein (the enzymatic fermentation of milk). Wherein, the casein in the form of clots precipitates together with the milk fat. This precipitate is longer retained in stomach, slowly ingested, and split by the pepsin. The milk and milk products have a high nutritional value largely owing to the casein. The casein is a rich source of available calcium and phosphorus. The casein preparations are widely used in medicine, especially, in parenteral nutrition. Due to the balanced amino acid composition and high digestibility, the milk-derived casein often acts as a basis of the athletes' diet. However, because of the relatively slow process of splitting in stomach, its intake is appropriate during the long periods of rest between the workouts, for example, at night [7, 8].

The whey proteins (albumins and globulins) contain the optimal set of essential amino acids and, in terms of nutritional physiology, approach the amino acid scale of "perfect protein", wherein the ratio of amino acid corresponds to the needs of the organism, and according to the content of essential amino acids and branched-chain amino acids (valine, leucine, and isoleucine), surpass all other proteins of animal or vegetable origin [9, 10]. The whey proteins stimulate the immune system, increase the level of insulin-like growth factor, and reduce the cholesterol content better than the casein and soy protein. Moreover, the whey proteins have a low glycemic index, which optimizes the insulin secretion by regulating the blood glucose level and, thereby, prevents the occurrence of the type II diabetes. The use of the whey proteins for the fortification of dairy products is a physiologically sound and priority direction. A whey protein isolate, which is produced through micro- and ultrafiltration of whey and has a mass fraction of protein over 90%, is almost free of fat, cholesterol, and carbohydrates (lactose). A whey protein isolate is very rapidly ingested by an organism and contains a high concentration of amino acids with branched side chains, which prevail in the metabolism of muscle tissues. A whey protein concentrate is the most advantageous and common form of the whey protein containing a certain amount of fats, cholesterol, and carbohydrates (lactose) – 20% of the product weight and above.

The purpose of this work is to develop the new types of dairy products with increased protein content, and to assess the functional and textural characteristics of the products developed. The new technologies involve the application of whey protein and caseinates with the justification of the methodological principles for producing dairy products using the principle of products gradation according to the content of protein. The use of this concept will allow producing the products, the proteins in which act as a structure-forming agent and replace a part of the fat component and a source of the branched-chain amino acids, which will ultimately lead to the production of products with a full range of biological properties.

OBJECTS AND METHODS OF STUDY

The commercially available milk ingredients were used to prepare test samples: cream with 51.5% of fat, skim milk with 0.12% of fat, whole milk (with 3.8% of fat and 3.2% of protein), and low-fat milk (with 2% of fat and 4.2% of protein).

In order to prepare the samples of cream with increased protein content, the following ingredients were used: low-esterified pectin (LEP) and sodium alginate (CP Kelco, USA), λ-carrageenan (Swift and Company Limited, Australia), guar gum (Lotus Foods Pty Ltd., Australia), ι-carrageenan (SBI, the Netherlands), xanthan (Langdon, Australia), agar (RYP Foods PTY Ltd., Australia), gelatin (Bloom 225, Gelita, Germany), and whey protein isolate with a mass content of protein - 90 g, fat - 1.5 g, carbohydrates - 0.5 g, and sodium - 0.7 mg per 100 g of product (Asceno Sport, Australia).

The concentration of fat content in cream was reduced by adding an appropriate proportion of skim milk. The confirmation of the final fat content of 35% in the modified cream was carried out using the method of Babcock. In order to prepare the final samples of cream with increased content of protein, the aqueous solution of the whey protein isolate with the specified stabilizers were added to the cream with 35% of fat content, which reduced the fat content from 35% down to 20%.

To prepare the samples of drinks, the following ingredients were used: Alanate 180 sodium caseinate (Fonterra, New Zealand), carrageenan (FMC, USA), Cetrolex G lecithin (Langdon, Australia), inuline (Orafti, Belgium), cocoa powder (Maltra Foods, Australia), food coloring agents - chocolate brown (Hanson, Australia), cherry pink (Queen Food Colouring Company, Australia), and natural vanilla flavoring agent (Queens Fine food, Australia).

To prepare the samples of milk drinks, the dry ingredients and the skim/whole milk were separately weighted, and then stirred for 20 minutes at room temperature. In order to ensure the proper dissolution of the ingredients, the system temperature was increased up to 50°C followed by a four-step homogenization (FT9, Armfild, UK). The heat treatment of drinks was carried out at 85°C during 5 minutes using a pasteurizer, as well as through the ultra-high temperature treatment (UHT) with the plate heat exchanger system (FT74X, Armfild, UK) at 140°C during 2 to 5 seconds. The drinks were filled into the sterilized glass containers (250 ml) in aseptic conditions and stored at 4°C for 24.0 ± 1.0 hours prior to the physical and chemical analysis.

To prepare the samples of desserts, the following ingredients were used: Lacprodan DI-7017 whey protein concentrate (WPC) (Arla Foods Ingredient Group, Denmark), Alanate 385 calcium caseinate, Alanate 180 sodium caseinate, whey protein isolate (WPI) (Fonterra, New Zealand), Peptiplus XB hydrolyzed gelatin (Gelita, Germany), Gelcarin GP379 carrageenan (FMC, USA), xanthan (CP Kelco, USA), hydroxypropyl distarch phosphate (National starch, USA), High Bloom 25 gelatin (Gelita, Germany), Cegepal TG 186 vegetable fat (BASF, Australia).

To prepare the samples of the commercially frozen desserts (4 kg), the dry ingredients and the skim/whole milk were separately weighted, and then stirred for 20 minutes at room temperature. In order to ensure the proper dissolution of the ingredients, the system temperature was increased up to 50°C followed by a four-step homogenization at 70 bars. The heat treatment of desserts included heating up to 85°C and holding at this temperature for 5 minutes. The samples were cooled to 55°C, filled into plastic containers (120 ml), and stored at –20°C, which is a commercial product storage temperature. For conducting the physico-chemical and sensory tests, the dairy desserts were thawed at 4°C for 24.0 ± 1.0 hours prior to testing.

The relationship of the system's viscosity versus shear rate of the yoghurt samples was determined by a AR-G2 rheometer (TA Instruments, USA) using a parallel geometry of 40 mm in diameter at 4 and 22°C.

The functional properties of the cream foam were determined through measuring the fold and stability of foam. The fold was determined as the percentage of the foam volume increase to the volume of initial mixture due to the inclusion of air [11]. The foam fold was determined by whipping the cream on a low (first) speed (by a Breville Wizz Mix mixer). The foam fold was measured every 30 seconds when whipping, which lasted until the maximum fold was achieved (about 3 min). However, the whipping continued until the clearly separated phases of fat and liquid were obtained. In order to determine the foam fold percentage, the following formula was used [12]:

$$\text{Fold (\%)} = [(\text{fixed volume after whipping} / \text{fixed initial volume}) - 1] \times 100. \quad (1)$$

After achieving the maximum foam fold, its stability was assessed during 7 hours. According to the data obtained, there were built the relationships of the foam fold percentage versus the time of whipping and the foam stability within the time specified. The experiments were performed in a three-fold repetition.

The light microscopy of cream and its whipped texture with full and reduced fat content was carried out using a Leica DM 2500 microscope (Wetzlar, Germany) with an attached Leica DFC400 digital camera with a 100× lens. The samples were prepared for a microscopic examination by spreading the cream on a slide in a not too thick and not too thin layer ensuring that the structure of the product was not destroyed. A cover glass was used to obtain a flat surface of images.

The hardness and adhesiveness of the samples were determined using a TA.XT2 texture analyzer (Stable Micro Systems, England) with a cell load of 5 kg. The measurements were carried out using an aluminum cylindrical probe (25 mm in diameter) submersible into a ring with a diameter of 40 mm for sample compression. The tests were conducted at a speed of 1 mm/s with a pressing force of 10 g until the sample compressive deformation of 80% of the original height was obtained. The mechanical properties (hardness) were assessed by interpreting the experimental data into stress (kPa) depending on the strain. After a compression cycle, the aluminum probe returned back to its original position. Since the dairy desserts are adhesive, the monitor displays a negative area taken as a measure of the sample's adhesiveness. This parameter has no units of measurement and is expressed in the internal units of the computer program. Each experiment was repeated three times. An average value of the hardness

and adhesiveness was accepted. All experiments were conducted at room temperature (22 ± 1°C).

The assessment of the milk drinks color was carried out by a Minolta colorimeter (CR-100, Japan). Various color gamut are given in scales L*, +a*, -a*, +b*, -b* representing the degree of white, red, green, yellow and blue colors, respectively [13]. Initially, the device was calibrated with the following values of color gamut: Y = 93.13, x = 0.3138, y = 0.3199. 40 ml of the drink sample were poured into the small plastic containers with the subsequent measurement of the color attributes in three repetitions. The color of the new milk drink compositions, as compared to a commercial sample, was determined by calculating the degree of luminance, color value (C^*), color hue angle (h_{ab}), and general characteristic of color ΔE using the following equations [14]:

$$\Delta E^*_{ab} = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}, \quad (2)$$

$$\% \text{ of } \Delta E^*_{max} = (\Delta E^* \times 100) / \Delta E_{max}, \quad (3)$$

where ΔE_{max} is the standard value (ΔE_{max} = 196.98).

$$C^*_{ab} = ((a^*)^2 + (b^*)^2, \quad (4)$$

$$h_{ab} = \tan^{-1} (b^*/a^*), \quad (5)$$

$$\Delta H = ((\Delta E^*_{ab})^2 - (\Delta L^*)^2 - (\Delta C^*)^2)^{1/2}. \quad (6)$$

Usually, the ΔE value indicates the general difference between the two products without specifying the direction of the changes (due to *L*, *a*, *b* or their combinations). Thus, by detecting a color hue angle (h_{ab}), it is possible to claim the absolute difference in color, while ΔH describes the Euclidean color difference between the two samples.

RESULTS AND DISCUSSION

The functional and technological properties of cream with a protein content of about 4%

Many studies in Russia have found that there is not enough complete protein in the diet of Russians. Quite often this fact causes decrease in immunity, heart muscle disorders, hormonal disorders, digestive system disorders, etc. The protein is an organic compound comprising 22 amino acids that are the basic building material for a human organism. The proteins are involved in many biological processes and perform many diverse functions. In order to investigate the potential of protein, the principle of protein gradation was chosen as a basis for producing structured dairy products. This principle is based on the production of dairy products comprising about 4% (whipped products), 6% (milk drinks), and 12% (dairy desserts) of protein. The choice is substantiated by an analysis of structural characteristics of various systems comprising an increased amount of protein as well as by the assessment of texture formation of the structured dairy products depending on the protein content.

A commercial sample containing gelatin was taken as a basis for developing whipping cream with increased protein content. The increase in the protein content and the decrease in the content of a part of fat component in the products under study were carried out by adding a whey protein isolate possessing a high content of essential amino acids. In order to determine the textural properties of the developed products, their viscosity-shear rate relationship was measured (Fig. 1). The rheological characteristic of behavior of the samples with reduced fat content and with increased protein content indicates the imitation of textural properties of the cream with gelatin and full fat content, which confirms the appropriateness of applying the advanced technologies of cream production according to the nutrition concept.

It is confirmed that the whipping cream with reduced fat content and with increased protein content produced the texture similar to that of the cream with full fat content and with gelatin. Since whipping is the designated purpose of the products, the functional and technological properties of foam of the developed cream samples were assessed as compared to the samples of the cream with full fat content and with gelatin. The foam fold of the cream with increased protein content was similar to the foam fold of the cream with full fat content (Fig. 2). This indicator is

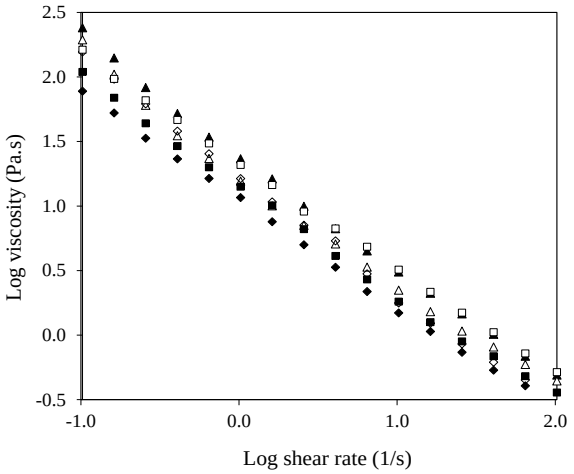


Fig. 1. The viscosity-shear rate relationship for the samples of whipping creams with full fat content with gelatin (▲); and reduced fat content with whey protein isolate and agar, LEP, and guar gum (Δ); ι-carrageenan, λ-carrageenan, and guar gum (♦); sodium alginate, λ-carrageenan, and guar gum (■); ι-carrageenan, LEP, and guar gum (◊) at 5°C.

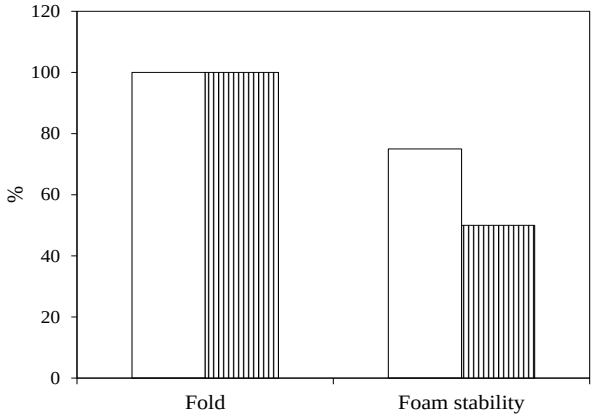
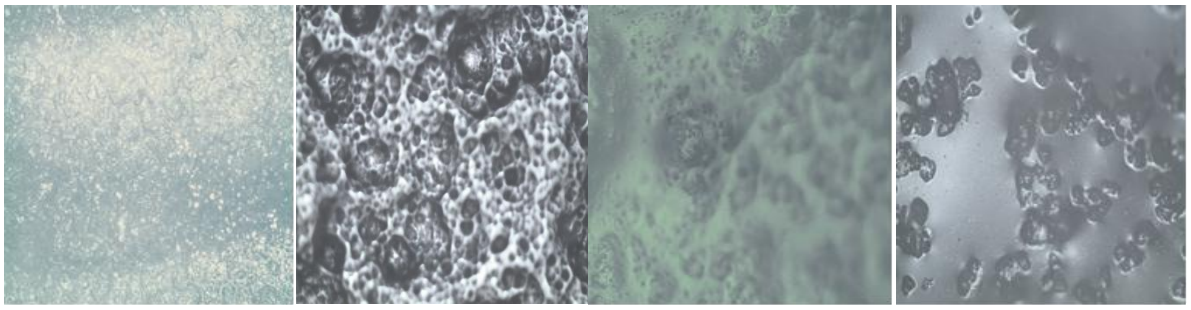


Fig. 2. The fold and stability of foam during 7 hours of the samples of whipping creams with full fat content and gelatin (left), and with full fat content with whey protein isolate, ι-carrageenan, LEP, and guar gum (right).



(a) (b) (c) (d)

Fig. 3. The photomicrographs of the gelatin-stabilized cream with full fat content, and of the polysaccharide-stabilized cream with reduced fat content with whey protein isolate (formulation of ι-carrageenan, λ-carrageenan , and guar gum) (a and b, respectively), and of the whipped cream of these systems (c and d, respectively). The photomicrographs are obtained with a 100×lens.

desirable since it is known that the currently existing cream with reduced fat content do not produce the foam fold similar to that of the cream with full fat content. The foam stability of the cream with reduced fat content was 30% lower than of the cream with full fat content and with gelatin. This indicator is expectable since in the cream with full fat content, the fat is the primary foam-forming and foam-stabilizing agent, which allows obtaining systems with high content of dry substances. After storage, the whey separation was not observed in all samples.

Four photomicrographs of the cream before and after whipping are presented in Fig. 3. These photomicrographs are shown for identifying the difference in the microstructure of the cream with full fat content and with gelatin, and of the cream with reduced fat content and with increased protein content stabilized by ι-carrageenan, λ-carrageenan, and guar gum. The cream with gelatin before and after whipping demonstrated a significant share of fat phase in their content (Fig. 3a, 3c). This product is homogeneous and single-phase with uniformly dispersed air bubbles. Unlike this system, the polysaccharide-stabilized cream with reduced fat content and with increased protein content demonstrated a three-phase system on a microscopic level. This behavior is related to the term of "thermodynamic incompatibility", which leads to the formation of a homogeneous system with a delicate, pliable, and air structure (Fig. 3b, 3d).

Thus, this study allows us to assess the functional and technological properties of the products with a reduced fat content and a protein content of about 4% additionally stabilized by the dietary fibers. The present results have shown the appropriateness of applying a whey protein isolate as a substitute for a part of the fat component, which, in turn, corresponds to the modern trends in food production.

The assessment of the rheological and color parameters of drinks with a protein content of 6%

The casein contains all the essential amino acids, and is therefore an important dietary protein. Unlike the whey, which is soluble and therefore rather quickly digestible and absorbable, the casein takes the form of tiny micelles or globules, which are almost insoluble and form clots in the stomach. The recent studies have shown that the slow digestibility of casein stops the process of catabolism (cleavage) of the muscle protein, thereby contributing to the intensification of the muscle growth. In addition, the scientific studies of the Texas University Medical Branch have recently shown that the casein is nearly as effective in stimulating the muscle protein synthesis as the whey protein.

Besides the caseinate usage in the technology of milk drinks production, the purpose of this study is to include the natural inuline prebiotic. The inuline promotes the

growth of the beneficial intestinal microflora, and thus has a positive effect on the digestive process. Moreover, the inuline helps our organism to better ingest calcium and magnesium, contributes to improvement of the human immune system and to the enhancement of the lipid metabolism (cholesterol reduction). In addition to its healing properties, the inuline has other useful properties. Thus, it is capable of providing the products with a creamy saturation and texture, but also increases the feeling of satiety, which is especially important for the people who take care of their diet.

During the course of study, the various technological solutions on the protein and prebiotic inclusion into the new milk drinks were generated. In particular, the authors stated the technological solutions on the inclusion of sodium caseinate, lecithin, carrageenan, and inuline into the technology of the milk drinks production, which allows obtaining a product that contains about 6% of protein and 2% of prebiotic, as compared to a prebiotic-free commercial product that contains only 3.3% of protein. The rheological properties of the new milk drinks produced in two ways, as compared to a commercial sample, indicated the reproduction of the trend in the change of viscosity depending on the sear rate, as shown in Fig. 4. The curves of the viscosity versus shear rate relationship for all tested samples were similar and indicated the identical values of viscosity, for example, η~66 mPa·s (0.1 s^{-1}) and η~27 mPa·s (100 s^{-1}).

The UHT treatment makes it possible to process a product at a very high temperature (140°C) with holding for a short period of time (2–5 sec.) in the heating section of the plate heat exchanger system followed by the immediate cooling of a product to the room temperature in the cooling section. The pasteurization, which was also applied in the production of drinks during the course of study, also allowed processing the products at 85°C with holding for 5 minutes without the immediate process of cooling. It is shown that the UHT treatment is preferable for developing the food products of long-term storage, which was additionally analyzed by studying the physical changes in a product, and in particular, the variations in color.

In order to assess the color of the new products, as compared to the commercial ones, we applied the CIE modified in 1976 colorimetric systems with the new standardized color spaces called as the CIE 1976 *L*a*b** color space. A CIELAB color model is currently the main color space for the analysis and description of the physical colors. The formulas for calculating the *L*a*b** and their obtained polar coordinates *L*C*h** were defined in 1990 with the new version of the DIN 5033-3 standard. The *L*a*b** are the color gradations in a color space, which are defined as follows: *L** for the luminance, *a** for the gradation of the red-green hues, *b** for the gradation of the yellow-blue hues, *C** describes saturation, and *h** describes the color hue in the CIELAB circle [4, 5]. For many years, it was recognized that the values of *L**, *a**, *b** showed the most appropriate changes in color in the dark area, which is of especial importance for the chocolate drinks. Usually, the "*L*" values are used to denote the difference in the whiteness of a sample, while the "*a*" and "*b*" values characterize the redness and yellowness of the tested materials, respectively [15].

The color differences between the commercial and developed samples in Table 1 show that the milk drinks fortified with protein and prebiotic are lighter than a commercial sample, i.e. the *L** value of the new drinks is higher. However, the difference of 5 units in the *L** value between the commercial product and our developed product cannot be determined visually. Such color parameters as *a** and *b** possess positive values for both products, though it should be noted that the values of redness and yellowness are higher in the test sample, as compared to the commercial product. For the determination of whiteness, the differences of 1 or 2 units in the values of *a** and *b** are not significant and cannot be determined visually.

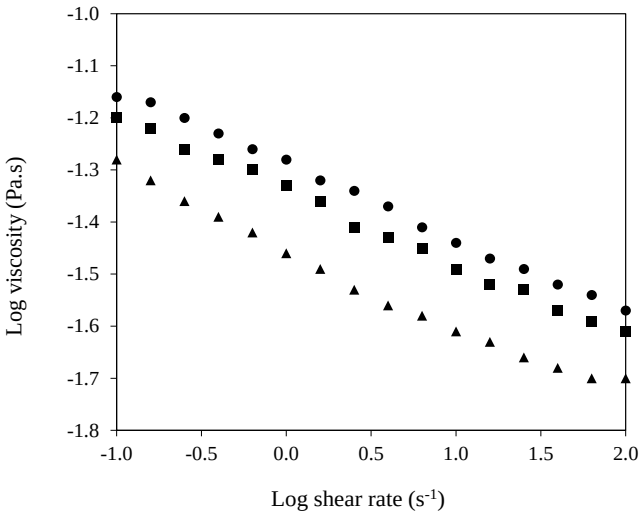


Fig. 4. The viscosity-shear rate relationship for a commercial sample of the milk drink containing 3.3% of protein (▲), and of the developed milk drinks with more than 6% of protein and 2% of prebiotic obtained through the UHT treatment (■) and pasteurization (●) at 4°C.

In general, the color distinctiveness (E^*_{ab}) between a commercial product and a protein/prebiotic-fortified drink was 5.43 ± 0.53, while the maximum possible color distinctiveness (E^*_{max}) between the two materials was 2.75%, which is in the area between the low (<0.5%) and high (5%) distinction in color. The color values in Table 1 for a commercial product and an experimental sample were determined as 13.49 ± 0.16 and 15.56 ± 0.04, respectively. This means that the product produced in this work is 15.34% more luminous than the commercial milk drink [(15.56–13.49)/13.49]. The actual color of the milk drinks was shown in the measurement of the color hue angle (h_{ab}) in Table 1 in order to demonstrate a small difference in the measured values of a^* and b^*. Thus, the milk drink fortified with protein and prebiotic was closer to the pale red color with yellow hue (h_{ab} = 57.66 ± 0.06), while the commercial product (h_{ab} = 55.58 ± 0.04) had a slight yellow hue. The difference between the hues of the tow milk drinks was rather small (ΔH = 0.53 + 0.03).

The results of this study indicate that both milk drinks (a commercial product and a test sample) showed the similar values of color attributes and the flux behavior, as shown in Fig. 1 and Table 1, which is very promising from the standpoint of developing the new functional technological solutions. The further development of these products should include the output of a larger batch of the product, the assessment of its general consumer acceptability, and the characteristic of some sensory properties using the human sense organs.

The rheological and textural characteristics of the developed desserts with a protein content of about 12%

In order to determine whether the technological parameters of the desserts production are approximated to the production parameters applied in this study, a prototype of a commercial dessert was reproduced, which was subjected to the tests on the viscosity versus shear rate relationship within the study concept and comparison of the textural properties of the produced counterpart and the commercial sample. The work was carried out using the parameters described in the "Objects and Methods of the Study" section.

Fig. 5 shows the rheological properties of the commercial sample and the counterpart of the commercial sample with the increase in the shear rate from 0.1 up to 100 s^{-1}. The trend of the steady decline in the viscosity values at shear demonstrates the linear descending profile of the textural properties indicating the dilution. The results show that our attempts to reproduce the textural characteristics of the commercial product were quite successful with the viscosity values for the two systems within the range of 100 to 1 Pa·s at the beginning and at the end of the experiment, respectively.

A texture analysis complements the above-mentioned small shear strains focusing on the instrumental properties of hardness and adhesion. The results indicate that the dairy desserts produced in a production workshop have similar values of hardness and adhesiveness indicating that these materials possess a "rich" texture (Fig. 6, 7).

The experimental work on the study of textural properties of the commercial dessert sample and its prototype developed in the laboratory allowed suggesting the new technological solutions on the protein fortification of desserts using a whey protein isolate (WPI) and a whey protein concentrate (WPC, Alacen 392 and Lacprodan). The data of the technological solution are based on the improvement of the product's nutritional characteristics along with preserving the pleasant organoleptic profile typical of dairy desserts. As noted earlier, it is known that the leucine is one of the most important amino acid for people over 60 years due to its specific action on the production of ribosomes, which are the place for the protein production in cells. Thus, it was noted that the additional fortification of food products with leucine improves the muscle protein synthesis in the adult population [16]. In this context, the dairy ingredients are an excellent source of protein with the high levels of branched-chain amino acids including the leucine; they are available in flexible formats, easily digested and can be included in a diet [17].

It should be noted that in this work, in order to increase the content of protein in desserts, about 150 formulations were developed, which were exposed to the thorough sensory and textural control. Some of these were inappropriate in terms of texture, as compared to the commercial sample. Therefore, only those samples of desserts, the properties of which were similar to the commercial product, were selected for the further study and included into the work. Thus, four samples with the content level of about 12% of protein and 1.2% of leucine were developed and compared according to the textural and nutritional properties to the commercial sample containing only 6.7% of protein and less than 0.5% of leucine.

Table 1. The color parameters for the commercial and test samples of milk drinks

Sample	Visual assessment of color	L^*	a^*	b^*	C	h_{ab}	ΔE^*_{ab}	ΔH
A commercial sample	Chocolate brown color	54.36 ± 0.40	7.63 ± 0.09	11.14 ± 0.12	13.49 ± 0.16	55.58 ± 0.04	5.43 ± 0.53	0.53 ± 0.03
A test sample fortified with protein and prebiotic	Chocolate brown color	59.35 ± 0.13	8.33 ± 0.03	13.15 ± 0.03	15.56 ± 0.04	57.66 ± 0.06		

Note. N = 3; L^* = axis of luminance (0 – black, 100 – while); a^* = red-green axis ("+"– value of red, "-" – value of green, 0 is neutral); b^* = blue-yellow axis ("+"– value of yellow, "-" – value of blue, 0 is neutral); C = color value; h_{ab} = color hue angle; E^*_{ab} = total difference in color; ΔH = difference between hues.

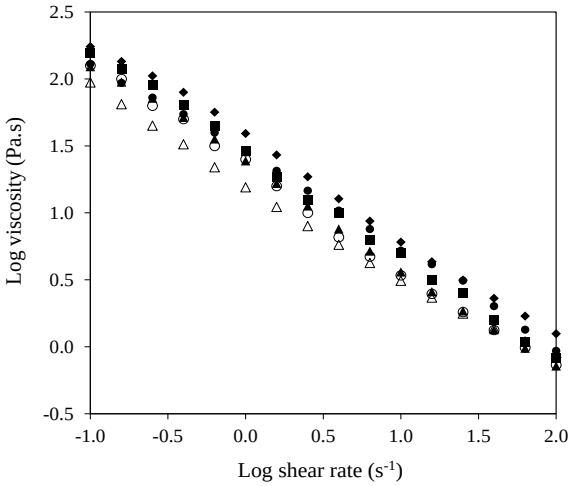


Fig. 5. The viscosity-shear rate relationship for a commercial sample of a dessert (Δ), a counterpart of a commercial sample prepared in the laboratory (●), and of the desserts fortified with protein with 2.6% of WPI and 7.8% of Lacprodan (♦), 3.2% of WPI and 7.5% of Lacprodan (○), 3% of WPI and 7.1% of Alacen 392 (■), and with 3% of WPI and 7% of Alacen 392 (▲) at 22°C.

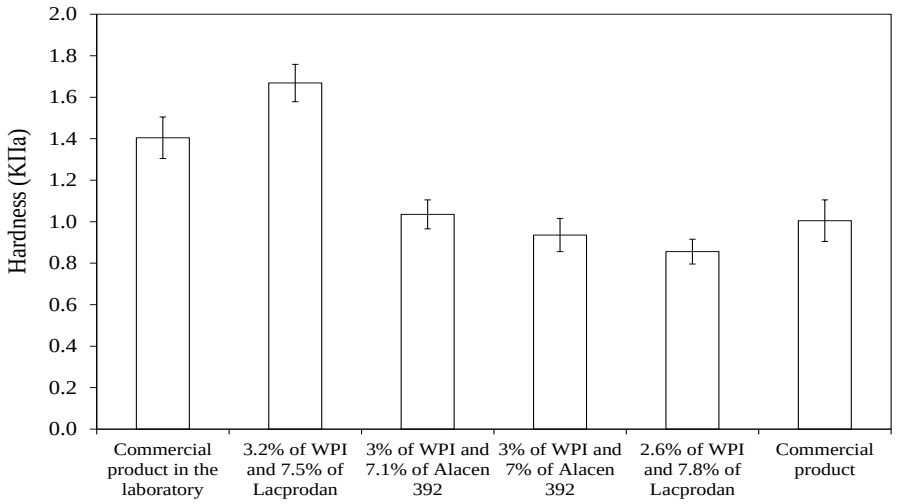


Fig. 6. The hardness of a commercial sample of a dessert, a counterpart of a commercial sample prepared in the laboratory, and of the desserts fortified with protein with 2.6% of WPI and 7.8% of Lacprodan, 3.2% of WPI and 7.5% of Lacprodan, 3% of WPI and 7.1% of Alacen 392, and with 3% of WPI and 7% of Alacen 392 at 22°C.

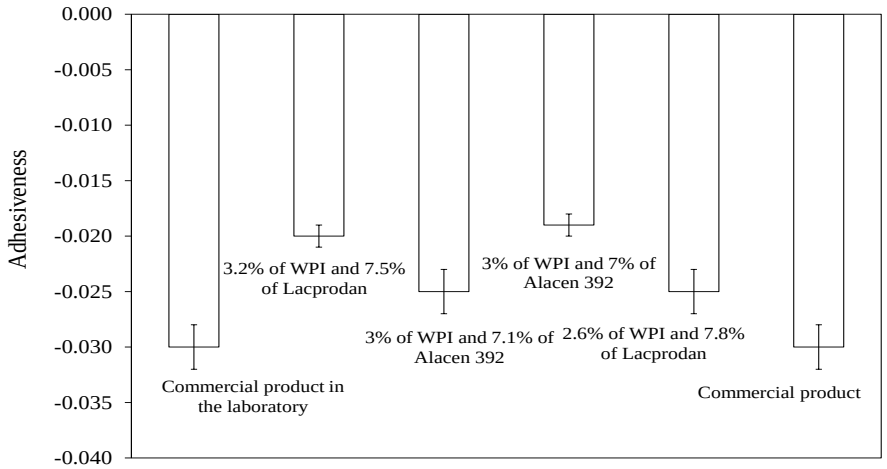


Fig. 7. The adhesiveness of a commercial sample of a dessert, a counterpart of a commercial sample prepared in the laboratory, and of the desserts fortified with protein with 2.6% of WPI and 7.8% of Lacprodan, 3.2% of WPI and 7.5% of Lacprodan, 3% of WPI and 7.1% of Alacen 392, and with 3% of WPI and 7% of Alacen 392 at 22°C.

Fig. 5 shows that the commercial prototype of the dessert and the four developed products with the high protein content demonstrated similar behavior of dilution at the increase in shear indicating the structure of a soft gel for the studied dairy desserts. Three developed desserts containing from 2.6 to 3% of WPI and from 7 to 7.8% of WPC (Lacprodan or Alasen 392) demonstrated the similar values of hardness (about 0.9 kPa), shown in Fig. 6. The figure also shows that the gel-like structure of these samples is relatively weaker than of the developed dessert with 3.2% of WPI and 7.5% of WPC of the Lacprodan type with the hardness values of about 1.7 kPa. The obtained data on the increase in the hardness of the test samples, as compared to the commercial sample, are associated with the higher protein content forming a three-dimensional lattice in the product structure, which affects the gel strength. The change in adhesiveness under compression of the developed samples is shown in Fig. 7. It is shown that the experimental samples of desserts demonstrate the comparable adhesion values within the range of –0.02 to –0.03, which is indicative of the creamy and "rich" texture of the dairy desserts with a protein content of about 12%.

The biological value of the developed food products with the increased content of protein

As noted, one of the main directions of the government policy concept in the field of healthy eating involves the development of the mass-consumption products, the technologies of the functional purpose products differentiated for the prevention of diseases and improvement of the protective functions of an organism, and reduction in the risk of exposure to the harmful substances. Considering the fact that at present, the natural products demonstrate a reduced amount of nutrients, including the protein, it is necessary, when developing the new products, to purposefully achieve the enhancement of their biological value by fortifying these products with the functional ingredients, which contain the substantial amounts of vitamins, minerals, amino- and fatty acids.

Table 2 presents the amino acid composition of the developed cream, drinks, and desserts with the increased protein content. The table shows that the sum of the essential, non-essential, and conditionally essential amino acids in the developed samples exceeds the values of the reference samples with the full fat content. Thus, the amount of the essential amino acids in the new cream increased by 76.9%, in the drinks – by 80%, and in the desserts – by 80.7%, which ultimately increases the total content of amino acids in the cream by 82.2%, in the drinks – by 82.9%, and in the desserts – by 65.7%, as compared to the reference samples.

As already noted, the leucine refers to the group of branched-chain amino acids, which play a core role in the protein synthesis in a cell. Its increase averaged 61.9%, while the increase in another branched-chain amino acid – the isoleucine, which is necessary for

Table 2. The amino acid composition of the developed products with an increased content of protein as compared to the commercial products (g/100 g of product)

Amino acid	Cream (protein content – 4%)		Drinks (protein content – 6%)		Desserts (protein content – 12%)	
	Commercial sample	Developed cream	Commercial sample	Developed drink	Commercial sample	Developed desserts
EAA	1.0706	1.8934	1.2462	2.2793	2.6934	4.4622
Lysine	0.1986	0.3485	0.2294	0.4196	0.4958	0.8214
Methionine	0.0624	0.1178	0.0775	0.1418	0.1675	0.2775
Phenylalanine	0.1219	0.2120	0.1395	0.2552	0.3015	0.4995
Valine	0.1625	0.3062	0.2015	0.3686	0.4355	0.7215
Isoleucine	0.1385	0.2591	0.1705	0.3119	0.3685	0.6105
Leucine	0.2415	0.3909	0.2573	0.4706	0.5561	0.9213
Threonine	0.1094	0.2072	0.1364	0.2495	0.2948	0.4884
Tryptophan	0.0358	0.0518	0.0341	0.0624	0.0737	0.1221
CEAA	0.296	0.5888	0.3875	0.7088	0.8375	1.3875
Arginine	0.0871	0.1743	0.1147	0.2098	0.2479	0.4107
Histidine	0.0676	0.1319	0.0868	0.1588	0.1876	0.3108
Cystine	0.0202	0.0202	0.0093	0.0170	0.0201	0.0333
Tyrosine	0.1211	0.2685	0.1767	0.3232	0.3819	0.6327
NAA	1.233	2.2420	1.4756	2.6989	3.1892	5.2836
Alanine	0.0813	0.1272	0.0837	0.1531	0.1809	0.2997
Glycine	0.0473	0.1130	0.0744	0.1361	0.1608	0.2664
Proline	0.2496	0.4804	0.3162	0.5783	0.6834	1.1322
Serin	0.1365	0.2685	0.1767	0.3232	0.3819	0.6327
Aspartic acid	0.1807	0.3014	0.1984	0.3629	0.4288	0.7104
Glutamic acid	0.5376	0.9514	0.6262	1.1453	1.3534	2.2422
All amino acids	2.5996	4.7241	3.1093	5.6870	6.7201	11.1333

Note. EAA – essential amino acids; CEAA – conditionally essential amino acid; NAA – non-essential amino acids.

for the hemoglobin synthesis, amounted to 87%. Such an increase in the amount of the essential amino acids, especially of the branched-chain amino acids, is beneficial for the use of these products by all groups of population, since the developed structured products have the entire spectrum of biological properties required for the protein synthesis. Besides, the principle of protein gradation successively increased the concentration of the essential amino acids with preservation of the acceptable texture of the structured products.

CONCLUSION

This work is devoted to a detailed justification of the use of protein preparations rich in essential amino acids within the technology of the dairy products production. The new technological concepts in protein fortification enabled obtaining a range of products with the high nutritional and consumer characteristics. The optimal technical functionality in combination with the recommended nutritional value in the developed products has been instrumentally confirmed by assessing the functional and rheological characteristics of the structured products, which demonstrate similarity to the commercial counterparts containing less amount of the total protein. Using the whey protein and caseinates, this study on the fortification of the dairy products with the high-quality protein allows developing compositions comprising up to 12% of protein and 1.2% of leucine.

The inclusion of such products into the diet of the adult population will provide real benefits substantiated by the high level of leucine, an amino acid needed for the efficient muscle protein synthesis. In addition to the nutritional properties, the new types of the structured products possess a "rich" texture and pleasant sensory characteristics, which makes it possible to consider the developed technological concepts as the acceptable for the future implementation.

REFERENCES

1. Valeriol A., Antona G. and Nisoli E. Branched-chain amino acids, mitochondrial biogenesis, and health span: an evolutionary perspective. *Aging*, 2011, vol. 3, no. 5, pp. 464–478.
2. Burton L.A. and Sumukadas D. Optimal management of sarcopenia. *Clinical Interventions in Aging*, 2010, no. 5, pp. 217–228.
3. Loenneke J.P. and Pujol T.J. Sarcopenia: an emphasis on occlusion training and dietary protein. *Hippokratia*, 2010, vol. 15, no. 2, pp. 132–137.
4. Bannikova A.V. and Evdokimov I.A. Dairy products fortified with whey proteins. Technological aspects of production. *Dairy Industry*, 2015, no. 1, pp. 46–48. (In Russian).
5. Rom O., Kaisari S., Aizenbud D. and Reznick A.Z. Lifestyle and sarcopenia – etiology, prevention, and treatment. *Rambam Maimonides Medical Journal*, 2012. vol. 3, no. 4, pp. 1–12.
6. Katsanos C. S., Kobayashi H., Sheffield-Moore M., Aarsland A. and Wolfe R. R. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *American Journal of Physiology Endocrinology and Metabolism*, 2006, vol. 291, no. 2, pp. 381–387.
7. Abou-Samra R. and Keersmaekers L. Effect of different protein sources on satiation and short-term satiety when consumed as a starter. *Nutrition Journal*, 2011, vol. 10, pp. 139–145.
8. Aguilera J.M. and Rademacher B. Protein gels. *In: Proteins in food processing* (R. Yada (Ed.)). Cambridge: Woodhead Publication, 2004, pp. 468–482.
9. Remeuf F., Mohammed S., Sodini I. and Tissier J.P. Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. *International Dairy Journal*, 2003, vol. 13, no. 9, pp. 773–782.
10. Fujita S. and Volpi E. Amino acids and muscle loss with aging. *The Journal of Nutrition*, 2006, vol. 136, no. 1, pp. 277–280.
11. Ostroumova T.L. and Prosekov A.Yu. The effect of protein substances on the foaming properties of milk. *Transactions of Higher Educational Institutions, Food Technology*, 2007, no. 2, pp. 43–46. (In Russian).
12. Balerin C., Aymard P., Ducept F., Vaslin S. and Cuvelier G. Effect of formulation and processing factors on the properties of liquid food foams. *Journal of Food Engineering*, 2007, vol. 78, no. 3, pp. 802–809.
13. Jafarpour A., Sherkat F., Leonard B. and Gorczyca E.M. Colour improvement of common carp (cyprinus carpio) fillets by hydrogen peroxide for surimi production. *International Journal of Food Science and Technology*, 2008, vol. 43, no. 9, pp. 1602–1609.
14. Iserliyska D., Chinnan M.S. and Resurreccion A.V.A. Physicochemical and sensory properties of a peanut drink. *Agricultural Engineering International: CIGR Journal*, 2012, vol. 14, no. 2, pp. 49–56.
15. Bayoumi H.M., Mohamed A.G., Sheikh M.M.E., Farrag A.F. and Eissa H.A. Effect of ultrafiltration permeates on the quality of chocolate milk. *Journal of American Science*, 2011, vol. 7, no. 7, pp. 609–615.
16. Newton J.P., Yemm R., Abel R.W. and Menhinick S. Changes in human jaw muscles with age and dental state. *Gerodontology*, 1993, vol. 10, no. 1, pp. 16–22.
17. Bannikova A.V. and Evdokimov I.A. *An Innovative Approach to the Production of Fortified Dairy Products with Increased Content of Protein*. Moscow: DeLi Plyus Publ., 2015. 136 p. (In Russian).

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OBTAINING AND IDENTIFICATION OF INULIN FROM JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) TUBERS

T. V. Barkhatova, M. N. Nazarenko*, M. A. Kozhukhova, I. A. Khripko

Kuban State Technological University,
Moscovskaya Str. 2, Krasnodar, 350072 Russian Federation

* e-mail: nazarenko.krd@gmail.com

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Abstract: The growing demand of the Russian population for healthy food dictates the need in functional ingredients production increase. Inulin, the polysaccharide of natural origin, has a wide range of functional activity. This article grounds the expedience of inulin obtaining from Jerusalem artichoke tubers and considers effective technological methods of ensuring high yield and quality of the target product. It was demonstrated that application of vibration with frequency 24 Hz for 60 min at temperature 30–35°C intensifies the extraction process, and fractionation of the extract on membranes with pore diameter 2, 3 and 5 kDa allows to obtain inulin with certain physicochemical properties. The membrane separation results in three inulin fractions: low molecular (DP = 2–10), medium molecular (DP = 11–18) and high molecular (DP = 19–35) fraction. The medium molecular fraction of inulin, which is used as prebiotic and fat substitute in food technology, was studied using FTIR spectroscopy and 1H-13C NMR spectrometry. The obtained spectral characteristics have led to a conclusion that the investigated sample of inulin is highly competitive with the best world analogues. The authors thoroughly describe the method of determining the degree of polymerization and average molecular weight of the investigated polysaccharide using ^{1}H-^{13}C NMR spectroscopy. It has been established that inulin obtained by improved technology has the degree of polymerization DP = 13–14 and molecular weight 2124–2286 Da. The results of this work have practical value for production of inulin from Jerusalem artichoke tubers and theoretical value for the chemistry of natural compounds.

Keywords: Functional ingredients, Jerusalem artichoke, inulin, degree of polymerization, FTIR, NMR spectroscopy

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INTRODUCTION

According to analytical agencies worldwide, the most promising area of food industry development is the production of functional food. Total consumption of these product in Europe is about 100 000 tons per year and in Russia – 1 400 tons per year.

Relevance of healthy nutrition is confirmed by studies indicating direct correlation between human immune status and consumed food. Manufacturers are expanding the range of prophylactic products by means of various functional ingredients: dietary fiber, vitamins, antioxidants, polyunsaturated fatty acids, pro- and prebiotics [1, 2].

Inulin, polysaccharide of natural origin, is an effective prebiotic. Inulin is a polymer consisting of several fructose residues (from 10 to 36) in furanose form (β, D-fructofuranose) and one glucose residue in pyranose form (α, D-glucopyranose), connected through β-2,1 glycoside bonds. Molecular weight of inulin is 5000–6000. Inulin produces D-fructose and a small amount of glucose during acid or enzymatic hydrolysis. Inulin and the intermediate products of its lysis (inulids) do not have reducing abilities [1, 3].

According to several studies, inulin belongs to reserve carbohydrates [4]. It is formed in leaves of plants during photosynthesis and accumulates in stems and roots of composite plants (mainly). Inulin is stored in vacuoles in form of spherocrystals.

Inulin is considered a soluble dietary fiber and a functional ingredient. Because inulin undergoes fermentation by microflora of the large intestine and is not absorbed in the stomach or small intestine, regular consumption of inulin with food provides the following health effects:

– Creating of optimal conditions for growth and development of normal intestinal microflora; prevention of goiter; increased resistance to bacterial and viral infections of the digestive system, as well as to the introduction of various parasites;

– Regulation of carbohydrate metabolism: the acidic gastric juice environment ensures the hydrolysis of inulin yielding fructose, which is absorbed by the body without insulin, reducing the hunger;

– Normalization of at metabolism: decrease of cholesterol and triglycerides level in blood, which prevents the development of blood vessel atherosclerosis;
– Reduction of body weight (overweight problems) through activation of fat disposal process associated with the processes of glucose digestion;
– Normalization of sugar level in blood: the molecules of inulin, unsplitted by hydrochloric acid in stomach, adsorb a large amount of alimentary glucose and hinder its absorption into the bloodstream, thereby reducing the sugar level in blood after meal.
– Stable glucose level decrease leading to the normalization of insulin production by pancreatic cells;
– Promotion of energy production: the major part of energy required for normal human life is obtained by glycolysis. Since fructose is much more easily absorbed by the body, the cells do not develop energy hunger. Moreover, short fragments of inulin molecules incorporated into cell membrane facilitate the passage of glucose inside the cells. Inulin promotes glycogen synthesis by improving the glucose utilization. This provides a higher level of energy metabolism;
– Normalization of metabolism: unlike non-utilized glucose, which turns into products of fat metabolism, fructose is used by the body entirely, preventing the development of obesity, vessel atherosclerosis, coronary heart disease, arterial hypertension;
– Integrated effect on the functional activity of the liver. Inulin stimulates the synthesis of protein, cholesterol, bile acids by improving the glycose utilization. Due to disposal of toxic substances in the intestines and blood, inulin significantly relieves the liver and keeps its fighting potential against various diseases and environmental factors [1].

Inulin possesses both physiological and technological functionality. It forms a creamy gel with a short fat-like texture together with water and thus simulates the presence of fat in the diet products, ensuring the full flavor and texture. In addition, inulin improves stability of air-concentrated product, such as ice cream and food emulsions (spreads, sauces) [5].

Dietary norm of inulin intake is 5–8 g per day. One portion produced for nutrition purpose contains 10–50% of recommended daily intake. If addition of inulin has technological purpose, the dosage may be higher, because inulin starts to work as a texture and flavor enhancer at concentrations more than 2% [6–8].

The application of inulin in the food industry is constantly growing. There is information about usage of inulin in production of various kinds of bread made from wheat and rye-wheat flour, shortbread products and cakes, waffles and gingerbread. It has been proved that adding of inulin makes the bread healthier and improves a number of technological effects – shape stability, porosity, oven and moisture losses, yield. Moreover, inulin increases the consumer qualities of bread: improves its external appearance and flavor, slows down hardening. Recommended dosage is 2.5–3.0% to weight of flour [9].

Thanks to its versatility inulin has found application in dairy industry for production of milk, milk products, butter, cheese, ice cream. The most famous inulin-containing products: kefir "Biomax effektivnyj" by Vimm-Bill-Dann Company and yogurt line "Ermigurt prebiotics" by Ermann Company. The Group of companies "Galaktika" designed a new product – pasteurized inulin-enriched milk. This is a healthy functional product, ideal for dietic nutrition – the milk with inulin contains only 1% of fat. One glass or 200 g of such milk provides 20% of recommended daily need in inulin for the organism [10].

Inulin is widely distributed in plants, but its content and degree of polymerization can be different. The data on quantitative and qualitative inulin content in different crop plants are presented in Table 1.

The main current source of inulin in the European countries is root chicory. Chicory (*Cichórium íntybus*) is a genus of biennial or perennial herbs of Asteraceae or Compositae family. The genus includes two cultivated species and from four to six wild species. Chicory contains up to 20% of inulin. The cultivation of chicory in Russia is limited [11].

Another representative of inulin-containing root vegetables is yacon (*Smallanthus sonchifolius*). Yacon tubers vary in shape and size. One tuber can weigh up to 850-900 g and contain up to 19% of inulin on a wet weight basis. Yacon application perspectiveness for inulin production is differently estimated by the experts [12].

Jerusalem artichoke is one of the most promising natural sources of inulin.

Jerusalem artichoke (*Helianthus tubersrosus L*) belongs to the Asteraceae family and is an annual plant [13]. According to biological characterization it is a plant with direct folious stem. The stem can grow up to 5 m depending on growing conditions. Stooling of

Table 1. Weight fraction and degree of polymerization of inulin from different sources [1, 9, 10]

Plant	Weight fraction of inulin, g/100 g of raw material	Mean degree of polymerization
Banana	0.3–0.7	≤ 5
Onion	1–1.75	≤ 12
Leek	3–10	≤ 12
Garlic	16	≥ 5
Yacon	15–19	≥ 30
Chicory	15–20	≥ 40
Jerusalem artichoke	17–22	≤ 40

Jerusalem artichoke varies between 1 and 5 stems. Inflorescence is a multi-flowered head with bright yellow flowers. Head diameter varies from 7 to 11 cm with consideration to ray flowers [13].

Tuber coloring is white, purple-red, light brown. The dominant shape is pear, but it can also be oblong, oval and fusiform. The tubers of some cultivars have uneven surface caused by the presence of growths. The average weight of tubers varies from 10 to 100 g (usually 30–80 g) depending on the cultivar and growing region. The high farming level allows to obtain 500 g tubers [13]. Jerusalem artichoke tubers contain about 22% of inulin on a wet weight basis [14, 15].

Thanks to its agrobiological properties including high cold- and drought-resistance, low demand towards soil and fertilizers, Jerusalem artichoke is widely cultivated in Russia. The Russian breeders have bred inulin-containing cultivars of Jerusalem artichoke with regular geometric shape that are suitable for industrial processing.

The State Register of breeding achievements currently include 4 cultivars of Jerusalem artichoke: "Vyl′gortskij" (Institute of Biology of Komi Science Center, Ural Division, Russian Academy of Sciences), "Interes" (Maykop station of VIR, KFH "Topinambur" LLC, Rodnik Zdorov'ya "IKSI" LLC), "Nakhodka" (Maykop station of VIR) and "Skorospelka" (Rodnik Zdorov'ya "IKSI" LLC and KFH "Topinambur" LLC) and one cultivar of topinsolnechnik (Maykop station of VIR) [16–18].

Analysis of literature sources revealed that Jerusalem artichoke is a multifunctional agricultural crop and can serve as raw material for production of many different common, functional and dietary products, biologically active and food supplements, medicines. Jerusalem artichoke also has significant biotechnology and bioenergy potential. Tubers and overground parts are used for ethanol production and microbiological synthesis of protein, glycerin, organic acids. At the same time, chemical composition, biochemical and technological properties of Jerusalem artichoke are affected by various environmental factors and are characterized by considerable variability. For example, the content of inulin, degree of its polymerization, qualitative and quantitative composition of oligofructose and free sugars depend on cultivar, growing conditions, time of harvesting, post-harvest ripening and storage conditions [1].

The comprehensive processing of tubers with sorting of raw materials according to technological indicators and further effective usage of sorted parties should be considered the most rational, taking into account the physiological and biochemical features of artichoke .

The Department of food of animal origin technology of Kuban State Technological University developed the concept of deep and complex Jerusalem artichoke processing. It allows to organize a flexible and stable production of food and ingredients from Jerusalem artichoke tubers, quick and cost-effective adjustment of product range depending on the quality of incoming raw materials.

Jerusalem artichoke can become the basis for creation of a large-scale industrial production of inulin in Russia. However, deep processing of Jerusalem artichoke and obtaining of high-quality target products according to the world standards require to resolve a number of technological problems.

The aim of this work is to improve the identification methods and technology of production of inulin from Jerusalem artichoke tubers.

OBJECTS AND METHODS OF STUDY

Jerusalem artichoke tubers of "Interes" cultivar were used for this study. They were washed, cleaned, blanched and crushed into particles with size approx. 1–2 mm. Then the water extraction using vibration with following technological parameters was conducted: water duty 1 : 2, frequency of vibration 24 Hz, process time 60 min, extragent temperature 30–35°C.

The extract obtained from Jerusalem artichoke tubers was fractionated using membranes with selectivity 5, 3, 2 kDa. It yielded four fractions, two of them contained high molecular and medium molecular inulin. After that, the concentrate was evaporated under vacuum and dried. Commercial inulin powder was obtained as a result.

Identification of medium molecular fractions of inulin was performed by IR spectroscopy on IR spectrometer Spectrum Two with ATR (United States). ^{1}H-^{13}C NMR spectra of inulin samples were recorded on Agilent/54 400 spectrometer (United States) at operating frequency 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei.

RESULTS AND DISCUSSION

Existing technologies of inulin production from Jerusalem artichoke tubers can be represented as the process flow diagram shown on Fig. 1. Extraction is the primary process determining yield and quality of the final product. Hot water (approx. 80°C) is currently used as extragent in most cases. It is added to the previously crushed tubers in various amounts (water duty from 1 : 2 to 1 : 10). The process is usually conducted in tanks with stirrer, continuous extractors are also used: screw, rotary pulsed and others. We have proposed and grounded an effective method of extraction using vibration with the following parameters: vibration frequency 24 Hz, process duration 60 min, extragent temperature 30–35°C. These parameters allow to obtain inulin yield about 90–96% from the theoretically possible [2].

The increasing of target product yield with application of vibration occurs because of the fact that vibration causes increasing particle motion relative to each other, as well as to their center of mass. As a result, interaction surface of the components involved in these processes increases; heat and mass transfer become more intensive. Besides, the classic extraction applied previously heated water. To increase the temperature of extragent additional energy costs are required, which increases the cost of the final product. There is no need in preheating when conducting mass transfer using vibration. This positively affects both quality and cost of the target product [18–21].

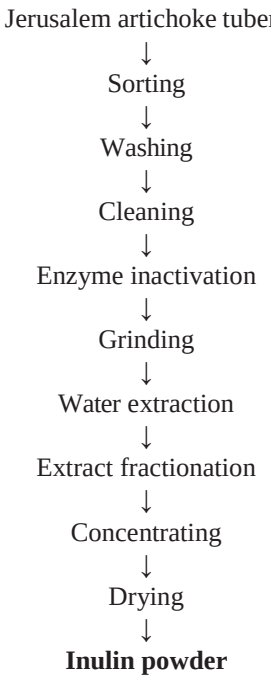

Fig. 1. The process flow diagram of production of inulin from Jerusalem artichoke tubers.

The extract obtained from tubers of Jerusalem artichoke contains inulin with various molecular weights, fructooligosaccharides, proteins, amino acids and other water-soluble substances. The ratio between different carbohydrate factions can vary depending on the raw material, growing and storage conditions and other factors. Therefore, we offer to fractionate the Jerusalem artichoke extract obtained using vibration into separate fractions. Conducted scientific and technical search revealed that the fractionation using ultra and nanofiltration is the most appropriate method. The usage of membranes with selective 5, 3, 2 kDa yielded three fractions for various purposes, including high molecular inulin for medicine and medium molecular inulin for food industry.

We applied FTIR spectrometry and ^{1}H-^{13}C NMR spectroscopy for identification and researching the quality of inulin powder (medium molecular fraction) obtained using improved technology.

The Raftiline ST inulin from Belgian company Beneo Orafti derived from chicory was taken as the reference model.

The IR spectra of reference and experimental samples (Fig. 2 and 3) contain broad absorption bands in the region 3 150–2 800 cm^{-1} that indicates the stretch vibrations of hydroxyl groups of inulin.

Bands in the region 1 636–1 628 and 1 427–1 403 cm^{-1} indicate the presence of esterified carboxyl groups, peaks 1 629 and 1 404 cm^{-1} signalize about the hydroxyl groups. The presence of glycoside bonds typical for inulin in reference and experimental samples is confirmed by the presence of peaks in the region 1 245–1 115 cm^{-1}. Absorption band in the region 1 170–950 cm^{-1} with peak at 1 028 cm^{-1} reveals the presence of OH groups of glucose in inulin. Absorption bands with maxima at 933, 870, 819, 937 and 818 cm^{-1} are typical for 2–1 bonds of fructofuranose residues that serve as links between α,D-glycopyranose and β, D- fructofuranose in the inulin molecule [22, 23].

IR spectra of experimental and reference samples are mostly identical. However, the experimental sample (Fig. 3) revealed a stronger absorption band in the range 1 245–1 404 cm^{-1}. It can be explained by high content of esterified carboxyl and hydroxyl groups that indicate the presence of pectin.

The comparison between spectrometry graphs of experimental and reference samples suggests that the experimental sample of inulin powder obtained using improved technology is identical to the reference, but has a slightly higher content of pectin.

We used ^{1}H-^{13}C NMR spectroscopy to determine the chemical structure of inulin. Data from the ^{1}H and ^{13}C NMR spectra allow to determine monomeric composition, presence and localization of functional groups, configuration of glycoside bonds, degree of polymerization and mean molecular weight of polysaccharide.

It is known that inulin is a polymer consisting of a single α,D-glycopyranose residue and several β,D- fructofuranose residues connected by glycoside bond [24].

The head chain of inulin molecule has a fragment of saccharose residue, where the ratio of α,D-glycopyranose and β,D-fructofuranose is 1 : 1, as shown in Fig. 4. The fundamental difference between the structure of saccharose and inulin lies in the number of fructofuranose residues per one glycopyranose residue. It is possible to determine the ratio of α,D-glycopyranose and β,D-fructofuranose residues using ^{1}H NMR method: each H atom in polysaccharide has characteristic integral peak indicators.

Hence, we researched in detail the spectra of saccharose using NMR 1H (Fig. 5), ^{13}C NMR (Fig. 6) first.

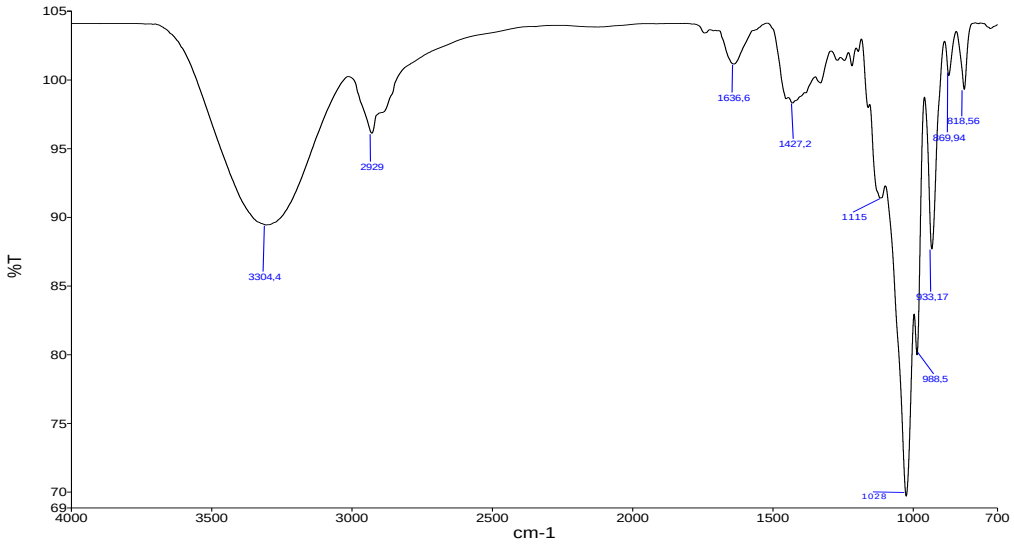

Fig. 2. IR spectrum of commercial inulin from chicory (reference model).

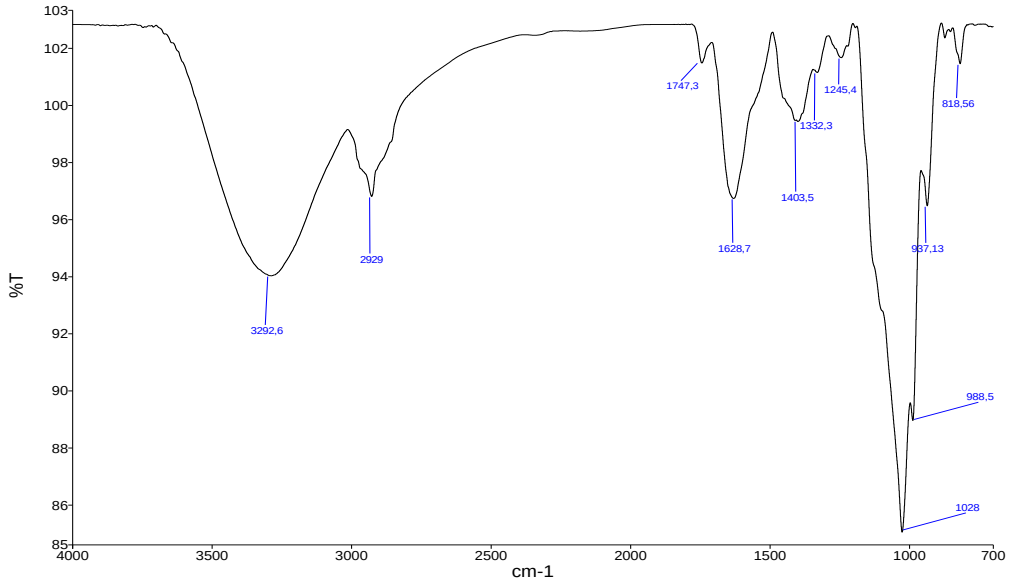

Fig. 3. IR spectrum of inulin from Jerusalem artichoke (experimental sample).

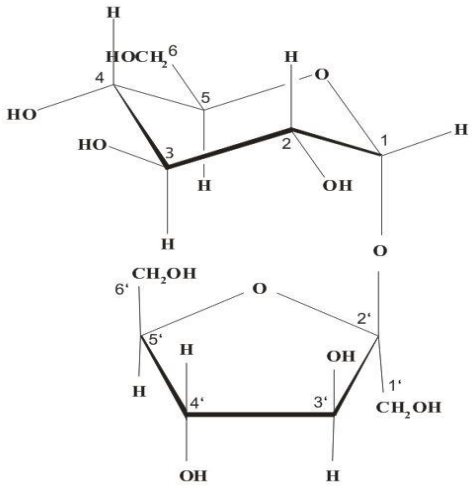

Fig. 4. Chemical structure of saccharose.

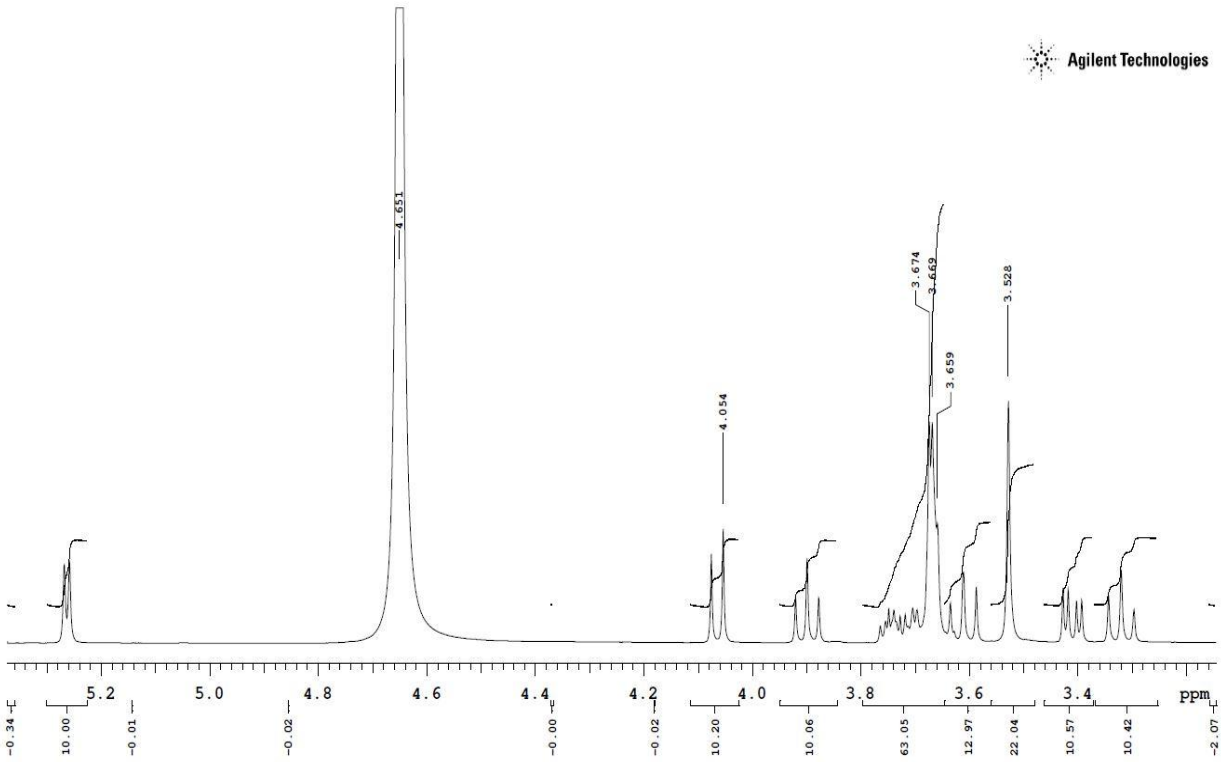

Fig. 5. [1]H NMR spectrum of saccharose.

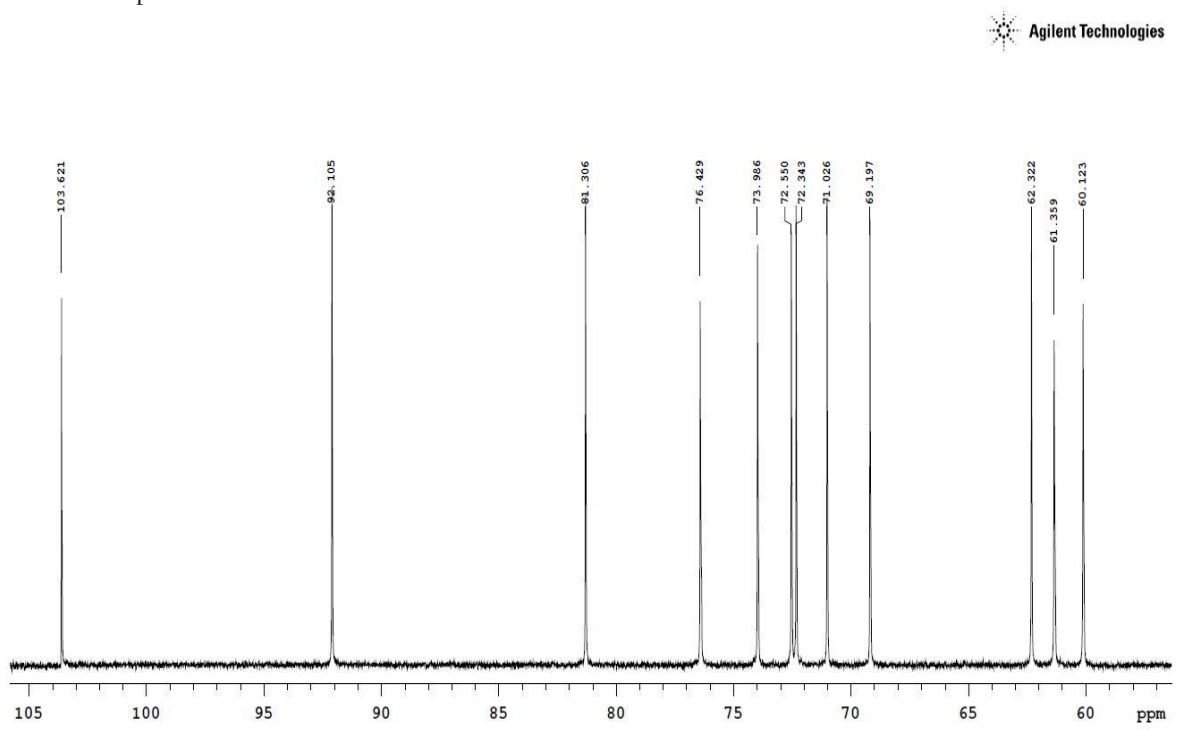

Fig. 6. [13]C NMR spectrum of saccharose.

Combination of these methods allows clear identification of all signals of hydrogen and carbon atoms in the saccharose molecule. Description of signals derived from [1]H and [13]C NMR spectroscopy is presented in Table 2.

One can see in [1]H NMR spectrum the signals of equatorial 1-H glycopyranose residue in the region 5.3 part per million (ppm) and 3′-H proton of the fructofuranose residue in the region 4.1 ppm that are suitable for further analysis. They are isolated and do not mix with the other signals. Their surface is easily interpretable, which allows to use these signals for further research of inulin samples.

The next step was examination of spectra of commercial inulin from Beneo Company (reference) and inulin obtained using improved technology. Results of [1]H and [13]C NMR spectrometry are presented in Fig. 7–10.

Table 2. Chemical shifts of hydrogen and carbon atoms in ^{1}H and ^{13}C NMR spectrum of saccharose (chemical shifts relative to the internal standard TMS)

Number of hydrogen atom	1H, δ, ppm	13C, δ, ppm
1-H	5.27	92.1
3'-H	4.06	76.4
4'-H	3.90	74.0
5'-H	3.73	81.3
5-H	3.69	72.5
6-CH2	3.68	62.3
6'-CH2	3.67	60.1
4-H	3.62	72.5
1-CH2	3.53	61.4
2-H	3.41	71.0
3-H	3.32	69.2

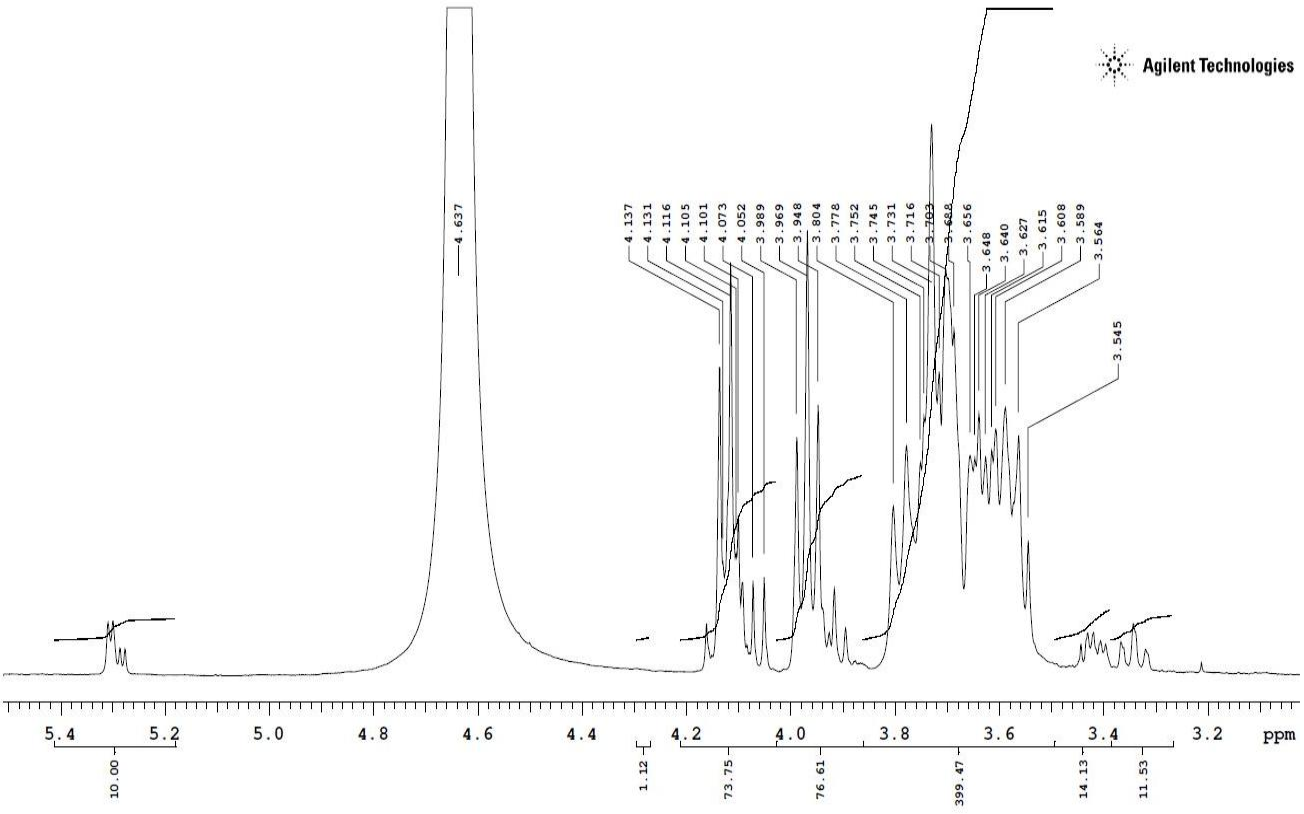

Fig. 7. ^{1}H NMR spectrum of commercial inulin from Beneo Company.

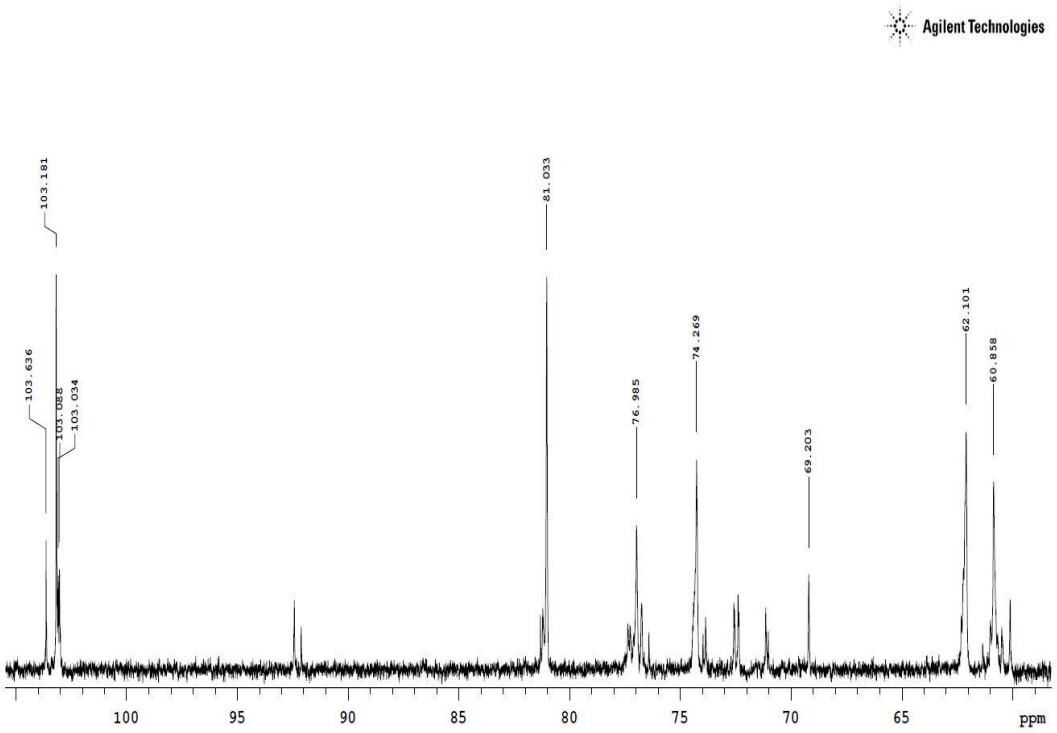

Fig. 8. ^{13}C NMR spectrum of commercial inulin from Beneo Company.

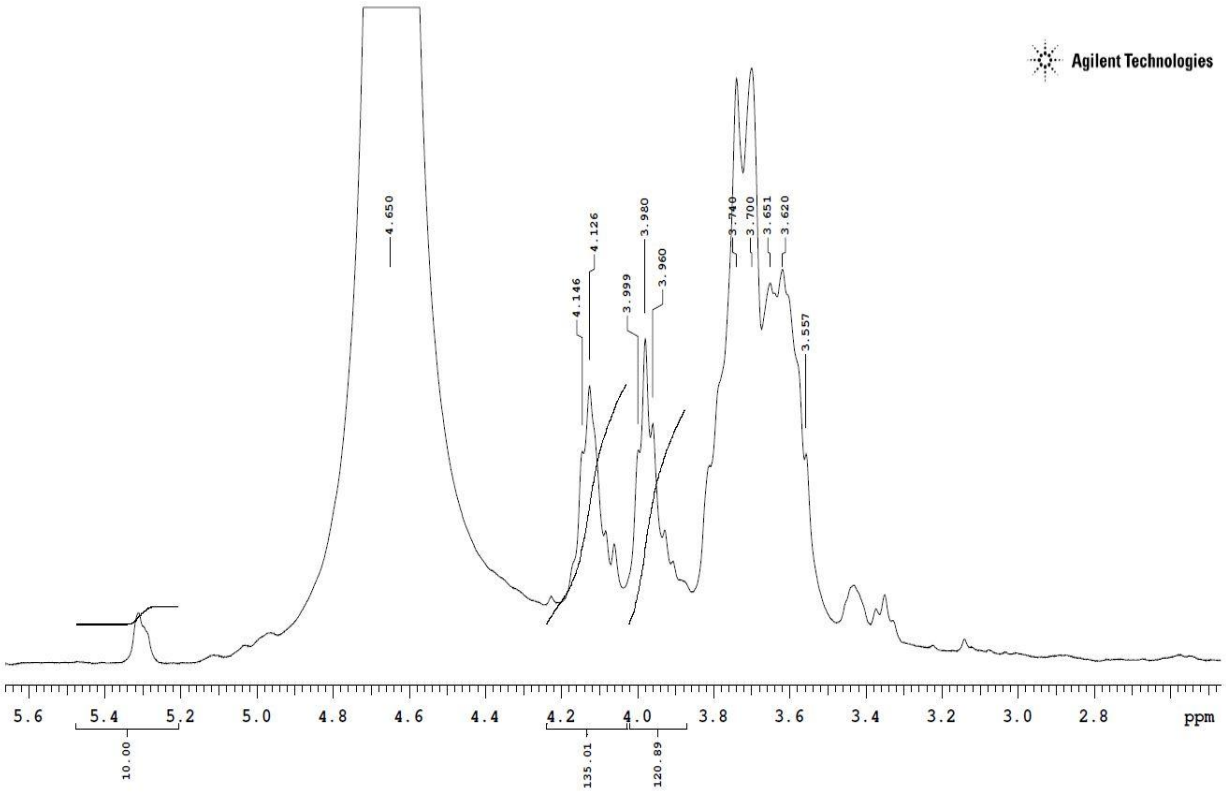

Fig. 9. ^{1}H NMR spectrum of inulin obtained using improved technology.

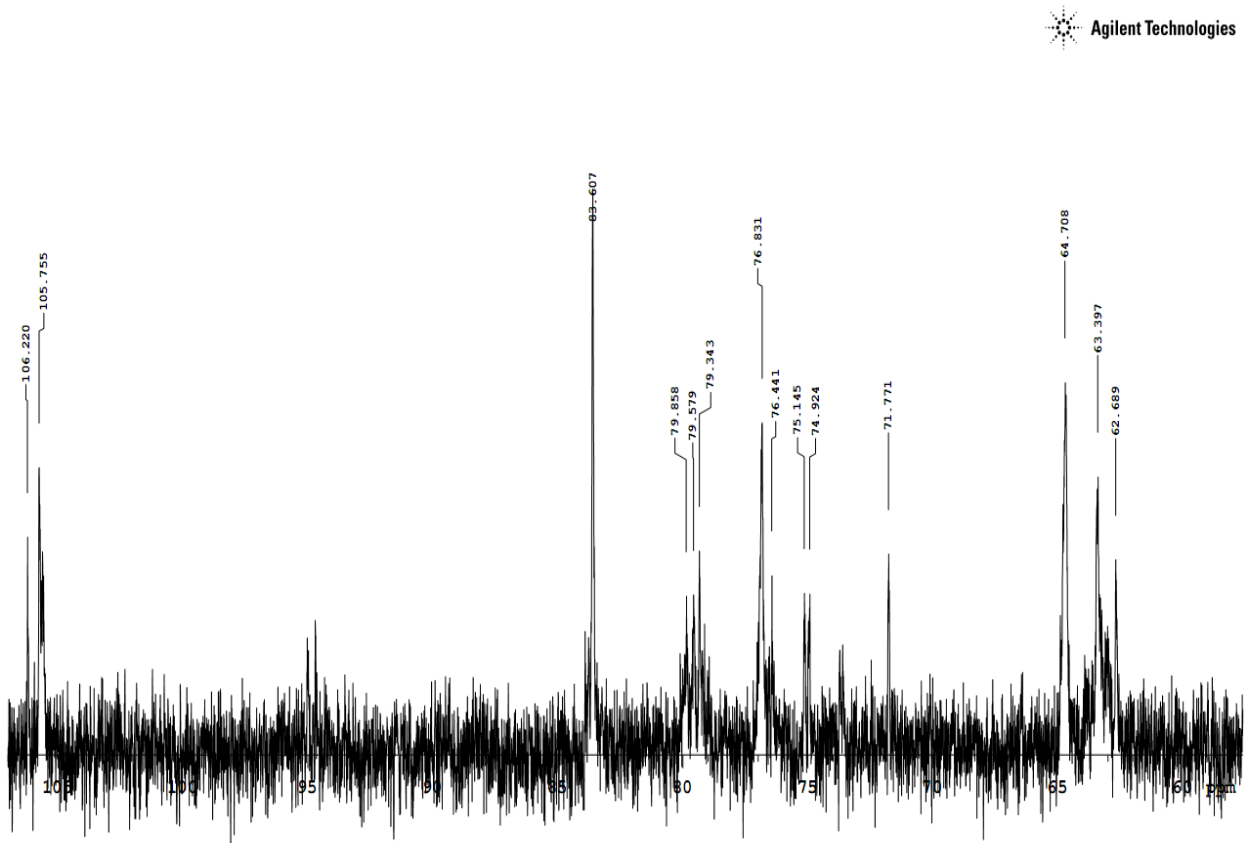

Fig. 10. ^{13}C NMR spectrum of inulin obtained using improved technology.

One can see that presented spectra of commercial inulin from Beneo Company and inulin obtained by improved technology differ by the integral intensity of signals, which belongs to α,D-glycopyranose and β,D-fructofuranose residues.

For the sample of commercial inulin from Beneo Company the ratio of integrated signal intensity of hydrogen atoms in 1-H glycopyranose form and 3′-H fructofuranose form is 1 : (7–8), i.e. one glycopyranose fragment in polysaccharide has 7–8 fructofuranose residues. Molecular weight of this compound lies between 1 314 and 1 476 Da.

For the inulin obtained using improved technology, the ratio of the integrated intensity of the same signals is 1 : (12–13), i.e. molecular weight is 2 124–2 286 Da. The higher molecular weight of experimental sample is indicated by integral surface increase in 1H NMR spectrum. This is associated with increased viscosity of the sample solution in comparison to the sample of commercial inulin from Beneo Company at the same solution concentration.

CONCLUSION

Experimental data indicate that the inulin derived from Jerusalem artichoke using improved technology has a fairly high degree of polymerization of DP = 13–14 and molecular weight 2 124–2 286 Da and therefore possesses good technological properties and physiological activity.

This allows to draw a conclusion about the prospect of its application in the food production as gelation agent, fat substitute and sustained action prebiotic.

Thus, obtaining of inulin from Jerusalem artichoke tubers using vibratory extraction and fractionation with ultra- and nanofiltration methods provides high yield of the target product and excellent quality that is highly competitive with the best world analogues.

REFERENCES

1. Roberfroid M.B. Inulin – type fructans: functional food ingredients. *J. Nutr.*, 2007, vol. 137, no. 11, pp. 2493–2502.
2. Nazarenko M.N., Barkhatova T.V., Kozhukhova M.A., Khripko I.A. and Burlakova E.V. Inulin changes in Jerusalem artichoke tubers during storage. *Scientific Journal of KubSAU*, 2013, vol. 10, no. 94. Available at: http://ej.kubagro.ru/2013/10/pdf/17.pdf. (accessed 14 September 2015). (In Russian).
3. Ladnova O.L. and Merkulova E.G. Application of inulin and stevia for development of new generation products. *Advances in Current Natural Sciences*, 2008, no. 2, pp. 46–47. Available at: www.rae.ru/use/?section=content&op=show_article&article_ id=7778866. (accessed 14 September 2015). (In Russian).
4. Kochnev N.K. and Kamenecheva M.V. *Jerusalem artichoke-bioenergy culture of the XXI century*. Moscow: Ares Publ., 2002. 76 p. (In Russian).
5. Molochnikov V.V., Orlova T.A. and Suyunchev O.A. Processing dairy raw materials using polysaccharides according to “Bio-Ton” technology. *Food Industry*, 1996, no. 5, pp. 34–35. (In Russian).

6. Perkovets M.V. Inulin and oligofructose-universal functional ingredients. *Oils&Fats*, 2008, no. 5, pp. 2–4. (In Russian).
7. Fedorenchenko L.A. and Tuzhilkin V.I. Method of determining the fractional composition of carbohydrate complex of inulin-containing raw materials. *Storage and Processing of Farm Products*, 1999, no. 12, pp. 24. (In Russian).
8. Yuta V.I. Inulin as the ingredient for soft drinks. *Beer and Beverages*, 2000, no. 6, pp. 24. (In Russian).
9. Koryachkina S.Ya. and Bajbasheva D.K. Influence of the degree of polymerization of inulin and oligofructose molecules on their residual content in rye-wheat malt bread with functional purpose. *Transactions of Higher Educational Institutions, Food Technology*, 2010, no. 1, pp. 28–30. (In Russian).
10. Anisimov S.V., Grishina A.S. and Papina M.V. Kefir – tasty, healthy, therapeutic product from "Stavropolsky" dairy factory. *Dairy Industry*, 2009, no. 7, pp. 75. (In Russian).
11. Yatsenko A.A., Kornienko A.V. and Zhuzhalova T.P. *Root chicory*. Voronezh: VNISS, "Istoki" press, 2002. 135 p. (In Russian).
12. Sheremetova S.G., Gasanova E.S., Ponomarev A.N., Sheremetova S.G., Kotov V.V., Polyanskiy K.K. and Nenokhov D.V. Use of stevia and yacon extracts and syrups in fermented milk products. *Food Industry*, 2007, no. 11, pp. 73. (In Russian).
13. Dzantieva L.B., Tsugkieva V.B. and Tsugkiev B.G. Nutrients of Jerusalem artichoke tubers. *Zemledeliye*, 2006, no. 4, pp. 33. (In Russian).
14. Zelenkov V.N. and Shahin S.S. *Many faces of Jerusalem artichoke in past and present*. Novosibirsk: Concern "OIT" – NTF "ARIS", SB RAMS Publ., 2000. 241 p. (In Russian).
15. Polyansky K.K., Rodionova N.S. and Glagoleva L.E. *Jerusalem artichoke: prospects for use in dairy industry*. Voronezh: VGU Press Publ., 1999. 104 p. (In Russian).
16. Bagautdinova R.I. and Fedoseeva G.P. Productivity and fractional composition of carbohydrate complex from Jerusalem artichoke cultivars with different precocity. *Agricultural Biology*, 2000, no. 1, pp. 55–63. (In Russian).
17. Dzantieva L.B., Tsugkieva V.B. and Tsugkiev B.G. Nutrient content in the green mass of Jerusalem artichoke cultivar "Interes". *Kormoproizvodstvo*, 2006, no. 6, pp. 27–29. (In Russian).
18. Nazarenko M.N., Barkhatova T.V., Kozhukhova M.A., Khristyuk V.T. and Babenkova M.A. Intensification of inulin extraction from Jerusalem artichoke tubers using vibration. *Scientific Journal of KubSAU*, 2013, vol. 10, no. 94. Available at: http://ej.kubagro. ru/2013/10/pdf/18.pdf. (accessed 14 September 2015). (In Russian).
19. Perov A.G., Kosachev V.S. and Koshevoy E.P. Modeling of working schedule of extraction production with specialized units. *News of Institutes of Higher education. Food Technology*, 2008, no. 5–6, pp. 55–58. (In Russian).
20. Sergienko M.A., Khristyuk V.T. and Uzun L.N. Intensification of brewing industry processes. *Storage and Processing of Farm Products*, 2008, no. 8, pp. 24–25.
21. Tkachenko R.N. and Khristyuk V.T. Influence of vibration of pure yeast culture on kinetics of wort fermentation and chemical composition of wine. *Transactions of Higher Educational Institutions, Food Technology*, 2009, no. 5–6, pp. 50–52. (In Russian).
22. Abou-Arab A.A., Talaat H.A. and Abu-Salem F.M. Physico-chemical properties of inulin produced from Jerusalem artichoke tubers on bench and pilot plant scale. *Australian Journal of Basic and Applied Sciences*, 2011, vol. 5, no. 5, pp. 1297–1309.
23. Xue B.L., Wen J.L., Sun S.L., Xue B.L. and Sun R.C. Recent advances in characterization of lignin polymer by solution-state nuclear magnetic resonance (NMR) methodology. *Materials*, 2013, vol. 6, no. 1, pp. 359–391. Available at: http://www.mdpi.com/ journal/materials. (accessed 14 September 2015).
24. Coudray C., Demigné C. and Rayssiguier Y. Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. *Eur*. J. Nutr., 2003, vol. 42, no. 2, pp. 91–98.

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STUDY OF THE IMPACT OF THE PREPARATION OF CREAM FOR CHURNING USING VACUUM ATOMIZATION ON THE STRUCTURE OF BUTTER

V. V. Chervetsov[a],*, V. G. Kulenko[b], E. V. Rattur[b]

[a] All-Russian Research Institute of Dairy Industry,
Lyusinovskaya Str. 35, building 7, Moscow, 115093 Russian Federation

[b] Vologda State Dairy Farming Academy named after N.V. Vereshchagin,
Shmidta Str. 2, Molochnoye Village, Vologda, 160555 Russian Federation

* e-mail: conservlab@mail.ru

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Abstract: In the process of the production of butter, glycerides of butterfat crystallize with the formation of different space lattices. Depending on the conditions of cooling, crystals of various sizes and configurations with different physical properties are formed, which determines the crystalline structure of solidified fat, its physical and chemical properties, and therefore, the consistency of the finished product. The paper explores the impact of the flow two-stage method for physical aging of cream on the pattern of the crystallization of glycerides of butterfat. Comparative X-ray crystallographic and differential-scanning studies of butter, produced using the method of churning, were carried out. The method of differential scanning calorimetry was used to study thermal effects of the test sample of butter (received by churning cream which was aged using the flow method) and the control sample (received by churning cream which was aged using the conventional method). When analyzing data, no exothermic peaks, corresponding to processes that release heat, were observed. The polymorphism and the type of crystalline lattice of glycerides in the butter samples were analyzed using the method of X-ray diffraction. The separate group character of solidification of butterfat – low-melting, medium-melting and high-melting glycerides – was determined. X-ray crystallography did not reveal fundamental differences in the pattern of the crystallization of glycerides and formation of polymorphic modifications with different types of crystalline structure. This indicates the uniformity of type of successive phase changes of butterfat in butter, both after fast cooling of cream by vacuum atomization, followed by aftercooling in a scraped heat exchanger, and in case of using the conventional method of physical aging of cream.

Keywords: Aging of cream, butter, differential scanning calorimetry, thermogram, X-ray diffraction, polymorphism, crystalline structure

DOI 10.12737/13124 *Foods and Raw Materials*, 2015, vol. 3, no. 2, pp. 23–28.

INTRODUCTION

The crystalline structure of solidified fat, its physical and chemical properties, and hence the consistency of butter depend on the presence of certain polymorphic forms and their relationship. Glycerides of butterfat possess the properties of monotropic polymorphism, i.e. the ability to crystallize with the formation of different space lattices. Depending on the conditions of cooling, crystals of various sizes and configurations with different physical properties are formed.

According to research, conducted by G.V. Tverdokhleb [1], in the solid phase of butterfat and its fractions there are four modifications: γ, α, β' and β. The transition of one modification to another is irreversible and occurs in one direction, from a metastable form to a stable form γ→ α→ β'→ β. The transition point is close to the melting temperature of a metastable modification.

In order to obtain objective data on the impact of the flow two-stage method of aging of cream [2] and its implementation on the pattern of the crystallization of glycerides of butterfat, differential-scanning and X-ray crystallographic studies of butter, produced using the method of churning, were carried out.

OBJECTS AND METHODS OF STUDY

Differential scanning calorimetry is a method based on the registration of energy necessary to establish zero temperature difference between the studied sample and the reference sample in time or depending on temperature, when heating or cooling them in identical temperature conditions with a certain speed.

The results are recorded in the form of a DSC curve. The difference of energies between the cells with the studied sample and the reference sample ($d\Delta Q/dt$) is plotted on the ordinate, time or temperature are plotted on the abscissa [3–5].

DSC curves were obtained using the differential scanning calorimeter "Shimadzu DSC-60" (Fig. 1), with the use of aluminium crucibles having a diameter of 6 mm and a height of 5 mm, weighing 49 mg. The device was calibrated with indium, stannum and lead.

This differential scanning calorimeter has the following technical characteristics:
– temperature range of measurement: from room temperature to 600°C (without liquid nitrogen cooling); from –140°C to 600°C (with liquid nitrogen cooling);
– limits of DSC signal measurement: ±40 MW;
– noise level of DSC signal: 1 MW;
– programmable heating/cooling rate: from ±0.1 to 99.9°C/min;
– cooling conditions: cooling agent – liquid nitrogen; drying gas – nitrogen with feed rate of 200–300 ml/min;
– atmosphere: air, inert gas (nitrogen);
– pressure: atmospheric;
– amount of sample: depends on the type of crucible;
– form of sample: solid or liquid.

The polymorphism and the type of crystalline lattice of glycerides in the butter samples were analyzed using the method of X-ray diffraction. The analysis was carried out using the X-ray diffractometer Shimadzu XRD7000 (Japan) with the X-ray tube having a cobalt anode (characteristic radiation wavelength 0.178897 nm, Fe-filter). The appearance of the device is presented in Fig. 2. Curve recording was implemented in the Bragg–Brentano geometry at the tube voltage of 40 kV and the current of 30 μA. The slit width during the recording of curves was 0.3 mm. The recording was implemented at the angular rate of 2 degrees per minute.

Fig. 1. The appearance of DSC-60.

Fig. 2. X-ray powder diffractometer Shimadzu XRD7000.

The condition for obtaining diffraction maximum on the x-ray spectrum is the fulfillment of the Wulff–Bragg's condition:

$$2d\sin\theta=n\lambda, \quad (1)$$

where d is the interplanar distance, nm; θ is the grazing angle; λ is the wavelength, and n (n=1, 2 ...) is an integer, called the diffraction order.

The identification of the modifications of butterfat is based on the system proposed by E. Latton [6]. X-ray structural characteristics of the modifications of triglycerides according to Latton are presented in Table 1.

RESULTS AND DISCUSSION

Study of thermal effects using the method of differential scanning calorimetry

The test sample of butter (received by churning cream which was aged using the flow method) was placed into the crucible, weighed and transferred into the measuring cell. The device was cooled to –70°C using liquid nitrogen. After exposure to this temperature, the program of heating with the rate of 2°C/min was launched, which ensures almost complete separation of melting peaks of various components of butter. Aluminium oxide was used as a reference sample. DSK-thermogram of the test sample is presented in Fig. 3.

When analyzing data, no exothermic peaks, corresponding to processes that release heat, were observed. In the thermogram there is a sharp endothermic peak with a maximum at a temperature of –1.5°C, corresponding to the melting of plasma. In terms of intensity this peak is comparable with the similar peak of the thermograms of the control sample (received by churning cream which was aged using the conventional method) (Fig. 4), but has a more pronounced, "narrow" character: in the control sample the beginning of this peak corresponds to the temperature of approximately –12.9°C, and the peak is more diffused, while for the test sample the beginning of the melting corresponds to the temperature of approximately –3.9°C.

Table 1. X-ray structural characteristics of the modifications of triglycerides according to Latton

Modification	Characteristics of small intervals
α	A single diffraction peak corresponding to the interval of 0.414 nm
β'	Usually two (sometimes more) diffraction peaks corresponding to the intervals of 0.42 nm (of high intensity) and 0.38 nm
β	Sharp (usually very sharp) diffraction maximum corresponding to the interval of 0.46 nm, along with peaks of lesser intensity

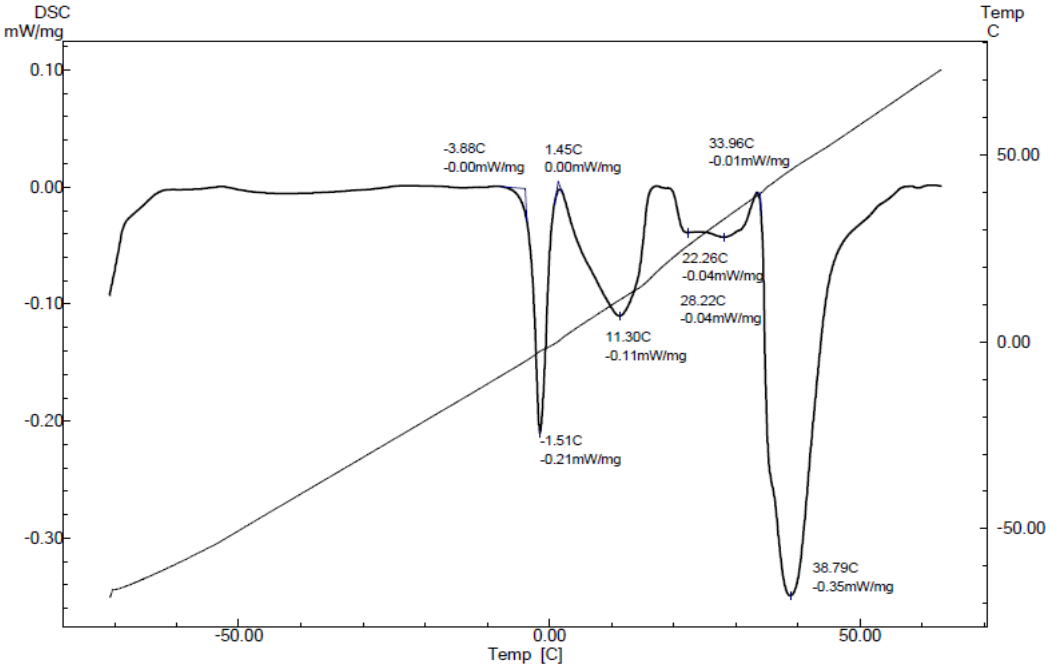

Fig. 3. DSC-thermogram of the test sample.

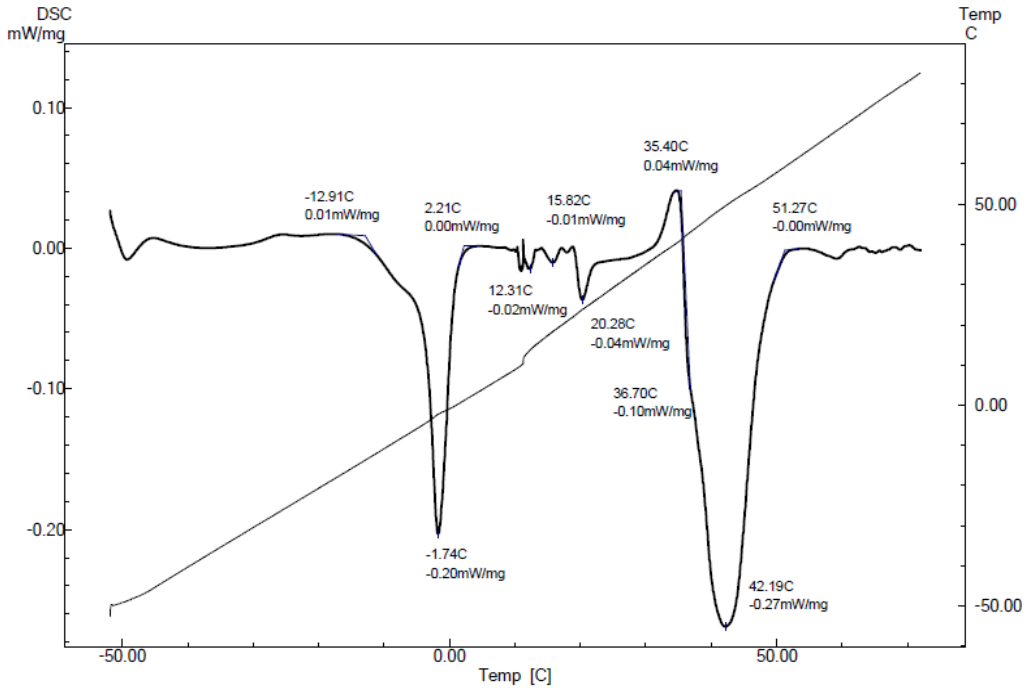

Fig. 4. DSC-thermogram of the control sample.

The test sample is also characterized by a more pronounced peak corresponding to low-melting glycerides (the peak corresponds to the temperature of approximately 11.3°C). As is the case with the control sample, the thermogram shows rather diffused peaks of low intensity corresponding to the glycerides with a melting temperature of about 22.3°C and 28.2°C.

The test sample is also characterized by the dominance of high-melting glycerides, identified by the most intense peak with a maximum at a temperature of 38.8°C (the beginning of the peak is around 34.0°C). The content of this high-melting group of glycerides is higher than in the control sample, which as evidenced by the greater intensity of the peak (–0.35 MW/mg vs –0.27 MW/mg).

Study of the structure of butter using the method of x-ray crystallography

Samples for recording curves were prepared by pressuring butter into flat open aluminum ditches. Butter samples for the study were prepared in four ways:

I – spectra were recorded at a temperature of 4 ± 2°C without additional heat treatment;

II – samples were heated to a temperature of 15°C and conditioned for 10 h before recording spectra;

III – samples were conditioned for 24 h at a temperature of –18° before recording spectra;

IV – samples were heated to a temperature of 70°C, conditioned for 20 minutes and recorded spectra immediately, after which the repeated recording of spectra was carried out in 2 hours.

As a result of X-ray studies, the following data were obtained. Both samples in the area of small interplanar distances have two peaks corresponding to the interplanar distances of 0.38 and 0.42 (0.43) nm, wherein the second peak is more intense. This combination of peaks characterizes the β'- polymorphous modification, while the offset and asymmetrical extension in the area of 0.46 nm can indicate the presence of tiny centers of crystallization of β-modification or is a consequence of a large amount of liquid fat phase.

In the test sample (Fig. 5), as opposed to the control sample (Fig. 6), along with β'-modification, β-modification was also clearly seen, which is evidenced by the clear peak of 0.46 nm.

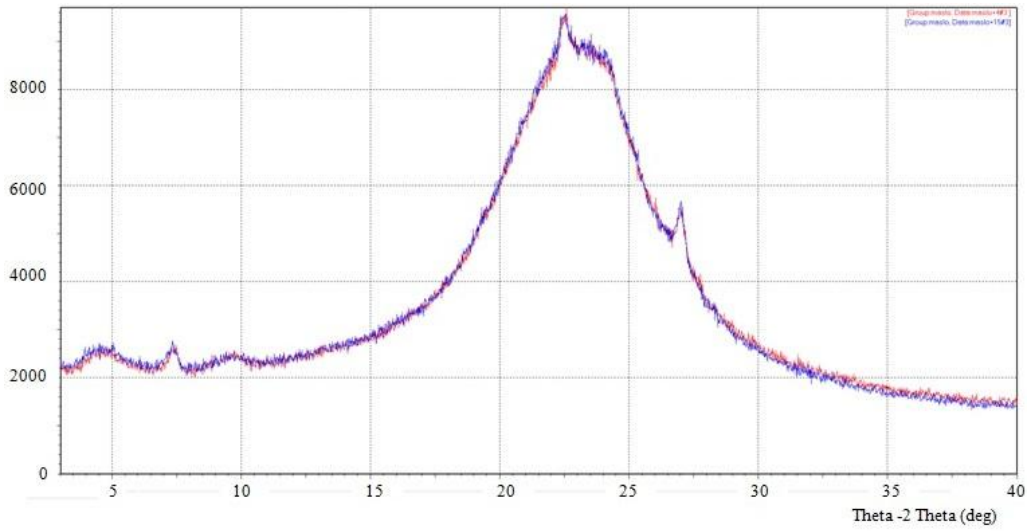

Fig. 5. The X-ray spectrum of the test sample at temperatures of 4 ± 2°C (red line) and 15°C (blue line).

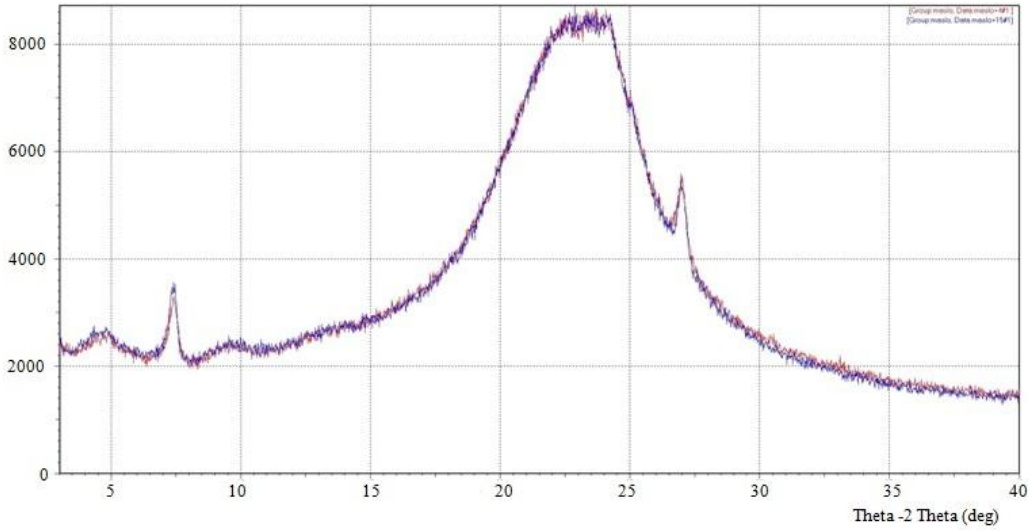

Fig. 6. The X-ray spectrum of the control sample at temperatures of 4 ± 2°C (red line) and 15°C (blue line).

In both samples, reflexes (of different order n) were identified in the area of large intervals, corresponding to the same interplanar distance of 4.1 (4.2) nm, which indicates the presence of the crystalline structure with double chain length (DCL).

When heating samples to a temperature of 15°C, no significant changes in the samples were identified.

When cooling samples to a temperature of –18°C there were also no significant differences in comparison with the samples, studied at 4 ± 2.1°C.

When heating samples to 70°C (Fig. 7 and Fig. 8) the following changes were identified: in the area of small angles the maximum of 0.43 nm transformed into the diffused halo within the boundaries of 0.40–0.47 nm, the maximum in the area of 0.38 nm also lost its sharpness, its intensity decreased.

In the area of large intervals, the intensity of reflexes, corresponding to the interplanar distance of 4.1 (4.2) nm, significantly decreased. These changes indicate partial melting and significant reduction of layered ordering of molecules in the structure with double chain length.

CONCLUSIONS

The studies determined the separate group character of solidification of butterfat – low-melting, medium-melting and high-melting glycerides. For mixed crystals of high-melting group of glycerides only structures with double chain lengths are typical. Low-melting glycerides are characterized by having structures with double and triple chain length, wherein the latter are rather stable.

The differentiation of mixed groups of solidified glycerides contributes to the formation of fat structure in individual fat balls and in butter, close to the structure of the control cream sample. X-ray crystallography did not reveal fundamental differences in the pattern of the crystallization of glycerides and formation of polymorphic modifications with different types of crystalline structure. This indicates the uniformity of type of successive phase changes of butterfat in butter, both after fast cooling of cream by vacuum atomization, followed by aftercooling in a scraped heat exchanger, and in case of using the conventional method of physical aging of cream.

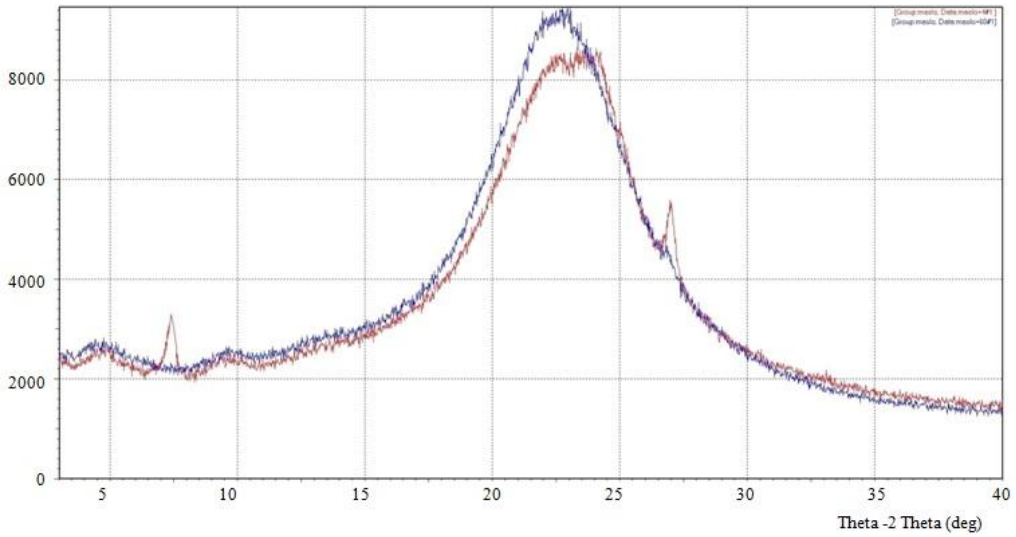

Fig. 7. The X-ray spectrum of the control sample at temperatures of 4 ± 2°C (red line) and 70°C (blue line).

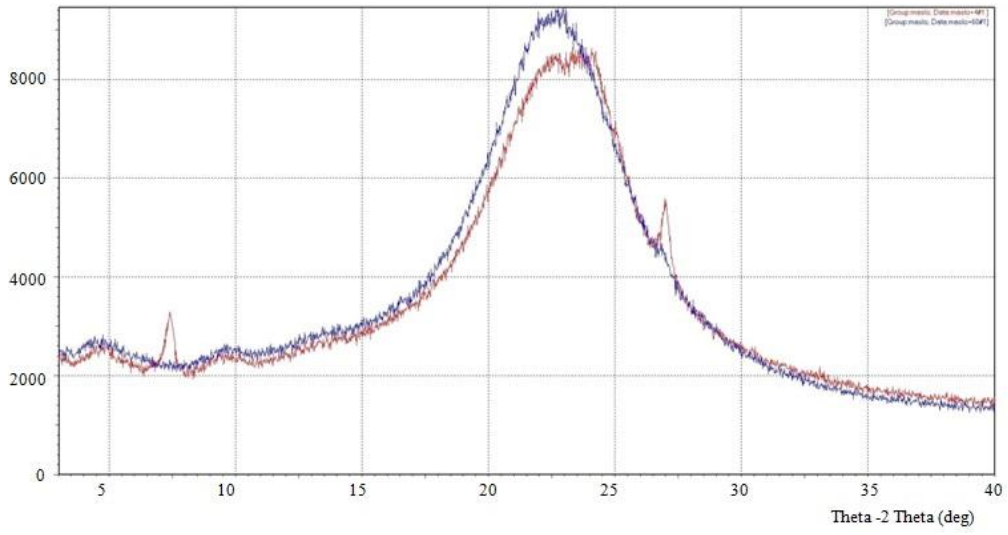

Fig. 8. The X-ray spectrum of the test sample at the temperatures of 4 ± 2°C (red line) and 70°C (blue line).

REFERENCES

1. Tverdokhleb G.V. *Fazovye izmeneniya molochnogo zhira v formirovanii konsistentsii slivochnogo masla*. *Avtoreferat dokt. diss.* [Phase changes of butterfat in forming the consistency of butter. Abstract of the doctoral thesis]. Moscow, 1962. 30 p.
2. Chervetsov V.V., Galstyan A.G., Kuzin A.A. and Rattur E.V. *Sposob potochnogo dvukhstadiynogo sozrevaniya slivok dlya polucheniya slivochnogo masla* [Method for flow two-stage aging of cream for butter production]. Patent RF, no. 2531239, 2014.
3. Hemminger W. and Hohne G. *Calorimetry. Fundamentals and practice*. Weinheim: Verlag Chemie, 1984. 176 p.
4. Hemminger W., Hohne G. and Flammersheim H. *Differential scanning calorimetry*. 2nd edn. Berlin: Springer, 2003. 298 p.
5. Brown M. *Introduction to thermal analysis. Techniques and applications*. 2nd edn. Dordrecht: Kluer Academic Publishers, 2001. 264 p.
6. Latton E. The polymorphism of glycerides progress in the milk fat. In *Lipids*. Vol. 8. London: Pergamon Press Ltd., 1954, pp. 18–56.

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USAGE OF CHITOSAN IN DAIRY PRODUCTS PRODUCTION

I. A. Evdokimov[a], L. R. Alieva[a], V. P. Varlamov[b], V. D. Kharitonov[c], T. V. Butkevich[d], V. P. Kurchenko[d,*]

[a] North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355009 Russian Federation

[b] Bioengineering Center of RAS,
60 years of October Avenue 7, building 1, Moscow, 117312 Russian Federation

[c] Institute of the Dairy Industry,
Lyusinovskaya Str. 35, building 7, Moscow, 115093 Russian Federation

[d] Belarusian State University,
Nezavisimosti Avenue 4, Minsk, 220030 Republic of Belarus

* e-mail: kurchenko@tut.by

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Abstract: The use of chitosan can significantly reduce energy costs in the processing of milk protein and carbohydrate raw materials, and is very promising for use in the dairy industry. In solution of serum proteins, the chitosan binds β-lactoglobulin and other proteins, thus forming an insoluble complex. The formation of complexes of proteins with the chitosan can be accompanied by both a change in the balance of forces that determine the nature of intra- and intermolecular interactions of protein globules, and formation of coacervates differing in size, shape, charge, degree of hydration. Electrostatic forces make the main contribution to the formation of the insoluble chitosan protein complex. In the study of the chitosan complexes formation conditions with the milk serum protein, the pH interaction was studied, as well as concentration of chitosan, the molecular mass of the polysaccharide, ionic strength, and other factors. These patterns of interaction of the milk serum proteins with the chitosan have formed the basis for the development of sorbents based on this polysaccharide. We studied the sorption properties of various forms of chitosan: granulated, as cryogels, as well as part of calcium tartrate gel. Using the chitozan containing sorbents allows us to select proteins from the milk serum and obtain purified preparations of β-lactoglobulin and lactoferrin. Inclusion of the chitosan in the milk beverages and dairy desserts allows us to create a functional food, where the polysaccharide acts as a technological, bactericidal and fungistatic agent.

Keywords: Chitosan, milk serum (whey), β-lactoglobulin, α-lactalbumin, bovine serum albumin, complexation of proteins

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INTRODUCTION

In recent years, intensive studies are being performed to assess the possibility of the chitosan usage in the manufacture of functional foods. Chitosan is a linear polysaccharide consisting of N acetyl 2 amino-2-deoxy-D-glucopyranose and preferably 2-amino-2-deoxy-D-glucose, which are in pyranose form and connected by 1–4 glycosidic linkages. This polysaccharide is second most abundant natural polymer after cellulose. It is biocompatible and biodegradable to conventional materials of the body, such as N-acetylglucosamine or glucosamine, has immunomodulatory, antimicrobial, fungistatic, antitumor, radioprotective, anti-inflammatory, anti-cholesteric action, and thus it has low toxicity [1, 2]. The chitosan can be attributed to the group of parapharmaceuticals, which are natural substances with specific pharmacological activity. Many states approved its use as a dietary supplement to food [3]. The combination of safety and biological activity of the chitosan creates prerequisites of its wide use as a food supplement. Lipotropic effect of chitosan is attached particular importance as an important factor contributing the confrontation to cardiovascular disease. In studying the effect of chitosan on lipid metabolism, we observe a significant reduction of total lipids, triglycerides, serum cholesterol, and reduced serum aminotransferase activity, indicating the positive effect of chitosan on liver function [4]. The chitosan useful qualities when used for food purposes are sorption properties and the ability to restore the intestinal

microflora. Primary amino groups of chitosan mediate binding of heavy metals and radionuclides. The ability of chitosan to form polyelectrolyte complexes with anionic biopolymers can be used for binding and excretion of the different toxins [5, 6]. The chitosan action mechanism on pathogenic microbial flora is associated with the integrity violation of their outer membrane composed of lipopolysaccharides, glycoproteins, phospholipids. It is shown that the chitosan enhances the nonspecific resistance to adverse environmental factors. In addition, it has the ability to stimulate growth of bifidobacteria and beneficial intestinal flora. Long-term studies on the the chitosan usage as a food additive found no contraindications of its use, it can be limited only when acidity, acute gastritis and peptic ulcer disease. The chitosan is one of the few enterosorbents, for which extensive biomedical research of security and preventive effects has been conducted [7–9].

The chitosan is becoming more widely used in the food industry, particularly in the production of dairy products. Milk processing is developing towards increasing the share of the production of cheese and cottage cheese. In connection with this, a volume of the resulting whey is increased. According to the International Dairy Association, the whey volume, which is obtainable in the world as a byproduct of processing, has reached 140 million tons per year. The Republic of Belarus is ranked 5th in the world in the export of dairy products, where the major share is made up of cheeses. The increase of production leads to an increase in the amount of serum, which remains within the Republic, which amounts to more than 4 million tons per year [10]. The whey contains 0.55% protein, 4.8% lactose, 0.05% fat, 0.5% minerals, and is a valuable product [11]. Its further use is not significant. The whey losses and waste discharge undermine the effectiveness of milk processing, and cause economic and environmental damage [12]. An urgent task is to complete the development of technologies and rational use of whey, which has a high nutritional and biological value. Proteins are an important component of whey, and are optimally balanced by amino acid composition. For years, the whey proteins have been the subject of intensive research, which found their physicochemical properties, structure, and some biological activities [11–15].

Among all the whey proteins, the largest number accounts for β-lactoglobulin (β-Lg) with content of 60%. By its structure, β-Lg is a globular protein consisting of 162 amino acid residues. In cow milk, the protein is present in two isoforms, which differ in their physicochemical properties. The biological function of β-Lg is still not clearly established. It is believed that this protein is involved in the regulation of phosphorus metabolism in the breast. Since β-Lg is resistant to proteolytic attack, it can cause allergic reactions in infants consuming cow milk in one form or another [13, 14].

The second quantitatively whey protein is α-lactalbumin (α-La) of 20%. It also has a globular structure stabilized by four disulfide bonds and consists of 123 amino acid residues [13, 14].

Among minor whey proteins, a content of bovine serum albumin (BSA) is 7%. It consists of 582 amino acids and has 17 intramolecular disulfide bridges. Due to its size and structural features, the BSA binds with the free fatty acids, lipids, and many other hydrophobic compounds [13].

It is known that the whey proteins, such as β-Lg, α-La, immunoglobulins, lactoferrin, exert their biological activity, either directly or after enzymatic hydrolysis. Antimicrobial peptides have been found in the partial hydrolyzate of the whey proteins together with antioxidant, antifungal, immunomodulatory, and other antihypertensive properties. In its natural form, they are present in fermented milk products, such as yogurt, kefir, feta cheese, and other cheeses [13, 15, 16]. Since the whey proteins have valuable biological properties, their isolation and purification are seemed to be an urgent task. To achieve it, ultrafiltration or various chromatographic techniques are used in order to provide protein preparations of high purity. However, the process of chromatography is time consuming, and needs special equipment. Simpler methods are selective precipitation of individual proteins using specific reagents. Such reagents include the chitosan and its various derivatives [17].

In view of the fact that the whey proteins are different in their structure and properties, they may have different affinities for the chitosan. In this regard, there is a great theoretical and practical interest of physical and chemical phenomena and laws of interaction of proteins with anionic and cationic polysaccharides. Such processes shall be useful for processing of various proteins, i.e. shall be "universal" and at the same time shall be simple and have manufacturability. The common proteins physicochemical nonequilibrium phenomena include complexation of proteins with anionic and cationic polysaccharides, anisotropic gels formation of two-phase systems, protein solutions concentration when establishing phase equilibrium between solutions of polysaccharides, proteins, and others [5].

It should be noted that in modern technologies of milk processing, a method for enrichment is widely used for traditional foods with dietary fiber. This approach is within the concept of functional food that provides for the development and production of products, which have not only a high nutritional value, but also are useful for human health. In this connection, in the dairy industry, the polysaccharide food additives are widely used as thickening and gelling agents. They are classified depending on the source of origin, structure of the polymer chain, the nature of the monomer residues, the charge. Depending on the charge, the polysaccharides are divided into the following types: Neutral, which are cellulose derivatives, amylopectin, galactomannans; anionic (acidic), which are alginates, carrageenans, pectins, xanthan, gum karaya, ghatti, and tragacanth, acacia, furcelleran, gellan gum; cationic, (basic), which are chitosan [18]. Among these polysaccharides, the chitosan is rarely used as a food additive. This is due to the fact that the mechanism of its interaction with the components of milk is not investigated yet.

The present author's review is devoted to the study of the chitosan interaction mechanism with the whey proteins, and its possible usage in the manufacture of dairy products.

CHITOSAN INTERACTION MECHANISM WITH MILK PROTEINS

The ability to interact with proteins is an important property of this polysaccharide, and it can form emulsions, gels, act as a stabilizer and an antioxidant [5]. In the dairy industry in the production of the series of products (lactose, syrups, beverage), removal of the proteins from the whey is necessary. The chitosan can be successfully used for the coagulation of the whey proteins. The mechanism of the whey proteins completing with chitosan is determined by the physicochemical properties of these biopolymers.

The chitosan is soluble in mineral and organic acids. At pH above 7, the amino group is deprotonated and shows nucleophilic properties. A preparation of water soluble chitosan derivatives is based on this characteristic of the chitosan, including chitosan succinate. At low pH (pK_a < 6.5), the amino group is protonated, chitosan is a water soluble cationic polyelectrolyte. Due to its chemical nature, it is able to various kinds of interaction forming 4 basic chelation types of connections, such as ionic, hydrogen, hydrophobic, wherein the chitosan acts as the complex nucleus. The ionic interactions occur due to the amino groups. The weak linkages (hydrogen, hydrophobic) are formed by interaction of the chitosan with a number of organic substances. This same type of interaction is observed with the introduction of excess chitosan to collagen solution [5].

The chitosan ability to form complexes is due to the presence of a lone electron pair on the nitrogen atom, and in some cases, the linkages are formed by a lone electron pair of the oxygen atom. This principle forms the formation of numerous types of chelate linkages with metals. The chitosan can form such links under certain conditions with all metals except the alkali and alkaline earth ones [9].

In these types of interactions is a separation of raw milk for protein and protein-free fraction by introducing a colloidal solution of the chitosan as an active whey protein cationic completing agent. The polycationic nature of the polysaccharide opens up broad prospects for its use for the separation of milk proteins that have low values of isoelectric points [14].

Based on the properties of the whey protein and chitosan, we have put forward a hypothesis, which is based on the assumption that the division of the milk raw materials on the protein and protein fractions with using the chitosan is based on the formation of the chitosan and proteins complexes. Such interaction of negatively charged ions of milk serum proteins with the protonated chitosan can occur in a narrow range of pH 5.0–6.5 (Fig. 1). Among the whey proteins, the largest number amounts for β-Lg with an isoelectric point, this is 4.9–5.4. The second quantitatively whey protein is a-La with an isoelectric point of 4.6. The bovine serum albumin has an isoelectric point of 4.8 [13]. At a pH above 5.0, these proteins will have a negative charge, and up to pH 6.5, the chitosan has a positive charge. This creates the possibility of the ionic interactions.

In this regard, we have previously studied the interaction process of the chitosan with whey proteins [14, 16, 19, 20]. We added a solution of chitosan to a 1% solution of demineralized whey at pH 6.2. Binding to the proteins, the chitosan formed insoluble protein-polysaccharide complex, and for 30 min there was an increase in absorbance at λ = 580 nm. For the separation of insoluble particles, the whey protein solution, mixed with chitosan, was centrifuged for 10 min at 9 000 g. We conducted an electrophoretic analysis of the whey proteins residue, and calculated their relative amount in the insoluble complex with the polysaccharide [14, 20].

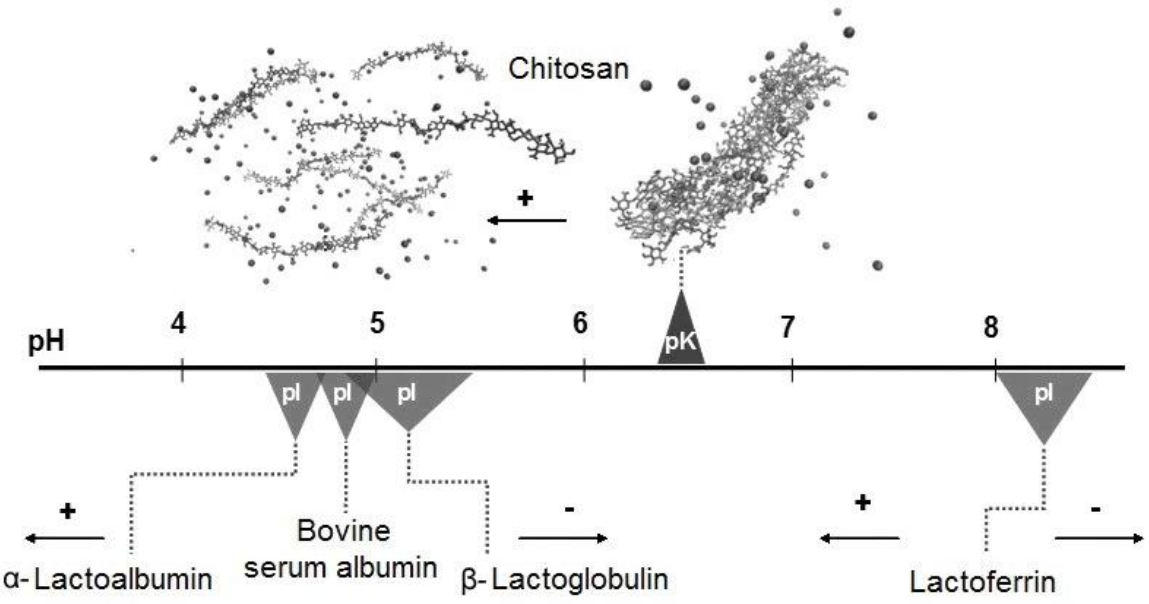


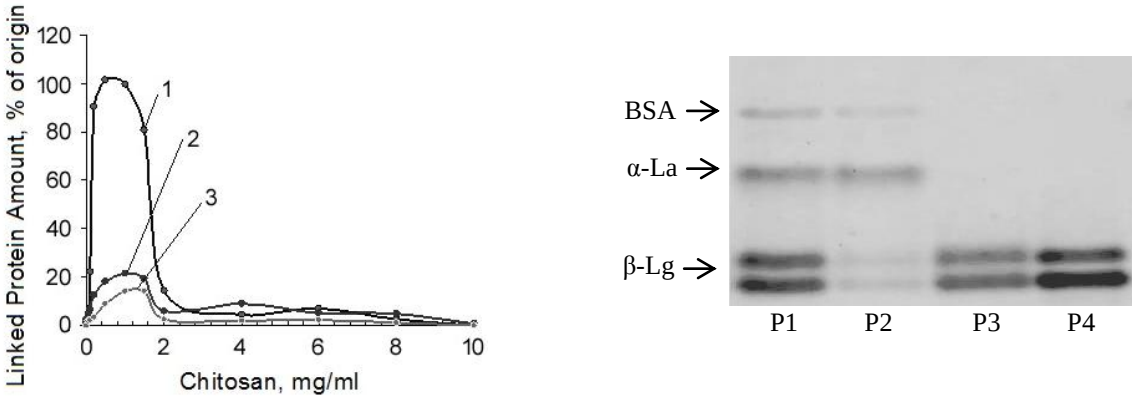
Fig. 1. pH Effect on chitosan and whey proteins charge.

The results of the authors' study showed that the major protein associated with the chitosan are α-Lg, α-La, BSA (Fig. 2). The lactoferrin and several minor proteins do not participate in the complex formation. These data suggest that maximal binding β-Lg and other serum proteins with the chitosan are observed in the content of polysaccharide in the reaction environment of 0.5 mg/ml. In this case, more than 90% of the whey protein is transferred in the precipitate. 100% of β-Lg, 20% of α-La and 10% of BSA are removed therefrom. With further increase of the chitosan content, an amount of generated sludge is considerably reduced in the solution (Fig. 2).

Thus, the chitosan is reacted with the whey at a pH above their isoelectric points, forms an insoluble complex. The Milk Protein – Chitosan Interaction comes under the influence of opposite charges in the pH range and a certain ratio of the reagents. A phenomenon of coacervation is fully observed in the system, i.e. decay into two phases, in one of which the chitosan coacervates and whey proteins are concentrated representing protein + chitosan complex. The system second phase is an equilibrium fluid comprising lactose, whey protein residues, and chitosan. In case where the amount of chitosan in a 1% solution of the whey protein is more than 3 mg/ml, the insoluble chitosan-protein complex is formed.

In the study of the chitosan-protein complexes formation, the pH interaction was studied, as well as the influence of ionic strength, concentration of chitosan with different molecular mass, temperature, and other factors.

The pH factor is very important for the implementation of many of intermolecular interactions, as it affects the ionization of certain functional groups of polymer compounds. Our study of the interaction process of the chitosan with a molecular mass of 200 kDa, 86% DM and β-Lg was carried out in the pH range from 5.2 to 6.2. The results of the experiment are shown in Fig. 3.



(a) (b)

Fig. 2. Dependence of the relative content of serum proteins in the precipitate on the chitosan content in the milk serum (a) and electrophoretogram (b) of WPC solution to (P1), after (P2) treating with the chitosan, complex proteins with the chitosan (P3), the standard β-Lg (P4).

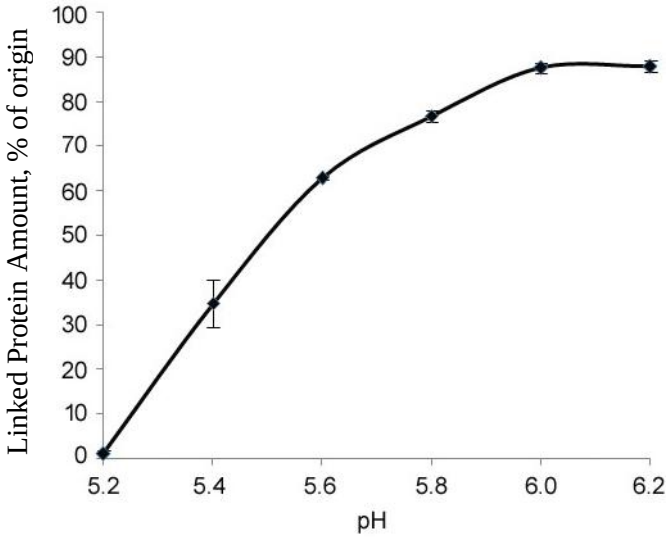


Fig. 3. pH Effect on the interaction effectiveness of β-Lg with chitosan.

At pH 5.2, there is no formation of a precipitate, indicating the formation of a complex between the protein and chitosan. The most effective complex formation occurs at pH 6.2. The latter value is close to the pH of the cheese whey, obtained by enzymatic treatment of cow milk. It should be noted that within the studied pH range, the chitosan is in the protonated form. At pH 5.2, β-Lg does not react with the chitosan as the pH value is close to the isoelectric point of 4.9–5.4, and its net charge is zero. With the increase of the pH value above the isoelectric point, the protein acquires a negative charge, which facilitates efficient ion interaction with the chitosan. Our findings suggest an important role of ionic groups in the interaction of the β-Lg with chitosan. Furthermore, these results indicate the impossibility of isolating the protein from "acid whey" obtained by isoelectric casein coagulation. Despite the important role of electrostatic interactions, one can not exclude the existence of other types of links arising from the formation of the protein- polysaccharide complex.

Our hypothesis of the ionic groups participation in the insoluble β-Lg and chitosan complex formation was confirmed by the change of the solution ionic strength. For this, the reaction is carried out in MES NaOH buffer at pH 6.2 containing sodium chloride at a concentration of from 0.01 M to 0.2 M. It was found that best the chitosan binds to the protein when the solution ionic strength is minimal, whereas at the NaCl concentrations of 0.2 M, the interaction does not occur (Fig. 4). These data support the role of electrostatic interactions in the formation of an insoluble complex between the chitosan and β-Lg.

The whey contains ions of various metals in its structure, including calcium, which is one of the major mineral components of the whey. We studied the effect of calcium ion concentration in the demineralized whey for formation of the insoluble chitosan-protein complex (Fig. 5).

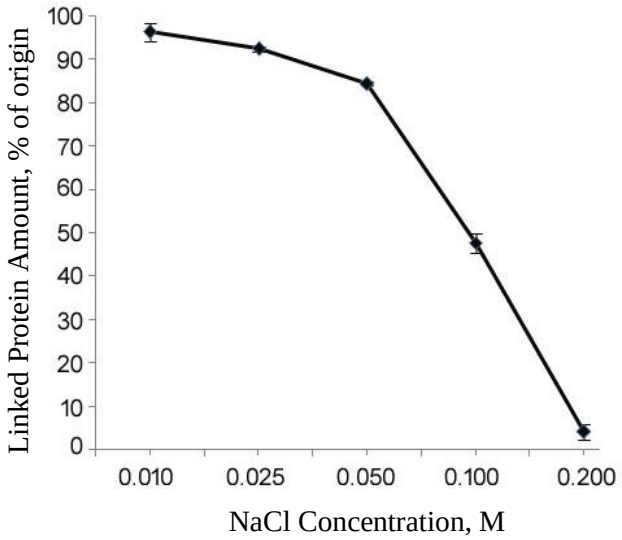


Fig. 4. Ionic strength effect on insoluble chitosan-protein complex formation.

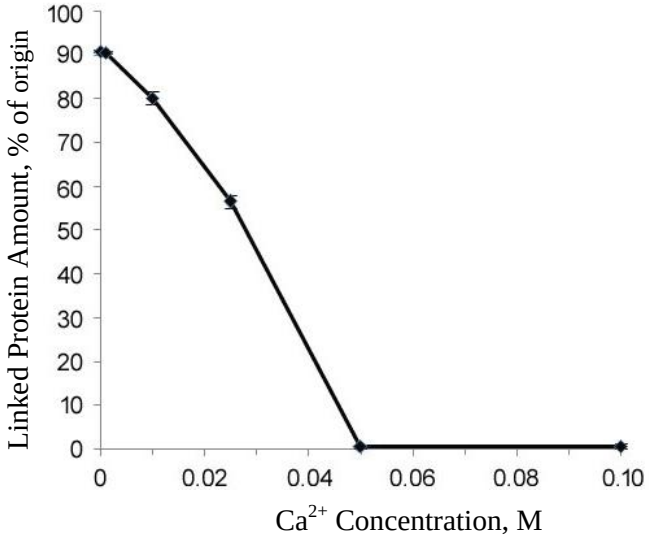


Fig. 5. Calcium ion concentration effect on insoluble chitosan-protein complex formation.

Upon reaching the calcium ion concentration of 0.05 M, the insoluble complex formation practically ceases.

An important factor in the formation of the chitosan-protein complex is the chitosan molecular mass, depending on which the effectiveness of its formation may change. Our study found that, regardless of the used chitosan molecular mass, the most complete extraction of the β-Lg occurs when the contents of polysaccharide in the reaction mixture was 0.5 mg/ml. The efficiency of the chitosan and β-Lg insoluble complex increases with increasing the chitosan molecular mass from 15 to 200 kDa (Fig. 6) [14]. Probably, this effect is due to the fact that in the use of chitosan with a higher molecular mass, a sedimentation capability of the complex formed becomes higher than when using a low molecular mass of the polysaccharide.

The size and morphology of the particles formed from the chitosan and β-Lg complex were determined by atomic force microscopy. It was shown that at the reaction with β-Lg of the chitosan with a molecular mass of 200 kDa, insoluble particles of (170 ± 50) nm are formed, followed by formation of larger aggregates (Fig. 7b), while the size of particles produced by using the chitosan with a molecular mass of 21 kDa does not exceed 100 nm (Fig. 7a) [14, 20].

This effect is due to the fact that in the use of chitosan with a higher molecular mass, a sedimentation capability of the complex formed becomes higher due to the formation of the larger aggregates than when using a low molecular mass polysaccharide. Thus, the high molecular mass chitosan binds most efficiently β-Lg and other milk serum proteins.

As a result of the complex formation between the chitosan and β-Lg, the solution became opalescent that is connected with the complex formation. It was shown that the formation of insoluble chitosan-protein complex occurred only on addition of 0.5 mg/ml of polysaccharide (Fig. 8).

It was found that the maximum protein fluorescence was observed in Zona II at 336 nm at 24°C, pH 6.2 in 0.05 M MES-NaOH buffer. By increasing the chitosan content in the protein solution (Zona III, Fig. 8), the protein fluorescence was reducing (Fig. 9). This phenomenon can be explained by intermolecular interaction of the chitosan and β-Lg, which leads to a change in the protein spatial conformation [14, 20, 21].

Using the method of isothermal calorimetrical titration, we observed that in Zona I (Fig. 8), there is a change in enthalpy of $\Delta H = -2.1$ kcal/mol per the chitosan unit link, whereas the subsequent addition of the chitosan in the protein solution (Zona II, Fig. 8) did not lead to a change in the energy component of the process. This may indicate that the complex dissociation and formation of new interaction types do not occur [22, 23].

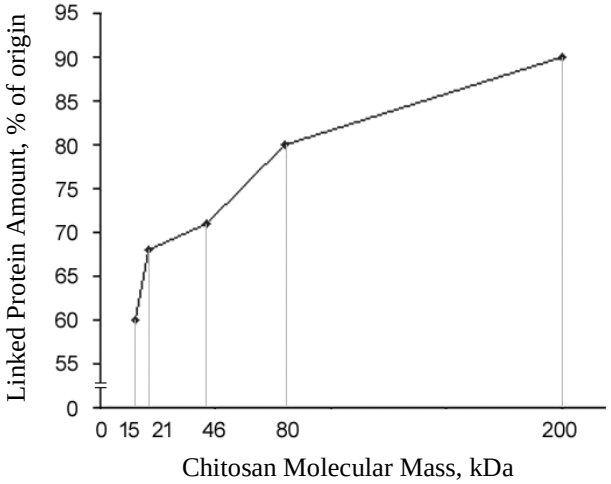
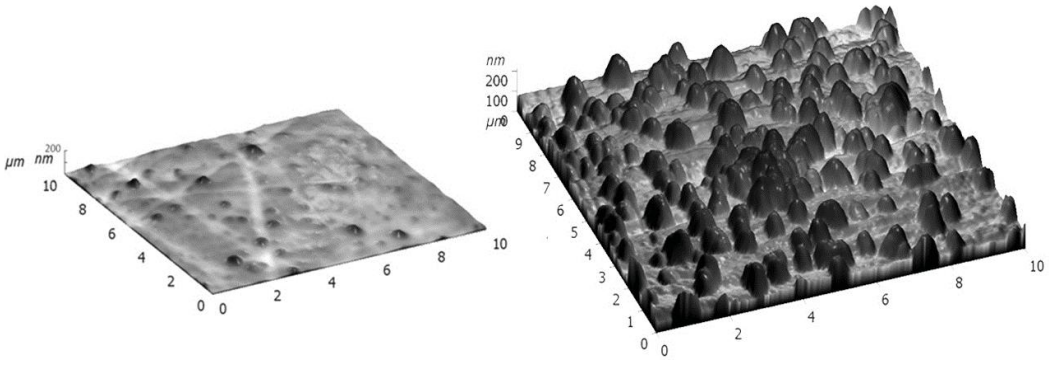


Fig. 6. Eefficiency of binding β-Lg depending on the chitosan molecular mass.



(a) (b)

Fig. 7. Image of insoluble complex of β-Lg with 21 kDa chitosan (a), and 200 kDa chitosan (b) obtained by atomic force microscopy.

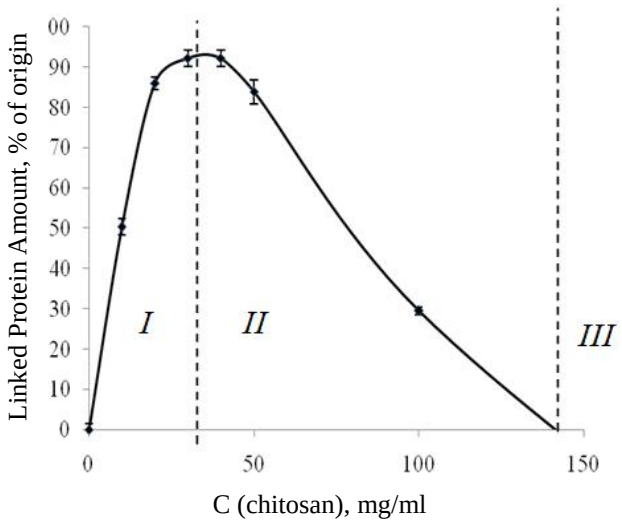


Fig. 8. Dependence of bound β-Lg amount on 200 kDa chitosan content.

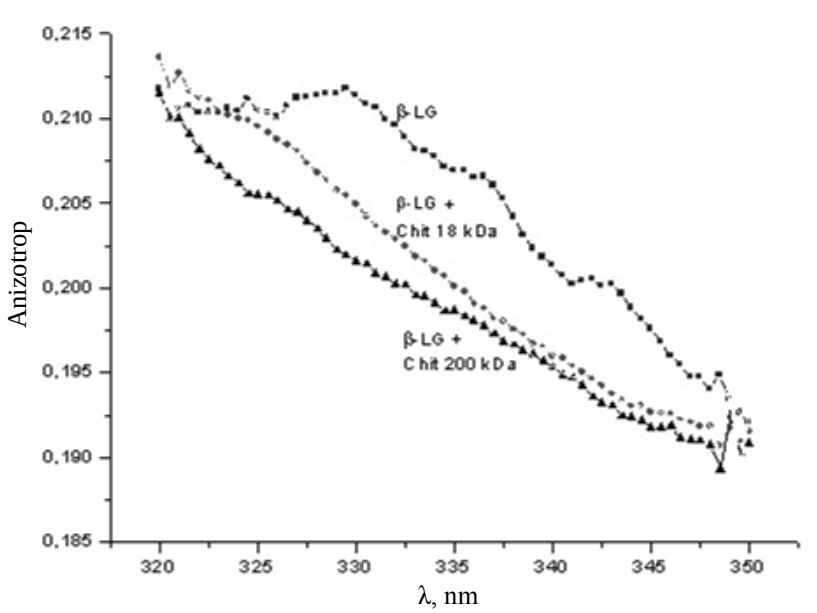


Fig. 9. β-Lg и β-Lg with chitosan fluorescence anisotropy.

Based on these data, we suggest that in the chitosan excess addition to the β-Lg solution, not all the polysaccharide functional groups are involved in the protein complex formation, which is why the ability to the complex sedimentation decreases, and the chitosan-protein complex remains in solution. The water-soluble complexes of globular proteins with chitosan are far from protein saturation and presented by an equilibrium colloid. The whey protein complexes formation with the chitosan can be accompanied by a change of their tertiary structure.

According to the hypothesis proposed, at the molecular level, the electrostatic complexes formation can be considered as the sequential addition of ligands, i.e. whey proteins macroions to the complex nucleus, i.e. the chitosan macroion. The protein can be considered as a ligand on the ground that a large number of smaller negatively charged protein macroion can bind with one chitosan macroion. The polyion complex charge decreases with each subsequent joining of the ligand. The result is the formation of electrically neutral chitosan whey protein complex. The electrically neutral complexes aggregation leads to their isolation in a complex coacervate. The complex coacervate phase composition is defined by stoichiometry of the electroneutral insoluble complex, and depends on the charge ratio of whey proteins. The aggregation of the complex particles is due to their hydrophobic interactions and hydrogen bonding. The insoluble complexes of globular whey proteins with the chitosan contain a relative surplus of proteins.

The findings suggest to consider the chitosan usage be promising for the milk processing enterprises in the treatment of liquid industrial waste containing serum proteins, and the use of these systems for the isolation of proteins [20].

CHITOSAN USAGE FOR MILK PROTEINS EXTRACTION

These patterns of interaction of the milk serum proteins with the chitosan solution have formed the basis for the development of a sorbent based on this polysaccharide. This sorbent is to have a high whey proteins sorption capacity, and differs for use in a column chromatography. To do this, we developed a technology for producing a chitosan hydrogel, modified with cross-linking agent, which is glutardialdehyde [17].

A reactive amino group in the chitosan anhydropyronific monomer unit allows to apply this biopolymer in order to obtain a covalently crosslinked hydrogels [2, 17]. The process of the system gelation, "solvent (water) – polymer (chitosan) – crosslinker (glutardialdehyde)", is possible not only for positive temperature values, but also in the frozen conditions, up to several tens of degrees below the crystallization point of the pure solvent. In such conditions, a frozen preparation, although it looks macroscopically solid, is microscopically heterogeneous, because it consists of polycrystalline of a chilled solvent, i.e. water, and so-called unfrozen liquid microphase, i.e. chitosan and glutardialdehyde. In this microphase, components of these substances are concentrated, and the reaction products, i.e. chemical processes occurring in it, are inherently liquid phase. Due to the effects of such cryotic concentration, we observe an apparent reduction of the critical gelling concentration, when the polymer gel, so-called cryogel may be formed at substantially lower concentrations of the starting precursors, at a temperature higher than the freezing point of the system [2].

Cryogel based on the chitosan was obtained by the ratio, which is 5/1 for the ammin groups of chitosan/ aldehyde groups of glutardialdehyde. The aqueous solution of the polymer and the crosslinking agent was placed in a chromatography column and frozen at 18°C. The gelation reaction was carried out by treating thefrozen solution in a microwave oven for 1 min. The water crystals did not pass into the liquid phase state, whereby the polymer chitosan gel was synthesized around them. We obtained the chitosan macroporous form using cryotechnology [16, 17, 19]; this form had a degree of the gel swelling of 250.5% and held up water of up to 3000 %. Its use as a sorbent for separation of proteins by column chromatography, allows us to perform the process at a high speed of up to 200 ml/ hour. To analyze the efficiency of the purified proteins adsorption, such as the BSA, β-Lg, α-La, α-casein (1S), β-casein, κ-casein, we passed individual proteins through a column of cryogel, after which the sorbent was washed with 0.01 M acetate buffer with pH 6.18, and eluted the protein bound to the chitosan using the linear gradient of 0–0.5 M NaCl in 0.01 M acetate buffer, pH 6.18. The protein content in the eluate was analyzed using denaturing electrophoresis and reverse phase HPLC. The results are given in Table 1.

The participation of the milk proteins ionic groups by reacting with the chitosan macroporous cryogel is confirmed by chromatographic separation of the whey proteins when changing the ionic strength of the eluting solution. Eluting the proteins using the NaCl linear gradient at a salt concentration of 0.01 M to 0.5 M, there are significant differences in their binding with the chitosan cryogel. The analysis of the results presented in Table 1 indicates that the β-Lg and α-La most effectively bind with the sorbent. They are eluted out of the chitosan cryogel with a lower ionic strength.

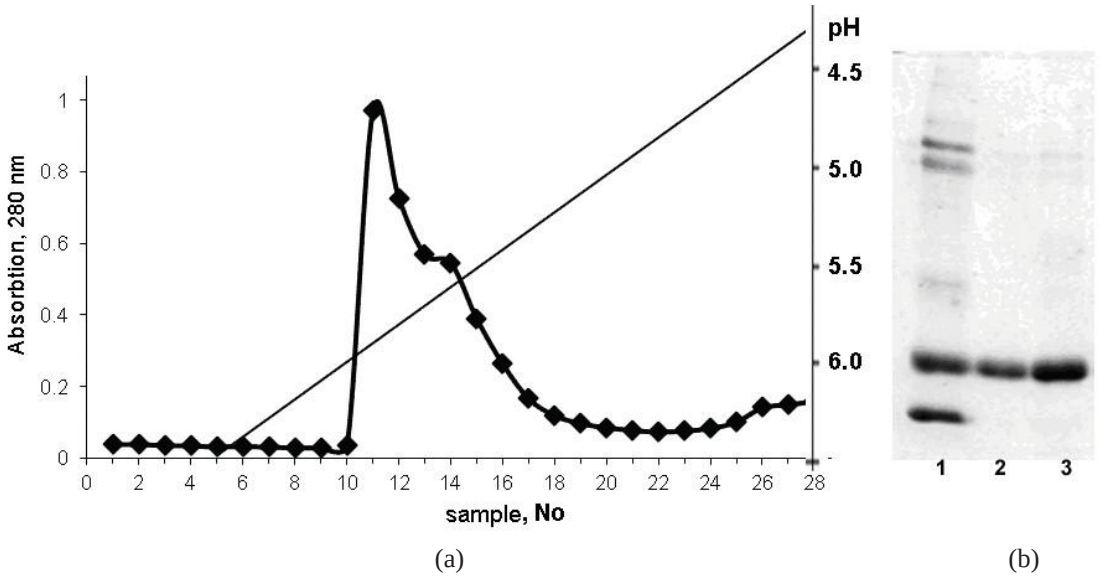
An important result, confirming the ionic character of the interaction of the whey proteins with the chitosan cryogels is represented by the results of chromatography using the pH linear gradient of proteins elution (Fig. 10).

The resulting patterns of the whey proteins interaction with the chitosan macroporous cryogel depending on the ionic strength of the solution, the calcium ions and pH gradient allowed to use the crosslinked chitosan hydrogel based on this polysaccharide as a sorbent suitable for the α-La, β-Lg and BSA isolation and prepare up to 90% of protein from milk serum. The production technology development for the sorbent granular form from the chitosan became the consequence of the research, which showed a high separation efficiency of proteins from the whey. This sorbent is easy to use and possible to regenerate of the chitosan granular form without the use of expensive equipment [19].

Thus, using the chitosan sorbents makes it possible to remove the proteins from the whey. The resulting protein can be used in individual form, as well as an additive that improves the bioavailability of foodstuffs.

Table 1. Efficiency of binding of purified milk proteins with chitosan cryogels

Protein / Feature	BSA	β-Lg	α-La	α-casein (1S)	β-casein	κ-casein
Molecular Mass, kDa	66.0	18.3	14.2	23.0	24.0	19.0
Isoelectrical Point, pI	4.8	5.3	5.1 (4.8)	5.1 (4.7)	5.3 (4.9)	4.1
Protein Sorption mg/mg of Cryogel	0.6	20.0	11.5	2.0	1.5	5.5
NaCl Elution Concentration, M	0.1	0.16	0.2	> 0.5	0.26	0.36



(a) (b)

Fig. 10. β-lactoglobulin elution chromatographic profile with chitosan cryogel of pH gradient (a) and isolated protein electrophoretogram (b). B: 1 – initial whey; 2 – sample 11; 3 – sample 14.

USAGE OF CHITOSAN IN DAIRY PRODUCTS PRODUCTION

The functional products become especially popular in the area of supply that have high organoleptic properties, and also have a preventive effect. A promising direction in this area is the creation of functional foods based on milk and whey, which are sources of complete protein, vitamins, and minerals.

Since the chitosan is a structurant, emulsifier, thickener and clarifier, it has antibacterial, antifungal and antiviral properties, and is able to heal gastric mucosa; it can be assumed that its use in acidified milk beverages and dairy desserts will create a functional product, in which the polysaccharide will function as a technological, functional, bactericidal, or bacteriostatic agent.

Due to the chitosan high activity in the process of complexation with milk proteins, its using in the technology of soft drinks allows to obtain the clarified whey with low proteins and organoleptic neutral features (no serum undesirable sharp taste and smell). The technology is simple in hardware design and does not require significant capital expenditures [24].

A carbonated beverage production process using the chitosan is exercised as follows. Remnants of fat and casein are removed from fresh whey. The prepared serum is added a colloidal chitosan solution. After thorough mixing, the mixture is incubated, centrifuged. The clarified serum is combined with prepared blend that is then fed by the glass in the carbonated form. The best organoleptic characteristics have such drinks as "Apple", "Peach", "Orange" [25, 26].

The use of the colloidal chitosan solution as a composite structurant provides a protein complex to produce next-generation products that have curative properties. It can be used to produce enriched curd as the source of the native protein having high biological value in the production of cream cheese as the raw material, as well as the formulation of sour and milk beverages. In addition, the protein complex obtained is advisable to apply as a protein fortifier in the production of milky beverage from serum [25, 26].

To confirm the possible role of the chitosan as the structurant, the lactic system (with 0.5% fat) properties were studied after introduction of the polysaccharide in concentration of 1–4%.

The analysis of the results shows that increasing the amount of contributed chitosan solution in the lactic system results in a slight increase of the effective viscosity of all samples. When the content of the chitosan is higher than 3%, there is a significant deterioration of the organoleptic characteristics of the product, expressed in acute unpleasant astringent aftertaste, bitter aftertaste. After 30 minutes, there is a precipitation. Thus, in future studies, we used the chitosan solution with a concentration of 3%, which provides relatively acceptable organoleptic characteristics, and does not lead to a process of complexation of the chitosan with milk proteins [26].

Given that the chitosan has sufficiently strong antibacterial and bacteriostatic properties, we studied the relationship between a chemical structure and its biological effects on microbial cells in the lactic model system. Table 2 presents the results of high molecular chitosan effect experiments on the growth of test cultures *E.coli* DSM 396 in the lactic model system.

The results showed that for three days the number of *E.coli* cells in the samples with the addition of the chitosan was reduced from 10^6 to 10^4 CFU/g, while in the control sample prepared with addition of E.coli, but without using the chitosan, the cell number in contrast, increased to 10^7 CFU/g. Thus, the chitosan concentration of 3% added to the lactic model system exhibits bactericidal activity against Gram-negative *E.Coli* DSM 396 microorganisms.

The research also was investigated for fungi; these data demonstrate a pronounced fungistatic action of the chitosan and allow its use as a natural preservative in production technology of unfermented dairy desserts.

Table 2. *E.coli* DSM 396 dynamics in lactic model systems

Sample	Sowing time from the samples preparation, days			
	After preparation	1st day	3rd day	8th day
	The number of detected cells (CFU/g)			
Sterilized Milk (0.5% fat) + 1% Broth with *E.coli*	$(3.8 \pm 0.2) \times 10^6$	$(6 \pm 0.2) \times 10^6$	$(1.2 \pm 0.2) \times 10^7$	$(1 \pm 0.2) \times 10^7$
Sterilized Milk (0.5% fat) + 3%, 3% chitosan solution + 1% Broth with *E.coli*	$(6.5 \pm 0.2) \times 10^6$	$(9 \pm 0.2) \times 10^5$	$(9 \pm 0.2) \times 10^4$	1×10^4
Sterilized Milk (0.5% fat)	Not detected			$(4 \pm 0.2) \times 10^1$
Sterilized Milk (0.5% fat) + 3%, 3% chitosan solution	Not detected			1×10^1

The dairy desserts were being prepared with the use of the chitosan, sodium alginate and a sweetener (fructose). It was found that the developed dairy desserts had a protective effect against erosive and ulcerative lesions caused by using aspirin gastric damage model that allows us to assign a product to a group of functional foods [25, 26].

Thus, the use of the chitosan in the manufacture of dairy products at this stage is very promising because it allows the development of new functional products possessing curative properties.

RESULTS

The modern milk processing technology is directed towards the development of functional foods that have not only a high nutritional value, but also are useful for human health. For their creation, a method of enrichment is widely used regarding the traditional foods using dietary polysaccharide fiber. Neutral, which are cellulose derivatives, amylopectin, galactomannans; anionic (acidic), which are alginates, carrageenans, pectins, xanthan, gum karaya, ghatti, and tragacanth, acacia, furcelleran, gellan gum; cationic, (basic), which are chitosan, have received widespread usage. The chitosan is the only natural cationic polysaccharide, which is used primarily because of its hypocholerestic action. It can be used in recycling of dairy industry wastes for the removal of proteins from serum. Such use of the chitosan is based on the fact that the whey proteins are characterized by low values of the isoelectric points, and at pH 4.8–6.2 have a negative charge, thereby are capable for ionic interaction with the positively charged chitosan molecules. The proteins complexation efficacy with the chitosan depends on the pH, ionic strength, chitosan concentration, and its molecular mass. It should be noted that using one chitosan macroion you can bind a large number of smaller negatively charged protein macroions, whereby a the polyion complex charge decreases at each subsequent attachment of the ligand. This leads to the formation of electrically neutral chitosan complexes with serum proteins and their aggregation. The phenomenon of the insoluble complex formation of the globular whey proteins with the chitosan can be used in the removal of cheese production waste and recovering the valuable protein products.

The chitosan-based adsorbents are developed that permit their use for the chromatographic separation of proteins from the whey, which can be used in individual form, as well as an additive that improves the bioavailability of foodstuffs.

As dietary fiber, the chitosan is included to the biscuits, crisps, pasta, and other foods. With the use of the chitosan, dairy desserts, a variety of beverages are prepared, as well as milk-based functional products.

CONCLUSION

Nowadays, the chitosan use in the dairy industry is very promising because it allows to profitably process milk protein and carbohydrate raw materials, excluding significant energy costs. The processed products have curative properties, which make them attractive for a consumer and, as a consequence, competitive at food market.

REFERENCES

1. Skryabin K.G., Mikhaylova S.N. and Varlamova V.P. (Eds.). *Chitosan*. Moscow: Bioengineering Center of RAS Publ., 2013. 593 p. (In Russian).
2. Varlamov V.P., Nemtsev S.V. and TikhonovV. E. *Chitin and chitosan: nature, production and usage*. Moscow: The Russian Chitin Society Publ., 2010. 292 p. (In Russian).
3. Aranaz I., Mengíbar M., Harris R., Paños I., Miralles B., Acosta N., Galed G. and Heras Á. Functional characterization of chitin and chitosan. *Current Chemical Biology*, 2009, vol. 3, no. 2, pp. 203–230.
4. Kean T. and Thanou M. Biodegradation, biodistribution and toxicity of chitosan. *Advanced Drug Delivery Reviews*, 2010, vol. 62, no. 1, pp. 3–11.

5. Tolstoguzov V.B. *Protein food new forms (technological issues and prospective)*. Moscow: AgroPromIzdat Publ., 1987. 303 p. (In Russian).
6. Zhao Y., Park R.D. and Muzzarelli R.A.A. Chitin deacetylases: properties and applications. *Marine Drugs*, 2010, vol. 8, no. 1, pp. 24–46.
7. Dutta P.K., Tripathi S., Mehrotra G.K. and Dutta J. Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry*, 2009, vol. 114, no. 4, pp. 1173–1182.
8. Jayakumar R., Prabaharan M., Nair S.V. and Tamura H. Novel chitin and chitosan nanofibers in biomedical applications. *Biotechnology Advances*, 2010, vol. 28, no. 1, pp. 142–150.
9. Bhatnagar A. and Sillanpää M. Applications of chitin- and chitosan-derivatives for the detoxification of water and wastewater – a short review. *Adv. Colloid Interface Sci*., 2009, vol. 152, no. 1–2, pp. 26–38.
10. Gets A.A. Production process modification trends at milk processing enterprises. *Sustainable economic development: state, problems, prospects: Proceeding of the 3rd international research and practice conference*. Pinsk: PolesSU Publ., 2009, pp.14–15. (In Russian).
11. Kurchenko V.P., Golovach T.N., Kruglik V.I., Kharitonov V.D. and Agarkova E.Yu. Milk proteins allergic properties reduction. Technological approaches. *Dairy Industry*, 2012, no. 4, pp. 40–42. (In Russian).
12. Khramtsov A.G., Polyanskiy K.K., Vasilisin S.V. and Nesterenko P.G. *Secondary dairy raw materials processing*. Voronezh: VSU Press Publ., 1986. 159 p. (In Russian).
13. Halavach T.N. and Kurchenko V.P. Allergenic potency of milk proteins and its reduction. *Proceedings of the Belarusian State University*, 2010, vol. 5, part 1, pp. 9–55. (In Russian).
14. Kurchenko V.P., Alieva L.R., Butkevich T.V. and Gavrilenko N.V. Chitosan interaction mechanism with whey proteins. *Proceedings of the Belarusian State University*, 2013, vol. 8, part 1, pp. 45–51. (In Russian).
15. Kurchenko V.P., Golovach T.N., Chervyakovskiy E.M., Simonenko S.V. and Kharitonov V.D. Whey protein partial hydrolysates for specialized and infant nutrition. *Russian Agricultural Sciences*, 2011, vol. 37, no. 1, pp. 90–93. (In Russian).
16. Butkevich T.V., Varlamov V.P., Evdokimov I.A., Alieva L.R. and Kurchenko V.P. Usage of chitosan in dairy products production. *Proceedings of the Belarusian State University*, 2014, vol. 9, part 2. (In Russian).
17. Butkevich T.V., Ivanov A.A. and Kurchenko V.P. Use of chitosan various forms for environmental complex whey utilization from milk processing wastes. *Proceedings of the Samara Scientific Center of the Russian Academy of Sciences*, 2013, vol. 15, no. 3(5), pp. 1575–1578. (In Russian).
18. Nechaev A.P., Kochetkova A.A. and Zaytsev A.N. *Food Additives*. Moscow: Kolos Publ., Kolos-Press Publ., 2002. 256 p. (In Russian).
19. Varlamov V.P., Shcherbinina T.S., Bakulin A.V., Butkevich T.V., Kurchenko V.P., Kharitonov V.D., Agarkova E.Yu. and Botina S.G. β-lactoglobulin extraction from whey: use of various chitosan forms. *Dairy Industry*, 2013, no. 10, pp. 11–12. (In Russian).
20. Bakulin A.V., Gavrilenko N.V., Chervyakovskiy E.M., Kurchenko V.P. and Varlamov V.P. Use of chitosan for β-lactoglobulin extraction from whey protein mixture. *Biotechnology*, 2011, no. 1, pp. 34–41. (In Russian).
21. Bakulin A.V., Gavrilenko N.V., Chervyakovskiy E.M., Kurchenko V.P. and Varlamov V.P. Use of shellfish chitosan in whey processing technology. *RybProm*, 2010, no. 4, pp. 64–67. (In Russian).
22. Bansal V., Sharma P.K., Sharma N., Pal O.P. and Malviya R. Applications of chitosan and chitosan derivatives in drug delivery. *Advances in Biological Research*, 2011, vol. 5, no. 1, pp. 28–37.
23. Ur'yash V.F., Larina V.N., Kokurina N.Y., Bakulin A.V., Kashtanov E.A. and Varlamov V.P. Chitin and chitosan orderliness degree and thermochemical characteristics dependence form biological origin. *Russian Journal of Physical Chemistry* A, 2012, vol. 86, no. 1, pp. 5–12. (In Russian).
24. Alieva L.R., Butkevich T.V. and Kurchenko V.P. Whey protein interaction with chitosan macroporous cryogel. *Modern achievements of biotechnology: Materials of the 4th International Scientific and Practical Conference, Minsk – Stavropol*. Stavropol, 2014, pp. 3–8. (In Russian).
25. Vasilisin S.V., Evdokimov I.L., Alieva L.R., Popova M.S. and Volodin D.N. Carbonated drinks with using chitosan. *New developments in the study of chitin and chitosan, Materials of the Sixth Intern. Conf.* Moscow: VNIRO Press, 2001, pp. 149–150. (In Russian).
26. Evdokimov I.A., Budkevich R.O., Buchakhchyan J.V., Alieva L.R. and Budkevich E.V. Functional food with chitosan in erosion damage of the gastric mucosa in rats. *2nd International ISEKI Food Conference*. Milan, 2011, pp. 200.

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FUNCTIONAL FERMENTED MILK DESSERTS BASED ON ACID WHEY

I. A. Evdokimov[a], D. N. Volodin[b], V. A. Misyura[c], M. S. Zolotoreva[c,*], M. I. Shramko[a]

[a] North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355009 Russian Federation

[b] LLC "MEGA ProfiLine",
Dzerzhinskogo Str. 158, Stavropol, 355003 Russian Federation

[c] JSC Dairy plant "Stavropol",
Dovatortsev Str. 36, Stavropol, 355036 Russian Federation

* e-mail: zolotoreva@mpline.ru

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Abstract: Given the shortage of raw milk, the problem of the rational use of whey as a source of biologically valuable milk components for functional products is urgent. The area of research is the use of acid demineralized whey for the production of functional fermented desserts. Acid whey is characterized by a high content of lactic acid and minerals, which makes its industrial processing difficult and limits the directions of use for food purposes. To solve these problems, the membrane methods area used, they allow regulating the composition and properties of raw materials. Demineralization of acid whey allows removing a significant part of minerals (50%) from it and providing the required acidity in the range, corresponding to raw milk. We set the following objectives: to determine the parameters of whey demineralization, the factors of thermostability; increase stability and improve the consistency of the dairy base from demineralized whey; the effect of the probiotic starter microflora on the characteristics of the final product. In the course of studies, we used a modern membrane equipment, standard and generally accepted research methods. We analysed the composition and properties of demineralized acid whey, considered the theoretical prerequisites to an increase in stability and the ways to improve the consistency of the dairy base. We examined the effect of the dose of stabilizers' salts, dose of the consistency stabilizer and the fat content on physicochemical, organoleptic and rheological parameters of the milk base, as well as the effect of the probiotic cultures ratio in the starter, which make the product functional, on the properties and qualities of the final product. We developed the formulas and technologies of functional desserts. We developed and approved technical specifications for the desserts, based on which the experimental-industrial production is conducted. The feasibility of using demineralized acid whey as a raw material for the production of dessert products is confirmed experimentally.

Keywords: Acid whey, demineralised whey, electrodialysis, membrane processes, functional dairy products

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INTRODUCTION

Due to the increase in production volumes of milk protein products in the Russian Federation such as cheeses and acid, the resources of dairy whey increase proportionally. Given the shortage of raw milk, the problem of using secondary raw materials rationally becomes particularly relevant, and the task of experts of the dairy industry is to come up with such solutions so that valuable dairy whey is used to the fullest, which will ensure the environmental safety and the resource conservation of milk production.

Recently, the sector of drinks and desserts based on dairy whey (around 50 thousands tonne) has become more popular, however, the share of whey, used for these purposes is small [1]. It is due to the features of its composition and properties. Dairy whey is characterized by high content of minerals and increased acidity (especially acid and casein whey), which limits its use for food purposes. However, its valuable composition, significant volume and accessibility predetermine the need for its industrial processing.

Whey includes around 50% of milk solids, 70% of which is accounted for lactose, around 13% is protein components, less than 5% – milk fat and about 11% is minerals. Whey protein is represented by β lactalbumin, β-lactoglobulin, whey albumin, immunoglobulins, lactoferrin, osteopontin, lactoperoxidase and the protease-peptone fraction. These proteins have the highest biological value by the content of essential amino acids among other food proteins. In this regard,

whey protein fractions can fully meet the need in protein in the diet of infants, the elderly, athletes, as well as people exposed to stress and engaged in heavy physical labour [2]. Nowadays, the intensive research and scientific works are conducted to create new kinds of dairy products, the raw material for which can be dairy whey as a source of nutrients with the important physiological value, in order to solve the problem of providing the population with biologically full, affordable and safe food products.

The nutrition structure of the Russian population is characterized by low consumption of biologically valuable products. In these circumstances, it is feasible to pay special attention to the production of products that enrich the diet with biologically active substances, bifidogenic additives, functional ingredients that have a beneficial effect on the general state and the metabolism of the body. High nutritional and biological value of dairy whey is well-known and due to the presence of valuable carbohydrates, minerals, enzymes, vitamins, organic acids, easily digestible whey proteins, which are an additional source of essential amino acids, predetermines the need to find the ways how to process it.

Milk processing enterprises are especially interested in acid whey. According to statistics, the share of acid whey in Russia accounts for more than 45% of the total volume of whey [3], due to the fact that acid is a traditional national product in Russia. However, the heat treatment processes, condensation, crystallization, drying of acid whey are complicated by its physical and chemical properties. Acid whey is characterized by an increased content of lactic acid and a significant mineral content, which is due to the characteristics of the acid technological production process. For the same reason, its use is limited in the production of dairy products. The use of new and innovative technological methods can improve the quality characteristics of the products based on acid whey.

One of the main development directions of the domestic branch of science is the development of fundamentally new, innovative processes and rational processing technologies of raw milk based on deep fractionation of its components. A priority direction for solving the above problems is the introduction of the membrane technologies, providing for efficient processing of acid (acid) whey, for the subsequent use of the obtained semi-finished products in the food technology. It can be both traditional dairy products, enriched with whey proteins, and new functional products with useful properties. It becomes possible to vary the ratio of milk components and composition, giving new physicochemical, organoleptic and structural and mechanical properties to dairy products.

Electrodialysis is already successfully used to process whey. It is one of the most effective processes for regulating the composition and properties of whey, which opens up possibilities to produce new kinds of functional dairy products. In electromembrane processes, filtration is carried out via the movement of dissociated ions through ion-selective membranes under the influence of direct current. As a result, the acidity and the mineral composition of raw materials are adjusted. Electrodialysis almost completely removes monovalent ions and a significant part of the divalent ions such as calcium, magnesium, as well as hydrophosphates, organic and inorganic acids.

Demineralization of acid whey allows obtaining the base with the necessary titratable (15–25°T) and active acidity (pH 6.0–6.7). As a result of the electrodialysis treatment, organoleptic characteristics significantly improve: the acid whey acquires a sweetish taste already at the desalting level of 50%, and a sweet taste – during further demineralization. This allows expanding the application range of demineralized whey as part of many food products significantly.

Improvement of technological processes, as well as wide processing of secondary dairy raw materials improves the nutritional value of food products, while addressing a number of the issues of rational raw materials use and environmental safety. In this regard, the studies, aimed at the development and introduction of food products based on the principles of comprehensive non-waste milk processing, are relevant.

Let us consider the promising direction of producing dessert products, based on the demineralized acid whey, for the functional use.

To achieve the research goals, it is necessary to solve the following problems:

– to examine the factors, influencing on thermostability of a dairy base;

– to study the possibility of increasing the stability of the dairy base from demineralized acid whey and improving the consistency of dairy products;

– to study the influence of the starter microflora on physicochemical and organoleptic characteristics of the dairy base;

– to develop technological modes for producing dairy desserts.

OBJECTS AND METHODS OF STUDY

The objects of research are the following:

– acid whey, obtained after the production of fat-free acid with the traditional technology, concentrated on the Nano filtration unit and demineralized with the electrodialysis method;

– cream, made from cow's milk, which complies with the requirements of GOST (State Standard) R 53435- 2009;

– stabilizers of consistency, designed for the production of fermented products and dairy desserts in compliance with the corresponding regulatory documentation;

– bacterial concentrates of lactic acid microorganisms: *Lactobacillus acidophilus* (BK-Uglich-AB), *Lactobacillus bulgaricus* (BK-Uglich-B), *Lactococcus lactis subsp. diacetilactis* (BK-Uglich-LD), *Lactobacillus casei* (BK-Uglich-K) TU 9229-369-00419785-04.

The research was carried out in the laboratories of the Department of Applied Biotechnology of

the North Caucasus Federal University (SKFU), as well as jointly with NCFU and "MEGA ProfiLine", the international research laboratory "Electro- and baromembrane technologies" of the Institute of living systems. Pilot testing and development of the technological parameters of desserts production were carried out on the basis of the Dairy plant "Stavropol" in the acid products department. In the experimental studies, we used the materials provided by the companies FSUE "Experimental biofactory" (Uglich), LLC "Wimm-Bill-Dann. Food products" (Moscow) and JSC Dairy plant "Stavropol" (Stavropol).

Laboratory and pilot units were used as the membrane equipment in the experimental studies. Approbation was carried out on the pilot and industrial membrane equipment. To concentrate acid whey, we used the Test Unit M20, universal baromembrane unit, equipped with Nanofiltration membranes. The baromembrane unit is designed for research. It can be used with a range of membranes to carry out the processes of reverse osmosis, nanofiltration, ultrafiltration and microfiltration. Nanofiltration membranes have a unique design and are made from a special material, approved for the use with food products and pharmaceuticals.

The main conditions of the Nanofiltration process (corresponding to the industrial operating conditions of the unit):
– temperature of the process – 10°C;
– pressure – 20 bar;
– duration of the process – not more than 20 hours;
– membrane module NF-2517/48.

The process of demineralization was carried out on the laboratory electrodialysis unit ED-mini (JSC "MEGA", the Check Republic) and the pilot electrodialysis unit ED-Epsilon (JSC "MEGA", the Check Republic), equipped with the heterogeneous ion-exchange membranes Ralex®, permitted for the food industry.

During the process, acid whey was subjected to demineralization up to the level of 50%. The process was controlled by the specific conductivity value and then by the value of ash in the final product.

The following parameters of the demineralization process are adopted:
– liquid temperature in all circuits – 10–15°C;
– maximum voltage applied to the module – 20 V;
– maximum current – 1.5 A;
– pumping speed in all the chambers – 60 l/h;
– Electrode solution – salt solution with the specific conductivity greater than the specific conductivity of milk whey (16–22 mS/cm).

In the process of electrodialysis, the following parameters were monitored: specific conductivity and active acidity in each circulation circuit. These two parameters were determined by pH/Cond 340i (manufactured by "WTW", Germany). The concentration of ash residue was determined by phase-by-phase combustion of the accurately weighed milk whey samples at a temperature of 550°C for at least 5 hours in a muffle furnace.

To determine the composition and properties of dairy raw materials and studies samples, as well as during experimental tests we used standard and accepted methods of research.

RESULTS AND DISCUSSION

As known, natural milk whey is characterized by low solids content, but increased salinity and acidity. Therefore, to use whey in the technology of many dairy products it is advisable to concentrate it and then adjust its composition to remove unwanted substances. The most effective way to concentrate whey is to use baromembrane methods such as Nanofiltration and reverse osmosis. These processes can significantly reduce energy consumption for the concentration compared with the most common method of vacuum evaporation. We chose Nanofiltration since during such processing, whey concentration and partial demineralisation of raw materials occur due to the permeability of Nanofiltration elements relative to monovalent ions. During this process, the mineral part of whey is reduced by 20–25%, the total amount of raw materials for further processing reduces at the account of the solids content concentration in the retentate up to 18–20%. Due to partial removal of salts and total increase in the specific conductivity as a result of the concentration solids in whey, the electrodialysis process is intensified.

Technical data and the possibilities of using electrodialysis units allow providing a demineralisation level of raw materials of 50, 70 and even 90% without additional auxiliary processes. Applied heterogeneous ion-exchange membranes Ralex® have high selectivity for removing mono-, divalent ions and lactic acid, which significantly improves the efficiency of electrodialysis. Ability to control the acidity in the process of electrodialysis allows getting the desired product with the required acidity indicators. During preliminary studies it was found that for the use of demineralized whey in the technology, for example, of whole-milk products, a sufficient demineralisation level is 50–70% and the pH range should match the natural pH of raw milk, i.e. 6.5–6.7.

In our experiments, the raw materials were concentrated to the 12–14% content of solids, which is comparable to a content of solids in raw milk, and in the process of electrodialysis treatment of acid whey, the demineralization level was brought to 50% and a pH of 6.7. Thus, when exiting the electrodialysis unit, the acid whey concentrate with the solids content of 11–13% had a titratable acidity not more than 18°T. The resulting semi-finished whey can be successfully used in the production of products such as "drinking" (thin) and "spoon" (thick) yoghurts, acid desserts, dairy drinks at the normalization stage in order to optimize the mixture composition and reduce the cost of raw milk.

The comparative composition and basic physicochemical and organoleptic indicators of non-demineralized and demineralized whey are shown in Table 1.

Table 1. Physicochemical and organoleptic properties of concentrated acid non-demineralized and demineralized whey

Indicator	Value	
	Concentrated acid whey	Concentrated acid whey, 50% wt.
Content of solids, %	12.0–14.0	11.0–13.0
Titratable acidity, °T	125–145	15–18
Active acidity pH	4.2–4.55	6.5–6.7
Density, kg/m^3	1040–1055	1040–1050
Specific conductivity, S/cm	11.0–14.0	3.2–4.0
Lactose, %	9.0–11.0	8.5–10.0
Protein, %	1.2–1.5	1.2–1.4
Fat, %	0.13–0.15	0.11–0.13
Ash, %	1.4–1.5	0.5–0.6
Appearance, consistency	Homogeneous low-viscosity fluid	Homogeneous low-viscosity fluid
Taste and smell	The taste is pronounced wheyish acidic, slightly salty. The smell is wheyish. Without foreign tastes and smells	Pure, sweetish, a bit wheyish taste. Slight wheyish smell. Without foreign tastes and smells
Colour	From light yellow to yellow	From light yellow to yellow

The study on the demineralization of acid whey showed that it is possible to remove up to 50% of minerals with insignificant losses of whey proteins and lactose with titratable acidity being reduced at the same time. As a result of electrodialysis processing, organoleptic indicators of milk whey improve significantly.

As the technologies of all dairy products envisage the process of pasteurization, at the next stage of the study, we examined the stability of demineralized whey in the course of thermal processing.

It is known that in the process of demineralization, monovalent ions (Na^+, K^+, Cl^-) are removed first, and then the anions of phosphoric and citric acids are removed. This leads to the partial dissociation of the complexes that connect Ca^{2+} and Mg^{2+} ions [5, 6], which in turn influence the stability of proteins. As the desalting degree increases, the rate of their removal from whey increases as well, which leads to a shift of the salt equilibrium and, as a consequence, the decrease in thermostability of milk whey at high temperature processing modes.

Typically, the following pasteurization modes are used in the production of dairy products: the product is held for 2–3 seconds at a temperature of 95 ± 2°C or 5–10 minutes at 85 ± 2°C [7, 8]. These modes provide the required microbiological safety and structure of the finished product. Taking into account the thermolability of milk-based whey proteins and the need to ensure the required safety indicators, we will adhere to the second mode of thermal treatment.

Thermal treatment has an important technological value and forms the quality of the finished product. During thermal treatment of raw milk in certain modes, the formation of a complex between casein and whey proteins occurs. The share of whey proteins in raw milk amounts to around 0.65%, the main part of which (0.4%) belongs to β-lactoglobulin. This whey protein has specific functional properties that are of great value in the food industry, as it is able to form complexes with casein micelles, increasing the water holding capacity of clots at higher temperatures. Denaturation of whey proteins under the temperature occurs by specific patterns. Individual fractions of whey proteins have different thermostability and the denaturation rate, depending on the temperature [9]. The increase in the whey protein concentration contributes to higher denaturation rate. The latter is influenced by other factors as well. For example, the increase in lactose concentration slows down denaturation, especially at temperatures lower that 90°C; treatment with ultrahigh pressure, decrease in pH accelerate denaturation. With the increase in pasteurization temperature from 63 to 90°C, the effective viscosity of the intact structure of a fermented milk clot becomes 4 times greater, the relaxation viscosity – more than 2 times, the intensity of whey separation reduces by half. However, in this case, the casein content in the milk base is quite insignificant, that is why mainly whey proteins form the structure [10, 11].

For the milk base with the solids content of around 14%, the denaturation degree of whey proteins, which does not exceed 70–75%, is achieved at 80–85°C with the holding of 5–10 sec [11].

Further studies were aimed at determining the conditions, at which the milk base would be stable with the chosen pasteurization method. To increase thermostability of raw milk, stabilizer salts are used. When choosing the type and doses of stabilizer salts, we applied the recommendations of manufactures and the data in literary sources [12]. However, in regard with the fact that the content of whey proteins in the concentrated demineralized whey is 2 times greater than in milk, we made a decision to increase the concentration of stabilizer salts up to 25%, as well as the dose of salt in the milk base. Then, we studied the effect of the introduced stabilizer salt – potassium citrate –

on the thermo-stability of acid demineralized whey. Potassium citrate was introduced into the samples of demineralized whey as a 25% solution. The resulting mixed were subjected to pasteurization at (85 ± 2)°C, with the holding of 5–10 sec.

After cooling, the amount of residues in the samples was determined by centrifugation. Data on the quantitative content of residues in the samples are presented in Fig. 1.

Based on the conducted study and according to the data in the presented graph, we chose the optimal dose of stabilizer salt. It is 0.8%. We observed the higher stability in the process of heat treatment and the least amount of residues after centrifugation with this dose. Larger amounts of potassium citrate shift the salt equilibrium, protein molecules are destabilized, and after pasteurization the amount of denatured whey proteins increases in comparison with the control sample. Smaller amounts of salt did not give the desired effect.

To obtain the required consistency and structure in the milk base composition, the stabilizer systems are introduced.

Recently, various companies have developed many stabilizers and stabilizer systems, which are specially selected authorized blends of hydrocolloids. Stabilizers are used in the production of dairy drinks, mainly yoghurt, to prevent whey separation, improve the consistency and viscosity of the product.

Based on the preliminary studies with stabilizer systems of various companies, we chose the GRINDSTED® stabilizers ("Danisco", Denmark), which showed the best results.

To carry out the experiments, we took samples of the acid demineralized whey (the solids content of 14 ± 2%, 50% demineralization level). The general characteristic of the used GRINDSTED® stabilizers is given in Table 2.

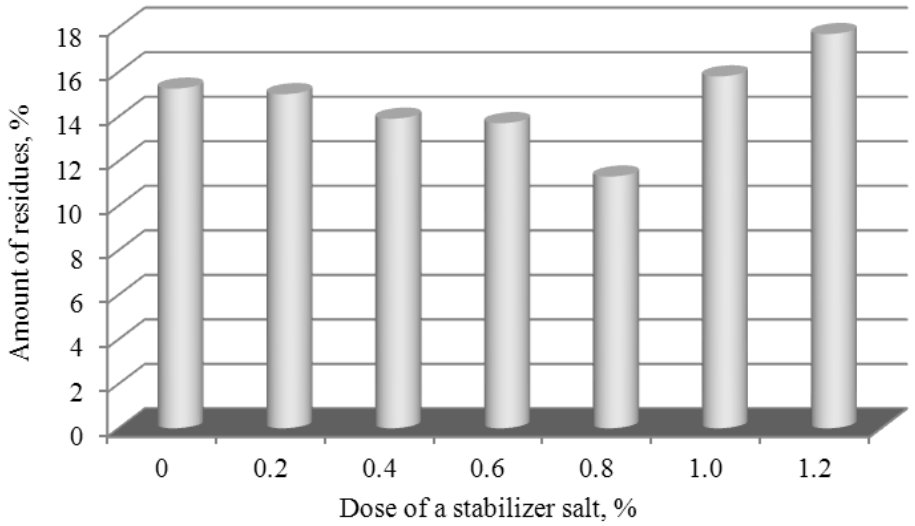


Fig. 1. The effect of dose of stabilizer salt on the thermostability of the milk base.

Table 2. General characteristic of the complex stabilizers ("Danisco")

Name	Application	Composition	Properties	Dosage
GRINDSTED® SB 251	Sour cream, yoghurt, dairy products (kefir, fermented baked milk, varenets (boiled fermented milk), etc.	Gelatine, modified starch (E1422), pectin (E440)	It increases viscosity, glossiness, binds free moisture, prevents whey separation, makes the texture creamier, replaces a part of non-fat milk solids	0.5–0.8%
GRINDSTED® SB 550A	Sour cream, yoghurt, dairy desserts	Modified starch (E1422), pectin (E440)	It binds free moisture, prevents whey separation and increases viscosity during cooling. Does not contain gelatine	0.9–1.3%
GRINDSTED® SB 271	Low-fat/non-fat yoghurt, dairy drinks (kefir)	Milk solids; modified starch (E1422), pectin (E440)	Enhances creamy taste, binds free moisture, prevents whey separation, replaces a part of non-fat milk solids and increases viscosity	0.9–1.3%
GRINDSTED® ES 258	Yoghurt, sour cream, thermostatic dairy products	Modified Starch (E1422), emulsifier (E471), pectin (E440)	A mixture of food emulsifiers and stabilizers. It increases viscosity, improves the consistency, binds free moisture, prevents whey separation, reduces the effect of spraying during packing	0.9–1.3%

We prepared milk-base samples with a different dose of each type of stabilizer. The amount of stabilizer in the samples was determined, based on the manufacturer's recommendations – we introduced a minimum, maximum and an average dose of stabilizer. Then these samples were pasteurized at a chosen mode: (85 ± 2)°C, with the holding of (5–10) s.

After pasteurization and cooling, separation in the milk base and its consistency was evaluated visually, the amount of residues was determined by centrifugation as well. The best result was achieved with the GRINDSTED® ES 258. After thermal treatment, there was no whey separation, the milk base structure was even, homogeneous, of white colour with a yellowish hue, tasted and smelled nice. This is due to the presence of emulsifies and stabilizing additives in the content of this stabilizer system, which allow getting even, smooth, homogeneous consistency, its emulsifying effect is also positive with the higher fat content in the base. The optimal average dose is 1.1%. With this does, the milk base had satisfactory indicator, smaller amount of stabilizer did not give a required pronounced affect, and the maximum dose gave the base an aftertaste of the stabilizer.

We conducted a series of experiments to normalize whey with cream to assess the influence of fat content on the structure and organoleptic indicators of the milk base. Concentrated demineralized whey was normalized with milk cream with 20% fat content and was homogenized on a laboratory dispersant. Then, the resulting mixtures were subjected to pasteurization at (85 ± 2)°C, with the holding of 5–10 s. Organoleptic and physicochemical indicators were determined in cooled samples. The results are given in Table 3 and Fig. 2.

Organoleptic indicators were determined on a scale from 1 to 5, by the total points of the following parameters: consistency, appearance, taste, smell and colour.

Organoleptic indicators of the samples with the increased fat content improve: whey aftertaste becomes less pronounced, pleasant milky smell, the products tastes more like a yoghurt, the consistency becomes more viscous.

The increase in the fat content of the product improves organoleptic indicators of the milk base, but does not give a necessary structure and consistency, similar to the structure of traditional yoghurt, as well as does not have a significant influence on thermostability of the milk base during thermal treatment, i.e. does not exclude the use of stabilizers, selected earlier. The optimal fat content of the mixture – (4 ± 0.2)%, it was determined by the results of the experiment. With this fat content, the milk base had a viscous consistency; sweet, pleasant, creamy taste; clean, creamy smell; creamy colour. Smaller amount of cream in the mixture did not give necessary organoleptic indicator, larger amount definitely improved the flavour profile of the milk base. However, the increased fat content, firstly, significantly increases the energy value of the product, and secondly, it increases its cost.

Table 3. Physicochemical indicators of the samples of demineralized whey, normalized according to fat content

Sample	Fat content, %	Acidity, °T	Solids, %	Viscosity, mPa×s
1	0 (Control)	15.6 ± 1	13.4 ± 0.5	18.3 ± 1.5
2	2.0	16.0 ± 1	15.1 ± 0.5	36.6 ± 2.0
3	4.0	16.0 ± 1	16.4 ± 0.5	108.6 ± 5.5
4	6.0	16.3 ± 1	17.2 ± 0.5	184.6 ± 10.0
5	8.0	15.3 ± 1	18.6 ± 0.5	355.0 ± 20.0

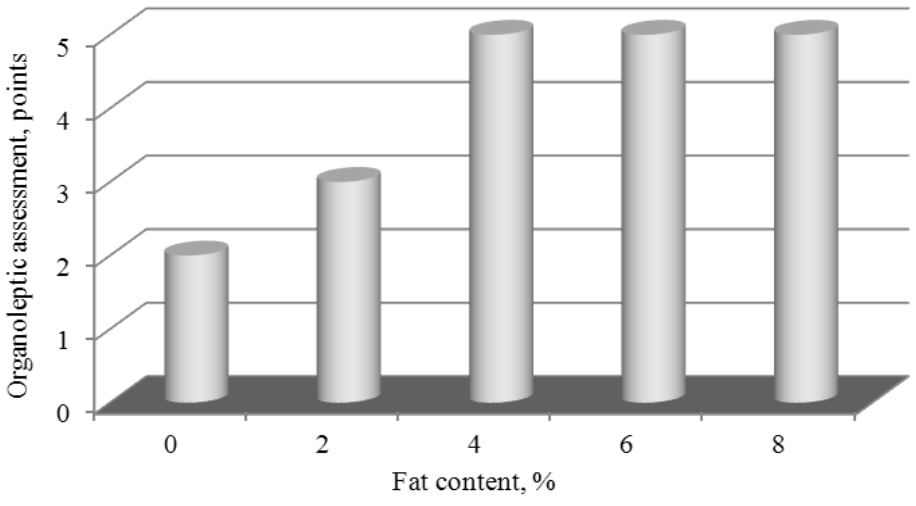


Fig. 2. Organoleptic indicators of demineralized whey, normalized by the fat content (on a scale from 1 to 5).

Thus, the series of conducted experiments led us to the following conclusions:
– demineralized whey can be a milk base for the production of dessert products;
– the optimal mode of heat treatment of the milk base is (85 ± 2)°C, with the holding of 5–10 sec;
– the optimal amount of the GRINDSTED® ES 258 stabilizer, that gives the milk base a homogeneous, viscous consistency, amounts to 1.1%;
– the optimal dosage of the stabilizer salt – (0.8 ± 0.05)%, it provides thermostability of the milk base during pasteurization;
– the optimal fat content of the milk base – (4 ± 0.2)%, it significantly improves its organoleptic indicators.

The goal of the experiments was to make a functional fermented dessert based on demineralized whey. Its functionality is determined not only by the valuable content of the milk base, which contains larger amount of biologically full whey proteins, but also by the composition and properties of the starter's microflora, which provides probiotic properties of the product.

The composition and properties of starters have a great impact on the structure and indicator of fermented products. Depending on the type of a starter, fermented microorganisms form clots with different consistency types during milk ripening: Prickly, more viscous or with a different degree of ductility [10, 13].

The main requirements to the clot imply high viscosity after ripening; moderate degree of destruction during mixing; ability to recover the structure after mixing maximally; ability to retain whey during storage. Structured systems that are formed in raw milk during production of fermented products, contain both strong bonds of the condensation type, that irreversibly break down, and weak thixotropic reversible bonds of the coagulation type that give elasticity and plasticity [9, 14].

Content of bacterial starters is an important factor that determines the density and other structural and mechanical properties of acid protein clots. Many studies showed that there is a close interconnection between the strength of the clot, the degree of its recovery after a break down, water holding capacity and the content of a bacterial starter, development conditions of its microflora, the accumulation rate of milk acid and some other factors. Such cultures as *Lact. lactis (subsp. lactis, biovar diacetilactis, subsp. cremoris), Lb. delbrueckii subsp. bulgaricus, Str. swalivarius subsp. thermophilus* are able to form extracellular polymers that are carbohydrate-protein complexes [10, 15, 16].

Adding high-energy acidifiers to the content of bacterial starters helps making a dense clot with intensive whey separation, adding low-energy acidifiers (aroma-producing streptococci) – a more gentle clot. The use of *Lac. cremoris* and *Lb. acidophilus* in starters gives the clot elastic properties and hinders whey separation. Thus, with a certain combination of fermented bacteria it is possible to obtain the product with a desired consistency.

For further studies, the concentrated demineralized whey was normalized by milk cream to 4% fat content, 1.1% wt. of GRINDSTED® ES 258 was introduced into the mixture, as well as (0.8 ± 0.1)% of the stabilizer salt. Then the mixture was homogenized on the laboratory dispersant, pasteurized at the temperature of (85 ± 2)°C with the holding of 10 seconds. Five percent wt. of starter was introduced into the mixture, cooled down to a required temperature. Preliminary studies showed that the increased content of whey proteins in raw milk contributed to the intensification of the milk fermentation process at the account of the increased buffer capacity of the medium. Noted features of the lactic acid bacteria, including those produced as bacterial concentrates with the cryo- frozen microbial mass [17], are taken into account in the development of the probiotic fermented milk desserts, and the buffer capacity increase – in the regulation of physico-chemical indicators of the finished product [3].

Based on the preliminary studies on the ripening process of the mixture by different monotype lactic acid microorganisms, we selected representatives for the combines ripening that showed the optimal results on acid-forming and water-holding capacity, viscosity, organoleptic and probiotic properties.

The analysis of rheological and organoleptic indicators showed that samples with the best characteristics are ripened with the following cultures: *Lb. casei*, *Lb. acidophilus* and *Lac. lactis subsp. diacetilactis*. A milk base, ripened with *Lb. acidophilus* and *Lb. casei* had a more viscous, homogeneous structure and a pleasant sourish taste and the *Lac. diacetilactis* culture gave the product a lactic acid aroma, characteristic for diacetyl. Samples, ripened with *Lb. delbrueckii subsp. bulgaricus*, tasted a bit too sour.

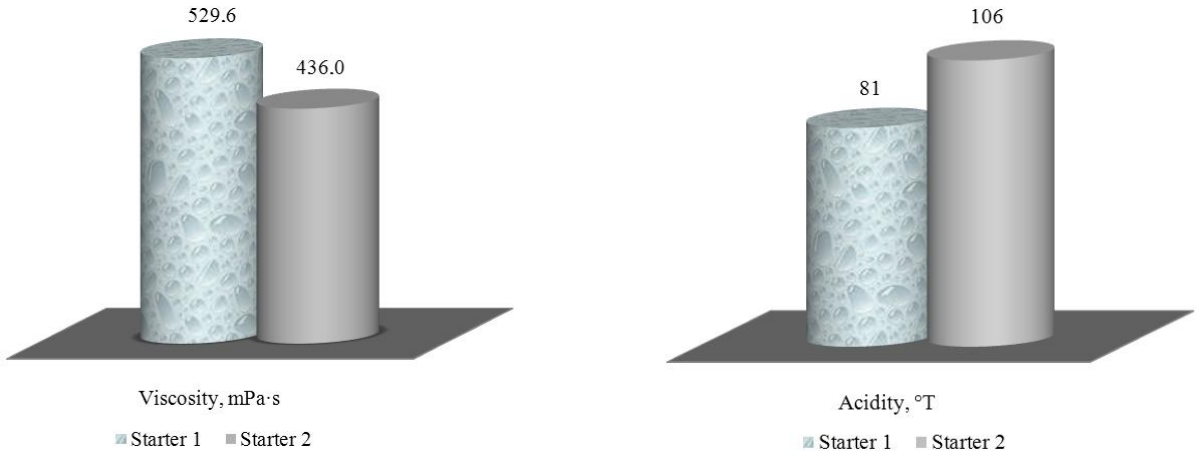
Pure whey has a specific aftertaste, which negatively affects the product's organoleptic indicators. That is why, in the selection of a starter, the preference should be given to the cultures that mask this aftertaste or make it less pronounced after ripening. *Lb. acidophilus, Lb. casei, Lac. diacetilactis* meet these requirements in the best way possible. They mask whey smell, give the product a pleasant lactic acid taste and specific aroma, the required consistency. Then, we matched the optimal ratio of cultures in the starter.

The combination of cultures in the starter (in percentage) was as follows:

Starter 1: *Lb. acidophilus* : *Lb. casei* : *Lac. diacetilactis* = 30 : 40 : 30;

Starter 2*: Lb. acidophilus : Lb. casei* : *Lac. diacetilactis* = 50 : 30 : 20.

Temperatures of fermentation and ripening were determined by the content of a starter. For the first sample, it amounted to (35 ± 1)°C, for the second (40 ± 1)°C. The choice of the indicated temperature values is due to the fact that in the composition of each starter there were mesophilic cultures, the optimum for which is 35°C, and thermophilic cultures (lactobacilli) with the optimum of (40–42)°C. At the same time, the percentage of each type of cultures in the starter was taken into account. The starter was introduced into the prepared samples in the amount of 5% of the mixture volume. Ripening was conducted at the specified temperatures during 7–8 hours until the milk base gets to the (80–90)°T titratable acidity for the starter 1 and (100–110)°T – for the starter 2.



(a) (b)

Fig. 3. The value of viscosity (a) and titratable acidity (b) of repined samples, depending on the content of a starter.

The results of the experiment on mixture fermentation with mixed starters are given in Fig. 3.

The obtained data confirms that exopolysaccharides, produced by lactic acid microorganisms (in particular, *Lac. lactis subsp. diacetilactis*), influence the consistency of the finished product. Temperatures of starters' cultivation also influences the structure of fermented milk drinks. The ripening temperature decrease to 35°C (even with the use of thermophilic cultures in the starter content) contributes to smaller amount of lactic acid and larger amount of exopolysaccharides, which leads to a product with the more pronounced stability of consistency and viscosity, as well as to the accumulation of a large amount of aromatic compounds, improving the taste and aroma of the product. However, both combinations of starter cultures can be used, depending on necessary organoleptic indicators of the finished product. Starter 1 is best suited for a functional fermented milk dessert, to make a products with a softer taste and aroma, a more viscous and denser consistency. Starter 2 can be used to produce a "drinking" (thin) dessert product. Obtained compositions can be successfully combined with different fruit and berry fillings to expand the range of products and meet the consumer preferences. Based on the conducted studies, we developed formulas of functional fermented milk desserts, tested technological modes for producing demineralized whey, milk mixture and finished products first on the pilot equipment and then under industrial conditions. It served as a basis for developing technical documentation of the product and obtaining a patent of the Russian federation No. 2493718 "Production method of the product based on milk whey".

As a result of the conducted studies, we showed a fundamentally new possibility of using demineralized acid whey for the production of functional milk desserts, which leads to a broader range of this product category, and opens up an opportunity of using secondary raw milk rationally, reduces the risk of environmental pollution by run-offs of a dairy plant. Demineralization of milk whey transforms this type of raw material from the problematic to a high-quality and cost-effective product, which is in demand in the food industry. The use of membrane equipment opens up new possibilities for enterprises. It allows processing raw materials, adjusting its composition, but maintaining its biological value and improving technological characteristic of raw materials, as well as producing new functional products with regulated composition and properties.

Milk desserts perfectly fit into the idea of a healthy lifestyle and nutrition. Even if the product is not enriched with functional ingredients, the buyer is convinced that a milk-based dessert does not only bring pleasure, but also a certain benefit to the body. This way, buyers form a belief that they take care of their health and improve quality of life. With the population being more and more aware of healthy nutrition, consumers prefer natural dessert products of high quality without dyes and preservatives, even despite their high cost. Experts consider the market of milk desserts as one of the most dynamically developing and marginal markets.

REFERENCES

1. Sviridenko Yu.Ya. and Volkova T.A. Secondary raw milk as an effective resource for the production of cheese and butter. *Cheesemaking and Buttermaking*, 2013, no. 3, pp. 34–38. (In Russian).
2. Affertsholt T. and Fenger M. *Whey book 2014 – the global market for whey and lactose ingredients 2014-2017/3A Business Consulting*, 2014. 146 p.
3. Mikhneva V.A., Zolotoreva M.S., Bessonov A.S., Volodin D.N., Shramko M.I. and Evdokimov I.A. An efficient method of processing acid whey. *Dairy Industry*, 2011, no. 1, pp. 40–41. (In Russian).

4. Zolotoreva M.S., Topalov V.K., Volodin D.N. and Evdokimov I.A. Processing problems of sour milk whey. *Cheesemaking and Buttermaking*, 2014, no. 6, pp. 46. (In Russian).
5. Evdokimov I.A., Barsukov V.A., Kulikova I.K., Volodin D.N. and Bessonov A.S. Combination of nanofiltration and electrodialysis for processing milk whey. *Proceeding of the Russian Conference with international participation "Ion transfer in organic and non- organic membranes"*. Krasnodar: CubSU Publ., 2008, pp. 102–103. (In Russian).
6. Evdokimov I.A., Dykalo N.Ya. and Permyakov A.V. *Electrodialysis of milk whey: a monograph*. Georgiyevsk: STI (branch) North-Caucasus STU Publ., 2009. 248 p. (In Russian).
7. Tverdokhleb G.B., Dilanyan Z.H., Chekulayeva L.V. and Shiler G.G. *Milk and milk products technology*. Moscow: Agropromizdat Publ., 1991. 463 p. (In Russian).
8. Varnam A.H. and Sutherland J.P. *Milk and milk products. Technology, Chemistry and Microbiology*. London: Ed. Chapman & Hall, 1994. 452 p.
9. Gorbatova K.K. *Physico-chemical and biochemical basis of dairy products production*. St. Petersburg: GIORD Publ., 2004. 352 p. (In Russian).
10. Gorbatova K.K. *Biochemistry of milk and milk products*. Moscow: Light Industry Publ., 1984. 344 p.
11. Zobkova Z.S. and Fursova T.P. On the consistency of fermented milk products. *Dairy industry*, 2002, no. 9, pp. 31–32. (In Russian).
12. Stepanova L.I. *Guide of the milk production technologist. Technology and formula. Vol. 1. Whole-milk products*. 2nd edn. St. Petersburg: GMORD Publ., 2003. 384 p. (In Russian).
13. Glazachev V.V. *Technology of fermented milk products*. Moscow: Food industry Publ., 1974. 118 p. (In Russian).
14. D'yachenko P.F. *Technology of milk and milk products*. Moscow: Food industry Publ., 1974. 447 p. (In Russian).
15. Walker W.A. and Duffy L.C. Diet and bacterial colonization: role of probiotics and prebiotics. *J. Nutr. Biochem*., 1998, vol. 9, iss. 12, pp. 668–675.
16. Salminen S., von Wright, A. and Ouwerhand, A. *Lactic Acid Bacteria Microbiology and Functional Aspects*. 3rd edn. New York: CRC Press, 2004. 656 p.
17. Kharitonov D.V., Shramko M.I. and Belova O.I. The principles of creating the bacterial concentrates technology with microbial mass cryo-freezing. *Proceedings of the 1st International scientific-practical conference "Modern science: theory and practice"*. Stavropol: NorthCaucSTU Publ., 2010. (In Russian).

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APPLE PECTIN AND NATURAL HONEY IN THE CLOSED MILK PROCESSING CYCLE

A. N. Fedosova*, M. V. Kaledina

Belgorod State Agricultural University named after V.Ya. Gorin,
Vavilova Str. 1, Belgorod, 308503 Russian Federation

* e-mail: fedosova.anna2011@yandex.ru

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Abstract: The paper is based on the methodological approach to using pectin and natural honey in the technology of production of new dairy products. The purpose of the study is to use products of the fractionation of milk by apple pectin – natural casein concentrate (NCC) and whey-pectin fraction (WPF) in natural form for the development of new functional products enriched with natural bee honey. Study of the specifics of the fractionation of whole and skim milk by Russian-manufactured apple pectin with the substantiation of the technological parameters of receiving WPF and NCC was conducted. It was established that the process runs effectively under the following conditions: preliminary heat treatment of milk at a temperature of 76°C, introduction of a 5% polysaccharide solution into milk, concentration of pectin of 0.6% of the weight of milk, temperature of fractionation of 4–6°C. The technological properties of the received fractions were studied. It was determined that NCC is relatively thermostable (withstands heat treatment at a temperature up to 80°C), beats well at a temperature of 5°C (overrun 50–60%), adding honey has a positive effect on the structure of the beaten NCC and reduces the likelihood of the separation of whey during storage. At the pectin content within 0.6–0.7% of the weight of milk (on a dry basis), the yield of WPF from skim milk was within 80–81%, from whole milk – 72–73%, solids content was 6.2–6.3%. The total protein content in WPF was 0.9–1.0%, of which whey proteins – 0.45–0.50%. When adding honey to the WPF, a dense gel was formed after a while, having a tendency to syneresis; in 96 hours the degree of syneresis was 10–12% for the studied concentrations of honey from 1 to 10%. On the basis of the obtained data, the closed milk processing cycle is provided by the technology and the formulations of the two products with honey on the basis of WPF and NCC – a pudding and a "Smoothie".

Keywords: Pectin, natural honey, fractionation, flocculation, natural casein concentrate, whey-pectin fraction, pudding, smoothie

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INTRODUCTION

Virtually all food industry branches, medicine and cosmetology show interest in pectins. Pectins have unique versatile properties due to the structural characteristics of the molecule.

In terms of the chemical nature, pectins are biopolymers of carbohydrate nature, the structural part of the polymer is ά- galacturonic acid. Combining the properties of acids and polyatomic alcohol, pectins can form stable insoluble complexes with polyvalent cations of metals, including toxic, heavy and radioactive metals. Pectins also form similar complexes with organic toxins getting into the human body or forming within it. Pectins are used as an antidote in case of intoxication with heavy metal salts and other toxins. They are figuratively called "a gift of the vegetable kingdom" and "an attendant of human body". Pectin as a dietary supplement is approved by the World Health Organization (WHO) and can be used in all countries of the world without restrictions. The preventive daily dose of pectin, recommended by the World Health Organization, is 4–5 g on a dry basis, 15–16 g in conditions of radioactive contamination. Pectin does not cause side effects and can be used in food on a long-term basis [2].

Properties of pectins as polyhydrophylic colloids are widely used in the food industry: pectins are used as thickeners and stabilizers, for the purpose of the improvement of the rheological properties of products. In dairy products, pectin is used not only as a stabilizer, but also with a view of enhancing health-promoting properties.

Gelling power is characteristic of all high molecular weight hydrocolloids and is shown by each of them at a certain concentration in the system. It should be noted that under certain conditions pectin can, on the contrary, destabilize the polydisperse system of milk with its separation into fractions. This ability of pectin is of scientific and practical interest, since both fractions – natural casein concentrate (NCC) and whey-pectin fraction (WPF) – have high biological value,

which makes it possible to use this process in a non-waste milk processing technology.

NCC contains up to 70% of high quality milk protein, which has a complete set of essential and non-essential amino acids with the preservation of native molecular structures, up to 20% of carbohydrates, 7–8% of mineral substances and vitamins. It is a natural casein-calcium- phosphate complex, completely soluble in water. This makes it an especially attractive raw material for the food industry [3, 6].

WPF contains whey proteins, complete in terms of their amino acid composition, lactose, mineral salts, vitamins, and due to the presence of pectin it has a prebiotic effect on the intestine, so any food products, obtained on the basis of WPF, will be functional.

Both fractions (NCC and WPF) are soluble in water in all proportions even in dry form, are well mixed with any of the components of milk and other food raw materials, which makes it possible to use them for the production of liquid, pasty and structured products.

Fundamental research in the field of fractionation and concentration of the proteins of raw milk by pectin were conducted under the supervision of Professor V.V. Molochnikov [3–5]. The biotechnological principles of the production of functional dairy products using polysaccharides were thoroughly studied by T.A. Orlova [6].

The present work is based on the methodological approach to the substantiation of technological parameters of obtaining NCC and its subsequent use for the production of dairy desserts, which are in demand in the market and are popular with consumers due to their organoleptic characteristics.

High nutritional and biological value, as well as the attractive taste characteristics of the new dessert, are ensured by using natural honey in its formulation.

Honey is the oldest sweet delicacy and a very useful concentrated product. Natural honey is especially rich in monosaccharides – fructose and glucose. Mass fraction of fructose is 33–42%, mass fraction of glucose is 27–36%. Monosaccharides are quickly absorbed into the blood stream, replenishing the energy stores of the body. Honey also contains amino acids, essential oils, hormones, enzymes, organic acids, minerals, vitamins, antibiotics, antifungal, antidiabetic and other beneficial substances in favourable combination. In total, honey contains about 400 different substances, necessary for the human body [1].

Honey has no equal in terms of the amount of mineral substances. The average content of mineral salts in honey is 0.62%. Though they have a low absolute concentration, they are characterized by high physiological activity. In total, honey is found to contain 37 minerals substances, containing essential macro-and microelements. Perhaps, this fact explains the healing properties of honey. Each of the elements plays its own important role in the vital activities of the human body. The concentration of biologically active substances in honey is directly related to the type of honey, pollen and nectar composition [1].

Honey is an excellent dietary and medical food product, used for the prevention and treatment of metabolic disorders in the human body. Vitamins are contained in honey in small amounts, but they also play an important role, because their effect increases in combination with fructose, glucose, dextrines, mineral salts, and organic acids.

Honey falls into the category of functional foods necessary for optimal functioning of the human body as a whole. Honey is widely used as a restorative, tonic and recuperative agent.

Prebiotic properties of honey are associated with the high content of fructose, which is an easily accessible source of energy for the enzyme system of bifidobacteria. In the present work we studied the physical and chemical properties of Russian-manufactured apple pectin and the specifics of the fractionation of whole and skim milk, with the substantiation of the technological parameters of receiving fractions of NCC and WPF.

The purpose of the study is to use products of the fractionation of milk by apple pectin – natural casein concentrate (NCC) and whey-pectin fraction (WPF) in natural form – for the development of new functional products enriched with natural bee honey.

OBJECTS AND METHODS OF STUDY

Research objects were skim and whole cow's milk, apple pectin, manufactured in the Belgorod Region according to TU-9199 012-01014470-04 "Apple pectin, dietary food supplement", natural bee honey produced in the Belgorod Region, products of fractionation − natural casein concentrate (NCC) and whey-pectin fraction (WPF) in natural form, milk (raw milk), produced in farming enterprises. Fractionation of milk (raw milk) by apple pectin was implemented in the field of gravitational forces.

To obtain physical and chemical and microbiological research results, standard methods and methods found in the specialized literature [7, 8] were used.

Study of the impact of the concentration of pectin on the process of flocculation of casein was implemented as follows. A 5% aqueous solution of pectin was added to milk until its concentration in milk amounted to 0.5–1.0 g per 100 g (on a dry basis). The temperature of components in the process of mixing was 20–22°C, the amount of milk was 100 cm^3. The mixture was quickly stirred and dispensed into three rows of biological tubes, having the capacity of 30 cm^3, to ensure three replications for each concentration. The time of completion of the process was determined on the basis of the stabilization of the NCC layer height. The dynamics and efficiency of fractionation were estimated by the height of the whey layer, expressed as percentage of the original height of the mixture in test tubes, and by the solids content in the received fractions. When the volume of the mixture was 30 cm^3, the height of the mixture in tubes reached 25 cm (100%).

PH measurement was implemented using the pH meter/ionomer IPL-201 (MULTITEST "Semiko").

The measurement of the solids content was carried out using the method of drying to a constant weight at a temperature of 102–105°C.

The total protein content and the content of whey proteins were determined by the refractometric method using the refractometer IRF-464.

The impact of acidity on the process of fractionation was evaluated according to the same pattern, by modeling the pH systems by a lactic acid solution.

To determine the impact of temperature on the casein flocculation process, 100 cm^3 of milk were mixed with a pectin solution in the amount of 0.6% of the weight of milk (on a dry basis) at a temperature of components from 10 to 70°C in increments of 10°C. The mixture was stirred thoroughly and poured into seven tubes. The total volume of the mixture in the tubes was 30 cm^3, the height of the mixture layer was 25 cm (100%).

The heat treatment of milk, WPF and NCC was carried out in the laboratory of the Belgorod State Agricultural Univerisity named after V. Gorin using a tank with a heat exchange jacket. Gorin by using tanks with heat jacket.

The whipping of the NCC solution was carried out at the laboratory disperser IKA RW 20 digital at a mixing speed of 2 000 rpm.

The evaluation of taste, consistency and consumer properties of the product was carried out through the experts' tasting assessment on the 10-point scale.

RESULTS AND DISCUSSION

Study of the functional and technological properties of cream with a protein content of about 4%

Pectin was used in the study in the form of an aqueous solution. Physical and chemical properties of pectin are presented in Table 1.

Pectin almost did not dissolve in cold water. The optimal solubility was observed at a water temperature of 70°C. The concentration of the work pectin solution was selected on the basis of the results of the study presented in Table 2.

The optimal concentration of pectin for the fractionation of milk is a 5% solution.

Organoleptic and physical and chemical properties of the 5% aqueous solution of pectin:
– colour – cream white;
– smell – refreshing, apple;
– taste – slightly astringent, slightly sour;
– pH of the solution within 2.48–2.52.

When heated to a temperature of 20–30°C, the 5% aqueous solution of apple pectin was a dense homogeneous liquid, at a lower temperature the solution turned into nonfluid, homogeneous, very dense system.

The impact of pectin on the acidity of skim milk was noted (Table3).

An increase in the acidity of milk is attributed to the chemical nature of apple pectin, which, though it is a methoxilated (esterified methyl alcohol) biopolimer, has free carboxylic groups, which determine its pronounced acid properties (pH of the solution is within 2.48–2.52).

Study of the technological arameters of the fractionation of skim and whole milk

The impact of the mass fraction of pectin on the efficiency of the process of flocculation of casein in skim milk was studied. The impact of the pectin concentrations of 1, 2, and 4% of the weight of milk (on a dry basis) at the fractionation temperature of 20–22°C was also analyzed (Table 4).

At the pectin content of 1%, milk was divided into two factions. At the polysaccharide concentration of 2 and 4%, the fractionation of milk did not occur, a more viscous homogeneous liquid was formed. Thus, the concentration of pectin in the mixture with skim milk shall be not more than 1 g per 100 g of milk.

The dynamics of the process and the effectiveness of the fractionation of milk at the pectin content within the range of 0.6 to 1.0 g per 100 g of milk are shown in Table 5.

Table 1. Physical and chemical properties of apple pectin (TU 9199-012-01014470-04)

Degree of etherificafion, %	Molecular weight, ×103	Gelling power, Tarr-Baker degrees
75 ± 1	25 ± 2	190 ± 10

Table 2. The impact of the pectin concentration on the solution consistency

Pectin content, g per 100 g of solution	Characteristics of pectin solution at a temperature of 70°C
1.0	The fluidity of the solution is close to that of water, the color is pale yellow
3.0	The fluidity of the solution is close to that of milk, the color is yellow
5.0	The solution is thick, viscous, the color is intense yellow
6.0	The solution is not fluid, very viscous, the color is intense yellow

Table 3. The impact of pectin on the acidity of skim milk

Pectin content, g per 100 g of milk	Acidity of the initial mixtures	
	titratable, °T	active, pH
0.25	22 ± 1	6.47 ± 0.04
0.50	23 ± 1	6.38 ± 0.04
0.75	24 ± 1	6.30 ± 0.04
1.0	24 ± 1	6.22 ± 0.04

Table 4. The impact of different concentrations of pectin on skim milk

Pectin content, g per 100 g of milk	Acidity of the mixture		Visual effect	Time from the beginning of the process, h
	titratable, °T	active, pH		
Control	20 ± 1	6.5 ± 0.04	Cream coloured liquid	0
1	28 ± 1	6.1 ± 0.04	Visible separation of the phases. At the bottom there was concentrated solution of bright white colour, at the top – yellow liquid	1
2	30 ± 1	5.8 ± 0.04	Homogeneous viscous cream coloured liquid	2
4	31 ± 1	5.5 ± 0.04	Homogeneous viscous cream coloured liquid	2

Table 5. The dynamics and the effectiveness of the fractionation of milk at the pectin content within the range of 0.6 to 1.0 g per 100 g of milk

Time from the beginning of the process, min	Pectin content (on a dry basis), g per 100 g of milk					
	0.6		0.75		1.0	
	The height of the whey layer, cm	% of the original height of the mixture	The height of the whey layer, cm	% of the original height of the mixture	The height of the whey layer, cm	% of the original height of the mixture
30	3	12	2	8	0	0
45	16	64	4	16	0	0
60	19	78.1	7	28	3	12
90	19	78.1	10	40	5	20
150	Fractionation process ends after 60 min		12.5	50	10	40
180			14	56	11	44
210			16	64	12	48
240			16	64	12	48
300			Fractionation process ends after 210 min			

It was determined that pectin concentration in the process of fractionation of skim milk should be close to 0.6–0.7 g per 100 g of milk (on a dry basis).

To test the observed effect and to determine the optimal concentration of pectin, additional studies were conducted using pectin concentrations of 0.5%, 0.6% and 0.7% (on a dry substance basis). The yield of NCC and WPF and the solids content in NCC and WPF at different concentrations of pectin is presented in the diagrams (Fig. 1).

The accuracy of determining the yield of fractions was within the range of 0.3–0.5%.

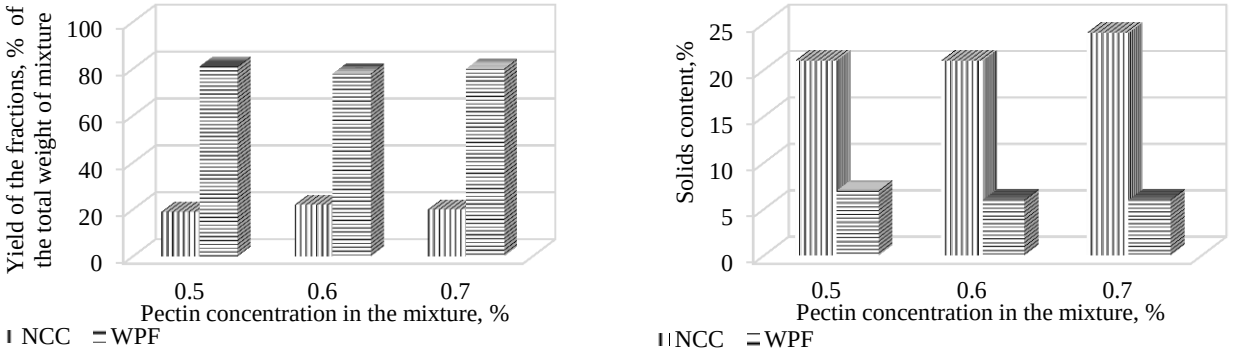
The concentration of pectin in the mixture below 0.5% and over 0.7% resulted in a decrease of solids content and of the yield of NCC, while within 0.6–0.7% these figures changed insignificantly.

The forecast of the commercial yield of NCC and WPF on the basis of experimental findings is presented in Table 6.

The yield of WPF was 79%; the yield of NCC was 20%, respectively, while the production losses amounted to about 1%.

The impact of temperature on the efficiency of the concentration (flocculation) of casein was studied. Concentration of pectin in skim milk was 0.6% (on a dry basis). The acidity of skim milk was 21–22°T.

The experimental data are presented in Table 7.



(a) (b)

Fig. 1. Yield (a) of NCC and WPF and solids content (b) in NCC and WPF.

Table 6. The forecast of the commercial yield of NCC and WPF

Raw materials	Pectin concentration 0.6% of the weight of milk			Solids content, %
	Yield, %	Acidity		
		°T	pH	
Base mixture (skim milk and pectin)	100	22 ± 1	6.26 ± 0.04	9.12 ± 0.5
WPF	79 ± 1	17 ± 1	6.25 ± 0.04	6.09 ± 0.5
NCC	20 ± 1	47 ± 1	6.31 ± 0.04	25.16 ± 0.5

Table 7. The impact of the temperature of the mixture on the flocculation efficiency of casein

Temperature, °C	After 30 min		After 60 min	
	Characteristics of the system	% NCC of the initial height of the mixture	Characteristics of the system	% NCC of the initial height of the mixture
10	No clear boundary between fractions	—	Clear boundary between fractions	24.0 ± 0.5
20	No clear boundary between fractions	—	Clear boundary between fractions	24.0 ± 0.5
30	No clear boundary between fractions	—	Clear boundary between fractions	24.0 ± 0.5
40	Clear boundary between fractions	24.0 ± 0.5	Clear boundary between fractions	24.0 ± 0.5
50	Clear boundary between fractions	24.0 ± 0.5	Clear boundary between fractions	24.0 ± 0.5
60	Clear boundary between fractions	26.0 ± 0.5	Clear boundary between fractions	26.0 ± 0.5
70	Clear boundary between fractions	22.8 ± 0.5	Clear boundary between fractions	22.8 ± 0.5

On the basis of the results of the study of the impact of temperature on the fractionation process following conclusions were made:

– In the temperature range of 10–30°C the concentration of casein ended after 60 min.

– With the rise in temperature of the mixture, the flocculation of casein proceeded faster and reached a maximum after 30 min.

– At temperatures above 60°C, due to the denaturation of whey proteins, the flocculation of casein decreased.

– Temperature did not have a significant impact on the fractionation of skim milk and the yield of NCC.

Important technological parameters, that can affect the process of fractionation, are the titratable and active acidity of the mixture of raw milk with polysaccharide. It was found that the studied range of acidity of the mixture has almost no impact on the yield of natural casein concentrate in case of using the studied type of apple pectin (Table 8).

However, there is considerable literature data on the significant increase of the mass fraction of protein in the WPF, when the initial acidity of raw milk is below 12 and above 20°T [3].

The stability of casein micelles, as well as the stability of any colloidal lyophilic system, depends on the charge magnitude, influenced by the salt composition, pH and the presence of various hydrophilic colloids in milk. When developing the methods of the separation of casein, it is necessary to take into account the specifics of its structure [10, 11].

Along with the fractionation of skim milk by apple pectin, the possibility of the fractionation of whole milk was analyzed.

A preliminary study on the impact of the pectin

Table 8. The impact of the acidity of skim milk on the flocculation of casein by apple pectin

Acidity of the mixture (milk + pectin)		% NCC of the initial height of the mixture	
Titratable,°T	Active, pH	After 30 min	After 60 min
24	6.52 ± 0.04	24.8 ± 0.5	24.8 ± 0.5
25	6.49 ± 0.04	24.2 ± 0.5	24.2 ± 0.5
26	6.38 ± 0.04	24.2 ± 0.5	24.2 ± 0.5
27	6.26 ± 0.04	23.8 ± 0.5	24.2 ± 0.5
28	6.13 ± 0.04	23.2 ± 0.5	23.2 ± 0.5
29	6.07 ± 0.04	23.0 ± 0.5	23.0 ± 0.5
30	6.00 ± 0.04	23.0 ± 0.5	23.0 ± 0.5

concentration on the fractionation of whole milk made it possible to conclude that the pectin content (on a dry basis) should be within the range of 0.6 to 0.7% of the weight of milk, as it is the case for skim milk.

The impact of heat treatment and temperature on the fractionation of whole milk is shown in Table 9.

Heat treatment causes a number of physical and chemical processes in the milk, resulting in the changes in the aggregative stability of casein micelles, which has a positive effect on the fractionation process. In raw milk, virtually no fractionation was observed. The fractionation process proceeded more efficiently in pasteurized milk and within the temperature range of 4–6°C. To provide a quantitative characteristic of the process of fractionation of whole milk, additional research was conducted. The following parameters were determined: yield of fractions, solids content, the acidity of the obtained fractions and the fat content in the WPF. The titratable acidity of the raw milk was 16°T. Whole milk was pasteurized at 76°C for 20 seconds.

The impact of the temperature of the mixture during fractionation on the yield of fractions and the solids content in the obtained fractions is shown in Fig. 2.

The fat content and the acidity of WPF and concentrate (protein-lipid fraction) for pasteurized milk at various temperatures are presented in Table 10.

Table 9. The impact of pasteurization and temperature on the fractionation of whole milk

Raw milk:	
Temperature 20–23°C	Temperature 4–6°C
Insignificant fractionation, at the bottom there is a bright white layer, having a height of less than 1 cm within the tube, there are flocs in the whey	Insignificant fractionation, at the bottom there is a bright white layer, having a height of less than 1 cm within the tube, there are flocs in the whey
Milk pasteurized at 76°C for 20 seconds:	
Temperature 20–23°C	Temperature 4–6°C
Complete separation of milk into fractions: transparent whey and bright white layer (NCC + Fat) at the bottom of the tube. The height of the concentrated layer amounted to 16.8% of the total height of the mixture layer.	Complete separation of milk into fractions: transparent whey and bright white layer (NCC + Fat) at the bottom of the tube. The height of the concentrated layer amounted to 20.0% of the total height of the mixture layer.

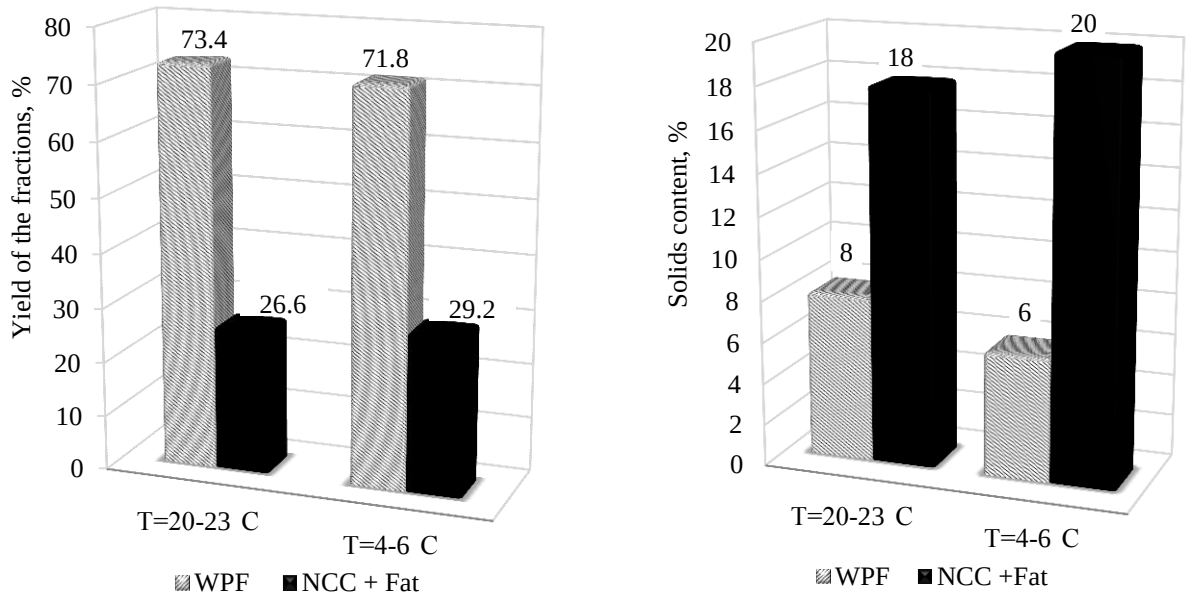


Fig. 2. The impact of temperature on the efficiency of the fractionation of pasteurized whole milk by apple pectin.

Table 10. The fat content and the acidity of the obtained fractions at various temperatures

Parameters	20–23°C		4–6°C	
	WPF	Concentrate (protein-lipid fraction)	WPF	Concentrate (protein-lipid fraction)
Mass fraction of fat,	0.1 ± 0.01	14 ± 0.05	0.05 ± 0.01	14 ± 0.05
Titratable acidity, °T	17 ± 1	37 ± 1	18 ± 1	35 ± 1

Apple pectin is able to efficiently fractionate whole pasteurized milk at a temperature of 4–6°C. The yield of concentrated fraction (protein-lipid fraction) amounted to 29% of the total weight of the mixture, with the solids content of 20%. Milk fat passed almost completely into the layer of concentrated casein. The fat content in the whey fraction was no more than 0.05%.

The closed milk processing cycle with the use of apple pectin was implemented in the development of two functional products with honey on the basis of the obtained fractions – WPF and NCC in their natural form.

Study of the technological parameters of producing NCC from skim milk and ITS functional and technological properties

Based on the research results, the technology of producing natural casein concentrate (NCC) from skim milk under the action of pectin was determined. The following technology was used: skim milk was pasteurized at a temperature of 76–78°C for 15–20 seconds, cooled to a temperature of 15–20°C, mixed with 5% aqueous solution of pectin in the amount of 0.6% of the weight of milk, calculated on a dry basis. After thorough stirring, the mixture was left for one hour at a temperature of 4–6°C. Upon the expiry of this time, the flocculation of casein was virtually fully completed, that is, skim milk was separated into two fractions: NCC and WPF. The upper fraction (WPF) was removed by draining, after which the remaining natural concentrate of casein (NCC) with solids content of 23–25% was used. The yield of NCC was approximately 20%.

The obtained NCC solution had a bright white color, a pleasant slightly sour taste, the titratable acidity was 45–47°T, pH was 6.31–6.34. High values of titratable acidity of NCC were attributable to the increased proportion of protein. The same factor also results in the increase of the density of protein to 1 050–1 055 kg/m^3.

The ability of natural concentrate of casein to withstand heat treatment (Table 11) was also noted, which has the following explanation. The basic protein in the NCC is casein – a thermostable protein, the content of whey proteins in the NCC solution does not exceed 1.0%.

Another important technological property of NCC is its whippability/foam-forming ability. The molecular structure of foaming agents has both hydrophilic and hydrophobic properties. This is characteristic of all soluble proteins; during whipping their molecular chains are distributed in a very thin layer on the surface of the bubbles. An important factor of the stability of foam is temperature. With the decrease in temperature, the solubility of gases increases, consequently, more air bubbles are formed. The study showed the whippability of NCC at a temperature below 10°C, the volume of the whipped mass increased by 1.5–1.6 times at a temperature of 5°C.

To ensure foam stability, NCC should contain sugars. Besides, using natural honey as a sweetener for the purpose of improving the biological and nutritional value of NCC is of particular interest. The diagram (Fig. 3) shows the impact of honey and sucrose on the stability of the structure of NCC after 24 hours of storage at a temperature of 4–6°C.

Introduction of carbohydrate components does not affect the degree of overrun, but introduction of honey in amount of 0.5 to 2% improves foam stability over time. The combination of honey and sucrose in various proportions gave the same effect as using pure honey.

To study the ripening ability of NCC, a curd cheese starter culture was used (*Lc. lactis*, *Lc. cremoris*, *Leu. cremoris*, *Str. thermophilus*), which was added in the amount of 3% of the weight. The samples were thermostated at a temperature of 32°C. The dynamics of the fermentation process is shown in the diagram (Fig. 4).

Table 11. The impact of heat treatment on the structure of NCC

Heating temperature, °C					
68	72	76	80	85	90
The NCC solution preserves the homogeneity				Minor structural changes The appearance of individual protein flocs	Noticeable structural changes The appearance of visible protein flocs

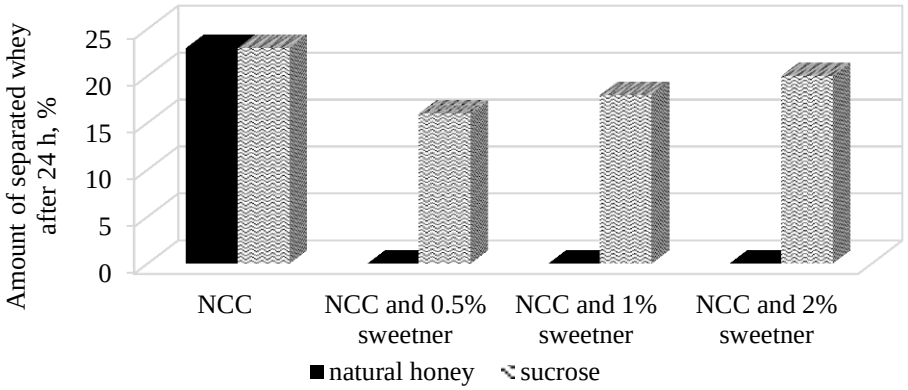


Fig. 3. The impact of natural honey and sucrose on the stability of the structure of NCC after 24 hours of storage.

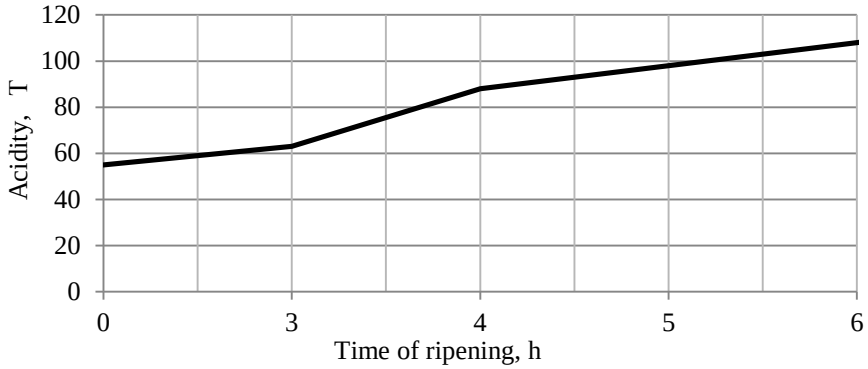


Fig. 4. The dynamics of the process of fermentation of NCC by lactic acid microorganisms.

NCC is efficiently ripened by lactic acid microorganisms with the formation of a dense clot with high water-holding capacity. The dynamics of the process is similar to the processes taking place during fermentation of any raw milk.

The studied technological properties of NCC make it possible to conclude that it is relatively thermostable, beats well at a temperature of 5°C (overrun 50–60%), adding honey has a positive effect on the structure of the beaten NCC and reduces the likelihood of the separation of whey during storage.

Thus, alongside with high functional characteristics, NCC also has good technological properties. Combination of NCC with other dairy or vegetable raw materials makes it possible to develop various forms of functional food products with adjustable organoleptic and consumer properties.

Development of the technology and formulation of a dairy dessert on the basis of NCC with honey

For the purpose of the practical implementation of the obtained results, the use of the NCC of skim milk in the technology of the production of a new dairy dessert with honey "Smoothie" is proposed.

In everyday understanding, "Smoothie" is a cooled dessert made from crushed pieces of fruits and berries with the addition of milk, yogurt, curd cheese, juice, honey, eggs, sugar, ice and other components. It contains dietary fiber, vitamins and antioxidants, can provide large amounts of energy and is a physiologically useful product.

The use of the following ingredients as basic raw materials for the obtaining of the product was envisaged: NCC solution, natural honey, berry fillers, apple pectin.

The technology of the production of the "Smoothie" product on the basis of natural concentrate of casein consisted of the following processes: obtaining NCC (according to the technology described above), formulation of the recipe and the development of manufacturing operations, ensuring safety and specific organoleptic properties of the "Smoothie" product.

Natural honey was added in the proportion of 3% of the weight of the produced NCC. After stirring, the mixture was pasteurized at a temperature of 76–78°C for 15–20 seconds and cooled to a temperature of 6–8°C. To improve the overrun, the NCC was kept at a temperature of 4–5°C for at least 16 hours. Cold mixture of NCC with honey was whipped until the volume was increased by not less than 50% (2 000 rpm, duration 3 min).

To improve the consumer properties of the product and its customer appeal, natural berry fillers were used: strawberry, cherry, plum and assorted (blackcurrant, strawberry, cherry). Fillers were previously prepared in the following way. Frozen berries were defrosted, poured onto the sieve and carefully washed with cold water from a shower. After draining the excess water from berries, an inspection for the rejection of low quality berries was conducted. Then berry raw materials were mixed with granulated sugar in the proportion of 30% of the weight of berries. The mixture was heated to 95°C and quickly cooled to 20–22°C.

A method for the adding of berry fillers in the product was studied. The overrun and the structure stability were significantly higher, when the filler was added into the previously whipped NCC. The proportion of berry filler in the product was determined experimentally (Table 12).

Table 12. The impact of the proportion of berries on the consistency and organoleptic properties of the product

The proportion of berries, g per 100 g of NCC	Consistency	The taste of the product	Color
70	Dense, nonfluid	Noticeable taste of berries	Pale, faint
100	Dense, nonfluid	Noticeable taste of berries	Characteristic of the type of added berries, not saturated
200	Dense, nonfluid	Noticeable taste of berries	Saturated, characteristic of the type of added berries

The ratio of the weight of berries and NCC was chosen to be 2 : 1.

To ensure the preservation of fluffy structure without signs of syneresis for the whole period of the product sales (7 days), berry filler was stabilized by apple pectin. This supplement also ensures pronounced functional properties of the product.

The optimal amount of apple pectin was determined experimentally. Pectin was added in dry form to the hot (80°C) berry filler under continuous stirring. Then the mixture was cooled to 20–25°C, and after 2 hours the consistency of the berry filler with various pectin content was determined. The impact of the pectin percentage on the consistency of the berry filler is shown in Table 13.

In the formulation of the product, the percentage of pectin was chosen to be 1% of the weight of berry filler.

The developed final formulation of the berry "Smoothie" with honey on the basis of natural concentrate of casein is provided in Table 14.

The product had a soft fluffy consistency, with a pleasant sense of berry pieces. The content of the components in the finished product is shown in Table 15.

The resulting dessert compares favorably with its dairy analogues by having high nutritional and biological value. The "Smoothie" contains casein in its native and easily assimilable form, contains virtually no fat, while adding apple pectin to the formulation ensures high functional properties. Natural honey and berry filler make the product not only attractive to consumers, but also beneficial to health.

Study of the functional and technological properties of WPF

Whey-pectin fraction, containing virtually no fat, was received both from skim and whole milk according to the above-described fractionation technology. WPF was a transparent, greenish-yellow liquid with a sweetish taste, with the titratable acidity of 17–18°T and pH of 6.27–6.25.

At the pectin content within 0.6–0.7% of the weight of milk (on a dry basis), the yield of WPF from skim milk was within 80–81%, from whole milk – 72–73%, solids content was 6.2–6.3%. The total protein content in the WPF was 0.9–1.0%, of which whey proteins – 0.45–0.50%.

The developed formulation of the new products included natural honey. Sucrose was used for the control purposes.

The organoleptic characteristics of WPF at the sugar content within the range from 1 to 5%, in increments of 1%, are shown in Table 16.

It was noted that after adding honey to WPF, a dense gel was formed after a while, having a tendency to syneresis; in 96 hours the degree of syneresis was 10–12% for the studied concentrations of honey from 1 to 10% (Table 17).

The ability of honey to transform WFP into gel can be associated with a complexing ability of polyvalent metal ions. Honey contains over 37 macro-and microelements; the high total concentration of sugar molecules (fructose – 33…42%, glucose – 31…36%, sucrose and other sugars – 10%), which possess high hydrophilic properties (can convert free water into the bound form), also contributes to the formation of gel [2, 9].

The primary role of the cations of metals in the formation of the gel of WPF with honey is evidence by the lack of sweet taste in the densifying gel and the presence of pronounced sweetness of the water phase. After adding the mixture of glucose and fructose (1 : 1) in the total amount of 5% of the weight of WPF (maximum amount of honey in the samples), no gel formation was observed. Neutral properties, pleasant smell, and the behavior of WPF when adding honey, were the reason for the selection of the product made on its basis – a pudding.

Table 13. The impact of the pectin percentage on the consistency of the berry filler

Percentage of pectin, %	Consistency of berry filler
0 (control)	Fluid, free moisture
0.5	Fluid, free moisture
1.0	Dense, retains moisture
1.5	Dense, retains moisture
2.0	Very dense

Table 14. The formulation of the berry "Smoothie" with honey on the basis of NCC

Component	Weight, kg
Natural casein concentrate, solids content = 24%	320
Berry filler in 30% syrup	640
Pectin, solids content = 95%	10
Honey, solids content = 83%	30
Total	1000
The solids content in the finished product is not less than 30%	

Table 15. The content of the components in the berry "Smoothie" with honey on the basis of NCC

Parameter	The content in the product
Mass fraction of protein, %	Not less than 7.0
Mass fraction of sucrose, %	Not less than 20.0
Mass fraction of honey, %	Not less than 3.0
Moisture content, %	Not less than 70.0

Table 16. Organoleptic characteristics of samples with honey and sucrose

WPF with honey	WPF with sucrose
The sweetness threshold rises until the honey concentration reaches 4%. A pleasant mild taste with a honey flavor. Further increasing of the concentration doesn't result in increasing sweetness. In WPF with honey, the formation of gel began after some time at a room temperature	The sweetness is more pronounced than that of WPF with honey. With the increase in concentration, the taste becomes too sweet (too sugary). The samples of WPF with sucrose remained liquid with time

Table 17. The impact of the honey concentration on the properties of WPF

Mass fraction of honey, g per 100 g of WPF	Weight of sample = 100 g			
	Consistency		Degree of syneresis, % fluid of the initial weight of gel at 4–6°C	
	after 1 hour	after 24 hours	12 hours	96 hours
0.5	liquid	jelly	3 ± 0.5	10 ± 0.5
1	liquid	dense gel	9 ± 0.5	11 ± 0.5
2	liquid	dense gel	10 ± 0.5	12 ± 0.5
3	slight solidification	dense gel	10 ± 0.5	12 ± 0.5
5	complete solidification	dense gel	10 ± 0.5	12 ± 0.5
10	thick, dense structure	dense gel	10 ± 0.5	12 ± 0.5

Development of a dairy dessert on the basis of WPF with honey

The selection of the components of the pudding formulation was carried out on a step-by-step basis stages, by choosing various ratios between the following components: WPF, skim milk powder, cream, starch and fillers [9]. This paper provides the final version of the formulation (Table 18).

CONCLUSION

Using apple pectin in a closed milk processsing cycle is of interest from both economic and biological perspective. The products obtained by fractionation of raw milk – NCC and WPF – have high nutritional, biological and technological value. On the basis thereof, it is possible to obtain a large variety of functional products. Using natural honey and vegetable raw materials in the manufacturing process ensures high consumer properties and improved functional properties of the products. The practical proof of this are the technologies and formulations of the products on the basis of WPF and NCC (pudding and "Smoothie"), presented in this paper.

Table 18. The formulations of a pudding on the basis of WPF

Components	A pudding on the basis of WPF, mass fraction of fat 3%		
	milk-honey	vanilla	chocolate
WPF, solids 6.2%	703	7	709
Cream, fat content 30%	100	1	94.3
Skim milk powder, solids content 96%	62.2	56.8	47.1
Honey, solids content 83%	50	25	25
Sugar, solids content 99.86%	35	60	60
Starch, solids content 96%	50	50	50
Vanilla, solids content 100%	–	0.5	–
Cocoa powder, solids content 96%	–	–	15
Total	1 000	1 000	1 000

REFERENCES

1. Danikov N.V. *Healing honey*. Moscow: Eksmo-Press Publ., 2012. 256 p. (In Russian).
2. Donchenko L.V. and Firsov G.G. *Pectin: basic properties, production and use*. Moscow: DeLi print Publ., 2007. 276 p. (In Russian).
3. Trukhachev V.I., Molochnikov V.V., Orlova T.A. and Ramanauskas R.I. *Concentrates of milk proteins: separation and application: A monograph*. Stavropol: AGRUS Publ., 2009. 152 p. (In Russian).
4. Molochnikov V.V. *Non-waste technology of milk processing using polysaccharides*. Moscow: Agropromizdat Publ., 2007. 320 p. (In Russian).
5. Molochnikov V.V., Orlova T.A. and Moreno V.V. A new insight into milk processing. *Food Industry*, 2009, no. 6, pp. 30-31. (In Russian).
6. Orlova T.A. *Technological principles of the production of functional dairy products using polysaccharides*. Dr. eng. sci. diss. Stavropol, 2010. 363 p. (In Russian).
7. Okhrimenko O.V., Gorbatova, K.K. and Okhrimenko, A.V. *Laboratory course on chemistry and physics of milk: A tutorial*. St. Petersburg: GIORD Publ., 2005. 256 p. (In Russian).
8. Merkulova N.G., Merkulov M.Yu. and Merkulov I.Yu. *Production control in the dairy industry. A practical guide*. S. Petersburg: Professija Publ., 2010. 506 p. (In Russian).
9. Fedosova A. N. and Kaledina, M.V. Functional dairy products with honey on the basis of fractionation of dairy raw material by pectin. *Modern Problems of Science and Education*, 2014, no. 4. Available at: http://www.science-education.ru/118-14238. (accessed 12 September 2015). (In Russian).
10. Singh H. and Ye A. *Interaction and functionality of milk proteins in food emulsions. In: Milk proteins: From expression to food* (A. Thompson, M. Boland, H. Singh (Eds)). London: Elsevier Applied Science, 2008, pp. 321– 346.
11. Kinsella J.E. Milk proteins: physicochemical and functional properties. *CRC Crit. Rev. Food Sci. Nutr*., 1984, no. 21, pp. 162–197.

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INVESTIGATION OF RHEOLOGICAL CHARACTERISTICS OF CONCENTRATED MILK PRODUCTS WITH A COMPLEX CARBOHYDRATE AND PROTEIN COMPOSITION

A. I. Gnezdilova*, T. Yu. Burmagina, L. A. Kurenkova

Vologda state dairy farming academy by N.V. Vereshchagin,
Schmidt Str. 2, Molochnoye, Vologda, 160555 Russian Federation

* e-mail: academy@molochnoe.ru

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Abstract: When developing new types of dairy products, the formulation of which includes components of both dairy and nondairy origin, it is necessary to study the rheological characteristics of these products. The paper studies the rheological characteristics of concentrated milk products with a complex carbohydrate and protein composition. The study was conducted at the premises of FSBEI HPE "Vologda State Dairy Farming Academy named after N.V. Vereshchagin" (Russia, Vologda Region, Vologda). By using the rotary viscometer "Reotest 2.1" the dependence of shear stress (τ, Pa) and effective viscosity (ηef) on shear rate (γ, s-1) was determined. Freshly made product samples were studied, as well as product samples in the process of storage. In the course of the processing of the obtained data, it was found that freshly made concentrated milk products with sugar, as well as the same products in the process of storage during a period of up to 3 months are classified as "Newtonian" liquids, by virtue of the fact that the dependence of shear stress on shear rate for these samples has a linear character. It is recommended to use a Höppler viscometer for the measurement of viscosity in these samples. Adding starch syrup, malt, or demineralized whey powder to the products results in the deviation of their rheological characteristics from the properties of "Newtonian" liquids. The dependence of shear stress on shear rate for these product samples follows a power-law relationship. On this basis it can be concluded that these products are classified as pseudoplastic bodies. During prolonged storage, consolidation of the structure and an increasing degree of deviation from the properties of Newtonian liquids was observed in all studied samples. This behavior is attributable to the formation of filamentary bridges between the casein micelles, which takes place in the microstructure of concentrated milk products with a complex carbohydrate and protein composition after long storage. These bridges are pseudo-polymers, formed by glucose monomers, and determine the microstructure of the product, its organoleptic and rheological properties. It is recommended to measure the viscosity of the developed products using a rotary viscometer.

Keywords: Rheology, structure, shear stress, effective viscosity, shear rate, "Newtonian" liquids, pseudo-plastic food products

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INTRODUCTION

For the purpose of designing and optimization of manufacturing processes, as well as monitoring quality of food products, it is necessary to study the rheological properties of these products. It is especially important in the process of developing new types of dairy products, the formulation of which includes components of both dairy and nondairy origin [1–3].

There is a view that condensed canned milk products with sugar are classified as weakly structured products and fall in between products with crystallization and condensation structures, and in case of maximum removal of liquid dispersion medium (water) the transition to the crystallization structure is possible [4, 5]. Currently, new conceptions of the microstructure of canned dairy products with sugar emerge, according to which, the general microstructure of freshly made products is characterized by loose, unbound to each other aggregates of casein micelles [6, 10]. After long storage, micelles are bound to each other by pseudo-polymer bridges (cords, strands, filaments).

According to GOST 27709-88 "Canned condensed milk. Viscosity estimation method" [11], it is recommended to measure the viscosity of canned condensed milk products with sugar using a Höppler viscometer. However, the authors of the work [12] demonstrated that the studied samples of canned condensed milk products are classified as abnormally viscous structured liquids, so for the purpose of monitoring viscosity of canned condensed milk products with sugar, the authors recommend to use a rotary viscometer with the preliminary breakdown of structure.

Besides, currently there are widely known concentrated milk products, which are produced from dry components by recombining. To manage the quality of these products, it is necessary to evaluate their structure on the basis of the study of their rheological properties.

The purpose of this work is to study the rheological characteristics of concentrated milk products with a complex carbohydrate and protein composition and to substantiate a method of measuring viscosity of these products.

OBJECTS AND METHODS OF STUDY

The most important rheological characteristics are shear stress τ and effective viscosity ηef. These characteristics are known to depend on shear rate γ.

The object of study in this paper were concentrated dairy products with sugar, with different percentage of substitution of sugar for starch syrup, skim milk powder for dry demineralised whey powder (DWP) or malt, as well as control samples (without substitution).

The studies were conducted at 20°C using the rotary viscometer "Reotest-2.1". The obtained data were processed using Microsoft Excel.

RESULTS AND DISCUSSION

Dependences of shear stress (τ, Pa) on shear rate (γ, s^{-1}) were determined for freshly made product samples, as well as for the product samples during storage. Research results are presented in Tables 1–3.

Table 1 shows that in the control samples of concentrated milk products with the storage period of up to 3 months the determined patterns follow a linear relationship with the correlation coefficient of 0.99, which makes it possible to classify them as Newtonian liquids.

In the control samples, product solidification takes place after 3 months of storage due to the formation of pseudo-polymeric filamentary bridges, which results in the strengthening of the spatial structure. The products behave as pseudoplastic liquids and follow a power-law relationship:

$$\tau = K \cdot \gamma^m, \quad (1)$$

where K is the consistency coefficient; m is the flow behavior index (characterizes the degree of "non-Newtonian" nature of liquids).

For pseudoplastic food products m < 1, for dilatant products m > 1.

The situation is somewhat different in the products with substitution of sugar for starch syrup (Table 2).

As can be seen from Table 2, in freshly made samples with substitution of 30% and 40% of sugar for starch syrup and in the sample with substitution of 30% of sugar, stored during 3 months, the dependence of shear stress on shear rate is linear with the coefficient of correlation of 0.99, that makes it possible to classify these products as "Newtonian" liquids. Based on the data of Table 2, it can be concluded that the product samples with substitution of 50% or more of sugar for starch syrup are classified as pseudoplastic bodies and are described by the equation (1).

It was determined [9, 10], that after long storage, filamentary bridges are formed between casein micelles in the microstructure of concentrated milk products with the substitution of sugar for starch syrup. These bridges are pseudo-polymers, formed by glucose mono-mers, and determine the microstructure of the product, its organoleptic and rheological properties.

The conducted studies showed that there is a critical concentration of starch syrup in condensed milk, which limits its applicability in the manufacturing of the product. The percentage of substitution of sugar for starch syrup should not exceed 40%.

The present work also explores the rheological characteristics of the developed concentrated milk product with sugar, in which skim milk powder is partly substituted for fermented malt [3]. Adding malt makes it possible to increase the content of vitamins and mineral substances in the product.

The equations of the dependence of shear stress on shear rate of the developed product are shown in Table 3.

Table 3 that shows that all the samples follow a power-law relationship and therefore are classified as pseudoplastic food products, which is confirmed by a rather high correlation coefficient equal to 0.99. The analysis of the equations shows that the increase in the percentage of substitution of skim milk powder for malt results in the increase in the consistency coefficient and the decrease in the flow behavior index. It gives evidence of the consolidation of the structure and the increase of non-Newtonian properties of the product.

We also developed a concentrated milk product in which skim milk powder is partially substituted for demineralized whey powder (DWP) [1] and studied its structure. The data on the impact of shear rate on shear stress in the product samples are shown in Table 4.

Table 1. The equations of the dependence of shear stress on shear rate in the control samples of concentrated milk products with sugar at various storage durations

Storage duration	Type of dependence	Correlation coefficient
Freshly made product samples	$\tau = 3.2476\ \gamma$	0.996
3 months	$\tau = 6.0942\ \gamma$	0.992
6 months	$\tau = 7.62 \cdot \gamma 0.8252$	0.991
14 months	$\tau = 10.834 \times \gamma\ 0.8659$	0.999

Table 2. The equations of the dependence of shear stress on shear rate in the samples of concentrated milk products with sugar with different percentage of substitution of sugar for starch syrup during storage

Storage duration	30%	40%	50%	100%
Freshly made product samples	$\tau = 3.49\ \gamma$	$\tau = 3.83\ \gamma$	$\tau = 14.32\ \gamma^{0.7064}$	$\tau = 44.19\gamma^{0.5856}$
3 months	$\tau = 6.06\ \gamma$	$\tau = 21.30\ \gamma^{0.6984}$	$\tau = 33.33\ \gamma^{0.5982}$	$\tau = 44.71\ \gamma^{0.5541}$
14 months	$\tau = 16.78\ \gamma^{0.7299}$	$\tau = 25.29\ \gamma^{0.7521}$	$\tau = 30.82\ \gamma^{0.7172}$	$\tau = 39.51\ \gamma^{0.6579}$

Table 3. The equations of the dependence of shear stress on shear rate in the samples of concentrated milk product with sugar on the percentage of substitution of skim milk powder for fermented malt at various storage durations

Storage duration	5%	10%	15%
Freshly made product samples	$\tau = 4.84\times\gamma^{0.9215}$	$\tau = 6.44\times\gamma^{0.8776}$	$\tau = 9.13\times\gamma^{0.8324}$
3 months	$\tau = 40.11\times\gamma^{0.6034}$	$\tau = 55.17\times\gamma^{0.5311}$	$\tau = 59.47\times\gamma^{0.5246}$
14 months	$\tau = 43.21\times\gamma^{0.6139}$	$\tau = 59.58\times\gamma^{0.5456}$	$\tau = 81.34\times\gamma^{0.4647}$

Table 4. The equations of the dependence of shear stress on shear rate in the samples of concentrated milk product with sugar with different percentage of substitution of skim milk powder for demineralized whey powder at various storage durations

Storage duration	10%	20%	25%
Freshly made product samples	$\tau = 6.74\times\gamma^{0.8521}$	$\tau = 7.7954\times\gamma^{0.8254}$	$\tau = 11.796\times\gamma^{0.7758}$
3 months	$\tau = 9.73\times\gamma^{0.7982}$	$\tau = 10.674\times\gamma^{0.7867}$	$\tau = 12.849\times\gamma^{0.7612}$
14 months	$\tau = 13.21\times\gamma^{0.7139}$	$\tau = 26.3\ \gamma^{0.5762}$	$\tau = 29.3\ \gamma^{0.4962}$

The determined patterns indicate that the product samples are classified as pseudoplastic fluids, which is confirmed by a rather high correlation coefficient of 0.99. It follows from the equations that the increase in the percentage of substitution of skim milk powder for demineralized whey powder results in the increase in the consistency coefficient (*k*) and the decrease in the flow behavior index (*m*). It gives evidence of the consolidation of the structure, an increase of its viscosity and of non-Newtonian properties in the presence of whey proteins, which constitute the major fraction of demineralized whey powder.

To describe the dependence of effective viscosity of shear rate in the studied samples, a well-known equation was used [13]:

$$\eta_{ef} = K\cdot\gamma^{-m}, \qquad (2)$$

where η_{ef} is the effective viscosity Pa·s; γ is the deformation frequency, s^{-1}; *K* is the consistency coefficient; *m* is the rate of structure breakdown.

The equations of the dependence of efficient viscosity on shear rate of the developed product are shown in Table 5.

The data of Tables 5–6 confirm that control samples of concentrated milk products and the products with substitution of 30% of sugar for starch syrup during storage for up to three months are classified as Newtonian liquids. Other dependencies follow a power-law relationship, which confirms that the samples are classified as pseudoplastic fluids (Tables 7–8).

The exponent in the equations, presented in Tables 5–8, describes the rate of structure breakdown. For a more complete description of the behavior of the products, the rate of structure breakdown was analyzed and presented in Fig. 1 and 2.

As can be seen from Fig. 1, a dramatic increase in the rate of structure breakdown is observed in all product samples in case of the substitution of 40% or more of sucrose for starch syrup.

More intense structure breakdown was observed in the products in which a part of skim milk powder was substituted by malt (Fig. 2). In freshly made product with the proportion of substitution of 5% the value of m remains almost unchanged, after which slightly increases. After three months of storage, the rate of structure breakdown in the control samples slightly increases, as compared to the freshly made product. Intense structure breakdown is observed in the samples after 3 months of storage in case of the substitution of 5% or more of skim milk powder for malt.

Table 5. The equations of the dependence of effective viscosity on shear rate in the control samples of concentrated milk product with sugar at various storage durations

Storage duration	Type of dependence	Correlation coefficient
Freshly made product samples	$\eta_{ef} = 2.6239\ \gamma$	0.995
3 months	$\eta_{ef} = 6.561\ \gamma$	0.993
6 months	$\eta_{ef} = 7.623\ \gamma^{-0.175}$	0.991
14 months	$\eta_{ef} = 11.102\ \gamma^{-0.157}$	0.959

Table 6. The equations of the dependence of effective viscosity on shear rate in the samples of concentrated milk product with sugar and starch syrup at various storage durations

Storage duration	30%	40%	50%	100%
Freshly made product	$\eta_{ef} = 3.29\ \gamma$	$\eta_{ef} = 10.309\gamma^{-0.234}$	$\eta_{ef} = 14.63\gamma^{-0.311}$	$\eta_{ef} = 46.223\gamma^{-0.412}$
3 months	$\eta_{ef} = 7.8768\ \gamma$	$\eta_{ef} = 21.304\gamma^{-0.289}$	$\eta_{ef} = 32.189\gamma^{-0.325}$	$\eta_{ef} = 42.557\gamma^{-0.423}$
14 months	$\eta_{ef} = 16.097\ \gamma^{-0.25}$	$\eta_{ef} = 23.65\ \gamma^{-0.321}$	$\eta_{ef} = 29.063\gamma^{-0.333}$	$\eta_{ef} = 0.378\gamma^{-0.444}$

Table 7. The equations of the dependence of effective viscosity on shear rate in the samples of concentrated milk product with sugar, depending on the percentage of substitution of skim milk powder for fermented malt, at various storage durations

Storage duration	5%	10%	15%
Freshly made product samples	$\eta_{ef} = 4.835\ \gamma^{-0.079}$	$\eta_{ef} = 6.436\gamma^{-0.122}$	$\eta_{ef} = 9.130\gamma^{-0.168}$
3 months	$\eta_{ef} = 40.114\gamma^{-0.386}$	$\eta_{ef} = 55.171\gamma^{-0.454}$	$\eta_{ef} = 59.474\gamma^{-0.475}$
14 months	$\eta_{ef} = 43.210\gamma^{-0.397}$	$\eta_{ef} = 59.580\gamma^{-0.469}$	$\eta_{ef} = 81.344\gamma^{-0.535}$

Table 8. The equations of the dependence of effective viscosity on shear rate in the samples of concentrated milk product with sugar, depending on the percentage of substitution of skim milk powder for demineralized whey powder, at various storage durations

Storage duration	10%	20%	25%
Freshly made product samples	$\eta_{ef} = 6.774\ \gamma^{-0.151}$	$\eta_{ef} = 7.868\ \gamma^{-0.181}$	$\eta_{ef} = 11.796\ \gamma^{-0.224}$
3 months	$\eta_{ef} = 9.725\gamma^{-0.202}$	$\eta_{ef} = 10.674\ \gamma^{-0.213}$	$\eta_{ef} = 12.849\gamma^{-0.240}$
14 months	$\eta_{ef} = 23.210\gamma^{-0.322}$	$\eta_{ef} = 26.325\gamma^{-0.424}$	$\eta_{ef} = 31.344\gamma^{-0.475}$

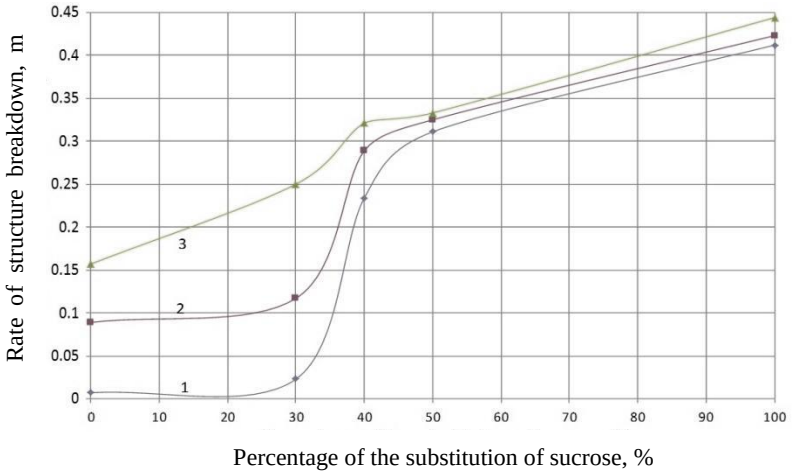

Fig. 1. The dependence of the rate of structure breakdown on the mass fraction of starch syrup: (1) freshly made product; (2) after 3 months of storage; (3) after 14 months of storage.

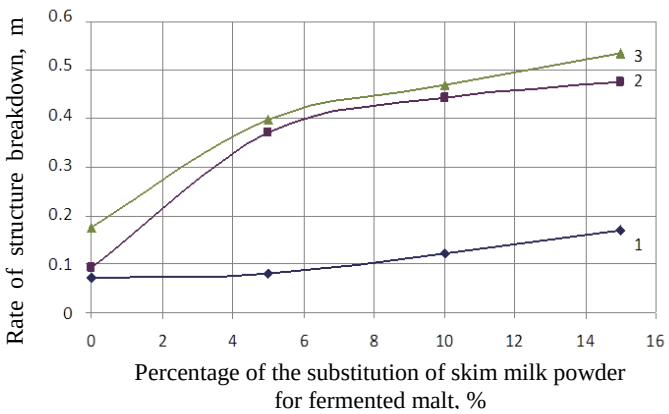

Fig. 2. The dependence of the rate of structure breakdown on the percentage of substitution of skim milk powder for fermented malt: 1 – freshly made product; 2 – after 3 months of storage; 3 – after 14 months of storage.

CONCLUSIONS

1. Freshly made concentrated milk products with sugar, and the same products in the process of storage during a period of up to 3 months, are classified as "Newtonian" liquids. It is recommended to use a Höppler viscometer for the measurement of viscosity in these samples.

2. Changing the composition of the developed products results in the deviation of their parameters from the properties of Newtonian liquids. These products are classified as pseudoplastic bodies.

3. During prolonged storage, consolidation of the structure and increased deviation from the properties of Newtonian liquids was observed in all studied samples.

4. It is recommended to measure the viscosity of the developed products using a rotary viscometer.

5. The obtained results were used to estimate the duration of storage of the developed products.

REFERENCES

1. Gnezdilova A.I., Kulenko V.G. and Glushkova A.V. *Sposob proizvodstva molokosoderzhashchego kontsentrirovannogo produkta s sakharom* [Method for production of concentrated milk-containing product with sugar]. Patent RF, no. 2407347, 2009.
2. Gnezdilova A.I., Kulenko V.G., Vinogradova Yu.V., Kurenkova L.A. and Burdeynaya O.S. *Sposob proizvodstva sgushchennogo molochnogo produkta s sakharom* [Method for production of condensed milk product with sugar]. Patent RF, no. 2490920, 2012.
3. Gnezdilova A.I., Sharova T.Yu. and Kulenko, V.G. *Sposob proizvodstva molokosoderzhashchego kontsentrirovannogo produkta s sakharom* [Method for production of concentrated milk-containing product with sugar]. Patent RF, no. 2525666, 2012.
4. Zavarin Yu.A. and Chekulaeva L.V. Structure formation in the production of condensed milk with sugar. *Dairy Industry*, 1977, no. 9, pp. 11–13. (In Russian).
5. Dobriyan E.I. and Chekulaeva L.V. Changing of the colloid-dispersed properties of milk proteins in the process of the production of condensed milk with sugar using a flow process. *Dairy Industry*, 1985, no. 4, pp. 27–29. (In Russian).
6. Araki K., Takasawa R. and Yoshikawa I. Design, fabrication, and properties of macroscale supramolecular fibers consisted of fully hydrogen-bonded pseudo-polymer chains. *Chemical Communications*, 2001, no. 18, pp. 1826–1827.
7. Roy N., Saha N., Kitano T. and Saha P. Novel hydrogels of PVP-CMC and their swelling effect on viscoelastic properties. *J. of Applied Polymer Science*, 2010, vol. 117, no. 3, pp. 1703–1710.
8. Jones O.G. *Fabrication of protein-polysaccharide particulates through thermal treatment of associative complexes*. PhD dissertation. University of Massachusetts-Amherst, 2009. 287 p.
9. Smykov I.T., Gnezdilova A.I., Vinogradova Yu.V. and Kurenkova L.A. The impact of long-term storage on the structure of condensed milk. *Storage and Processing of Farm Products*, 2014, no. 4, pp. 9–14. (In Russian).
10. Smykov I.T., Gnezdilova A.I., Vinogradova Yu.V. and Kurenkova L.A. The impact of temperature on the rheological properties of condensed milk with starch syrup. *Bulletin of the Russian Academy of Agricultural Sciences*, 2014, no. 3, pp. 68–71. (In Russian).
11. *GOST 27709-88. Konservy molochnye sgushchennye. Metod izmereniya vyazkosti* [State Standard 27709-88. Canned condensed milk. Viscosity estimation method]. Moscow: Standartinform Publ., 2009. 5 p.
12. Pirogov A.N., Pirogova N.A. and Shilov A.V. Method for determining the viscosity of canned milk products using a rotary viscometer. *Storage and Processing of Farm Products*, 2006, no. 4, pp. 46–48. (In Russian).
13. Machikhin Yu.A (Ed.). *Rheometry of food raw materials and products: a reference guide*. Moscow: Agropromizdat Publ., 1990. 271 p. (In Russian).

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AN APPROACH TO THE CHOICE OF ALTERNATIVES OF THE OPTIMIZED FORMULATIONS

O. N. Musina[a,*], P. A. Lisin[b]

[a] Siberian Research Institute of Cheesemaking,
Sovetskoi Armii Str. 66, Barnaul, 656016 Russian Federation

[b] Omsk State Agrarian University named after P.A. Stolypin,
Institutskaia Square 2, Omsk, 644008 Russian Federation

* e-mail: musinaolga@gmail.com

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Abstract: The scientific direction of the food product designing with a specified set of indicators of nutritional value is currently topical in the world. The mathematical bases for solving formulation problems are well studied. The problems concerning the multi-objective optimization of formulations for multicomponent products are frequently met. At the same time, only one, the most important, criterion is to be optimized, and the rest criteria act as the additional constraints, since the intersection of sets of the optimal solutions for all single-objective problems usually turns out to be an empty set. As a result, several formulation alternatives are obtained, which are optimized according to any single or several (but not all) criteria. The purpose of the work is to theoretically substantiate a universal approach to choosing out of the set of alternatives of the optimized formulations of food products. The authors suggest reasserting the problem of choice as the problem of assessing the degree of the product's composition conformance with the recommended physiological standards. When assessing the balanced state of the formulation alternatives, the conclusions are made by comparing the relative degree of conformance of the generalized Harrington's desirability function value with the reference standard, and not of the absolute value of the generalized desirability function. To select from a variety of the optimized formulation alternatives of the multicomponent food products, it is proposed to use the following 6 criteria: a balanced state index of the product's macronutrient composition; a balanced state index of the vitamin composition; a balanced state index of the mineral composition; a balanced state index of the amino acid composition; a balanced state index of the fatty acid composition; and a balanced state index of the energy value. Wherein, it is proposed to calculate the generalized Harrington's desirability function as a geometric mean of the partial balanced state indices. A universal approach is suggested for making a choice out of the variety of the optimized formulation alternatives. At the same time, the subjectivity is eliminated in choosing the nomenclature and numerical values of the physical indicators of quality of the compared variants of multicomponent products.

Keywords: Formulation, food product, multicomponent product, mathematical design of a product composition, optimization of formulation

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INTRODUCTION

Since the human diet consists of a variety of products, this generally compensates for the shortage of any substances in a daily diet, though it is still desirable to provide people with food products balanced in chemical composition. Therefore, the design of food products with a given set of nutritional value indicators is currently topical.

The fundamental principles for designing products and diets with the specifiable nutritional value are inherent in the works written by the academics I.A. Rogov and N.N. Lipatov (junior). They stated the basic principles of composition design of the balanced products with the required set of indicators and of the diets containing such products [4–6, 13]. Later, this methodology was developed in the works by A.B. Lisitsin, E.I. Titov, S.B. Iudina, Iu.N. Nelepov, Yu.A. Ivashkin, A.M. Brazhnikov, G.I. Kas'ianov, A.E. Krasnov, A.T. Diplock, A. Wollen, Ruguo Hu, and other scientists [1, 3, 8, 19, 21]. The method of neural networks is proposed by the A.G. Khramtsov School [16].

Currently, this trend retains its relevance and not only in the scientific, but also in the applied aspects. For many years, the domestic (Fig. 1) and foreign scientists [17–21, 22–26] have been working on solving this problem including through the methods of mathematical modeling [1, 2, 7, 9–10, 20]. The basic methodological principles and approaches have been developed for designing quality and balanced state of the food products according to the main macro- and micronutrients [14, 15].

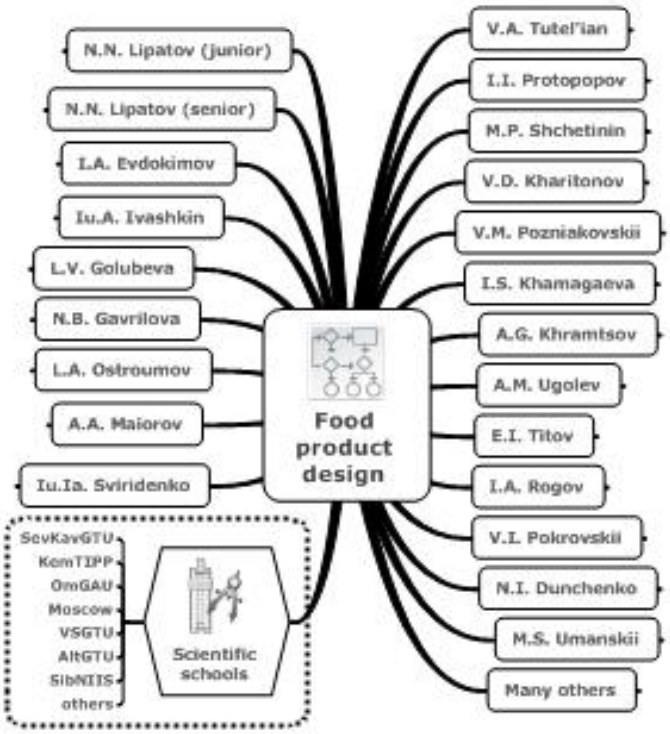

Fig. 1. The Russian leaders in the trend of the food product design.

A key objective in designing the multicomponent food products is to establish a preferred set and ratio of components. The difficulty in solving a formulation problem consists in frequent usage of a large number of ingredients in designing, while the optimization can involve more than two criteria.

At the present stage of the science and technology development, it is impossible to solve this problem without involving the formal approaches, wherein the numerical information is used and the desired properties of composition are provided. Moreover, the solution of this problem is very complicated without applying appropriate software, since the manual solution of the system of linear equations and inequalities with a large number of variables is of significant difficulty, at which the computational errors cannot be excluded [9, 10].

The mathematical framework for solving such problems is well known. Among the various models of the technological processes, the so-called linear models hold a specific place, i.e. the models, wherein the mathematical relationships (equalities or inequalities) are linear with respect to all the variables included into the model. The essence of such problems consists in choosing according to the specified criterion an optimal alternative out of the variety of possible alternatives of the process studied. The development of general methods for their solution was started in 1939 by the Russian mathematician and academician L.V. Kantorovich. Later, in the works by the American scientist G. Dantzig, this method was called a simplex method. The simplex method is a universal method for solving linear programming problems. The simplex method is based on the algorithm of simplex transformations of a system, which is supplemented by the rule ensuring a transition to the best basic solution, and not just to the any solution. That is, at first, an admissible alternative is obtained, which satisfies all the constraints, but it is not necessary to be the optimal alternative (an initial basic solution). The optimality is achieved through a consistent improvement of the initial alternative of a certain number of steps (iterations).

The application of the simplex method in the dairy industry was considered by Yu.P. Markin and Yu.A. Ivashkin. To implement the simplex method, either the specially written programs (KSIMP, ESIMP, ISIMP) or the universal mathematical software packages (MathCAD, Maple) are applied. The Solver Microsoft Excel add-in uses the Generalized Reduced Gradient, a nonlinear optimization algorithm developed by Leon Lasdon and Allan Waren. The simplex method algorithms for solving linear and integer problems with constraints are developed by John G. Watson and Daniel Fylstra [7]. In the United States, this approach with reference to designing the food product formulations is perfectly described in the book by Ruguo Hu [21]. The work abounds in practical examples from various areas of the food industry, is well illustrated, and, in our opinion, is of great applied interest even now.

It should be noted that in practice, we often meet problems that require finding the best solution in the presence of different irreducible criteria of optimality – the problems of multi-objective optimization. For example, when designing a multicomponent dairy product, it is necessary to take into account such

frequently controversial facts as the quantity and ratio of the essential amino acids, the balance of fatty acids, the low energy value, the minimum cost, the technologically or organoleptically limited content of vegetable components, and many others. In other words, there are several objectives that cannot be reflected by a single criterion. Some partial criteria may be controversial, others may act in single direction, and others can be indifferent to each other.

Usually, in order tocope with such a situation, we have to make the following compromises: the optimization of single criterion recognized as the most important one, whereas all the rest criteria act as the additional constraints (in particular, the authors have implemented this approach in the "Minimum-Maximum" computer program); the ordering of a given set of criteria and a consistent optimization of each of them.

Theoretically, in an ideal case, it is possible to search for such a solution that belongs to the intersection of the sets of optimal solutions for all single-objective problems. However, it is known that such an intersection usually appears to be an empty set [7]. Therefore, a set of effective solutions should be considered at the time when the optimization means the enhancement of some indicators provided that the others do not deteriorate.

As a result, we obtain a set of alternatives optimized according to any single criterion of formulations. The purpose of this work is to theoretically substantiate a universal approach to choosing out of the set of alternatives of the optimized formulations of food products.

OBJECTS AND METHODS OF STUDIES

The problem of mathematical design of the food product formulations can be interpreted as the problem concerning the optimal use of the limited resources. The essence of the formulation optimization for a food product consists in finding such a solution $\overline{X} = (x_1, x_2, ...x_n)$, wherein x_j are the formulation components, which would be the best to take account of the optimality criteria. An optimality criterion can be represented for example by a minimum cost, a maximum macronutrient content, etc. In addition, a number of conditions are superimposed on the solution, i.e. the choice $\overline{X}$ is carried out from a certain area of possible solutions *R*. Within the framework of this article, the term "to optimize a formulation of a food product" means to solve a problem of the following type: *max(min)* f $(\overline{X})$, $\overline{X} \in R$, where $f(\overline{X})$ is an objective function (the mathematical notation of an optimality criterion).

In solving an optimization problem, an uncertain system, i.e. a set of non-negative solutions of a system of linear equations, is of practical interest. From a technological point of view, this means finding a set of formulation alternatives, which correspond to the predetermined constraining conditions. It should be emphasized that the objective of the present article involves not just a discussion of the methods for solving optimization problems, but the scientific substantiation of the methodology for choosing a product formulation out of the set of possible optimized formulation alternatives.

One of the known methods for choosing an optimal formulation out of the set of formulation alternatives consists in using the generalized Harrington's desirability function. The construction of a generalized Harrington's desirability function is based on the idea of converting the natural values of partial responses into a dimensionless scale of desirability or preferability.

The partial response value converted into a dimensionless scale of desirability is denoted by d_i (i =1, 2, …, n) and called a partial desirability. The formulation of a designed product should be evaluated in the units of partial desirability function (d_i). All the partial desirability functions d_i are to be combined into the generalized desirability function. The generalized index of desirability (D_i) is calculated as a geometric mean according to the following formula:

$$D_i = \sqrt[n]{\prod_{i=1}^{n} d_i} = \sqrt[n]{d_1 \cdot d_2 ... d_n}\ . \qquad (1)$$

The scale of desirably has a range from zero to one. The $d_i = 0$ value corresponds to the absolutely unacceptable level of this property, and the $d_i = 1$ value corresponds to the best value of the property. The $d_i = 0.37$ value usually corresponds to the lower boundary of the permissible values.

The desirability function reflects the dependence of assessments or indicators of the desirability (d) on the dimensionless indicators (y), into which the dimensional (physical) quality indicators are converted. If the top or bottom unilateral constraints are imposed on a parameter, then the desirability function is to be calculated according to the following formula:

$$d_i = \exp(-\exp(-y_i)). \qquad (2)$$

If the optimization parameters possess the bilateral constraints, i.e. they are of the $y_{min} \le y \le y_{max}$ form, then the desirability function is to be calculated according to the following formula:

$$d_i = \exp\left[-|y_i|^n\right], \qquad (3)$$

where n is a positive figure.

By choosing different values of n, it is possible to specify different curvature of the desirability curve. This provision allows taking into account the particular importance of the individual parameters of optimization: therefor n will make a big value, and thus, a small change of the optimization parameter near the limits will correspond to the sharp change in desirability.

The dimensionless parameter y_i is to be calculated according to the following formula:

$$y_i = \frac{2y - y_{max} + y_{min}}{y_{max} - y_{min}}. \qquad (4)$$

The exponent n is to be calculated by specifying the value d (preferably in the range of $0.6 \le d \le 0.9$) followed by the calculation of y_i according to the expression (4). Then the exponent is to be calculated according to the following formula:

$$n = \frac{\ln \ln \frac{1}{d}}{\ln |y_i|}. \quad (5)$$

The conversion of the values of dimensional (physical) indicators (x) of the product quality into the dimensionless indicators (y) in a linear relationship there between can be carried out according to the following formula:

$$y = a_0 + a_1 x, \quad (6)$$

where a_0 and a_1 are the equation coefficients.

Having taken the logarithm of the equation (2) for the second time, we will obtain as follows:

$$\ln \ln \frac{1}{d_i} = -y, \text{ or } y = \ln \frac{1}{\ln \frac{1}{d_i}}. \quad (7)$$

Let us substitute the values of y (7) into the equation (6):

$$a_o + a_1 x = \ln \frac{1}{\ln \frac{1}{d_i}}. \quad (8)$$

Let us set up a system of equations for the boundary values of the desirability indicators d_1 and d_2 (a distinct and satisfactory value):

$$\begin{cases} a_o + a_1 x_1 = \ln \frac{1}{\ln \frac{1}{d_1}} \\ a_o + a_1 x_2 = \ln \frac{1}{\ln \frac{1}{d_2}} \end{cases}. \quad (9)$$

The values of the partial desirability indicators d_1 and d_2 are to be chosen independently (for example, a distinct value equal to $d_1 = 0.8$; and a satisfactory value $d_2 = 0.37$). By simultaneously solving the system of equations (9), the values of the a_o and a_1 coefficients are to be found.

The obtained solution should result in the equation of linear dependence between the studied indicator (x) and the dimensionless values (y). By using this equation, the value of y can be found for any value of x, followed by the calculation of the partial indicator of desirability d according to the formula (2) and of the generalized indicator of desirability D_i according to the formula (1).

According to the authors, the major challenge in using the Harrington's desirability function when solving a problem concerning the choice of the product formulation out of the set of possible alternatives of the optimized formulations is the lack of a common approach and, therefore, the subjectivity in selecting the nomenclature and numeric values of the physical quality indicators of the compared product alternatives. The following describes the authors' approach to solving this problem.

RESULTS AND DISCUSSION

For a clear understanding of the properties of the object studied (a multicomponent food product), it is necessary to identify the relations between the elements of such an object. The aggregate of the elements' interrelations ensuring the integrity of the system is called the system structure. The model of the structure of a designed product is a list of the relations being essential for the solution of a specific problem. For example, generally, when optimizing the formulation of a multicomponent product, the raw material wasted while moving through the pipelines is not taken into account, though such losses exist. At the same time, the loss of the nutritional value of a raw material during the mechanical or thermal treatment can be considered. It is possible to construct the block diagrams, wherein only the elements and their interrelations as well as the difference between the elements and the relations are marked. Such diagrams are called graphs.

The diagram demonstrating the interrelations between the elements of the designed product with a specified composition is shown in Fig. 2 in the form of a planar graph with 7 elements and 6 relations. This interrelation determines the energy, nutrition, and biological value of a multicomponent product. The change in values (mass fractions) of one of the formulation mixture elements leads to the change in values of the interrelated elements. For example, the vitamins-related optimization of a product will lead to the change in its energy value as well as in the formulation-based, mineral, fatty acid, and amino acid composition.

In its general view, the design of a multicomponent product formulation involves the implementation of the stages shown in Fig. 3. This article observes only the fourth stage.

The implementation of the third stage is possible both by using the following computer programs developed by the authors: "Minimum-Maximum" (Certificate of Registration No. 2010612628 of April 15, 2010), "Ideal Protein" (Certificate of Registration No. 2010616153 of September 17, 2010), "Design of Formulations" (Certificate of Registration No. 2011611470 of February 14, 2011), and through any other automated means for calculating and optimizing the formulations, which are currently known in a sufficient quantity.

As known, a diet should contain such an amount of energy and nutrients, which corresponds to a daily rate of the physiological standard for a certain group of the population. The content of a micro- and macronutrient, both below and above the permissible rates, indicates the unbalanced state of the diet. The problem of formulation optimization consists in choosing the components and determining their ratios, which ensure the maximum conformance of the nutrients' mass fractions with a physiological standard.

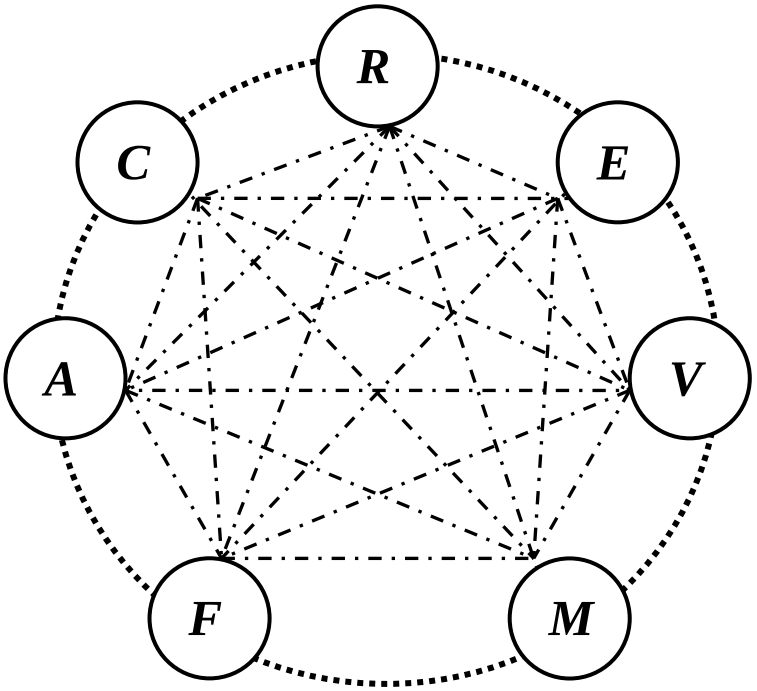

Fig. 2. A planar graph of the formulation design of a multicomponent food product: *R* – the macronutrient composition of the designed product; *A* – the amino acid composition of the product; *F* – the fatty acid composition of the product; *C* – the cost of the product; *E* – the energy value of the product; *V* – the vitamin composition of the product; *M* – the mineral composition of the product.

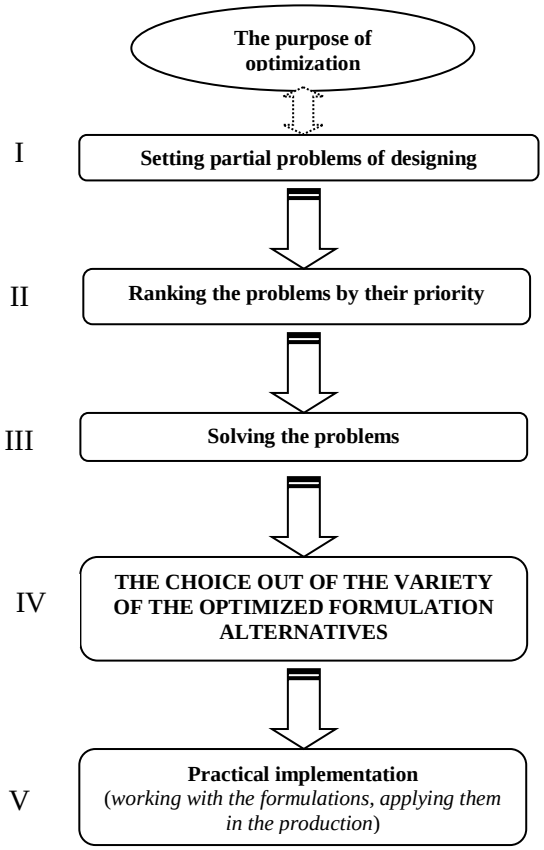

Fig. 3. The stages of a multicomponent product design.

All the developed countries provide the recognized norms of such physiological standards. For example, in the United States, the Food, Nutrition and Consumer Services Division established in the U.S. Department of Agriculture and its agencies (www.cnpp.usda.gov, www.fns.usda.gov) are engaged in dealing with these issues. In Russia, there are the "Standards of Physiological Needs of the Energy and Nutrients for Various Groups of the Population of the Russian Federation" approved in 2008 by the Federal Service for Supervision of Consumer Rights Protection and Human Welfare. The "Standards" are the effective national regulations that define the values of the standard rates of consumption of the essential nutrients and energy sources, which are physiologically substantiated by the modern science of nutrition. The World Health Organization also regularly publishes the recommendations on diet and health [12].

The theoretical calculations and practical experiments show that in most cases it is impossible to achieve simultaneously by all indicators the standard level of balance when designing the multicomponent food products. This complicates the task of choosing one or more multicomponent products out of the variety of formulation alternatives optimized according to a certain partial indicator or even a set of indicators. Therefore, we suggest reasserting the problem of choice as the problem of assessing the degree of the product's composition conformance with the recommended physiological standards.

To make a scientifically substantiated choice out of the variety of the optimized formulation alternatives of the multicomponent food products, it is proposed to use the following 6 criteria:

– A balanced state index of the product's macronutrient composition BNCI (will be denoted in the formulas as U_n);
– A balanced state index of the vitamin composition BVCI (U_v);
– A balanced state index of the mineral composition BMCI (U_m);
– A balanced state index of the amino acid composition BACI (U_a);
– A balanced state index of the fatty acid composition BFCI (U_f);
– A balanced state index of the energy value BEVI (U_e).

These partial criteria allow us to comprehensively assess the level of the formulation balanced state of a product designed for a specific group of the population.

The criteria calculation is carried out as the geometric mean. Thus, a partial criterion for assessing the balanced state of the BNCI macronutrient composition is to be calculated according to the following formula:

$$U_n = \sqrt[3]{\prod_{j=1}^{3}\left(\frac{N_j}{N_{ej}}\right)}, \qquad (10)$$

where N_j is the content of the j-th macronutrient (fat, protein, carbohydrate) in the formulation of a product, g; N_{ej} is the standard of physiological needs of the j-th macronutrient, g; *3* is the number of standardized macronutrients (proteins, fats, carbohydrates).

The balanced state index of the vitamin composition is to be calculated according to the following formula:

$$U_v = \sqrt[n]{\prod_{j=1}^{n}\left(\frac{V_j}{V_{ej}}\right)}, \qquad (11)$$

where V_j is the content of the j-th vitamin in the formulation of a product, mg; V_{ej} is the standard of physiological needs of the j-th vitamin, mg; n is the number of standardized vitamins (the list depends on a group of the population).

The balanced state index of the mineral composition BMCI is to be calculated according to the following formula:

$$U_m = \sqrt[n]{\prod_{j=1}^{n}\left(\frac{M_j}{M_{ej}}\right)}, \qquad (12)$$

where M_j is the content of the j-th mineral substance in the formulation of a product, mg; M_{ej} is the standard of physiological needs of the j-th mineral substance, mg; n is the number of standardized mineral substances (the list depends on a group of the population).

The balanced state index of the amino acid composition BACI is to be calculated according to the following formula:

$$U_A = \sqrt[8]{\prod_{j=1}^{8}\left(\frac{A_j}{A_{ej}}\right)}, \qquad (13)$$

where A_j is the content of the j-th essential amino acid in the formulation of a product, mg/g of protein; A_{ej} is the content of the j-th essential amino acid in 100 g of ideal protein, mg/g of protein; 8 – is the number of essential amino acids.

The balanced state index of the fatty acid composition BFCI is to be calculated according to the following formula:

$$U_F = \sqrt[n]{\prod_{j=1}^{n}\left(\frac{F_j}{F_{ej}}\right)}, \qquad (14)$$

where F_j is the content of the j-th fatty acid in the formulation of a product, mg%; F_{ej} is the physiologically substantiated standard of the j-th fatty acid, mg%; n is the number of the taken into account fatty acids.

A partial criterion for assessing the balanced state of the product formulation according to the energy value:

$$U_E = \frac{E_J}{E_{EJ}}, \quad (15)$$

where E_j is the energy value of 100 g of a product, kcal; E_{ej} is the desired level of the energy value of 100 g of a product, kcal.

Theoretically, the ideal formulation will be the product formulation with all indices equal to "1": $U_n = 1$, $U_v = 1$, $U_m = 1$, $U_f = 1$, $U_a = 1$, $U_e = 1$. Thus, we propose to perform the calculation of the generalized Harrington's desirability function (D_i) as the geometric mean of the partial balanced state indices:

$$D_i = \sqrt[6]{\prod_{i=1}^{6} U_i} = \sqrt[6]{U_n \cdot U_v \cdot U_m \cdot U_f \cdot U_a \cdot U_e}. \quad (16)$$

The ideal balanced state of the formulation is achieved at $D_i = 1$. Since the criteria calculation is carried out basing on the daily rate of the physiological needs of nutrients and energy, then theoretically, the "1" can be achieved only by analyzing a daily human diet for its balanced state. When assessing the balanced state of the formulation alternatives, the conclusions should be made according to the comparison of the relative degree of conformance of the D_i value with the standard, and not of the absolute value of the generalized desirability function.

Let us consider a specific example. The task is to design the formulation of a dish for the out-of-school feeding of the children aged 7-11 years – cottage cheese with fresh berries. The "Standards of Physiological Needs of the Energy and Nutrients for Various Groups of the Population of the Russian Federation" are taken as a standard of the children's needs. The initial stage of designing is the calculation of the nutritional composition, i.e. the substantiation of the nomenclature of the ingredients and of their quantitative ratio. In this example, the nutritional compositions are based on the low-fat cottage cheese of the industrial production. Given the prevalence among children and adolescents of the nutritional imbalance, including the lack of essential micronutrients in diet [11], the most important task is to increase the nutritional value of the composition. With regard to the dairy products, the major method for enhancing the nutritional value is the fortification with such essential nutrients as vitamins, minerals, dietary fibers, probiotic and prebiotic components. A common thing is to fortify products with a vitamin-mineral premix containing a complex of vitamins and mineral substances, the lack of which is of top priority for this region. Since we design a dish to feed children outside the organized teams (at home), another way to enhance the nutritional and biological value of products is considered to be more substantiated, which is a special selection of raw materials and the scientific substantiation of the formulations. In developing the formulations of dairy products for the schoolchildren feeding, our task is to limit the content of fat and easily digested carbohydrates as well as the sodium chloride in the dairy products [11]. Therefore, our nutritional compositions based on the cottage cheese do not contain salt, while a stevia extract (stevioside) can be applied as a sweetener. Such products as sour cream and cream are limitedly used in the children's diet due to a sufficiently high content of fat. Therefore, in order to improve the organoleptic characteristics of the low-fat cottage cheese and the nutritional balance of the designed nutrient compositions, their content is enriched with the following fresh berries: cowberry, blueberry, bilberry, cranberry, and black currant.

Using the simplex method, we carried out the formulation optimization of the nutritional compositions of the low-fat cottage cheese with fresh berries. The optimization criteria were the maximum balanced stated indices of BNCI, BVCI, BMCI, BACI, and BEVI. The balanced state indices were calculated according to the formulas (11–16) based on the a priori information on the ingredients content retrieved from the official reference books. As a result, 5 formulation alternatives have been obtained (Table 1).

Table 1. Formulation alternatives

Ingredients	Variants of nutritional compositions, % (without losses)				
	1	2	3	4	5
Low-fat cottage cheese	75.0	95.9	60.0	85.0	60.0
Fresh berries	25.0 (black currant)	1.3 (cowberry) + 1.5 (bilberry) + 1.3 (blueberry)	9.4 (bilberry) + 30.6 (black currant)	8.1 (cowberry) + 6.9 (cranberry)	2.7 (bilberry) + 37.3 (black currant)
BNCI	0.027	0.022	0.027	0.023	0.027
BEVI	0.034	0.036	0.032	0.033	0.033
BVCI	0.090	0.044	0.112	0.055	0.108
BMCI	0.065	0.041	0.063	0.049	0.068
BACI	1.570	1.900	1.320	1.640	1.350
Generalized desirability function *Di*	0.097	0.077	0.096	0.078	0.098

The difficulty of choosing out of five variants consists in the fact that the formulations appear to be balanced according to different indicators – one composition is better in vitamin content, another – in mineral, etc.; there is no evident leader. Using out methodology, it is possible to substantiate the choice of a specific formulation out of the number of alternatives. The calculation of the generalized Harrington's desirability function taking into account the proposed by us partial criteria (balanced state indices) has shown that the integral level of the formulation balance according to the fifth variant is the maximum out of the variants analyzed: 0.098. Of course, this figure is far from "1", but, nevertheless, now the choice among the variety of alternatives becomes objective and substantiated.

Such a nutritional composition contains 0.5% of fat, 11.4% of protein, 7.0% of carbohydrates, and 76.5 kcal per 100 g. The ratio in the composition of the polyunsaturated fatty acids, monounsaturated fatty acids, and saturated fatty acids is 1.0 : 0.6 : 0.1. One portion of the product (200 g) according to the fifth variant satisfies the daily demand of a schoolboy/schoolgirl aged 7–10 years for vitamin A by 20.6%, C – by 226.0%, B3 – by 95.4%, B2 – by 24.2%, and B6 – by 17.4%; for micro- and macro-elements such as potassium – by 42.6%, calcium – by 16.8%, phosphorus – by 24.6%, magnesium – by 18.8%, and iron – by 10%. There is no shortage of any of the essential amino acids.

Thus, when designing the formulations for the out-of-school feeding of the children aged 7–11 years, it can be recommended to take as a basis the composition of the low-fat cottage cheese with the addition of the fresh bilberry and black currant as well as of the stevioside, a natural sweetener, according to one's taste. It should be noted herein that the given example observes only the initial stage of the formulation designing – the nomenclature and the ratio of ingredients are substantiated. However, this does not mean the end of formulation designing. Although such a composition is considered to be balanced according to the nutritional content, it is still required to complete the rest stages of the formulation designing before making a final decision. In particular, it is necessary to assess the organoleptic characteristics of the dish (it is possible that the ratio of 60% of cottage cheese and 40% of berries would fail the tasting assessment), the technological compatibility of ingredients, the need for the additional preparation of the vegetable raw materials and its influence on the raw material content, the stage and the form of adding the fruit fillers, the storage capabilities of the product, etc.

However, the purpose specified by the authors has been reached – the choice out of the variety of the formulation alternatives optimized according to any criterion becomes understandable and is based on the formalized clear scientific data.

CONCLUSION

The universal approach, proposed by the authors, to the choice out of the variety of the optimized formulation alternatives allows us to eliminate the subjectivity when selecting the nomenclature and the quantitative values of the physical indicators of quality of the compared variants of products, to integrally assess the efficiency of optimization, and to scientifically substantiate the choice of a specific formulation out of several variants being optimal according to different criteria.

REFERENCES

1. Borisenko A.A., Kas'yanov G.I., Borisenko A.A. and Zaporozhskiy A.A. Designing the balanced multicomponent food products based on their nutritional composition. *Transactions of Higher Educational Institutions. Food Technology*, 2005, vol. 3, no. 2, pp. 106–107. (In Russian).
2. Donskikh N.V., Muratova E.I., Tolstykh S.G. and Leonov D.V. The development of an automated information system for calculating and optimizing formulations. *Transactions of Higher Educational Institutions. Food Technology*, 2011, vol. 3, no. 2, pp. 122–123. (In Russian).
3. Ivashkin Yu.A. and Nikitina M.A. The modelling and optimization of an adequate nutrition taking into account the individual medical and biological requirements. *Storage and Processing of Farm Products*, 2007, no. 2, pp. 71–74. (In Russian).
4. Lipatov N.N. The background of the computer design of products and diets with the specifiable nutritional value. *Storage and Processing of Farm Products*, 1995, no. 3, pp. 4–9. (In Russian).
5. Lipatov N.N. and Bashkirov O.I. The methodological aspects of quality optimization of the multicomponent baby food products of a new generation (in the light of the nutritional combinatorial theory). *Storage and Processing of Farm Products*, 2000, no. 6, pp. 6–8. (In Russian).
6. Lipatov N.N., Neskromnaya L.V. and Bashkirov O.I. The information-algorithmic and terminological aspects of improving the quality of the multicomponent food products of special purposes. *Storage and Processing of Farm Products*, 2002, no. 9, pp. 25–28. (In Russian).
7. Lisin P.A. *The computer technologies in the formulation calculations of dairy products*. Moscow: DeLi Print Publ., 2007. 130 p. (In Russian).
8. Lisitsyn A.B., Ivashov V.I., Zakharov A.N., Kapovskiy B.R. and Kozhevnikova O.Ye. The intelligent quality control system for minced meat. *All about Meat*, 2013, no. 6, pp. 32–36. (In Russian).
9. Musina O.N. and Lisin P.A. The system modeling of the multicomponent food products. *Food Processing: Techniques and Technology*, 2012, no. 4, pp. 32–38. (In Russian).

10. Musina O.N. and Lisin P.A. Improving the quality of life of the population of the Altai region by improving the nutritional status at the expense of introduction in a diet of dairy products with added nutritional value. *Proceedings of Academic Science: Materials of the X International Scientific and Practical Conference (August 30 – September 7, 2014, Sheffield, S. Yorkshire, England). Volume 6. Mathematics. Physics. Modern information technologies. Technical sciences. Construction and Architecture. Agriculture*. Sheffield: Science and Education LTD, 2014, pp. 92–96.
11. Mosov A. The dairy products in the diet of children and adolescents: recommendations of a sanitary engineer to the manufacturing enterprises. *Milk and Dairy Products: Production and Sale*, 2011, no. 2, pp. 31–35. (In Russian).
12. Robertson A., Tirado C., Lobstein T., Jermini M., Knai C., Jensen J.H., Ferro-Luzzi A. and James W.P.T. Diet and health in Europe: a new basis of action. *WHO Regional Publications: European Series (Geneva)*, 2005, no. 96, pp. 506.
13. Rogov I.A., Zharinov A.I. and Voyakin M.P. Functional products: composition, properties, purpose. *Meat Technologies*, 2010, no. 2, pp. 6. (In Russian).
14. Tutel'ian V.A. The laws of the science of nutrition. *Modern Medical Technologies*, 2010, no. 4, pp. 98–100. (In Russian).
15. Shazzo R.I. and Kulieva R.G. The qualimetric aspects of optimization of the multicomponent products for infant nutrition. *Storage and Processing of Farm Products*, 2010, no. 9, pp. 44–46. (In Russian).
16. Khramtsov A.G., Selimov T.V., Sadovoy V.V. and Shchedrina T.V. The parametric modeling of the composition of food products for individual nutrition. *Storage and Processing of Farm Products*, 2011, no. 6, pp. 8–10. (In Russian).
17. Berner L.A., Keast D.R., Bailey R.L. and Dwyer J.T. (2014). Fortified foods are major contributors to nutrient intakes in diets of US children and adolescents. *Journal of the Academy of Nutrition and Dietetics*, 2014, vol. 114, no. 7, pp. 1009–1022.
18. Casala E., Matthys C., Péter S., Baka A., Kettler S., McNulty B., Stephen A.M., Verkaik-Kloosterman J., Wollgast J., Berry R. and Roe M. (2014). Monitoring and addressing trends in dietary exposure to micronutrients through voluntarily fortified foods in the European Union. *Trends in Food Science & Technology*, 2014, vol. 37, no. 2, pp. 152–161.
19. Diplock A.T., Aggett P.J., Ashwell M., Bornet F., Fern E.B. and Roberfroid M.B. Scientific concepts in functional foods in Europe: consensus document. *British Journal of Nutrition*, 1999, vol. 81, no. 1, pp. 1–27.
20. Gulati T. and Datta A.K. Enabling computer-aided food process engineering: property estimation equations for transport phenomena- based models. *Journal of Food Engineering*, 2013, vol. 116, no. 2, pp. 483–504.
21. Hu R. *Food product design: a computer-aided statistical approach*. CRC Press, 1999. 240 p.
22. Jiménez-Colmenero F. Potential applications of multiple emulsions in the development of healthy and functional foods. *Food Research International*, 2013, vol. 52, no. 1. pp. 64–74.
23. Oliviero T.A., Verkerk R. and Dekker M. Research approach for quality based design of healthy foods. *Trends in Food Science & Technology*, 2013, vol. 30, no. 2, pp. 178–184.
24. Ronteltap A., Sijtsema S.J., Dagevos H. and de Winter M.A. Construal levels of healthy eating. Exploring consumers' interpretation of health in the food context. *Appetite*, 2012, vol. 59, no. 2, pp. 333–340.
25. Sacco J. and Tarasuk V. Limitations of food composition databases and nutrition surveys for evaluating food fortification in the United States and Canada. *Procedia Food Science*, 2013, no. 2, pp. 203–210.
26. Vannice G. and Rasmussen H. Position of the Academy of Nutrition and Dietetics: dietary fatty acids for healthy adults. *Journal of the Academy of Nutrition and Dietetics*, 2014, vol. 114, no. 1, pp. 136–153.

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THE STUDY ON THE INFLUENCE OF THE ELECTROHYDRAULIC EFFECT ON THE DIFFUSION COEFFICIENT AND THE PENETRATION DEPTH OF SALT INTO MUSCLE TISSUES DURING SALTING

N. P. Oboturova*, I. A. Evdokimov, A. A. Nagdalian, Yu. I. Kulikov, O. A. Gusevskaya

North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355000 Russian Federation

* e-mail: geniando@yandex.ru

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Abstract: Currently, promising methods for intensifying the salting technology of raw meat are those based on pulsed energy effects, accompanied by a variety of physical and chemical effects. One of these methods is a discharge-pulse technology, developed by the scientists of the department of meat and canning technologies of the North Caucasus Federal University. When a short high voltage electrical pulse forms in the brine-meat system, high pressure forms in the working tank, the increase in pressure is accompanied by a set of physical and chemical phenomena, such as ultrasound and electromagnetic radiation, ultraviolet glow, cavitation, etc. Taken together, all these phenomena have a beneficial impact on both the brine and the meat itself, accelerating the process of salting. In the present study, we tried using the method of determining the diffusion coefficient of salt in beef muscle tissues empirically by creating a concentration difference in two communicating chambers, the liquids in which are separated by the studied raw material of a given thickness. The main goal of the work was to evaluate the effectiveness of the discharge-pulse technology of salting meat raw materials by determining the NaCl diffusion coefficient and the penetration depth of salt into meat samples. The experimental results showed that with the discharge-pulse treatment of meat, the penetration rate of salt into muscle tissue increases and is proportional to the number of pulses transmitted to the system. The diffusion coefficient in test samples was higher that in the control meat samples at each time period. Based on the obtained results, we came to the conclusion that the discharge-pulse meat treatment contributes to the intensification of diffusion osmotic processes in the wet salting technology.

Keywords: Electrohydraulic effect, pulsed discharges technology, the diffusion coefficient, meat salting

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INTRODUCTION

Meat salting is a major operation in the salty piece goods technology and is carried out to achieve the desired organoleptic characteristics, inhibition of microbiological spoilage, meat maturation, give meat such important properties as tackiness, plasticity, high moisture content [1]. Three classic methods underpin all the various options of meat salting: dry (dry salting mixture), wet (with brine) and mixed. With any method of salting, the mass exchange occurs between the salting substances and soluble components of the product in the forming system of "brine-meat". With the dry salting, in the beginning, due to the hygroscopic qualities salt and moisture in raw materials, brine is produced. Upon contact of salt with the surface of raw material, the exchange diffusion occurs between them, which leads to a redistribution of salting substances, water and soluble components of the product. Penetration of salt into the tissue and the redistribution between tissues and brine occurs in at least two ways:

– osmotically, through membranes, covering the outer surface of the treated part of the tissue;

– through a system of macro- and micro-capillaries penetrating the tissue in all directions, followed by subsequent salt and water redistribution between this system and the cellular elements of the tissue.

At the same time, the penetration of salt in the second way occurs firstly and at a higher rate [4]. With the wet salting of raw materials, salt and water redistribution consists of three simultaneous processes:

1. Water and salt redistribution between the brine and the product.
2. Water and salt redistribution in the brine.
3. Water and salt redistribution in the product.

All three processes of water and salt redistribution occur in a diffusion-osmotic way. This suggests that despite the complexity of salting in detail, the kinetics of the process can generally be described by the equations adopted to describe diffusion processes [7].

In case of an isotropic environment, the second law of Fick describes the exchange diffusion:

$$\frac{dc}{d\tau} = D\frac{d^2c}{dx^2}, \quad (1)$$

where c is the concentration of diffuse substances, %; τ is the duration of the process, seconds; D is the diffusion coefficient of substances in water, m^2/s; $\frac{d^2c}{dx^2}$ is the concentration gradient towards diffusion.

However, meat is an anisotropic composite material, formed by a volumetric combination of chemically dissimilar colloidal components. In anisotropic bodies, the diffusion coefficient is a function of the crystallographic direction. In this case, D is no longer a "scalar" and becomes a tensor by its geometrical properties, i.e. the diffusion occurs by each coordinate axis with its diffusion coefficient D_x, D_y, D_z. Accordingly, for the non-stationary process, which occurs in three dimensions, the rate of diffusion will be determined by the Fick equation which is more complex:

$$\frac{dc}{d\tau} = D\left(\frac{d^2c}{dx^2} + \frac{d^2c}{dy^2} + \frac{d^2c}{dz^2}\right), \quad (2)$$

where $\frac{d^2c}{dx^2} + \frac{d^2c}{dy^2} + \frac{d^2c}{dz^2}$ is the Laplace operator.

In the brine-meat product heterogeneous system, the distribution process of salting substances depends on the value of resistance, offered to the diffusion flux by the meat product tissues. The permeability coefficient serves as a criterion for the process. The value of this indicator depends on specific salting conditions: parameters of brine and properties of raw meat. The quantitative ratio between the permeability of muscle, connective and adipose tissues is about 8 : 3 : 1. Therefore, the presence of adipose tissues in the product slows down the accumulation and redistribution of the salting substances therein. As already mentioned, muscle tissues have anisotropic properties: permeability along muscle fibres is approximately 11% greater than across the fibres, which indicates that salting substances mainly move by the intercellular space of a tissue [10]. In other words, the drop coefficient of the diffusion rate is lower along the fibres than across the fibres. The drop coefficient of the diffusion rate also depends on the initial water supply of fibres, water activity and greatly affects the rate of the salting ingredients distribution within the muscle system.

The duration of salting process can be determined by the equation of A.S. Bolshakov:

$$\tau = \frac{dh^2}{D\lg\frac{c_b}{c_i}}, \quad (3)$$

where τ is the duration of salting (diffusion), days; d is the constant equal to 1.08; h is the depth of salting substances penetration into the product, m; (for homogeneous raw materials $h = H/2$, where H is the thickness of product, m); D is the diffusion coefficient, m^2/s; c_b is the substance concentration in brine, %; c_i is the substance concentration in the brine in the tissue at a depth of h, %.

Theoretically, the diffusion rate can be elevated by increasing the temperature, solution concentration, the kinetic energy of the system (usually by stirring) or by changing the structure of raw materials (loosening, destruction, defrost, electro-stimulation, enzyme treatment, etc.) [2].

Indeed: permeability depends on the temperature rise of brine – the temperature gradient causes additional mixing of salting substances towards the heat flow – thermo diffusion [1]. Furthermore, there is an empirical connection, which takes into account the temperature conditions of the salting process, for determining the time that is required for salting meat with NaCl (without applying external actions):

$$lg\mathrm{T} = 0.0515 \times (23.5 - t), \quad (4)$$

where T is the time of salting, hours; t is the salting temperature, °C.

However, with the increase in the brine temperature, the risk of unwanted microbial processes appears. Increasing the salt concentration in the brine intensifies the exchange diffusion, but the use of high concentrations of sodium chloride (14–25%) with prolonged exposure leads to denaturation and desalting of sarcoplasmic proteins that leads to the decrease in WBC (water-binding capacity) and the formation of a denser consistency in the surface layer.

During mixing, the diffusion boundary layer, which is located on the boundary of the brine-product system separation, offers the main resistance to the diffusion flux in the brine. The acceleration of the brine movement and the transition from a laminar flow to a turbulent flow lead to a decrease in the thickness of this layer and increase the speed of the salting process [6].

Effects, leading to the increase in permeability of the sarcolemma and membrane structures of muscle fibres, causes a more rapid and more even distribution of salting substances therein. Changes in the tissue permeability during the salting process are associated with the structural changes (loosening) of tissues and increased permeability of tissue membranes.

If the brine is mixed artificially due to convection, product overhauling or for other reasons, the diffusive salt transfer in the brine is displaced with the molecular (convective) transfer. With vigorous stirring, sufficient for rapid equalization of the salt concentration in the brine, the concentration that sets in is practically close to the average concentration [4]. In this case, the diffusion salt transfer in the brine will occur only within the boundary layer, the thickness of which depends on the speed of the brine movement. Various concentration gradients are established: in the boundary layer, between the boundary layer, the surface layer of the product and inside the product (Fig. 1). At the same time, the transfer of salt from the brine into the product under other identical conditions is carried out at a maximum speed, which depends on the intensity of mixing.

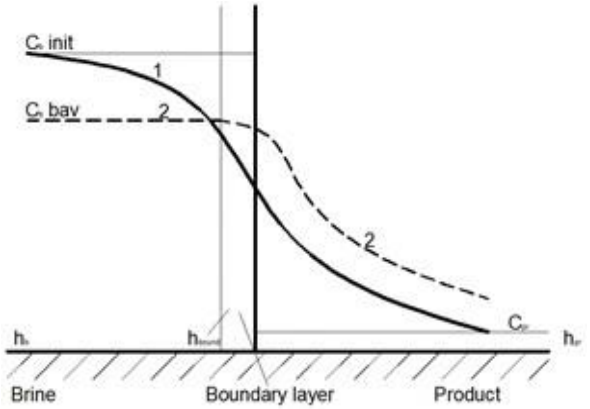

Fig. 1. Changes in the salt concentration in the brine, the boundary layer and in the product thickness: 1 – for a state of rest; 2 – stirring; $C_b init$ – initial concentration of brine; C_{pr} – product; C_{bav} – average brine concentration achieved by stirring; h_b – thickness of the brine layer; h_{bound} – thickness of the boundary layer; h_{pr} – thickness of the product layer

In practice, the boundary layer thickness may vary within wide limits. In a state of complete rest, the whole brine layer essentially acquires the properties of the boundary layer for the brine-product system. With vigorous stirring, its thickness decreases to an insignificant value [9]. Salting under the conditions of active physical (mechanical) influence – massaging, tumbling, vibration, electro-massaging – can significantly accelerate mass transfer processes, as the alternating mechanical effect, along with the diffusion exchange, causes intensive mechanical movements of the brine (and salting substances), directed to their uniform distribution through the product volume.

Lipatov S.M. solved the differential equation for the case of salt diffusion in animal tissues [1]:

$$ln\frac{C_1}{C_2} = \frac{h_2^2 - h_1^2}{4D\tau}, \quad (5)$$

where h_1 and h_2 is the distances from the body surface, between which the diffusion occurs, m; C_1 and C_2 is the concentrations at h_1 and h_2 distances from the body surface, %; τ is the maturation period in brine, days.

Provided that the diffusion process is considered on the interval between the body surface and the h depth point, i.e. if $h_1 = 0$, the equation (5) becomes:

$$ln\frac{C_1}{C_2} = \frac{h^2}{4D\tau}. \quad (6)$$

Equation (6) describes the salt concentration distribution in meat with the wet salting

The distribution process of the brine and its components in the muscle tissue under mechanical stress obeys the non-stationary filtration law and is described by the formula:

$$\frac{dp}{d\tau} = \varepsilon\frac{d^2c}{dx^2}, \quad (7)$$

where p is the pressure, Pa; τ is the duration of the process, seconds; ε is the diffusivity coefficient, m^2/s; x is the depth of brine movement, m.

The driving force of the process is produced by the pressure gradient, which occurs under the mechanical impact and an intensive filtration transfer of brine in the tissues. At the same time, the process of salting can be characterized as diffusion-filtration-osmotic.

Thus, the flow rate of salting and the intensity of salting components penetration into the product are largely dependent on the values of the diffusion coefficient D in the brine-meat system and the ε diffusivity coefficient of the tissues that most often are not known. The change in the diffusion coefficient in the salting dynamics is still poorly understood and usually limited to mathematical modelling.

Even in this case there is no uniform approach to solving the problem.

In the present study, we attempted the method of determining the diffusion coefficient of salt in beef muscle tissue empirically by creating a concentration difference in two communicating chambers, the liquids in which are separated by the studied raw material L metres thick.

The method was developed for the study on the effect of the pulsed discharges meat treatment in the brine on the permeability of muscle fibres and the penetration rate of salting components into meat, i.e., in other words, duration of the salting process.

The pulsed discharges technology for salting meat raw materials has been developed by the scientists of the department of the meat and canning technology of the North Caucasus Federal University in order to intensify the process and improve the quality and safety of meat. This technology is characterized by multifactorial physical and chemical effects on raw meat, which together were named as the electrohydraulic effect [8]. The electrohydraulic effect is accompanied by the formation of high and ultra-high hydraulic pressure, acoustic vibrations, liquid mixing intensity, the impact of powerful electromagnetic fields [11, 12].

For the formation of the electrohydraulic effect, it is necessary to form a high-pressure pulse in the transmission medium [12]. The simplest way to implement this process is to use low-inductive high-voltage energy storage devices with small capacity: high voltage provides the required amount of energy, but small inductance and capacitance of the electrical circuit – fast input of energy in the discharge gap.

The main goal of the work was to evaluate the effectiveness of the pulsed discharges technology of salting meat raw materials by determining the NaCl diffusion coefficient and the depth of salt penetration into meat samples.

OBJECTS AND METHODS OF STUDY

The object of research is the pieces of rear leg piriformis muscles (m. Piriformis) of chilled beef (+1…+2°C). The geometrical parameters of the pieces were equivalent and amounted to 50×50×100 mm. To conduct the experiment, we prepared the model brine, containing 7% NaCl solution. The main objective of the experiment was to determine the diffusion

coefficient and the depth of salt penetration into the meat in the control samples (intact or salted by the classical method) and treated samples. The pulsed discharges meat treatment was carried out in a specially designed working tank with the required strength characteristics.

To ensure purity of the experiment on determining the salt diffusion coefficient in meat, the samples were isolated from contact with the brine with a thin polyethylene film. I.e. NaCl concentration in the pieces after treatment was equal to the initial value – close to 0%. Meat isolation from contact with salt was motivated by the need to accurately calculate the concentration differences in the experiment. The control samples were intact, i.e. without any treatment.

In the course of the study on the penetration depth with the classic wet salting and pulsed discharges treatment, the test samples were exposed to the direct electrohydraulic effect deep in the brine volume, and then in parallel with the control samples had been kept in the identical brine for 24 hours.

The pulsed discharges treatment took place at a given voltage of the electric current (10 kW) and capacitance of condenser batteries (100 μF), i.e., the energy released by condensers in the established mode equalled to:

$$W = \frac{c\,u^2}{2} = 5\ kJ. \qquad (8)$$

In both cases, for determining the diffusion coefficient and for the study on the penetration depth of salting substances, a different quota of electrical pulses was applied to the test samples: 100, 200 and 300.

The method of determining the diffusion coefficient

The diffusion coefficient was determined by creating the sodium chloride concentration difference in two equivalent communicating tanks, using the studied muscle tissue as a porous membrane, through which the salt ions are migrating isothermally towards the lower concentration. The method was developed in the course of joint research by the staff of the Department of the meat and canning technology of the North Caucasus Federal University (Stavropol, Russia) and the Institute of meat technology and quality "Max Rubner-Institute" (city of Kulmbach, Germany) within the framework of the Development programme of the North Caucasus Federal University.

To carry out the studies aimed at determining the diffusion coefficient, we constructed a prototype of the laboratory setup shown in Fig. 2.

The samples of muscle tissue (2) were the sections of the control and test pieces of raw meat across the fibres with 5 mm step so that the linear dimensions of 5 × 50 × 50 mm were obtained. Then these sections were fixed between the walls of the tanks A and B via bolting (3.4) so as to completely close the opening, 20 mm in diameter. I.e. the active area of salt diffusion into muscle tissue amounted to 314 mm^2. Sequentially, after the muscle tissue was fixed, the tanks A and B were simultaneously filled with 7% NaCl solution and distilled water, respectively. The height of the liquid was adjusted to the same level to exclude an additional variable from the calculations of a pressure gradient. The risk of another variable – temperature gradient, was minimized by conducting the research in the areas with a constant temperature close to the temperature of standard physical conditions. Liquids were also kept at a room temperature until a constant temperature was reached.

Electromagnetic stirrers (6) rotationally drive magnetic beads (5) for artificial stirring and maintaining the turbulent flow in liquids. Stirring of the solution promotes continuous alignment of salt concentration, minimizing natural diffusion in the liquids and greatly reducing the diffusion salt transfer within the boundary layer, the thickness of which also depends on the rate of solution flow. Decrease in the salt diffusion factor in the boundary layer and in the solution allows determining the diffusion coefficient of the salt in the muscle tissue more accurately.

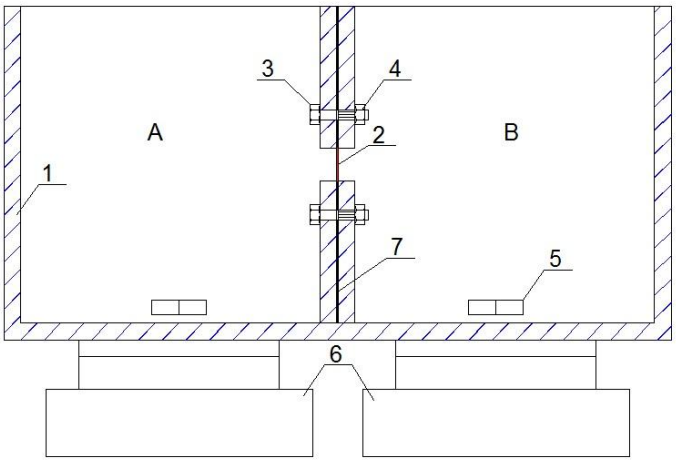

Fig. 2. A schematic view of the setup for determining the diffusion coefficient: A and B are the equivalent communicating tanks with the C_1 and C_2 salt solution concentrations; 1 – body of the device; 2 – sample of muscle tissue; 3 – stainless steel bolt; 4 – hexagonal nut made of stainless steel; 5 – magnetic beads; 6 – electromagnetic stirrers; 7 – rubber gasket between the walls.

The method of determining the depth of salt penetration

After overnight maturation in the brine, the control and test samples of raw meat were rinsed with tap water from residual salts on the surface, and then were divided into conditionally outer, middle and inner layers, 10 mm thick (Fig. 3). I.e. with the initial piece sizes 50 × 50 × 100 mm after the separation of the outer layer, the geometric parameters changed to 30 × 30× 80 mm, and the size of the inner layer made up 10 × 10 × 60 mm.

If we assume that the piece thickness is *H*, then the maximum depth of salt penetration is at a distance of $h = H/2$ – in the central plane of the inner layer.

The study on salt content was carried out using two conventional methods of Mohr and Folgard.

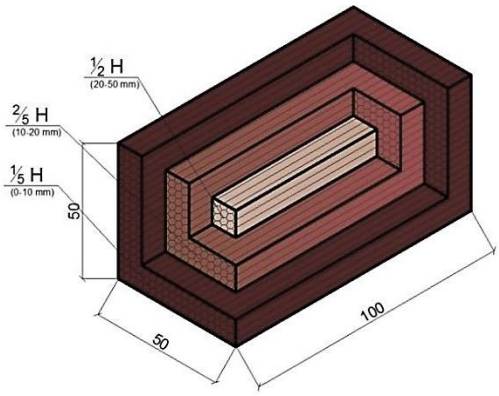

Fig. 3. Stereo metric interpretation of conditionally external, middle and inner layers of the studied pieces of meat**.**

RESULTS AND DISCUSSION

Since the determination of the diffusion coefficient of salt in the meat samples of muscle tissue had the shape of a plate with the area many times larger than the thickness, the mathematical description of the test process comes down to the solution of a one-dimensional non-stationary diffusion equation (1) with the following initial and boundary conditions: $C\ (x,\ 0) = 0$, i.e. at the beginning of the process, the muscle tissue contained no diffusible substance – NaCl.

$$V\frac{\partial C(x,0)}{\partial \tau} = -DS\frac{\partial C(x,0)}{\partial x}, \qquad (9)$$

where V is the volume of muscle tissue, through which the diffusion occurs, m^3; S is the active area of the muscle tissue, m^2; D is the diffusion coefficient.

The boundary condition shows that the intensity of salt concentration changes on the surface of the muscle tissue sample with the volume V causes the mass flow of NaCl into the thickness of the sample, the passage of which diffuses into the solution with a lower concentration [13].

$$\frac{C_{2n}-C_2}{C_1-C_2} = 1 + \sum_{n=0}^{\infty}\frac{2(a_n^2+b^2)}{a_n^2+b^2+b}\times\frac{\sin a_n}{a_n}\exp\left(\frac{Da_n^2\tau}{L^2}\right), \qquad (10)$$

where C_1 is the initial concentration in the tank A, 7%; C_2 is the initial concentration in the tank B, 0%; C_{2n} is the concentration in the tank B, % at the moment of τ, sec; $b = LS/V_2$, where L is the sample thickness, m; S is the the active area of diffusion, m^2; V_2 is the the volume of the tank B, m^3 at the moment of τ, sec; a is the nonzero positive root of the equation $a \times tga = b$.

The value C_{2n} was determined after 1, 2, 3, 4 and 24 hours after the start of diffusion. The study was conducted in triplicate for each sample, the average value of the obtained results was assigned to C_{2n}, except for the data which differed by more than 5%. The results of determining the salt content in the solution samples are given in Table 1.

Note that with each sampling, the volume of solution was reduced by 10 ml or 10^{-5} m^3. Thus, when calculating the b value, the V_2 parameter is of variable character, with the constant values $L = 0.05$ m and $S = 3.14\times10^{-4}$ m^2. In this case, the generalized formula becomes as follows:

$$b_n = \frac{LS}{V_2 - n*10^{-5}}, \qquad (11)$$

with the initial value $V_2 = 0.022$ m^3 the formula (11) comes down to the expression:

$$b_n = \frac{1.57\times10^{-5}}{0.022 - n\times10^{-5}}. \qquad (12)$$

The value of a, given that it is a nonzero positive root of the equation $a \times tga = b$, is determined graphically by the projection of the intersection point of the functions $f(a) = tga$ and $f(a) = b/a$ on the abscissa (Fig. 4).

Table 1. NaCl content in the solution of the tank B at the moment of τ, h

No. percentage point	The moment after the start of diffusion, h	Salt content in the sample, %			
		Control	Test		
			100 pulses	200 pulses	300 pulses
1	1	0.28	0.32	0.41	0.54
2	2	0.91	0.88	1.37	1.63
3	3	1.46	1.59	1.91	2.14
4	4	1.82	2.20	2.23	2.69
5	24	2.41	2.65	2.78	2.91

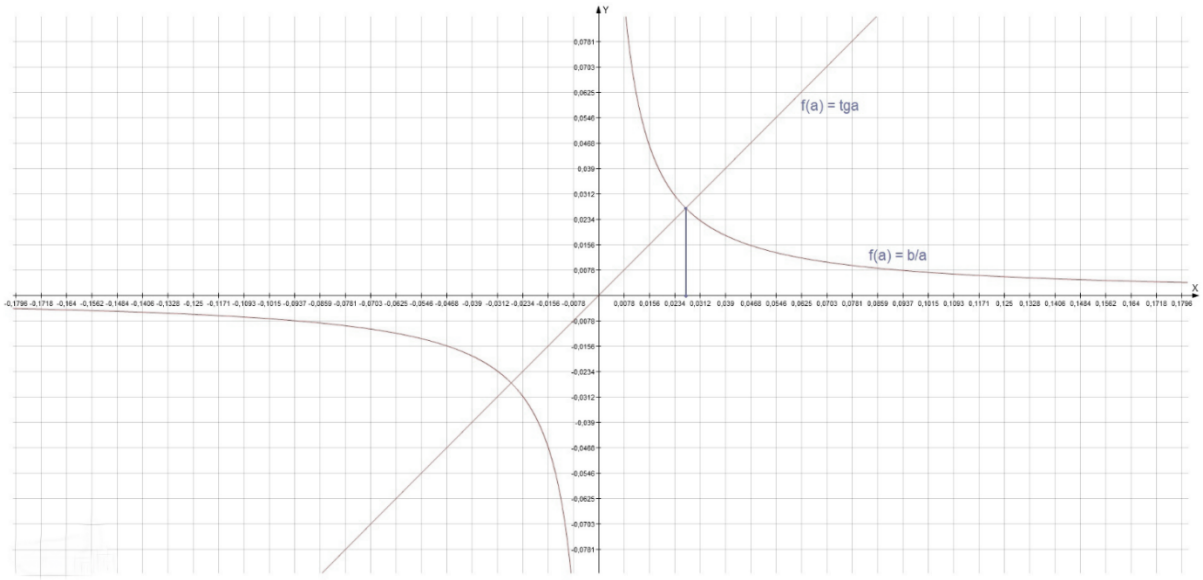

Fig. 4. Determination of the intersection point of the functions *f*(*a*) = *tga* and *f*(*a*) = *b/a* in the I coordinate quarter.

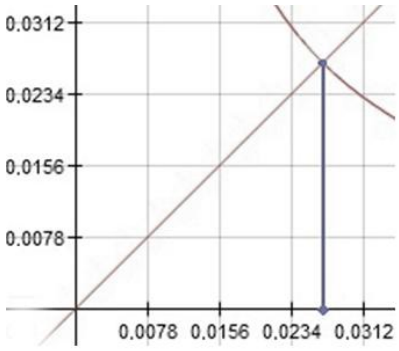

Fig. 5. Determining the value of *a* graphically.

The value of *a* with fairly close values of the dimensionless *b* parameter also varied within the narrow limits of the 0.026–0.028 range. 0.27 was taken for the average value (Fig. 5).

For all known parameters, the diffusion coefficient at the moment of τ (D_n) is determined by the formula (10):

$$\frac{Da_n^2\tau}{L^2} = \ln\frac{1+\frac{C_{2n}-C_2}{C_1-C_2}}{\frac{2(a_n^2+b^2)}{a_n^2+b^2+b}*\frac{\sin a_n}{a_n}}, \qquad (13)$$

$$D = \frac{L^2}{a_n^2\tau}\ \ln\frac{1+\frac{C_{2n}-C_2}{C_1-C_2}}{\frac{2(a_n^2+b^2)}{a_n^2+b^2+b}*\frac{\sin a_n}{a_n}}. \qquad (14)$$

The dependence of the diffusion coefficient from the process duration within the conducted experiment is given in Table 2. The studies were conducted in triplicate; deviations of more than 5% were not taken into account.

During the analysis of the data given in the table, we observed an expected tendency to the diffusion coefficient increase in the meat pieces, treated with electrohydraulic pulses. Moreover, the number of reported pulses is directly proportional to the value of the parameter under study. Thus, after the first hour, with meat pieces being treated with 100 pulses, D_1 almost equals to the diffusion coefficient in the control sample, but with a two hour interval there is difference of 0.4 mm^2/s. According to the obtained data, the diffusion of salt into the muscle tissue occurred the most effectively in the samples, treated with 300 impulses. In this case, already after 1, the diffusion coefficient of the test sample exceeded the one of the control sample by 1 mm^2/s. After 2 hours, the difference in *D* values amounted to more than 1.5 mm^2/s, after which it started to decrease slowly, which is due to the decrease in the concentration gradient. After 24 hours, the average value of the diffusion coefficient in the test samples was higher than in the control samples: by 0.03 mm^2/s if treated with 100 impulses, by 0.7 mm^2/s – with 200 impulses and by 0.13 mm^2/s – with 300 impulses.

Table 2. Results of the study for determining the diffusion coefficient of salt in the muscle tissue

No. percentage point	The studied sample	The value of the diffusion coefficient at the moment of τ, m^2/sec				
		D_1 (3 600 sec)	D_2 (7 200 sec)	D_3 (10 800 sec)	D_4 (14 400 sec)	D_{24} (86 400 sec)
1	Control	2.87×10^{-5}	1.49×10^{-5}	1.01×10^{-5}	8.37×10^{-6}	1.46×10^{-6}
2	Experimental (100 pulses)	2.88×10^{-5}	1.53×10^{-5}	1.09×10^{-5}	8.64×10^{-6}	1.49×10^{-6}
3	Experimental (200 pulses)	2.93×10^{-5}	1.61×10^{-5}	1.12×10^{-5}	8.66×10^{-6}	1.53×10^{-6}
4	Experimental (300 pulses)	2.97×10^{-5}	1.65×10^{-5}	1.15×10^{-5}	8.96×10^{-6}	1.59×10^{-6}

According to (1), with the diffusion along muscle fibres, the average penetration rate of salt into muscle tissues with the regular salting amounts to around 0.59 mm/h, with the diffusion across fibres – around 0.39 mm/h. With similar rates of the salt migration deep into muscle tissues, the changes in the diffusion coefficient by 0.15–1.5 mm^2/s will have a significant effect, as the salting rate increases proportionally to the diffusion coefficient.

It is known, that sodium chloride used for salting increases oxidation of the heme pigment of myoglobin. Oxidation of the heme centres leads to the formation of met-pigments, whereby the natural colour of meat disappears relatively quickly and it acquires the brownish-grey colour with different hues, and the more salt penetrates into the muscle tissue, the lighter the colour becomes. Fig. 6 clearly shows the trace of NaCl penetration into the muscle tissue samples.

The diameter of the grey circumference corresponded to the diameter of the opening between the walls of the A and B tanks, i.e. the trace from salt penetration equalled the cross section of the diffusion flux that proves the validity of the decision to use the one-dimensional equation of Fick, despite the anisotropic properties of muscle tissues. The area of muscle tissue, saturated with met-pigments, equalled to 3.14 cm^2. This fact confirms that made calculations are correct, with the active area of diffusion taken for 3.14×10^{-4} m^2.

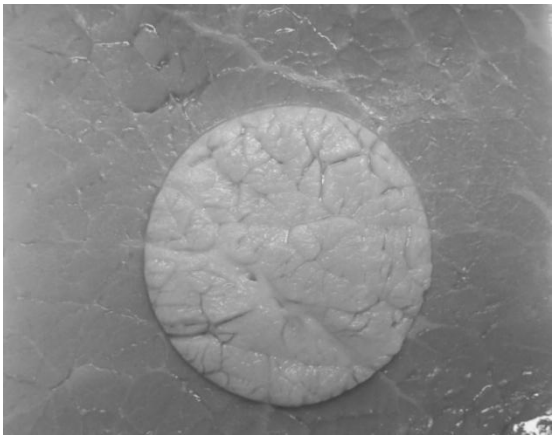

Fig. 6. The active area of NaCl diffusion deep into muscle tissue.

Thus, the use of the pulsed discharges treatment of meat pieces during wet salting resulted in a significant increase of the diffusion coefficient, which directly affects the penetration rate of salting substances into deeper muscle tissues. To obtain the empirical dependence of the salting rate from the value of effective diffusion coefficient, we conducted the study on the sodium chloride concentration at different depths of the control and test pieces after 24 hours maturation in brine. The research results are summarized in Table 3.

Electrohydraulic effect, occurring during the pulsed discharges treatment, has intense physical effect on muscle tissues. The effect causes muscle fibres to swell, increases the number of transverse slit-like ruptures, destruction of membrane structures and loosening of muscle tissues in general. Subsequent maturation of pieces in the brine causes muscle fibres to swell across the meat thickness. This salt fills transverse slit-like ruptures and actively penetrates the fibres through the ruptures in myofibrillar membranes. The salt content in test samples at a depth of 1, 2 and 3 cm was higher than in the control ones, which indicated the intensification of the sodium chloride penetration deep into meat and increase in the salting rate of meat.

Referring to the equation (6), which shows the distribution character of salt deep in the muscle tissue, the diffusion coefficient is as follows:

$$D = \frac{h^2}{4\tau \times ln\frac{C_1}{C_2}}, \quad (15)$$

Having calculated the diffusion coefficient of salt deep into meat pieces, we will obtain the values:
D of the control sample = 1.8×10^{-5} m^2/s;
D of the test sample (100 pulses) = 2.08×10^{-5} m^2/s;
D of the test sample (200 pulses) = 2.228×10^{-5} m^2/s;
D of the test sample (300 pulses) = 2.75×10^{-5} m^2/s.

The obtained values of the diffusion coefficient of salt in the control and test samples correlate with the values obtained in the experiment aimed at determining the D coefficient. The use of the pulsed discharges treatment in the wet salting technology for raw meat contributes to faster penetration and distribution of sodium chloride in the thickness of muscle tissue, i.e. intensification of the technological process.

Table 3. Sodium chloride content at different depths of the control and test samples

No. percentage point	Depth, mm	Salt content in the sample, %			
		Control	Test		
			100 pulses	200 pulses	300 pulses
1	0–10	1.80 ± 0.10	2.10 ± 0.12	2.30 ± 0.11	2.80 ± 0.13
2	10–20	1.40 ± 0.06	1.50 ± 0.09	1.60 ± 0.15	2.10 ± 0.08
3	20–30	0.90 ± 0.04	1.20 ± 0.11	1.30 ± 0.12	1.50 ± 0.12

REFERENCES

1. Kulikova V.V., Postnikov S.I. and Oboturova N.P. *Physicochemical and biochemical basis for the production of meat and meat products*. Stavropol: News Bureau Publ., 2011. 260 p. (In Russian).

2. Bratsihin A.A. *Scientific and practical aspects of the technological processes intensification with the use of nano-activated liquid media in the production of meat products*. Dr. eng. sci. thesis. Stavropol, 2009. 511 p. (In Russian).
3. Postnikov S.I. *Technology of meat and meat products. Course of lectures*. Stavropol, 2007. 112 p. (In Russian).
4. Miroshnikov E.P., Bogatov O.V. and Stadnikov S.V. *Physicochemical and biochemical basis for the production of meat and meat products.* Orenburg: GOU OG Publ., 2005. 248 p. (In Russian).
5. Filipović V., Ćurčić B.Lj., Nićetin M.R., Plavšić D.V., Koprivica G.B. and Mišljenović N.M. Mass transfer and microbiological profile of pork meat dehydrated in two different osmotic solutions. *Hemijska Industrija*, 2012, vol. 66, no. 5, pp. 743–748.
6. Dunayev S.A. and Popov A.A. *Methods for the intensification of technological processes in the meat industry: lecture notes*. Kemerovo: KemIFST Publ., 2006. 64 p. (In Russian).
7. Promtov M.A. *Machines and devices with the pulse energy impact on the substances to be treated: a textbook*. Moscow: Mechanical Engineering Publ., 2004. 136 p. (In Russian).
8. Yutkin L.A. *Electro-hydraulic effect and its application in industries*. Leningrad: Mechanical Engineering Publ., 1986. 208 p. (In Russian).
9. Jensen W.K., Devine C. and Dikeman M. (Eds.). *Encyclopedia of meat sciences, three-volume set*. New York: Elsevier, 2004. 1553 p.
10. Perkel T.P. *Physico-chemical and biochemical basis of the production of meat and meat products.* Kemerovo: KemIFST Publ., 2004. 100 p. (In Russian).
11. Oboturova N.P., Kozhevnikova O.N., Barybina L.I. and Nagdaljan A.A. Discharge-pulse effect for intensifying meat salting. *Meat Industry*, 2012, no. 12, pp. 32–35.
12. Nagdaljan A.A. and Oboturova N.P. Influence of electrohydraulic effect on the biopolymers hydration. *Current Problems of the Humanities and the Natural Sciences*, 2012, no. 12, pp. 74–78. (In Russian).
14. Djelveh G. and Gros J.B. Measurement of effective diffusivities of ionic and non-ionic solutes through beef and pork muscles using a diffusion cell. *Meat Science*, 1988, vol. 24, no. 1, pp. 11–20.
13. Bolumar T., Enneking M., Toepfl S. and Heinz V. New developments in shockwave technology intended for meat tenderization: opportunities and challenges. A review. *Meat Science*, 2013, vol. 95, no. 4, pp. 931–939.
15. Graiver N., Pinotti A., Califano A. and Zaritzky N. Diffusion of sodium chloride in pork tissue. *Journal of Food Engineering*, 2006, vol. 77, no. 4, pp. 910–918.

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EFFICIENCY OF ADDING ESSENTIAL MICRONUTRIENTS TO THE DIET OF BROILER CHICKENS

E. V. Potapenko*, I. A. Evdokimov, N. P. Oboturova, A. V. Serov

North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355009 Russian Federation

* e-mail: e_potapenko@list.ru

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Abstract: Selenium is an indispensable component for fattening broiler chickens of meat productivity. It normalizes their growth and metabolism by participating in the redox transformations of glutathione. The goal of this work was to examine the possibility of using a drug based on selenium nanoparticles and the "Ekstraselen+Vit" vitamins in the diet of broiler chickens. It is experimentally proved that supplementing the diet of poultry with a feed preventative additive leads to higher growth rates and lower mortality rates of young poultry, lower feed costs per unit of yield. During a scientific and economic experiment, we determined that there was an increase in the pre-slaughter weight, slaughter yield and the yield of certain carcass parts in accordance with anatomical butchering. Data on blood haematological parameters of broiler chickens is presented. We identified the improved chemical composition and functional-technological properties of raw meat, produced from broiler chickens, grown with the use of "Ekstraselen+Vit". The concentrations of selenium in the poultry processing products were determined. It was found that the use of the feed additive in the diet, based on selenium nanoparticles, contributes to the accumulation of this element in broiler chickens bodies. It is feasible to use enriched meat to produce medical and preventative food.

Keywords: Broiler chickens, selenium, vitamins, conversion, growth, anatomical butchering, chemical composition, microbiological parameters

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INTRODUCTION

Full feeding is an essential component that ensures high productivity of the birds, preservation of health, resistance to adverse environmental factors, quality improvement of raw meat and feather-down products. Without properly organized bird feeding, the genetic potential of poultry remains unfulfilled. Domestic and foreign experience shows that in the preparation of feed regimens for broiler chickens, a lot of attention is paid to the selection of vitamins and minerals.

Supplying poultry with the optimal amount of vitamins and minerals can improve the body's metabolism, ensure normal functioning of the immune system, and enhance the body's natural resistance.

In recent years, the research, aimed at identifying the needs of birds in certain mineral elements has become more active. Previously, these minerals were not taken into account in the preparation of food regimens; however, it is proved that they have a significant effect on the body. Selenium, which is recognized as an essential biotic ultra-microelement, also refers to these elements and their compounds that draw attention of experts and scholars in the field of poultry production.

Selenium is essential for the activity of people and animals. This biologically active microelement, which is found in a number of hormones and enzymes, is connected with all organs and systems.

The spectrum of biochemical effects of selenium in the bodies of humans and animals is quite broad, at the same time, the most studied function of selenium is the regulation of antioxidant processes in all organs and tissues, and, primarily, in the central nervous system. Selenium plays a very important role in the immune system activity; in particular, the levels of A, G and M immunoglobulin decrease in case of selenium deficiency.

It is now established that selenium deficiency is a cause of the increased risk of oncological and cardiovascular diseases, the development of arthritis, high infant mortality and various malformations in children (if women have selenium deficiency during pregnancy), cataract, coronary atherosclerosis, hypothyroidism, as well as increased risk of AIDS development. Selenium compounds are effective in preventive medicine. The introduction of selenium into a human body with the daily doses up to 200 mg during 4.5 years does not cause toxic effects. At the same time, the incidence of human skin cancer reduces

by 50%, prostate cancer – by 69%. The development of the hepatitis B and liver cancer is prevented, the risk of developing breast, stomach, colon and lung cancer is reduced. Selenium compounds protect cell membranes from free radicals damage and prevent their generation, reducing the risk of developing tumours, heart and blood vessels diseases, at the same time.

Selenium deficiency not only reduces immunity and working efficiency, but also leads to the development of cardiovascular and oncological diseases, the accumulation of heavy metals and premature aging, diabetes and joints diseases, male infertility and female uterine inertia.

Danish scientists have shown that selenium prevents the diseases of heart and arteries, and its deficiency increases the risk of the coronary heart disease by 70%. The results of the study, conducted by the US National Cancer Institute, shocked doctors. The mortality rate from the most common types of cancer of those people, who took selenium, reduced by 49%. In Finland, after the introduction of selenium into the diet of population, the number of cardiovascular pathologies decreased by 2.5 times, the number of cancer incidence reduced by 1.8 times, endocrine system diseases – by 77%, and the overall incidence rate decreased by 47.4%.

Selenium intake in most of the world is quite low, in the range of 10–70 Se micrograms per day, often in average less than 50 micrograms per day. Apparently, only the US citizens and some other countries in the Americas, take selenium in a higher range of 70–120 μg/day. The problem is that in some parts of the world the status of selenium decreases – there is a trend, which aggravates selenium deficiency.

With intensive cultivation technologies, under industrial conditions, modern high efficiency crosses have vitamins deficiency quite often. Vitamins act as catalysts for many metabolic reactions in birds, though they do not serve as a source of energy. They are necessary for normal functioning of tissues, organs and body as a whole in small doses.

Based on the above, we consider it expedient to enrich the diet of broiler chickens with the feed preventative additive "Ekstraselen+Vit", synthesized by a group of authors led by the Professor of NCFU, Serov A.V. This drug consists of selenium nanoparticles and vitamins E, A, B_1, B_2, B_6, B_{12}, PP, K.

A distinctive feature of the new product is its full compatibility with conventional feeds, vitamins and essential microelements, and high digestibility, as well as the retention in the body due to manifestations of a nano effect and synergistically low toxicity due to the zero oxidation degree.

"Ekstraselen+Vit" complex drug to the level of 100 micrograms of selenium per 1 litre of drinking water, in order to determine the level of its effectiveness when administered orally.

We consider it expedient to discuss the possibility of enriching drinking water of broiler chickens with the

The goal of our work is to improve the technology of producing raw meat with a high content of micronutrients and improved functional and technological properties.

OBJECTS AND METHODS OF STUDY

The experimental part of the work was carried out under the conditions of a private farm by the method of the All-Russian Institute of Scientific Research and Technologies of poultry farming (2008) on the broiler chickens of the "Ross 308" cross without gender division. Main research was conducted on the basis of the department "Technology of meat and conservation" of the North Caucasus Federal University in the laboratory of the Stavropol State Agrarian University, the ANO – Centre for Biotic Medicine (Moscow) and others specialized laboratories.

To carry out a scientific and economic experiment by the analogue method, we formed 2 groups of 18 days old chicks – control (C) and experimental (E) with 14 chicks in each group.

Breeding of hybrid meat young broiler chickens was carried out in the areas without pasture on deep litter in compliance with the necessary microclimate parameters (fan) and incandescent lighting with adjustable brightness. The main parameters of the microclimate were maintained according to the recommendations of the ARISRTPF (Table 1).

The "Ekstraselen+Vit" preparation was added to the drinking water of chickens. Water was changed daily. The control group drank regular water. For an experimental group, we used the drinking water with the concentration of selenium 125 μg/l. 1 ml of the drug was dissolved in 10 litres of water. Water was supplied in equal amounts to each group, with a subsequent increase [1].

Feeding of the birds was carried out to appetite with granulated and ground mixed feed ("Start", "Rost", "Finish"), produced at the feed plants of the Stavropol region, in accordance with the recommendations of the ARISRTPF.

RESULTS AND DISCUSSION

Feed was served in the equal amount of identical composition for each group.

During the experimental studies, we took into account the live weight of poultry, its liveability and food intake.

During the experiment, we accounted for the dynamics (Fig. 1) of live weight and the liveability of broilers. In the process of growing, the birds were weighed every 3 days individually on the electronic scales accurate to 0.5 g.

Table 1. Microclimate indicators during chicken broilers breeding

Age, weeks	Temperature, °C	Relative humidity,%	Duration of daylight hours	Intensity of the light, lux
3	27.0–26.0	60.0–65.0	23	15–17
4	26.0–24.0	60.0–65.0	23	10–12
5–6	18.0–22.0	60.0–65.0	23	20–25

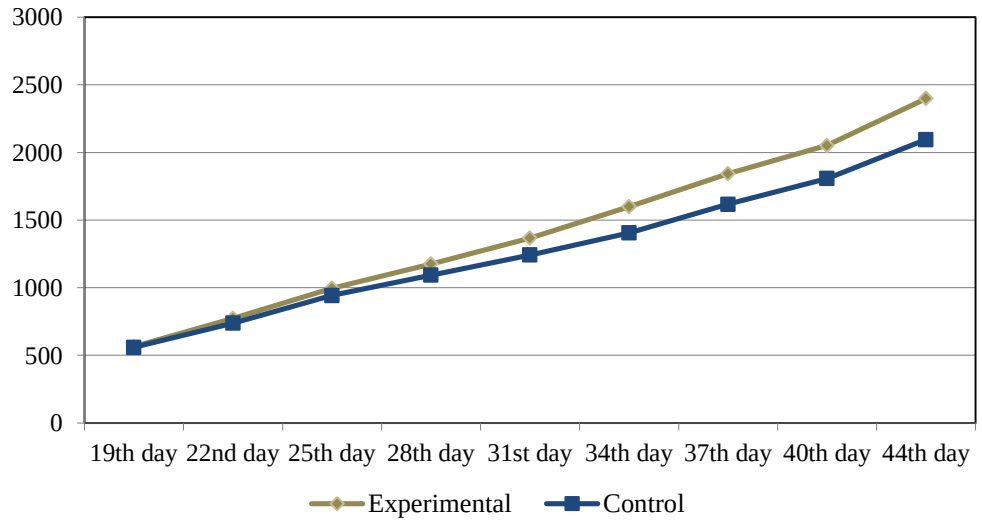

Fig. 1. Dynamics of broiler chickens' live weight gain in the control and experimental groups.

The data shows that by the end of breeding, the live weight of broiler chickens in the experimental group is 14.5% higher than the live weight of broiler chickens in the control group.

In order to give a comprehensive assessment of the broiler chickens' productive qualities, we determined such integrated value as the European Production Efficiency Factor (EPEF), calculated by the formula:

$$\text{EPEF} = \frac{L \times W}{\tau \times C} \times 100, \% \qquad (1)$$

where L is the liveability, %; W is the live weight of chicken broilers, kg; τ is the breeding period, days; C is the feed conversion ratio.

The results of the breeding effectiveness are shown in Table 2.

The experimental results showed that the youngsters who were given the "Ekstraselen+Vit" preventative additive with drinking water had the highest productivity rates.

Livestock mortality rate of both groups was equal to 0%.

The actual mixed feed intake during breeding was similar for both groups during the first four weeks,

Table 2. Indicators of the live weight gain of the broiler chickens in the control and experimental groups

24-hour period	Live weight, kg	Feed intake, g/24 hours	The average weight gain over the period to the next weighing, g	Feed conversion, kg/kg of live weight	EPEF
Control group					
19	0.559	98.1	–	–	–
22	0.738	128.6	179	1.652	203
25	0.943	136.3	205	1.879	201
28	1.093	142.1	150	2.720	144
31	1.242	144.3	149	2.876	139
34	1.406	156.3	164	2.628	157.4
37	1.617	151.3	211	2.153	203
40	1.808	170.0	191	2.669	169
44	2.096	158.6	288	2.202	216
Experimental group					
19	0.564	114.4	–	–	–
22	0.773	136.7	209	1.640	214
25	0.996	145.3	223	1.844	216
28	1.176	169.1	180	2.416	174
31	1.368	189.1	192	2.648	167
34	1.599	186.5	231	2.420	194
37	1.843	204.8	244	2.518	198
40	2.053	184.3	210	2.334	220
44	2.400	184.4	347.3	1.592	343

then there was a tendency to the increased intake in the experimental group. Therefore, by the end of breeding, young broiler chickens in the experimental group consumed 13.99% more feed than the birds in the control group, in which this indicator amounted to 158.6 g/day. It should be noted that the efficiency of feed use in the groups was different. Due to the high growth rate of chickens in the experimental group, the feed cost per 1 kg of live weight gain was lower by 27.7% and amounted to 1.592 and 2.202 kg/kg of live weight respectively. EPEF calculation showed that the birds of the experimental group compare favourably with the control group. Its value in the control group amounted to 216 units versus 343 units in the youngsters of the experimental group.

The above data shows that the method of growing broiler chickens using the "Ekstraselen+Vit" is significantly more efficient than the traditional one.

At the end of fattening, in order to determine the morphological, anatomical indicators of carcasses, physical and chemical parameters and functional-technological properties of raw meat, the control slaughter of 3 broiler chickens from each group was carried out.

Theeffect of the "Ekstraselen+ Vit" on haematological blood parameters of broiler chickens was studied.

Blood sampling was carried out after the experiment completion, 27 days after introducing the drug into drinking water. Blood in a test tube was stabilized with heparin and used for haematological studies.

As one of the most important physiological body systems, blood plays an important role in the body's vital functions. According to Stepanov V.I. et al. (1999), blood parameters are changed under the influence of internal and external factors. Since, body protective functions are regulated through blood, the intensity of metabolic processes in animals and their economically useful signs directly depend on it [3].

Haematological blood parameters of birds characterize its state (Table 3). Thus, haemoglobin, which is a blood pigment, acts as an oxygen carrier from the lungs to the tissues. The increased content of haemoglobin over the physiological norms occurs if blood thickens, during fluid loss due to diarrhoea, vomiting, oedemas, physiological stress, if animals are moved to mountains at high altitude, and the decreased content is observed in case of starvation, anaemia, blood loss, lack of iron, cobalt, vitamins in the feed and in case of chronic intoxications.

The analysis of the morphological composition of broiler chickens' blood showed that after the drug treatment in the experimental group haemoglobin increased by 1.1 times. We identified that the erythrocyte count in the experimental group increased 1.14 times, it indicates the presence of body compensatory mechanisms on the background of a balanced diet.

It is known that selenium promotes the synthesis of glutathione and the glutathione reductase enzyme, which constantly form in erythrocytes and protect the erythrocytes' components that protect from damage by the oxidation products of unsaturated fatty acids.

Established fluctuations in the erythrocytes count and haemoglobin content did not go beyond the physiological norm for broiler chickens.

A significant increase in erythrocytes, haemoglobin and total protein in the blood of the experimental birds suggests that essential microelements positively influence the protein metabolism in the body.

We took the carcass weight without blood and feathers as a weight of the non-eviscerated carcass. The slaughter yield was calculated as a ratio of the non eviscerated carcass to the pre-slaughter weight, expressed in percent.

The results of the slaughter are shown in Table 4.

The analysis of slaughter results indicates that the use "Extraselen+Vit" in the diet of chicken broilers contributes to the yield of an eviscerated carcass, and this complies with the data on an eviscerated carcass weight. In particular, the average yield of eviscerated carcasses of the control samples – 74.49%, the experimental ones – 75.15%.

Eviscerated carcasses from each group were butchered in the laboratory of the NCFU Department of

Table 3. Hematologic and biochemical blood parameters of broiler chickens

Indicators	Norm	Control group	Experimental group
Erythrocyte count, ×1012/l	3.2–4.5	3.15	3.59
Leucocyte count, ×109/l	20–40	36.9	29.7
Haemoglobin, g/l	100–135	115.4	126.5
Total protein, g/l	30–60	35.4	39.9
Glucose, mmol/l	11.0–27.5	12.86	12.82
Cholesterol, mmol/l	2.6–3.6	3.1	3.6

Table 4. The results of the poultry slaughter in the experimental and control groups

The studied sample / Indicator	Control group	Experimental group
Pre-slaughter weight, g	1 976 ± 200	2 370 ± 100
Weight of a non-eviscerated carcass, g	1 805 ± 200	2 193 ± 100
Slaughter yield of a non-eviscerated carcass,	91.35 ± 5.00	92.53 ± 2.00
Weight of an eviscerated carcass, g	1 472 ± 180	1 781 ± 90
Slaughter yield of an eviscerated carcass, %	74.49 ± 5.00	75.15 ± 2.00

meat and canning technologies. Wings were cut on the glenohumeral joint, legs on the hip joint and breasts parts on the coracoid line of ribs. Then, legs were divided into two components – drumstick and thigh [2].

Selected parts were boned manually with the anatomical clean-up of bones. The ratio of muscle and bone tissue, as well as the skin was determined by weighing (Table 5).

Table 5. The results of the anatomical butchering of broiler chickens

Indicators	Control group			Experimental group		
	Weight of carcass parts, g	% from the eviscerated carcass weight	% from live weight	Weight of carcass parts, g	% from the eviscerated carcass weight	% from live weight
Pre-slaughter (live) weight	1976			2370		
Weight of an eviscerated carcass	1472			1781		
Slaughter yield	74.49			75.15		
Breast:						
muscles	472.61	32.11	23.92	584.81	32.84	24.68
including fillet	343.00	23.30	17.36	457.22	25.67	19.29
skin	28.74	1.95	1.45	34.81	1.95	1.47
bones	52.13	3.54	2.64	61.50	3.45	2.59
Total	553.48	37.60	28.01	681.12	38.81	28.74
Thigh:						
muscles	270.13	18.35	13.67	351.28	19.72	14.82
skin	52.72	3.58	2.67	65.12	3.66	2.75
bones	84.72	5.76	4.29	101.76	5.71	4.29
Total	407.57	28.43	20.63	518.16	29.09	21.86
Drumstick:						
muscles	119.03	8.09	6.02	144.04	8.09	6.08
skin	21.71	1.47	1.10	17.65	0.99	0.74
bones	60.04	4.08	3.04	57.11	3.21	2.41
Total	200.78	13.64	10.16	218.80	12.29	9.23
Wing:						
muscles	68.17	4.08	3.45	83.87	4.71	3.54
skin	37.18	2.53	1.88	39.22	2.20	1.65
bones	45.23	3.07	2.29	55.31	3.11	2.33
Total	150.58	10.23	7.62	178.4	10.02	7.53
Back:						
muscles	22.31	1.52	0.79	28.37	1.59	1.20
skin	15.13	1.03	0.77	19.87	1.12	0.84
bones	84.09	5.71	4.26	91.76	5.15	3.87
Total	128.65	8.74	6.51	146.75	8.24	6.20
Internal fat	13.24	0.90	0.67	15.37	0.86	0.65
Kidneys	10.92	0.74	0.55	13.12	0.74	0.55
Lungs	6.78	0.46	0.34	9.28	0.52	0.39
Wastes	7.12	0.48	0.36	6.75	0.38	0.28
Edible parts						
muscles	952.25	65.17	48.55	1192.37	66.95	50.32
skin	155.48	10.56	7.87	176.67	9.92	7.45
Kidney + fat + lungs	30.94	2.10	1.57	37.77	2.12	1.59
Total	1138.67	77.36	57.63	1406.81	78.99	59.36
Inedible parts						
bones	326.21	22.16	16.51	367.44	20.63	15.50
wastes	7.12	0.48	0.36	6.75	0.38	0.28
Total	333.33	22.64	16.87	374.19	21.01	15.78
The ratio of the weight of edible parts to the weight inedible parts	3.42			3.76		
The ratio of muscle weight to bones weight	2.92			3.19		

As a result of the experiment, it was established that a live weight of the chickens in the experimental group was 16.63% higher than in the control group.

The anatomical butchering of carcasses showed an increase in the yield of the most valuable parts, from a technological point of view – breasts and thighs in the experimental chickens, of 18.8 and 21.3%, respectively.

Total content of muscle tissue in the test samples is significantly higher than in the control samples. In addition, the ratio of muscle weight to the weight of the eviscerated carcass amounts to 66.95%. This indicator exceeds the standard values for the species and the age groups of birds significantly.

It is noted that the internal fat content is at a quite low level, which suggests the dietary properties of the broiler chickens carcasses.

Thus, due to the conducted research, it is determined that the meat of broiler chickens, grown with the use of the "Ekstraselen+Vit" preventative feed additive, has better quality characteristics compared to the control chickens.

The chemical composition of the most valuable parts of carcasses from a technological point of view – skinless breast and thigh (Table 6).

To meet the needs of the population in poultry meat, its nutrition value, which depends on the chemical composition, is of great importance. The table above shows that the protein content in the test samples is higher than in the control samples. We identified a decrease of fat content in the thoracic and femoral muscles of test samples, which suggests the dietary properties of the raw meat.

Functional and technological properties of raw meat are shown in Table 7.

The value of active acidity (pH) is in the range of 6.3–6.5 for the control and test samples, which indicates a fairly high stability of the protein system.

Water binding capacity (BCC) is the most important functional characteristic, which determines the quality of minced meat and organoleptic, structural and mechanical properties, as well as the yield of finished products. The data analysis shows an increase in BCC of the test samples: BCC increase in breast – 7.11%, skinless thigh – 4.3%, drumstick – 0.55%. Due to the fact that from a technological point of view, the most valuable parts are breast (white meat) and thigh (red meat), in our opinion, the use of the "Ekstraselen+Vit" is quite effective.

The fact that the plasticity indicators of the test samples are increased, is also worth mentioning. This is due to a high protein content and decreased fat content in the test samples.

Based on the study of functional-technological properties of meat, we determined that is feasible to use this type of raw material in the production of meat products.

In the ANO centre for biotic medicine, the studies were conducted to determine the concentration of selenium in poultry processing products, obtained after slaughter of the control and test samples. The results of the study are presented in Table 8.

The data shows that the concentration of selenium in the muscles of the experimental chickens was significantly higher than in the control chickens. The difference in the selenium content in chickens' femoral muscles of the experimental and control groups amounted to 28.57%, and in the thoracic

Table 6. Chemical composition of raw meat

Studied indicators	Experimental group		Control group	
	breast	skinless thigh	breast	skinless thigh
Fat content, %	1.62	3.56	2.34	4.37
Water content, %	74.7	74.41	75.72	74.54
Salt content, %	2.45	1.99	2.19	1.93
Protein content, %	21.23	20.04	19.75	19.16

Table 7. Functional and technological properties of raw meat

Studied indicators	Experimental group			Control group		
	breast	skinless thigh	skinless drumstick	breast	skinless thigh	skinless drumstick
$pH_{\text{of the studied sample}}$ ($pH_{\text{of distilled water}}$ – 6.62)	6.53	6.39	6.37	6.42	6.36	6.29
WBC, % to the sample weight	60.48	56.55	54.90	56.18	54.12	54.60
Plasticity, cm2/g	3.57	2.59	2.06	2.46	2.14	1.95

Table 8. The concentration of selenium in poultry processing products

Name of the sample	Control group	Experimental group
Liver, mg/g	0.54 ± 0.100	0.84 ± 0.101
Heart, mg/g	0.36 ± 0.043	0.50 ± 0.059
Thigh, mg/g	0.28 ± 0.033	0.36 ± 0.043
Breast, mg/g	0.24 ± 0.028	0.26 ± 0.031

muscles – 8.33%. In the liver and heart, the selenium content increased significantly – by 55.56% and 38.89%, respectively.

Thus, the meat of broiler chickens, grown using the preventative feed additive, is a good source of selenium for humans. Consumption of the meat, enriched within the physiological range, will meet the daily needs of an adult in this microelement, which is 70 mg/day.

CONCLUSION

The studies determined that the introduction of the "Ekstraselen+Vit" feed preventative additive into the diet of broiler chickens increases the pre-slaughter weight and slaughter yield. Thanks to good feed bioconversion, it is possible obtain raw materials at reduced cost, but with high quality indicators.

The technology of growing broiler chickens under development can be used by farms as well as by small and medium-sized poultry processing businesses.

REFERENCES

1. Golubkina N.A. *Selenium in nutrition: plants, animals, people*. Moscow: Kolos Publ., 2006. 254 p. (In Russian).
2. Gmoshinsky I.V. and Mazo V.K. Minerals in human nutrition. Selenium: absorption and bioavailability. *Nutritional problems*, 2006, no. 5, pp. 15–21. (In Russian).
3. Gulyushin S.Yu. and Kovalev V.O. The state of antiradical protection system in broilers when using selenium-containing preparations against toxic feed. *Agricultural Biology*, 2009, no. 4, pp. 14–25. (In Russian).
4. Radchikov V.F., Gurin V.K., Tsay V.P., Shorets R.D and Lyundyshev V.A. *Sodium selenite in the diets of male calves during meat production*. Minsk, 2012. 25 p. (In Russian).
5. Yang G.W. and Li H.L. Sonochemical synthesis of highly monodishersed and size controllable Ag nanoparticles in ethanol solution. *Materials Letters*, 2008, vol. 62, no. 14, pp. 2189–2191.
6. Mishra B., Hassan P.A., Priyadarsini K.I. and Mohan H. Formation of redox active nanoselenium on reactions of oxidizing free radicals with selenourea. *BARC Newsletters*, 2006, no. 273, pp. 262–267.
7. Zhang J.S., Gao X.Y., Zhang L.D. and Bao Y.P. Biological effects of nano red elemental selenium. *BioFactors*, 2001, vol. 15, pp. 27–38.

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APPLICATION OF WHEY-DERIVED SYRUPS IN DAIRY PRODUCTS

V. Somov[a], I. Evdokimov[b,*], S. Knyazev[a], S. Perminov[a], Yu. Kurash[c]

[a] Wimm Bill-Dann/Dairy R&D,
Dmitrovskoe Shosse 108, Moscow, 127591 Russian Federation

[b] North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355009 Russian Federation

[c] PepsiCo/Long Term Research,
Skyline Drive 3, Hawthorne, NY, 10532 USA

* e-mail: ek-v-b@yandex.ru

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Abstract: Sugar substitution is a hot topic in current food and beverage development. Sugar substitute is a food additive with sugar-like taste and usually cheaper than sugar. We developed production of glucose-galactose syrup (GGS) from cheese whey to replace and lower sucrose content in dairy products. Nanofiltrated whey containing 15% lactose underwent enzymatic and demineralization processing, producing different levels of monosaccharaides and electrolytes. We hypothesized that the amount of glucose/galactose and minerals in GGS might mediate sweet taste transduction resulting in different perception of sweetness. Using cell-based approach we demonstrated a link between GGS composition, cellular response, and sensory data. GGS with 20% glucose and 16% galactose activated sweet taste transduction and had similar sweetness level compared to sucrose. Moreover, demineralization level of GGS mediated sweet perception and cellular responses. Taken together, our results provide opportunities to optimize production at low-cost GGS from whey to reduce sugar in various PepsiCo products.

Keywords: Glucose-galactose syrup (GGS), whey, lactose, nanofiltration, demineralization, dairy products

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INTRODUCTION

Sweet taste plays a critical role in the recognition of food and nutrition, and maintaining energy homeostasis. The sweet taste signals from presence of carbohydrates in solution triggers a pleasurable response. Over the past four decades, sugar substitute is the fastest growing segment of the sweetener market. A sugar substitute is a food additive that duplicates the effect of sugar in taste, usually with reduced calories.

Whey is a by-product resulting from dairy industry especially cheese production. It contains a good amount of a disaccharide, lactose that upon hydrolysis yields glucose and galactose leading to increased sweetness (Fig. 1).

Conversion of glucose to fructose and galactose to tagatose further increases sweetness and decrease calories (Fig. 1). Therefore, whey-derived syrups might be used as a sugar substitute.

We produced glucose-galactose syrups (GGS) with different amounts of monosaccharaides and electrolytes. We proposed that GGS composition may regulate sweet signaling pathways leading to different sweetness level. Recent molecular studies have revealed that the sweet receptor heterodimer T1R2/T1R3 is responsive to sweet tasting compounds [1]. Sweet ligands bind to the T1R2/T1R3 receptor and activate G-protein pathway transduction, which include receptor internalization, activation of secondary messengers and intracellular calcium mobilization [2]. Several studies demonstrate that there are T1R-independent mechanisms for sweet taste signaling [3, 4]. It was shown that T1R3-positive taste cells express glucose transporter GLUT4 [3] and glucagon receptor [4] suggesting that these signaling proteins may serve as mediators of sweet taste.

Recently, we have demonstrated that various sweet-tasting compounds selectively activate multiple receptors leading to different perception of sweetness [5]. Natural sugars activate T1R2/T1R3-mediated signaling cascades, whereas artificial sweeteners target both sweet receptors T1R2/T1R3 and GLUT4. Non-caloric sugars, i.e. rebaudioside A activate additional receptor, glucagon receptor, which mediates sweetness. Importantly, HFCS targets four receptors: T1R2, T1R3, GLUT4, and glucagon, indicating that activation of multiple signaling cascades is responsible for HFCS sweetness.

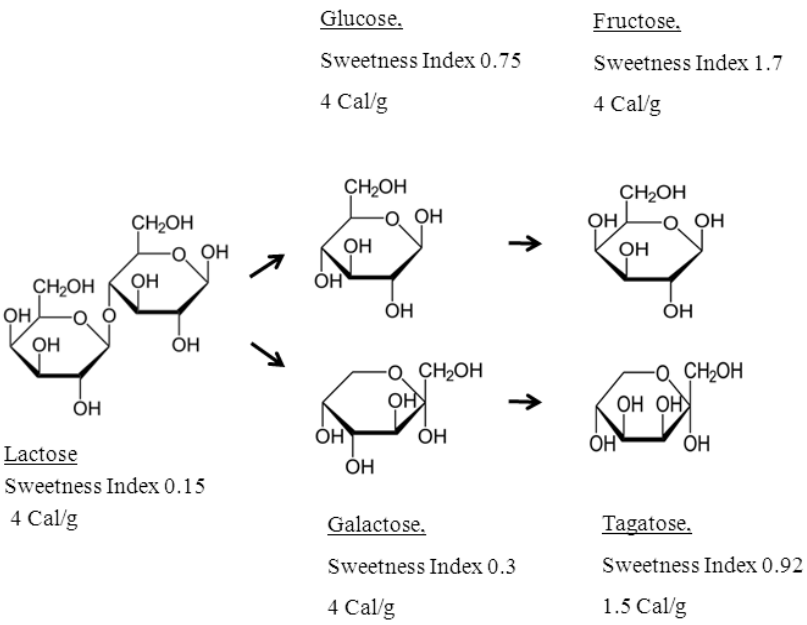

Fig. 1. Chemistry of lactose conversion to useful sweet molecules.

Using cell-based approach and bench top ranking sensory test we demonstrated that the amount of lactose in GGS mediated sweet taste transduction resulting in different perception of sweetness. Thus, GGS with 15% lactose, 20% glucose, and 16% galactose led to activation of T1R2/T1R3 and glucagon receptors mediating sweetness; whereas GGS with 20% lactose, 8% glucose, and 5% galactose significantly increased GLUT4 internalization resulting in aftertaste. Additionally, a correlation was observed between the demineralization level of GGS, receptor-recycling routes, and sensory data. Bench-top sensory studies demonstrated that GGS with low concentration of lactose and 70% demineralization did not affect sweetness in dairy products with 25% sugar reduction, thereby opening doors for utilization of a waste stream to deliver cost-effective sugar reduction in PepsiCo products.

OBJECTS AND METHODS OF STUDY

Production of GGS

GGS production from whey was conducted using baromembrane method. Dry whey was comprised of protein (0.73%), lactose (4.45%), fat (0.05%), and ash (0.55%). First, the whey underwent an ultrafiltration process that reduced the protein and fat content. Concentrated whey contained 0.03% protein, 4.4% lactose, 0% fat, and 0.5% ash. Then, whey was three-times concentrated down until the brix reached 19%. The final concentrations of whey components in nanofiltrated (NF) concentrate were 17% lactose, 1% protein, and 0.4% ash. NF concentrate was treated with β-galactosidase for four hours at 40°C to hydrolyze lactose into glucose and galactose at similar concentrations. Finally, NF concentrate was evaporated under vacuum at 0.8 bars until Brix reached 65–70%. Fig. 2 shows the process of GGS production from whey.

Chemical and sugar analysis

Chemical analysis of whey components and sugar concentration were determined using the following methods: (a) potentiometric method (pH measurement); (b) refract metric method (Brix measurement); (c) Duma's method (protein concentration); (d) enzymatic method (concentration of lactose, galactose, and glucose); (e) digestion at +520°C (ash content); (f) atomic absorption (cation content, i.e. K^+, Na^+, Ca^{2+}, Mg^{2+}); (g) IC method (anions content).

Materials for cell-based assay

Rabbit anti-T1R2 and rabbit anti-T1R3 antibodies were from Thermo Fisher. Rabbit anti-GLUT4, mouse anti-Glucagonreceptor, andrabbitanti-GLP1Rantibodies were from Sigma. Alexa488-conjugated antibodies and Hoechst 33342 were from Life Technology. NCI-H716 cell line was purchased from ATCC.

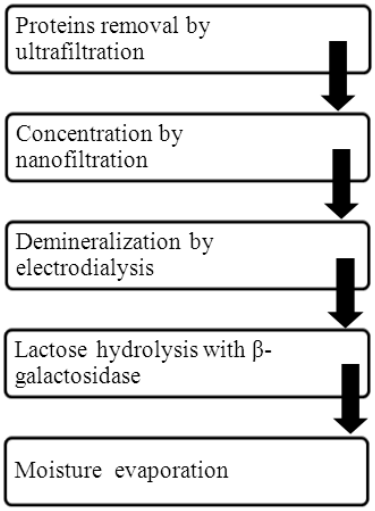

Fig. 2. Production of GGS from whey.

Cell-based assay

NCI-H716 cells were grown in RPMI1640 supplemented with 10% fetal bovine serum. Cells were seeded at density of 20 000 cells/well on PDL-coated 384-well plates. Then cells were treated with GGS at 6.5–7.0% brix or with control sweet molecules at 100 mM.

High-Content imaging and analysis

Cells were fixed in 4% formaldehyde and then permeabilized in 0.5% Triton X-100 in DPBS. For GLUT4 and Glucagon receptor staining cells were fixed in methanol/acetone. Primary antibody was added to each well for 16 h at +4°C. After washing the cells three times with D-PBS, secondary Alexa488-conjugated antibody was added for 30 min at room temperature. Nuclear staining was performed using Hoechst 33342 and incubated at room temperature for 15 minutes. Images were acquired using an ImageXpress Micro automated epifluorescence microscope (Molecular Devices Corporation). Images were analyzed with MetaXpress 4.0 Workstation software, utilizing the Multiwaves Translocation Scoring analysis algorithm for nuclear and cytoplasmic segmentation (Fig. 3). "Ring" positive cells were calculated by correlation coefficient for pixel values of Hoechst 33342 and Alexa 488 (receptor) signals [6]. Curve fitting and parameter estimation were analyzed with TIBCO Spotfire.

Bench top ranking sensory test

All sensory analyses were performed in Dairy R&D panel, using untrained employee personnel. Bench top yogurts were prepared using sucrose (100%), sucrose (75%), and 75% sucrose with GGS (Fig. 4). These experiments were replicated more than three times. Ranking analysis of sweetness was conducted as there are no perceivable sensory differences between exist sample and samples with 75% of sucrose with GGS.

RESULTS AND DISCUSSION

GGS composition

The disposal of whey remains a significant problem for the dairy industry. One possible approach to whey utilization is hydrolysis of lactose resulting in glucose-galactose syrup which might be used as a sucrose substitute. We developed production of GGS from NF concentrate using enzymatic approach. The lactose hydrolysis reactions were carried out using a commercial β-galactosidase. GGS generated from cheese whey contained varying amounts of monosaccharaides and lactose, depending on the β-galactosidase enzymatic activity. Produced GGS can be divided into two major types based on the level of lactose hydrolysis (Table 1). GGS type 1 contained high amount of monosaccharaides (20% glucose and 15% galactose), and 14% lactose, whereas type 2 had low amount of glucose-galactose (8% glucose and 5% galactose) and 20% lactose (Table 1).

We conducted to examine the effects of lactose hydrolysis level on sweet taste transduction using cell-based assay and sweetness level in dairy desserts with 25% sucrose reduction.

Cell-based assay results

Receptor internalization upon stimulation with ligand is considered to be a key component of a cellular response [1]. To determine whether GGS stimulates

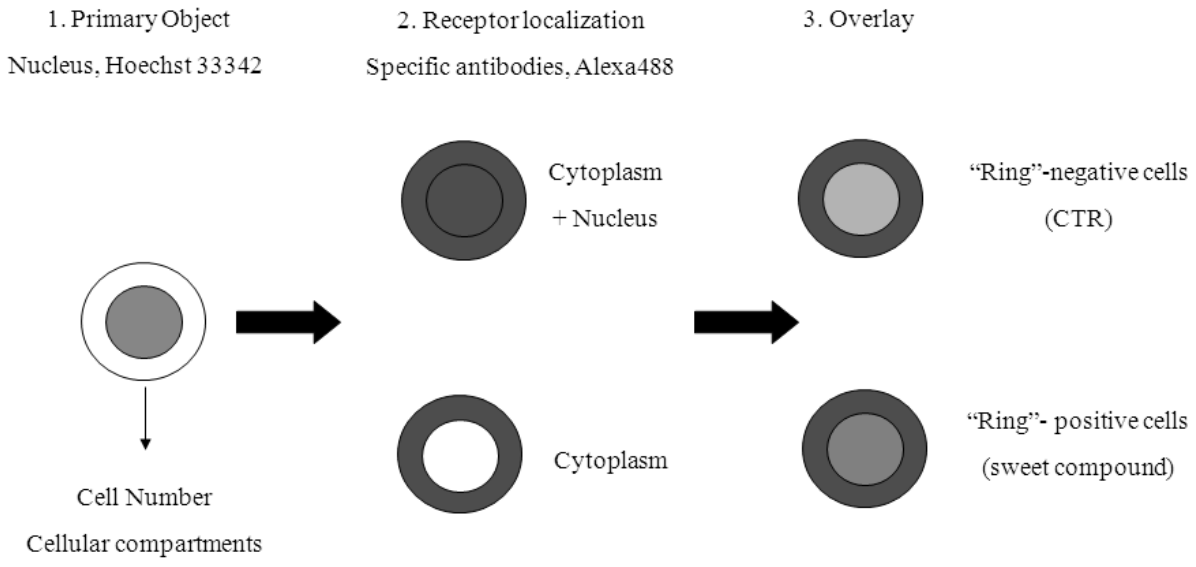

Fig. 3. Image analysis algorithm for quantification of "Ring"-positive cells.

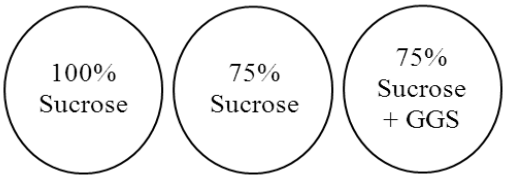

Fig. 4. Bench-top ranking sensory test.

internalization of receptors underlying sweet taste transduction, we developed a High-Content imaging assay using the human enteroendocrine L cell line NCI H716 that respond to sweet compounds [7].

First, we investigated the effects of GGS with various degrees of lactose hydrolysis on sweet receptor T1R2/T1R3 internalization. Recent molecular studies have revealed that the sweet receptor heterodimer T1R2/ T1R3 is responsive to sweet tasting compounds and activate G-protein pathway transduction [1]. We have demonstrated that untreated NCI-H716 cells expressed T1R2/T1R3 receptors at the cell-surface (Fig. 5, left). Treatment with sweet-tasting compounds induced T1R2/T1R3 receptor internalization and trafficking T1R2/T1R3 receptor internalization and trafficking from the target membrane to cytosolic vesicles, resulting in typical "Ring"-staining (Fig. 5, right). Using Molecular Devices Multiwaves Translocation Scoring Module, we quantitated internalization of endogenous T1R2/T1R3 in NCI-H716 cells treated with GGS (Table 2).

We have found that T1R2/T1R3 internalization increased after stimulation with GGS type 1 (Table 2). In contrast, GGS type 2 had a strong effect on T1R3 internalization only. Sample #1 of GGS type 2 slightly induced T1R2 internalization, whereas even minimal T1R2 internalization did not occur upon treatment with sample #2 of GGS type 2 (Table 2). Intriguingly, several other studies demonstrated distinct contributions of T1R2 and T1R3 taste receptor subunits in detection of sweet stimuli [8]. It was found that T1R3 requires co-expression with T1R2 to form a fully functional sweet taste receptor, whereas homomeric T1R3 receptor may act as low-efficacy sugar receptors [8]. Therefore, our data have demonstrated a link between GGS composition and activation of different subunits of sweet taste receptor.

Table 1. GGS composition (sugar)

Syrup type	Sample #	Glucose, %	Galactose, %	Lactose, %
1	1	21.1	17.0	13.7
	2	20.8	15.2	14.7
2	1	8.9	5.9	20.6
	2	8.1	5.2	22.4

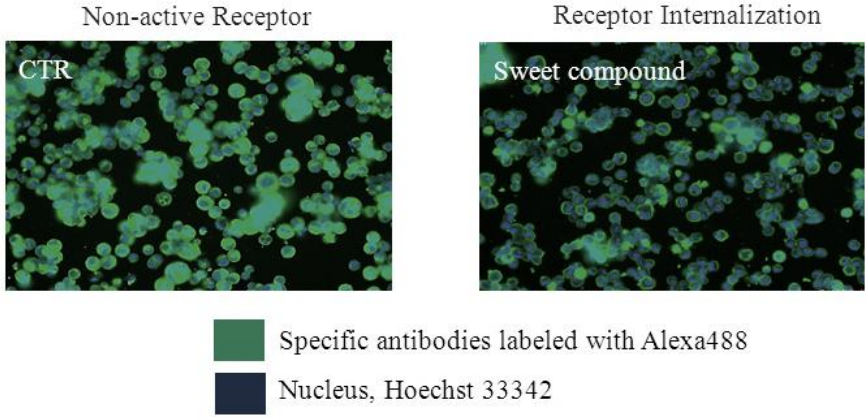

Fig. 5. "Ring"-formation assay.

Table 2. Link between GGS composition to cellular response and sensory

Syrup Type	GGS Samples				Cellular Response				Bench-top Sensory
	Sample #	Glucose, %	Galactose, %	Lactose, %	Sweet Receptor		Glucose Transporter GLUT4	Glucagon Receptor	
					T1R2	T1R3			
1	1	21.1	16.9	13.7	+	+	-	+	Sweetness match
	2	20.8	14.9	14.7	+	+	-	+	Sweetness match
2	1	8.3	5.2	22.4	+	+	++	-	Aftertaste
	2	8.9	5.9	20.6	-	+	-	+	Less sweet

Recently, we have demonstrated the existence of T1R-independent mechanisms for sweet taste signaling (personal communication). We showed that artificial sweeteners and non-caloric sugars, i.e. rebaudioside A activate GLUT4 and glucagon receptor, respectively in addition to sweet. GLUT4-mediated cellular response correlates with bitter and sweet aftertaste of artificial sweeteners, whereas glucagon receptor mediates sweetness (personal communication).

To further explore a link between GGS composition and cellular response, we investigated the internalization of GLUT4 and glucagon receptor in NCI-H716 cells. Both samples of GGS type 1 induced internalization of glucagon receptor and were not able to activate GLUT4 internalization (Table 2). In contrast, GGS type 2 robustly activated either GLUT4 internalization (sample #1), or slightly induced internalization of glucagon receptor (sample #2) (Table 2).

Recently, we have demonstrated that various sweet-tasting compounds selectively activated internalization of multiple receptors leading to different perception of sweetness (Table 3).

For example, glucose, or fructose activate T1R2 sweet receptor-mediated signaling cascade; galactose, or lactose stimulate GLUT4 and glucagon receptor internalization; treatment with sucrose leads to internalization of T1R2/T1R3 sweet receptor (Table 3). Interestingly, HFCS stimulates internalization of four receptors: T1R2, T1R3, GLUT4, and glucagon (Table 3), whereas mixture of 55% glucose+45% fructose induces internalization of T1R2 only, indicating that activation of multiple signaling cascades is responsible for HFCS sweetness.

Similar to HFCS, GGS type 1 activated multiple receptors, T1R2/T1R3 sweet receptor and glucagon receptor mediating sweet perception. In contrast, we did not observed a specific profile in cellular response upon treatment with GGS type 2. Taking together, our data demonstrated a relationship between level of lactose hydrolysis in GGS and activation of sweet taste signaling pathways.

Bench-top sensory data

To determine whether GGS composition and activation of specific sweet taste transduction receptors are responsible for GGS sweetness, we tested GGS type 1 and type 2 in bench-top ranking sensory studies. Experimental bench top yogurts were made using 100% sucrose, 75% sucrose, and 75% sucrose with GGS in fruit preparation recipe. In general, bench top yogurts with GGS type 1 were similar in sweetness level compared to control yogurts (Table 2). In contrast, bench top yogurts with GGS type 2 were significantly different compared to reference samples and possessed sweet aftertaste (Table 2). Thereby, sensory analysis data correlated with GGS composition and cellular response, providing useful experimental approach for further optimization of GGS production with different sensory properties.

Electrolytes composition in GGS

NF concentrate contains varying amounts of non-sugar substances, e.g. ash. During evaporation from 15 brix to 65–70 brix, there is a risk of scale formation. To prevent scale forming in the evaporation station and prevent precipitation during storage, a portion of the minerals have been removed by electrodialysis. Using GGS with ~24% glucose, ~20% galactose, and 0–2.9% lactose, we prepared GGS with 0%, 50% and 70% demineralization level (Table 4). Then, GGS samples were subjected to receptor internalization studies and sensory evaluation.

Cell-based assay and bench-top sensory results

Using High-Content imaging we have demonstrated that GGS with 0% and 70% demineralization levels, like HFCS activated four receptors, T1R2/T1R3, GLUT4 and glucagon (Table 5). In contrast, we did not observe

Table 3. Cellular response and sensory data, control samples

Control Samples	Sweet Receptor		Glucose Transporter GLUT4	Glucagon Receptor	GLP1 Receptor	Sweetness Index
	T1R2	T1R3				
Glucose	+	-	-	-	-	0.75
Galactose	-	-	+	+	-	0.3
Lactose	-	-	+	+	-	0.16
Sucrose	+	+	-	-	-	1.0
Fructose	+	-	-	-	-	1.7
45% Glucose+55% Fructose	+	-	-	-	-	<1.2
HFCS	+	+	+	+	+	1.2

Table 4. GGS composition (electrolytes)

Sample #	Sample name	Conductivity, mS	Ca^{2+}, mg/kg	Mg^{2+}, mg/kg	Na^{+}, mg/kg	K^{+}, mg/kg
1	Without demineralization	9	1 778	881	9 063	17 908
2	Demineralization 50%	3.8	1 365	563	2 613	3 760
3	Demineralization 70%	2.5	1 305	526	2 078	2 693

the effect on T1R2 internalization upon treatment with GGS with 50% demineralization level (Table 5), suggesting that GGS with 50% demineralization might have low sweetening potency and sweet aftertaste. Actually, bench-top sensory studies demonstrated that 50% demineralization of GGS affected sweetness level and possessed sweet aftertaste in dairy desserts with 25% sucrose reduction (Table 6).

At the same time, bench top yogurts with 0% and 70% demineralization of GGS were significantly different in sweetness level compared to each other (Table 6). GGS with 0% demineralization possessed sweet aftertaste, whereas GGS with 70% demineralization was similar in sweetness level compared to control yogurts and had like honey taste (Table 6).

Recently, we demonstrated a correlation between the molecular structures of sugars and T1R2-recycling routes. Thus, T1R2 recycled back to the cell membrane very quickly upon treatment with monosaccharaides, D- glucose and D-fructose, whereas slow T1R2-recycling pathway was observed with the disaccharide sucrose and its analog sucralose (personal communication). Moreover, we found that HFCS induced delayed T1R2/T1R3 sweet receptor internalization and slow T1R2/T1R3-recycling pathway compared to mixture of 55% glucose+45% fructose (Table 6), suggesting that internalization kinetics and recycling routes might be responsive for sweetness quality.

To explore the link between demineralization level of GGS, sweetness level and recycling kinetics of receptors mediating sweet taste signaling, we performed internalization kinetics studies. We found that GGS with 0% and 70% demineralization levels, like HFCS induced delayed T1R2/T1R3 sweet receptor internalization and slow T1R2/T1R3-recycling pathway (Table 6). However, we observed the significant difference in internalization kinetics and recycling routes for GLUT4 and glucagon receptor between GGS with 0% and 70% demineralization levels and HFCS. HFCS activated GLUT4 and glucagon receptor at 5 min and both receptors recycled back to the cell membrane at 15 min (Table 6). Treatment with GGS at 70% demineralization stimulated additional delay in GLUT4 and glucagon receptor internalization with quick recycling to the cell membrane, whereas GGS with 0% demineralization induced delayed internalization of GLUT4 and glucagon receptor and slow recycling pathway (Table 6).

Our results provided evidence that GGS with different sweetness level activated diverse patterns and kinetics of sweet taste signaling cascades and receptor trafficking routes, further supporting the conclusion that receptor internalization events mediate sweetness level of GGS and providing novel opportunities for optimization of GGS production from whey.

Practical application of whey-derived syrups

GGS was approved for use in Russia in various dairy products [9]. Table 7 shows the examples of fruits preparation recipes currently using in dairy desserts, such as mixed yogurts, drinkable yogurts and spoonable yogurts. Sucrose might be replaced with either HFCS or with GGS (Table 7). 25% replacement of sugar with GGS in fruit preparation recipe would result in saving of 6 MM USD per year. Application of GGS in dessert milk product recipes may bring additional 5 MM USD in savings.

Table 5. Link between demineralization level of GGS to cellular response (dose-response studies)

Sample #	GGS composition				Cellular response				Sensory
	Glucose, %	Galactose, %	Lactose, %	Demineraliza-tion level, %	T1R2	T1R3	GLUT4	Glucagon	
1	23.7	20.0	2.9	0	+	+	+	+	Sweet, Bitter aftertaste
2	24.4	15.9	0	50		+	+	+	Less sweet, Bitter aftertaste
3	26.3	21.6	2.6	70	+	+	+	+	Sweet, Honey taste

Table 6. Link between demineralization level of GGS to cellular response (kinetic study) and sensory

Sample #	Glucose, %	Galacto-se, %	Lactose, %	Demine-ralization level, %	Glucagon			GLUT4			T1R2			T1R3			Sensory
					5	15	30	5	15	30	5	15	30	5	15	30	
1	23.7	20.0	2.9	0		+	+		+	+		+	+		+	+	Sweet, Bitter aftertaste
2	24.4	15.9	0	50			+			+					+	+	Less sweet, Bitter
3	26.3	21.6	2.6	70			+			+		+	+		+	+	Sweet, Honey taste
HFCS					+	+		+	+			+	+		+	+	
45% Glucose + 55% Fructose											+			+			

Table 7. Sample of GGS application in fruit preparation recipe

Ingredients	Condition	kg/ton	kg/ton	kg/ton
Sugar	Bx 99	590.0	435.0	75.0
HFCS	Bx 71		219.0	0.0
GGS	Bx 75	0.0	0.0	690.5
Cranberry concentrate	Bx 64-66 TK (based on citric acid pH=8.1) 14.0–21.0%	40.0	40.0	40.0
Raspberry concentrate	Bx 64-66 TK (based on citric acid pH=8.1) 9.0–12.0%	27.0	27.0	27.0
Pectin	YM-115 H CP Kelco	4.0	4.0	4.0
Cranberry aroma	No. 321793 Symrise	6.0	6.0	6.0
Raspberry aroma	No. 648289 Symrise	1.0	1.0	1.0
Color carmine	9% Biocolor 180, Carmiliq, Naturex/Overseal	0.55	0.55	0.5
Water		331.45	267.45	156.00
Total		1 000.0	1 000.0	1 000.0

CONCLUSION AND NEXT STEPS

Taken together, we demonstrated a link between the amount of lactose/ monosaccharaides and minerals in GGS, cellular response, and sensory data. Additionally, we provided opportunity for PepsiCo to use GGS in dairy products. In general terms, replacing sugar with whey-based syrup, can produce savings with maintaining a high quality end-product. In the meantime, additional areas of commercial application of GGS will be explored.

For the next steps, optimization of enzymatic hydrolysis and demineralization process will be evaluated.

REFERENCES

1. Nelson G., Hoon M.A., Chandrashekar J., Zhang Y., Ryba N.J. and Zuker C.S. Mammalian sweet taste receptors. *Cell*, 2001, vol. 106, no. 3, pp. 381–390.
2. Linderman B. Receptors and transduction in taste. *Nature*, 2002, no. 413, pp. 219–225.
3. Yee K.K., Sukumaran S.K., Kotha R., Gilbertson T.A. and Margolskee R.F. Glucose transporters and ATP-gated K+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1R3)-expressing taste cells. *Proc. Natl. Acad. Sci. USA*, 2011, vol. 108, no. 13, pp. 5431–5436.
4. Elson A.E., Dotson C.D., Egan J.M. and Munger S.D. Glucagon signaling modulates sweet taste responsiveness. *FASEB J*., 2010, vol. 24, no. 10, pp. 3960–3969.
5. Somov V., Perminov S., Knyazev S., Kurash Y.K., Aglione A., Dragan S., Cassutt K. and Gravina S. Application of Whey-derived syrups in PepsiCo dairy products. *Global R&D Research Forum*. Chicago, 2013.
6. Kurash Y.K. and Gravina S. *Taste Receptor Internalization Assay*. Patent WO, no. 2014183041 A1, 2013.
7. Jang H.J., Kokrashvili Z., Theodorakis M.J., Carlson O.D., Kim B.J., Zhou J., Kim H.H., Xu X., Chan S.L., Juhaszova M., Bernier M., Mosinger B., Margolskee R.F. and Egan J.M. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc. Natl. Acad. Sci. USA*, 2007, vol. 104, no. 38, pp. 15069–15074.
8. Nie Y., Vigues S., Hobbs J.R., Conn G.L. and Munger S.D. Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. *Curr Biol*., 2005, vol. 15, no. 21, pp. 1948–52.
9. Somov V., Perminov S. and Knyazev S. *Fruit preparation with GGS, technology process*. Patent RF, no. 2011154292, 2013.

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PREPARATION AND USE OF WHEY PROTEIN MICROPARTICULATE IN SYNBIOTIC DRINK TECHNOLOGY

E. I. Melnikova[a], E. B. Stanislavskaya[a,*], E. G. Korotkov[b]

[a] Voronezh State University of Engineering Technologies,
Revolution Avenue 19, Voronezh, 394036 Russian Federation

[b] Voronezh State Agricultural University named after Emperor Peter the Great,
Michurina Str. 1, Voronezh, 394087 Russian Federation

* e-mail: tereshkova-katia@yandex.ru

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Abstract: A crucial task of the dairy industry is the modification of the composition and properties of cheese whey to level its organoleptic characteristics for use of qualitatively new food products in technology, including the one of functional use. The work purpose is the optimization of technological parameters of the microparticulation process of ultrafiltration cheese whey concentrate for its use in the production of low-calorie synbiotic drinks. The research objects are cheese whey, food composition based on it (whey protein microparticulate) and synbiotic drink. When performing work, the standard and commonly used in research practice physical and physical and chemical, chemical and biochemical, microbiological, physiological and technological methods of research were used. For mathematical support of experimental results different methods of statistics and optimization, including the method for artificial neural networks were used. The technology of producing milk fat simulator provides pre-cleaning of whey from casein particles and fat, fractionation and concentration of whey proteins using ultrafiltration, as well as thermomechanical processing of the obtained concentrate. The whey protein microparticulate is close to skimmed milk by physical and chemical properties and chemical composition, and its organoleptic properties simulate drinking cream. The new food composition is characterized by a pronounced prebiotic activity. During the development of synbiotic drink formulation the great importance was given to the selection of probiotic cultures able to synthesis of exopolysaccharides. The research results suggested the formulation and component solution of the synbiotic drink, which involves the replacement of 27% skimmed milk by the new food composition, with the exception of cream, stabilizer and skimmed milk powder. The main advantages of the new technology solution are the implementation of a closed cycle of production, the expansion of low-calorie products of high biological value and the reduction of economic costs.

Keywords: Cheese whey, ultrafiltration, microparticulation, food composition, synbiotic drink

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INTRODUCTION

The strategy of development of food and processing industry of the Russian Federation for the period up to 2020 provides for the development and implementation of innovative food technologies forming the agrofood market, food and economic security of the country. For the dairy industry, these objectives are very relevant, due to insufficient production of raw milk and stable dynamics of growth of prices for it.

It is advisable to consider the by-products of milk processing, particularly cheese whey, as a home-base industry resource for increase of production, improvement of economic performance and avoidance of environmental pollution [1, 2]. The main direction of cheese whey processing, implemented in our country – drying – does not allow to preserve the native properties and to fully realize the biotechnological potential of this raw material [3]. The significant volumes, high nutritional value, the presence of functional ingredients necessitate complete harvesting and rational use of whey in composition of food products [4, 5]. The problem of modification of the composition and properties of cheese whey to level its organoleptic characteristics for use of qualitatively new food products in technology, including the one of functional use remains urgent. The state policy in the field of healthy nutrition of the population

for the period up to 2020 provides for the growth of production of functional foods [6]. Taking into account the urgency, the implementation of biotechnological potential of cheese whey in technology of such products, particularly, synbiotic drinks is of great scientific and practical interest.

One of the latest trends in the development of new food products is the replacement of animal fats, including milk fat, by food compositions of protein nature which simulate the organoleptic properties of fat-containing components [7–12]. The whey protein microparticulates, obtained with the use of innovative methods of cheese whey modification, are characterized by such properties.

The work purpose is the optimization of technological parameters of the microparticulation process of ultrafiltration cheese whey concentrate for its use in the production of low-calorie synbiotic drinks.

OBJECTS AND METHODS OF STUDY

Cheese whey, food composition based on it and synbiotic drink were considered as the objects of research. When performing work, the standard and commonly used in research practice physical and physical and chemical, chemical and biochemical, microbiological, physiological and technological methods of research were used [13]. For mathematical support of experimental results various methods of statistics and optimization, including the method for artificial neural networks were used.

RESULTS AND DISCUSSION

The most valuable component of whey is whey proteins which can be modified to the food composition – milk fat simulator by physical and chemical modification of their properties. The technology of producing the simulator involves pre-cleaning of whey from casein particles and fat, fractionation and concentration of whey proteins using ultrafiltration, as well as thermomechanical processing of the obtained concentrate. Our proposed concentration factor (4–4.5) allows to obtain a food composition with mass fraction of dry solids of 9–9.6% and content of whey proteins of 2.9–3.6%. The composition is close to skimmed milk by chemical composition and, therefore, can replace it in technology of a wide range of low-calorie dairy products. For the formation of protein globule agglomerates, which create organoleptic sensation of creaminess, consistency smoothness and thus imitate the cream flavour, the method of thermo-induced aggregation was proposed. Its essence lies in denaturation and aggregation of proteins of UF cheese whey concentrate with consequent increase in the dispersity of the obtained agglomerates (Fig. 1). Technologically this operation is called micro-particulation and in practice can be implemented in the tubular heat exchanger and homogenizer.

According to the literature review data to obtain high quality fermented milk drinks, characterized by a viscous creamy texture, the particles of microparticulate should have a size of from 1 to 1.5 μm. For effective control of technological parameters of the microparticulation process an artificial neural network which with the help of learning algorithms was brought into accordance with the experimental data. On the basis of rational particle size of 1–1.5 μm by using the artificial neural network the optimal parameter values of the technological process were determined: heating temperature – 92.1°C, duration – 10.2 min, homogenization pressure – 24.8 MPa and homogenization temperature – 60.3°C.

The optimal conditions of microparticulation served as the basis for developing a method of technological cheese whey modification (Fig. 2). Much attention is paid to pre-cleaning of raw materials, which includes cleaning of whey from cheese dust with the use of vibrating screen and milk clarifying separator,

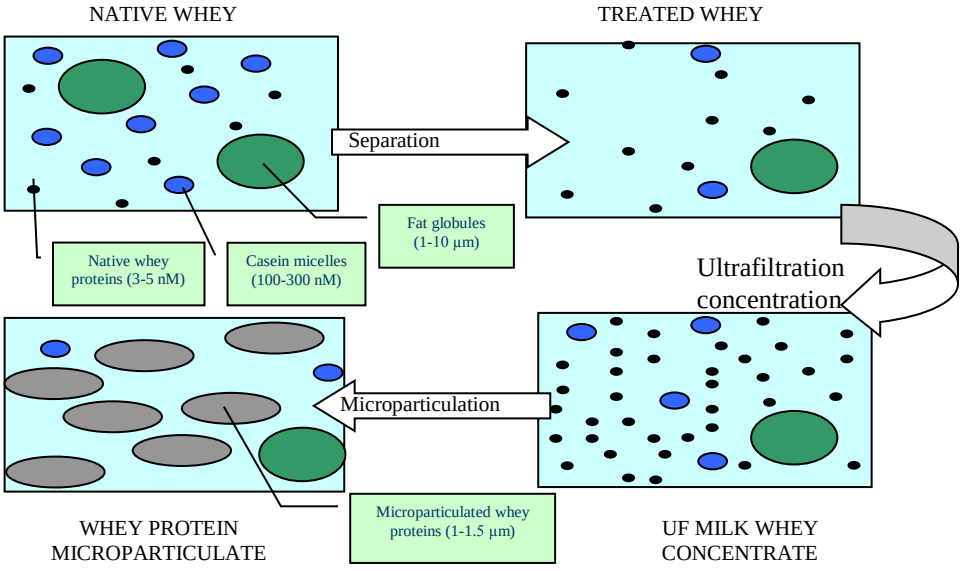

Fig. 1. Diagram of modification of whey cheese composition and properties.

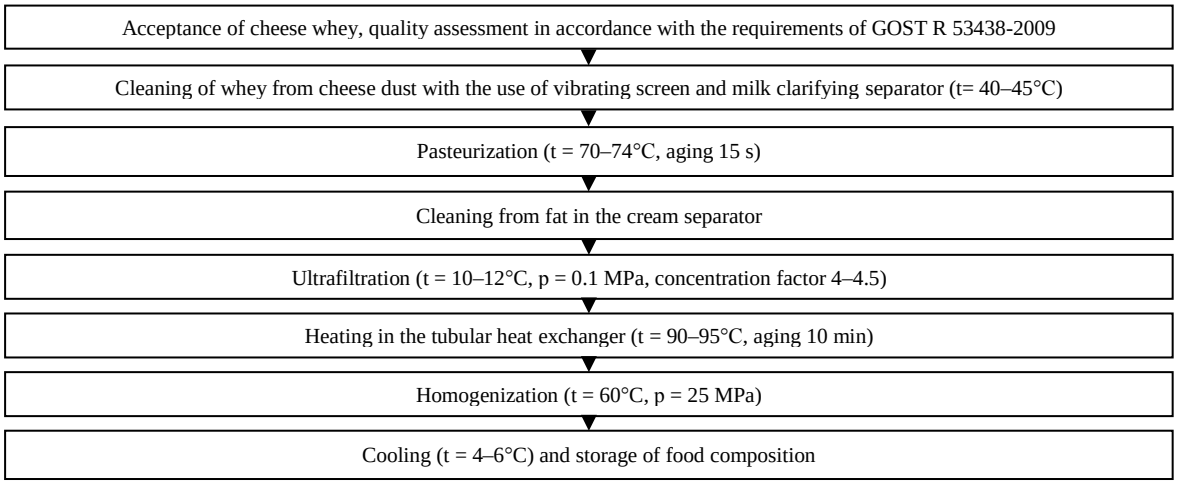

Fig. 2. Process flow diagram of obtaining whey protein microparticulate.

pasteurization, cleaning from fat in the cream separator, ultrafiltration with a concentration factor of 4.5, heating of the UF concentrate in the tubular heat exchanger, homogenization, cooling and storage.

Conducting thermo-induced aggregation under optimal conditions provided the food composition with a particle size of 1–1.5 μm, which is confirmed by the research data on granulometric composition. The obtained food composition is close to skimmed milk by physical and chemical properties and chemical composition (Table 1).

Both biological fluids are characterized by almost the same mass fraction of protein on a dry weight basis, titratable and active acidity.

This determines the feasibility of replacing skimmed milk by the new food composition in technology of a wide range of low-calorie food products with high biological value due to the change in the ratio of casein to whey proteins.

The organoleptic properties of the food composition simulate drinking cream: the new food composition is homogeneous, opaque, moderately viscous, white liquid with clean dairy flavour and aroma, pasteurization flavour.

Special attention was paid to the study of the aroma of the food composition, because it is known that cheese whey is characterized by unsatisfactory organoleptic properties, limiting its wide application in food technologies. Its specific taste and smell are due to the complex of heterogeneous by chemical nature substances, which are formed mainly as a result of the effect of enzymes on milk components at obtaining cheese. In the gas phase of cheese whey oleic, isobutyl, acetic and propionic acid, also acetone, methyl ethyl ketone, ethanol, propanol, acetaldehyde and methyl acetate were identified. Using the multi-touch installation with 9 sensors the visual images of cheese whey 2 hours after production and microparticulate were obtained (Fig. 3). The shape and size of the visual images differ from each other. In the new food composition the main contribution to the formation of aroma is made by propanol, isobutanol, acetaldehyde, propionic and oleic acid.

Table 1. Chemical composition and properties of the research objects

Indicator	Indicator value		
	cheese whey	skimmed milk	whey protein microparticulate
Mass fraction of dry solids, %	6.3	8.6	9.6
Mass fraction of protein, %, including: casein whey proteins nonprotein nitrogen	0.80 0.03 0.69 0.03	3.00 2.67 0.28 0.06	3.50 0.45 2.98 0.05
Mass fraction of fat, %	0.1	0.05	0.3
Mass fraction of lactose, %	4.7	4.8	4.5
Mass fraction of macroelements, %, including: calcium potassium magnesium phosphorus	 0.058 0.121 0.008 0.065	 0.125 0.150 0.013 0.086	 0.122 0.212 0.120 0.990
Active acidity, un. pH	6.5	6.1	5.7
Titratable acidity, °T	14.0	18.0	22.0
Viscosity, mPa·s	1.5	1.7	10.1

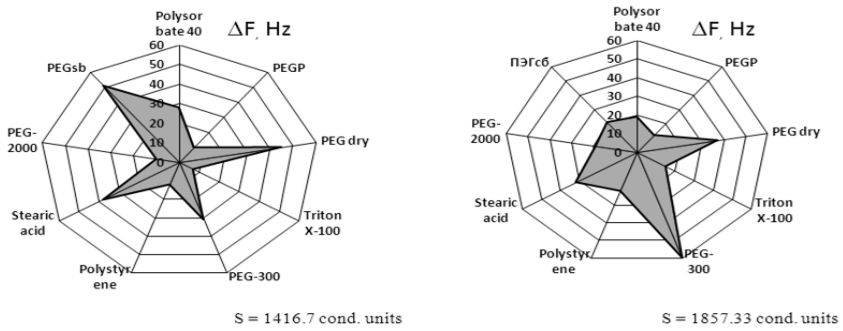

Fig. 3. Visual images of the aroma of cheese whey (a) and whey protein microparticulate (b).

The content of methyl ethyl ketone, methyl acetate, isobutyl alcohol is reduced, which is likely due to the chemical processes occurring during protein thermo- induced aggregation. On the whole, aroma became more pronounced, leveling the negative sensory properties of cheese whey, the new food composition acquired pleasant nutty flavour and aroma, due to the sulfur-containing and other substances. This opens up new opportunities for the application of whey protein microparticulate in technology of a wide range of food products.

The food composition with desired properties is characterized by higher biological value (Table 2) compared to cheese whey; its basic component is whey proteins containing all essential amino acids, the set of which is as close to scale FAO/WHO (Food and Agricultural Organization / World Health Organization) as possible (Fig. 4).

Lactose and the amino acids listed above are characterized by prebiotic properties. As a result of the synergistic interaction of these components the prebiotic effect of the new food composition enhances. To confirm this assumption we studied the bifidogenic activity of the microparticulate.

There was a significant increase in the physiological activity of bifidobacteria. The amount of biomass was several orders of magnitude greater than in the control medium (modified Blaurock medium without additives) (Table 3) and there was a more intense decrease in active acidity (Fig. 5).

Table 2. Value of indicators characterizing biological value

The indicator value for the product	Biological value, %	The amount of essential amino acids, g/100 g of protein	Balance (utility) ratio of amino acid composition	Imbalance ratio of amino acid composition	The comparable redundancy indicator, g/100 g of ethanol protein
Cheese whey	68	37.0	0.74	0.26	12.9
New food composition	77	43.9	0.82	0.18	7.9

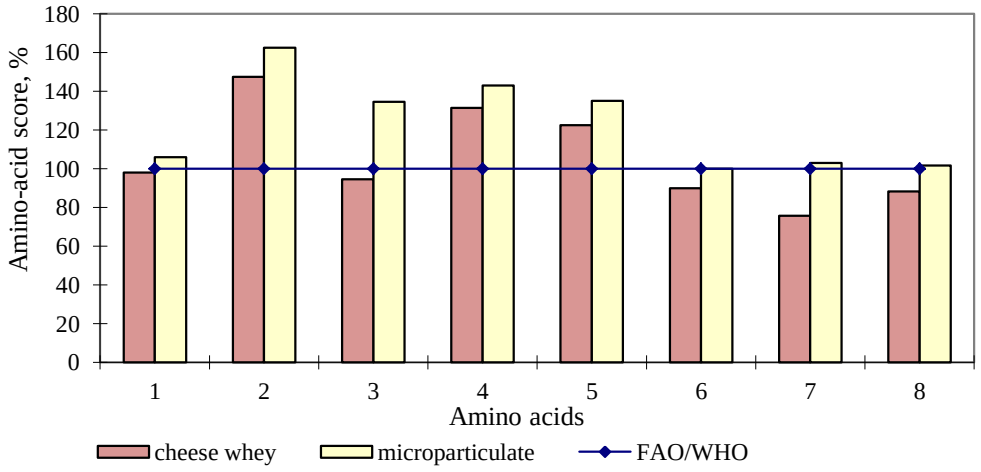

Fig. 4. Amino-acid score of whey protein microparticulate and cheese whey: 1 – valine; 2 – isoleucine; 3 – lysine; 4 – methionine + cysteine; 5 – threonine; 6 – tryptophan; 7 – leucine; 8 – phenylalanine + tyrosine.

Table 3. Changes in biomass of bifidobacteria

Duration of cultivation, h	Number of bifidobacteria in the media, CFU/g		
	control	with inulin	with food composition
18	$7 \cdot 10^5$	$6 \cdot 10^8$	$9 \cdot 10^9$
24	$1 \cdot 10^6$	$8 \cdot 10^9$	$1 \cdot 10^{10}$
48	$6 \cdot 10^7$	$7 \cdot 10^{10}$	$7 \cdot 10^{11}$

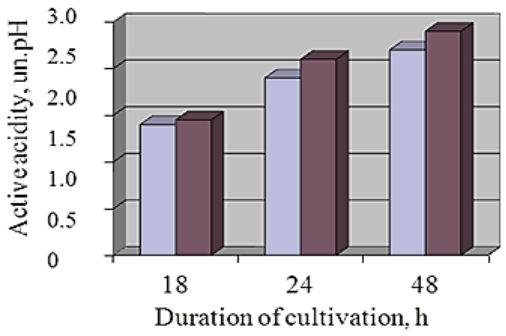

Fig. 5. Dynamics of changes in active acidity of culture medium.

Cells of bifidobacteria had a greater number of typical forms (sticks united in chains) than the ones in the control medium, indicating the usefulness of the nutrient medium composition. The new food composition was characterized by pronounced prebiotic activity comparable with the activity of a recognized growth stimulator of inulin bifidobacteria. Taking into account the results obtained, we have proposed the composition use in technology of fermented milk drinks to give them synbiotic properties. Its inclusion in the composition of functional synbiotic products is appropriate, will contribute to the correction of intestinal micro-biocenosis and enhance the immunocorrective effects of the products.

During the development of synbiotic drink formulation the great importance was given to the selection of probiotic cultures. We analyzed and conducted research on various ferments of mixed cultures, such as *Streptococcus thermophiles* (*S. thermophilus*) and *Lactobacillus delbrueckii of bulgaricus* (*L. bulgaricus*) subspecies. When comparing them, we investigated organoleptic, rheological and histological characteristics of the clot. Of particular interest was the study of the ability of ferment microorganisms to synthesize exopoly-saccharides (EPS), which is a strain-specific property. EPS are macromolecular polymers consisting of residues of sugars, which are secreted by microorganisms. These substances condense the texture of fermented dairy products due to binding free moisture and slowing whey separation, which is especially important in the manufacture of products with reduced fat content, in which the viscosity during fermentation is reduced.

EPS are the substances with prebiotic properties, which provides a synergistic effect in the final product. It is established that the use of Yo-Flex Mild 1.0 ferment, produced by the Christian Hansen company, helps to get the greatest amount of exopolysaccharides and thick, viscous consistency of the drink (Table 4).

When developing the formulation and component solution of the drink, yogurt with 3.2% mass fraction of fat, the formulation of which involves the use of skimmed and whole milk, cream, skimmed milk powder, stabilizer and ferment, was selected as a control sample.

The development of the formulation and component solution was carried out with account of:
– the retention of standard physical and chemical parameters and organoleptic properties of fat-containing products;
– the product rheological properties, the study of which is of particular importance in the development and practical implementation of the low-fat products technology;
– high synbiotic activity of the finished product.

We investigated several samples of the normalized mixture for yogurt with different mass fraction of whey protein microparticulate (Table 5). We obtained typical dependences of the effective viscosity and yield value of the shear rate for a product with mass fraction of microparticulate from 10 to 50% (Fig. 6). Samples No. 4–6, containing 30–50% of micro-particulate respectively, were characterized by the rational rheological properties. This correlates with the data of organoleptic and physical and chemical analysis, in particular, with the consistency (homogeneous, smooth, creamy). However, when adding more than 30% of the new food composition to the normalized milk mixture the taste of the product changed significantly, it was characterized as overly sour, not typical of fermented milk drink.

We investigated the influence of the normalized mixture composition on the ability of ferment microorganisms to synthesize exopolysaccharides. It was established that the symbiotic yogurt ferment was characterized by the best yield-producing power during ripening No. 4–6 samples (Fig. 7).

Table 4. Mass fraction of exopolysaccharides synthesized by ferment microorganisms

Ferment	Strain composition	Mass fraction of EPS, g/l
F-DVS Yo-Flex Mild 1.0	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Bifidobacterium bifidum*	0.3
F-DVS YF-L901	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus*	0.1
F-DVS Yo-Flex Harmony 1.0	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus fermentum*	0.08

Table 5. Characteristics of the normalized mixture samples

Sample No.	Mass fraction of food composition, %	Mass fraction of protein, %	Ratio of casein to whey proteins, %
1	0	3.00	82/18
2	10	3.05	75/25
3	20	3.10	68/32
4	30	3.15	61/39
5	40	3.20	54/46
6	50	3.25	47/53

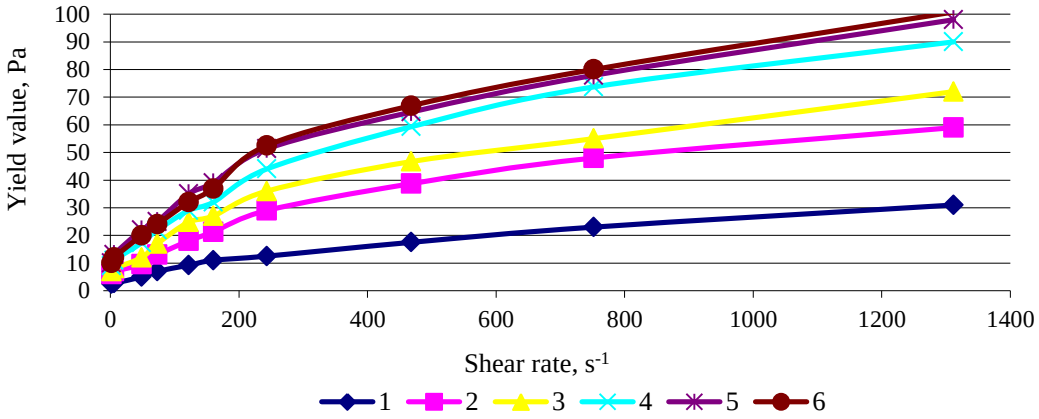

Fig. 6. The dependence of the yield value of the shear rate of drink samples.

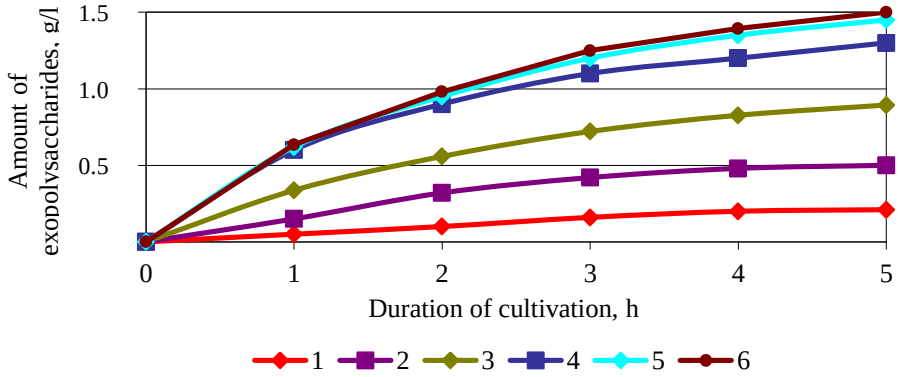

Fig. 7. The influence of normalized mixture composition on EPS synthesis.

To determine the rational composition of the normalized mixture we also investigated the process of acid formation and lactose-fermenting activity for successful fermentation and stable consistency of the clot. An intense increase of titratable acidity in the process of ripening was observed after 2–3 h in all samples of the drink. An increase of mass fraction of the food composition prolonged the duration of the lag phase, however, further it stimulated the process of milk mixture ripening.

According to the research results the formulation and component solution of the synbiotic drink, which involves the replacement of 27% skimmed milk by the new food composition with the exception of cream, stabilizer and skimmed milk powder, was suggested.

The developed drink is characterized by the standard quality indicators (Table 6). Its organoleptic properties are similar to fat-containing product (yogurt), the drink has a pleasant taste of milk, creamy, thick consistency without using stabilization systems.

Research of the product microstructureindicatesthat both in the control and in the test product microflora is distributed evenly throughout the volume. However, in the drink with the food composition there are plots with accumulated microorganisms, which can be explained by their association with whey proteins and products of their hydrolysis, displaying prebiotic properties. An electronic photo of the product structure indicates the presence of chains of EPS bound with agglomerates of protein particles. This gives the viscosity, density, malleability to the product and prevents syneresis.

Using the new food composition in the production of synbiotic drink increases the biological value of the product due to a good balance of its amino acid composition (Fig. 8).

We determined the nutritional value of the drink, in accordance with which we can conclude about the sufficient meet of the daily need of the human body in most nutrients through 100 g of the developed product. Besides, the developed drink has low calories – 39.7 kcal/100 g, which is by 46% less than in the control sample.

To determine the shelf life of the drink in the test sample, which was embedded in storage, the organoleptic, physical and chemical (Fig. 9) and microbiological properties (Fig. 10) were determined. The exception of the stabilization system from the drink formulation did not impair its consistency during storage. Owing to EPS, synthesized by ferment microflora, in the drink composition there was no separation of whey for 5 days of storage, the product was characterized by standard rheological properties. The shelf life of the drink, which was set with consideration of the reserve ratio for perishable products, is 5 days at temperature of (4 ± 2)°C.

For the production of the developed drink the traditional scheme, the modification of which implies the introduction of additional operations to obtain whey protein microparticulate (Fig. 11), was selected as the baseline technology.

The hardware configuration involves the use of commercially available equipment, does not complicate the technological cycle of production and facilitates complex processing of raw milk.

The results of chemical and toxicological research showed that the content of toxic elements, mycotoxins and pesticides in the drink does not exceed the minimum allowed levels and meets the safety requirements. According to the research results yogurt has no skin-resorptive and teratogenic effects.

Table 6. Physical and chemical properties of yogurt

Indicator	Indicator value		
	According to GOST R 51331-99: Dairy products. Yogurts. General technical specifications.	control	test
Mass fraction of dry skimmed milk residue, %	not less than 9.5	10.5	9.5
Mass fraction of fat, %	from 0.1 to 10	3.2	1.0
Mass fraction of protein, %	not less than 3.2	4.8	3.2
Acidity, °T	from 75 to 140	90–92	90–92
Viscosity, mPa·s	not regulated	82–84	78–82
Phosphatase	None		

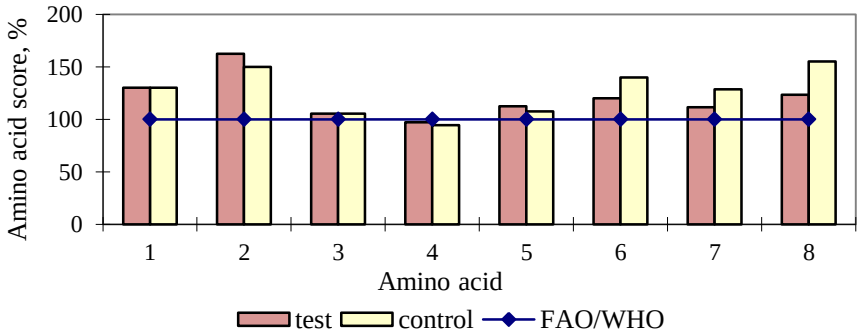

Fig. 8. Amino acid score of the test and control sample of yogurt: 1 – valine; 2 – isoleucine; 3 – lysine; 4 – methionine + cysteine; 5 – threonine; 6 – tryptophan; 7 – leucine; 8 – phenylalanine + tyrosine.

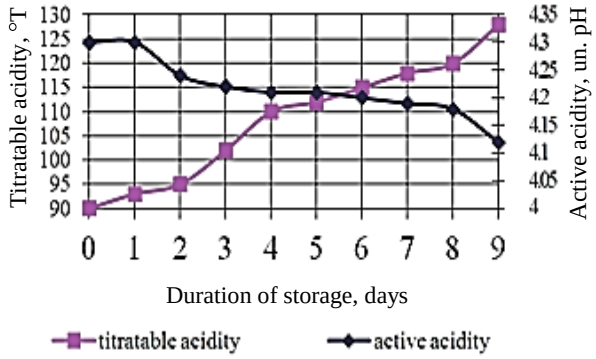

Fig. 9. Change of titratable and active acidity of the drink during storage.

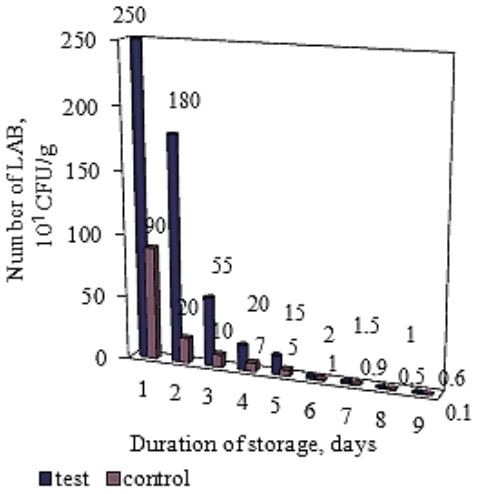

Fig. 10. Change of the number of lactic-acid bacteria (LAB) of yogurt during storage.

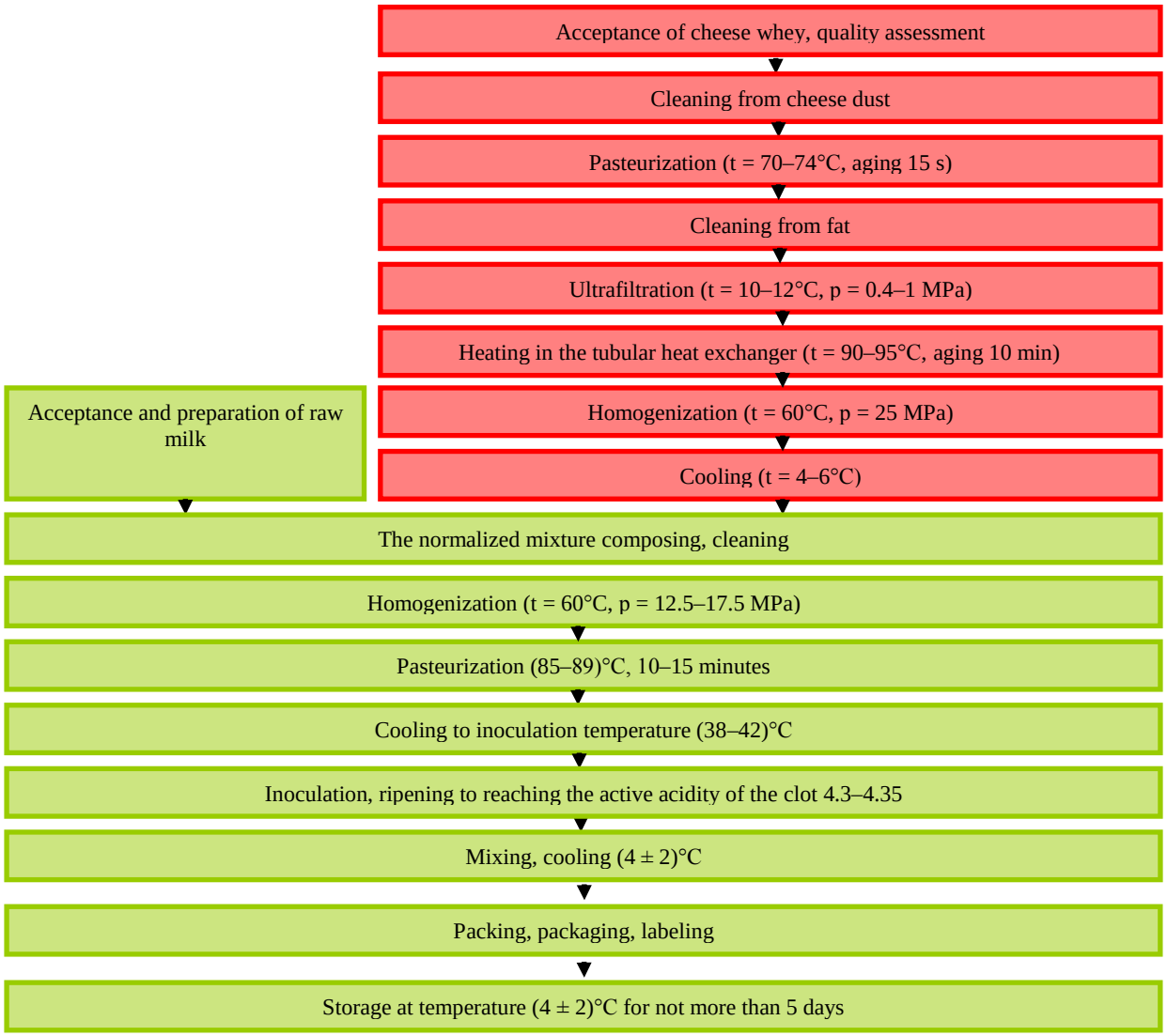

Fig. 11. Process flow diagram of yogurt production.

CONCLUSION

The main advantages of new technology solution are the implementation of a closed cycle of production; the expansion of low-calorie products of high biological value, the exception of stabilization systems while maintaining the required rheological characteristics of the drink; the reduction of technological cycle due to the stimulation of ripening; the replacement of skimmed milk, the exception of cream, stabilizer and skimmed milk powder in the traditional formulation and the reduction of economic costs. The developed technology allows to return a by-product to the production and to use it as the full-value raw material.

REFERENCES

1. Khramtsov A.G. and Nesterenko P.G. *Waste-free processing of raw milk: textbook*. Moscow: Kolos Publ., 2008. 200 p. (In Russian).
2. Khramtsov A.G., Evdokimov I.A. and Nesterenko P.G. Innovation priorities for the use of whey on the logistics principles of waste- free technology. *Dairy Industry*, 2008, no. 11, pp. 28–31. (In Russian).
3. Khramtsov A.G. and Nesterenko P.G. *Technology of whey products: textbook*. Moscow: DeLi Print Publ., 2004. 587 p. (In Russian).
4. Khramtsov A.G. *Whey phenomenon*. St. Petersburg: Profession, 2011. 900 p. (In Russian).
5. Mel'nikova E.I., Stanislavskaya E.B. and Golubeva L.V. *Curd whey: experience of processing and new technology solutions*. Voronezh: VSTA Publ., 2009. 236 p. (In Russian).
6. Kochetkova A.A. and Doronin A.F. *Functional foods: an introduction to technology*. Moscow: DeLi Print Publ., 2009. 286 p. (In Russian).
7. Mel'nikova E.I., Stanislavskaya E.B. and Podgornyy N.A. Milk fat simulator for synbiotic products. *Dairy Industry*, 2010, no. 7, pp. 55–56. (In Russian).
8. Mel'nikova E.I. and Stanislavskaya E.B. Whey protein microparticulates as butterfat imitators in food production. *Fundamental Research*, 2009, no. 7, pp. 23. (In Russian).
9. Mel'nikova I.E., Popova E.E. and Stanislavskaya E.B. Low-calorie ice-cream with whey protein microparticulate. *Food Industry*, 2012, no. 10, pp. 60–61. (In Russian).
10. Mel'nikova E.I., Stanislavskaya E.B., Podgornyy N.A. and Chunosova E.V. A synbiotic product based on whey protein microparticulates. *Cheesemaking and Buttermaking*, 2010, no. 6, pp. 26–28. (In Russian).
11. Sampson H.A., Cooke S. The antigenicity and allergenicity of microparticulated proteins: Simplesse. *Clinical & Experimental Allergy*, 1992, vol. 22, no. 10, pp. 963–969.
12. Roller S. and Jones S.A. *Handbook of fat replacers*. Boca Raton: CRC Press, 1996. 336 p.
13. Merkulova N.G., Merkulov M.Yu., Merkulov I.Yu. *Production control in the dairy industry. A practical guide*. Moscow: Profession Publ., 2010. 656 p. (In Russian).

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DETERMINATION OF PHYSICOCHEMICAL, IMMUNOCHEMICAL AND ANTIOXIDANT PROPERTIES, TOXICOLOGICAL AND HYGIENIC ASSESSMENT OF WHEY PROTEIN COMCENTRATE AND ITS HYDROLYSATE

T. N. Halavach[a,*], V. P. Kurchenko[a], V. G. Zhygankov[b], I. A. Evdokimov[c]

[a] Belarusian State University,
Nezavisimosti Avenue 4, Minsk, 220030 Republic of Belarus

[b] RUE "Scientific Practical Centre of Hygiene",
Academicheskaya Str. 8, Minsk, 220012 Republic of Belarus

[c] North-Caucasus Federal University,
Pushkin Str. 1, Stavropol, 355009 Russian Federation

* e-mail: halavachtn@gmail.com

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Abstract: Enzymatic hydrolysis of whey proteins is aimed to obtain products with low allergenic potential and high nutritional value. Whey peptides are protein fraction that possesses a variety of physicochemical, immunochemical and bioactive properties (antioxidant, antibacterial, immunomodulatory effects). Controlled parameters of enzymatic hydrolysates are the degree of hydrolysis of protein substrates, peptide composition, residual antigenicity, antioxidant capacity, etc. The purpose of this work is to characterize peptide profile, antigenicity and free radical scavenging properties of experimental hydrolysate sample, toxicological and hygienic assessment of its impact on a test object (infusoria *Tetrahymena pyriformis*). The research of properties of whey proteins and their enzymatic hydrolysates was conducted using classical and modern methodological approaches: SDS electrophoresis in polyacrylamide gel, HPLC, mass-spectrometry, competitive ELISA, TEAC (Trolox Equivalent Capacity Assay) technique. Peptide composition, antigenic and antioxidant properties of obtained enzymatic hydrolysate were determined, toxicity of raw and digested whey proteins were examined. We established that the investigated hydrolysate sample falls under the category of partial hydrolysates for functional products according to its physicochemical, immunochemical, free radical scavenging and organoleptic properties. According to the results of toxicological and hygienic assessment using *T. pyriformis* model, whey protein concentrate and its hydrolysates are non-toxic and do not possess cumulative properties. Thus, we obtained partial enzymatic hydrolysate of whey proteins from milk, which can be used as physiologically active component in the development of new specialized food.

Keywords: Allergenic milk proteins, protein hydrolysates, peptide composition, residual antigenicity, antioxidant properties, toxicological and hygienic assessment, infusoria *Tetrahymena pyriformis*

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INTRODUCTION

It is well known that the native proteins of cow's milk have diverse physiological properties: immunomodulating, antibacterial, antiviral and antifungal activities [1]. Caseins (α_{S1}-, α_{S2}-, β- and κ- form) are the predominant phosphoprotein fraction in milk of ruminants (80% of total protein). Whey proteins (20% of protein fraction) are presented by β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, lactoperoxidase and other minor components. During protein hydrolysis the cleavage of peptide bonds with the formation of amino acids and peptides of various lengths occurs. Hydrolyzed milk proteins have low antigenic potential due to destruction of antigenic determinant areas [2].

Caseins and whey proteins act as precursors of biologically active peptides that are produced during digestion or as a result of enzymatic cleavage during technological food processing. Biologically active peptides are particularly interesting for the nutritional science, because they possess opioid-like, immunomodulatory, hypotensive, antimicrobial, antiviral, antioxidant and antitumor effects [3].

The main controlled properties of enzymatic hydrolysates are molecular mass distribution of peptide fractions, degree of hydrolysis of substrates and residual antigenicity (AG) – the amount of undissolved protein that retains the ability to interact with antibodies [1, 4]. Milk proteins are divided into partial and extensive hydrolysates according to the degree of hydrolysis [5]. Partial hydrolysates used in prophylactic mixtures contain peptides of various lengths and a minimum amount of free amino acids. Extensive hydrolysates serving as components of therapeutic products are presented by short-chain peptides and amino acids. In severe cases of food allergy only amino acids without antigenic properties are applied. Significant disadvantage of extensive hydrolysates and mixtures of amino acids is their pronounced bitter taste. Partial hydrolysates with acceptable organoleptic properties are used in the production of baby food, medical, elderly and sports nutrition.

Along with this, a balanced diet requires products with antioxidant capacity or additional components with antiradical properties. Antioxidant activity (AOA) of proteins and peptides is caused by solvent available amino acids (restoring properties of amino acid radicals) [6]. The identified peptides with antiradical properties consist of tryptophan, tyrosine, methionine and histidine. Moreover, antioxidant activity is indicated for amino acid standards (tryptophan > tyrosine, methionine >> cysteine > histidine > phenylalanine) [7, 8]. We applied TEAC (Trolox equivalent antioxidant capacity) technique [9] for determination of the antioxidant properties of natural whey proteins and their corresponding enzymatic hydrolysate.

The safety assessment of certain food types, especially food for particular nutritional uses, is usually carried out using laboratory animals (rats, mice, guinea pigs). Alternative biomodels must meet several requirements: they must have similar physiological response as the higher animals, higher speed and efficiency in comparison with traditional methods and specific properties that enable to expand the activity spectrum of investigated factor on the biosystem. Infusoria *Tetrahymena pyriformis* is currently successfully applied for various biological studies including assessment of toxicity and biological value of food products and feed [10, 11]. Toxicity is usually determined by the time of death of 50% of exposed organisms in environment with a certain toxicant concentration (CL_{50}). Advantages of *T. pyriformis* as a test object are following: 1) it is both a cell and an eukaryotic organism – this fact allows evaluating food effects and drawing corresponding analogies on both cellular and organism levels; 2) it is much more similar to the higher organisms by main biochemical indicators and biological needs than other models; 3) many test functions of *T. pyriformis* correspond to the basic vital factors of the higher animals. Thus, *T. pyriformis* is a universal test organism suitable for studying of safety and biological value of food products and for biological evaluation of other natural and artificial objects.

Constantly growing application of enzymatic hydrolysates determines the relevance of toxicological and hygienic assessment of obtained sample of milk whey protein hydrolysate using *T. pyriformis* as a test object.

The purpose of this work is to characterize protein-peptide composition; to assess the antigenic and antioxidant properties of whey protein and its enzymatic hydrolysate; to carry out toxicological and hygienic assessment of sample on the test object (infusoria *T. pyriformis*).

OBJECTS AND METHODS OF STUDY

Obtaing of enzymatic hydrolysates of milk whey proteins

Whey protein concentrate derived using ultrafiltration method (WPC-UF-80, TNLA BY 100377914.550-2008) with protein m.f. 80% and serine protease (alcalase, EC 3.4.21.62, protease from *Bacillus licheniformis*, activity 2.64 u/g; Sigma, USA) were used for enzymatic hydrolysis. To obtain the experimental hydrolysate sample the 8% solution of WPC-UF-80 was made, protein substrate was thermally treated and then cooled to optimal hydrolysis temperature. The enzymatic agent was added in the resulting heat-treated solution; hydrolysis was carried out in thermostatic conditions. After proteolysis completion the agent was inactivated by heating. Finally, the resulting liquid hydrolysate was dried according to method [12].

Analysis of physicochemical and bioactive properties of hydrolysates

The basis for electrophoretic separation of milk proteins and their enzymatic hydrolysates was the methodology used in operational manual [13]. HPLC analysis of hydrolysis products was conducted on Agilent 1100 chromatograph (Agilent, United States) using Zorbax-300SB C8 column (4.6×250 mm, 5 μm; Agilent, United States) according to the technique [14]. Molecular mass distribution of peptides was studied using Bruker Microflex instrument (Bruker, United States). Techniques described in [15, 16] became the basis for competitive ELISA for determination of residual AG of whey proteins and their hydrolysates.

Whey protein hydrolysate was fractionized using filters Amicon Ultra-4 10K (Millipore, United States; permeability 10 kDa). Protein concentration (TN) in hydrolysate and ultrafiltrate was determined according to GOST 30648.2-99. The fraction with molecular weight ≤ 10 kDa (%) was evaluated as the ratio of TN value for ultrafiltrate to the concentration of protein in the original hydrolysate. The content of α-amino

nitrogen (AN) in hydrolysate samples was determined by formol titration according to GOST 13805-76 (p. 3.9). Degree of hydrolysis was evaluated as the ratio AN/TN.

TEAC (Trolox equivalent antioxidant capacity) technique was used to assess the AOA level. ABTS radical scavenging activity measurement required previously obtained cation radical of diammonium salt of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) [17]. $ABTS^{\bullet+}$ is a metastable radical, which can exist in solution for a long time. After introduction of various antiradical agents (trolox) into medium, quick reduction of this radical is observed. Reaction was monitored spectrophotometrically at λ_{734}: $ABTS^{\bullet+}$ radical (blue staining of solution) is converted back to its colorless neutral form during reduction. The described system has only one type of radical and antioxidant influence on its formation is impossible, therefore, the mechanism of direct interaction between the antioxidant and cation radical is carried out. AOA measurement was performed on the basis of modified technique described in article [9].

Toxicological and hygienic study of impact of whey protein concentrate and its enzymatic hydrolysate on *T. pyriformis* was based on principles and methods of hygienic regulation adopted in general toxicology [18, 19]. The principle of research on *T. pyriformis* is to analyze the nature of population growth in culture medium containing researched objects.

Primary toxicological assessment of objects on *T. pyriformis*

The study was carried out on the test object *T. pyriformis* in stationary growth phase, supported in standard nutrient medium at 25°C. Depending on toxicity of the researched object acute experiment duration was 0.5–4 h, subacute experiment – 24 h. The toxic effect was estimated by alternative state "life-death" [19]. Suspensions of the WPC and its hydrolysate containing 10–150 mg/ml of protein was prepared, the pH of suspension was adjusted to 7.1-7.2 units. Lethal and malfunctioning concentration was determined and then several intermediate concentrations were prepared. 1 ml of each concentration was put into two 10 ml flasks. Inoculate of infusoria in stationary growth phase (100 000 ± 1 000 organisms) was added to each sample. For acute experiment samples were incubated at 25°C for 30–240 min; for subacute experiment – for 24 h. Intoxication picture in native specimen was observed under the microscope after the incubation stage. The number of dead infusoria prior to fixation and the total number of infusoria after fixation (with 5% iodine solution) was counted in Fuchs-Rosenthal counting chamber. Lethality in % was calculates with consideration of lysed organisms. Probit analysis of direct lethality was carried out using general toxicology methods, in particular the method of V.B. Prozorovsky [20]. According to calculations of the test object lethality (in %) in acute and subacute experiments the basic toxicity parameters were determined (LD_{50}, LD_{16}, LD_{84}). Cumulation coefficient in acute experiment (K_{CUMas}) was calculated as ratio of subacute and acute experiment LD_{50}.

Chronic toxicity study on *T. pyriformis*

Study of toxicity in chronic experiment was carried out throughout the life cycle of *T. pyriformis* population. The chronic experiment setting was based on primary toxicological assessment results. Suspensions of investigated objects were poured into sterile tubes with gauze plugs. Each concentration was studied in at least three replications. Tubes with suspensions were sterilized at 85°C for 30 min. 20 000 of infusoria in stationary growth phase were added to samples after cooling. Samples were stored in thermostat at 25°C for 96 h. Registration of infusoria state and counting of organisms were carried out after 24 h (lag-phase), 48 hours (logarithmic phase), 72 h (slow growth phase), 96 h (stationary phase). For this purpose 1 ml sample was taken from each tube under sterile conditions. State of organisms in native sample was determined: the presence of dead organisms, nature of morphological and functional changes. After fixation with 1 drop of 5% iodine solution the number of organisms was counted in the Fuchs-Rosenthal counting chamber in 10 large squares. The number of organisms in 1 ml of culture were calculated as mean number of organisms in 1 square multiplied by 5 000 (if selected inoculate was not diluted) or by 20 000 (inoculate was diluted 4 times). The constant of instantaneous population growth rate, number of generations, generation time [20] were calculated using following formulas:

$$r = \frac{\ln \frac{N_t}{2000}}{t}, \quad (1)$$

$$n = \frac{\ln \frac{N_t}{2000}}{\ln 2}, \quad (2)$$

$$g = \frac{t}{n}, \quad (3)$$

where 2 000 is the number of organisms added to 1 ml of cultivation medium; N_t is the number of organisms grown in cultivation medium with the studied agent during time t; r is the constant of instantaneous growth rate; n is the number of generations; g is the generation time.

Chronic toxicity parameters were determined in the concentration range, where inhibition of population growth rate was proportional to the increase in agent concentration. Growth inhibition (ED) for each dose was calculated according to formula:

$$\mathrm{ED}(\%) = 100 - \frac{N_0}{N_C} \times 100, \quad (4)$$

where N_0 is the number of organisms in experiment; N_C is the number of organisms in control; ED_{16}, ED_{50}, ED_{84} were calculated using tables and formulas for determination of LD_{16}, LD_{50}, LD_{84}.

Acute experiment results were estimated with consideration for following parameters: ED_{50} – dose that causes 50% inhibition of generative functions in logarithmic (24–48 h) and stationary (72–96 h) phases of growth; K_{CUMchr} – cumulation coefficient at chronic exposure, calculated as ratio of ED_{50} determined in stationary phase to ED_{50} determined in logarithmic growth phase; cumulation coefficient value more than 1 suggests samples having adaptogenic properties; Z_{chr} – area of chronic action calculated as ratio of mean lethal dose in acute experiment to the dose, which inhibits population growth by 50% in the stationary phase of chronic experiment; MND – maximum non-effective dose determined by limitative indicator; LD_{50}/MND – hazard indicator calculated as ratio of mean lethal dose in acute experiment and maximum non-efficient dose determined in chronic experiment. The estimation results of investigated samples on *T. pyriformis* model allowed classifying them according to toxicity and hazard indexes (Table 1).

The researched object was ranged in a hazard class according to indicator, which corresponds to the highest hazard class.

Graph plotting and mathematical processing of research data were carried out using computer program "Microsoft Office Excel 2003" (Microsoft Corporation, United States).

RESULTS AND DISCUSSION

We performed integrated analysis of organoleptic, physicochemical and immunochemical properties of obtained partial hydrolysate of milk whey proteins (Table 2). According to results of SDS electrophoresis the hydrolysate exhibits almost full proteolysis of β-lactoglobulin, α-lactalbumin and BSA into peptides. HPLC study of sample profiles revealed that the sample does not contain native whey protein.

According to the experimental data, the sample contains 98% of fractions with $m_r \leq 10$ kDa, and the residual AG is reduced by 0.8×10^3 times (Table 2). According to literature, the residual AG of partial hydrolysates used in prophylactic mixtures is $\geq 10^{-3}$ rel. u. (or $\leq 10^3$ times smaller than AG of native proteins) [5]. Mass spectra study confirmed the absence of high molecular fractions in the obtained hydrolysate; peptides with $m_r < 5$ kDa dominate among proteolysis products. Thus, the investigated hydrolysate is proved to meet requirements for the category of partial enzymatic hydrolysates.

The antioxidant capacity of obtained hydrolysate sample was studied. Free radical scavenging efficiency of standard (trolox) upon inactivation of $ABTS^{\bullet+}$ was characterized during experiments. The degree of cation radical reduction within certain time interval (10 min) was determined. This indicator represents the actual decrease in concentration of free radicals in system caused by antioxidant. It was demonstrated that the process is characterized by rapid phase of $ABTS^{\bullet+}$ reduction during the first minute of reaction. Thus, $ABTS^{\bullet+}$ quickly reacts with active antioxidants, which allows to use it as a selective reagent for polycomponent sample analysis [17]. IC_{50} or trolox concentration was calculated (16.45 μM). At this concentration, speed of the process reduces 2 times. EC_{50} of the standard was used to calculate TEAC (free radical scavenging activity indicator, expressed in micromoles of trolox on 1 mg of protein).

Table 1. Hygienic classification of objects according to results of the study of their toxicity on *T. pyriformis*

Indicator	Classes (decreasing toxicity and hazard level)				
	1 (extra hazardous)	2 (highly hazardous)	3 (moderately hazardous)	4 (low hazardous)	5 (non-toxic)
LD_{50}, mg/ml	less than 0.1	0.1–1.0	1.1–20	21–50	more than 50
K_{CUMac}, K_{CUMchr}	less than 0.1	0.10–0.30	0.31–0.49	0.50–1.0	more than 1.0
Z_{chr}	more than 10	10.0–5.0	4.9–2.5	less than 2.5	–
MND, mg/ml	less than 10–6	10^{-6}–10^{-4}	10^{-4}–10^{-1}	more than 10^{-1}	–
LD_{50}/MND	more than 106	10^6–10^5	10^5–10^4	less than 10^4	–

Table 2. Organoleptic, physicochemical and immunochemical properties of milk whey protein hydrolysate

Parameter name	Parameter value
Appearance and consistency	Yellow-cream powder
Flavor and smell	Typical milk flavor. Weak bitter milk taste
Solubility	Soluble in water
Active acidity, u. pH (1% solution)	6.7
Weight fraction of total protein, %	80
Peptide profile: fragments with molecular weight >10% kDa, %	2*
Native whey proteins	Not found **
Decreasing of residual AG (compared to native WPC)	0.8×10^3

Note. * indicator values are set after determination of total nitrogen in hydrolysate and ultrafiltrate obtained using filters Amicon Ultra-4 10 K with permeability 10 kDa. ** According to SDS electrophoresis, HPLC and mass spectrometry.

The data on radical scavenging activity of initial substrate for production of hydrolysates (native whey protein concentrate WPC) were obtained. Study of the reaction kinetics of WPC cation radical reduction revealed the stage of rapid decrease in $ABTS^{\bullet+}$ amount during the first minute of reaction and relatively slow increase in degree of reduction within time interval 1-30 min. Rapid absorption inhibition stage (duration 1 min) is associated with the presence of highly active antioxidants. We estimated the overall free radical scavenging activity within 30 min of reaction to determine the total content of antioxidants with different $ABTS^{\bullet+}$ reduction efficiency. IC_{50} value for the WPC sample had been achieved at concentration 95.1 ± 2.8 μg/ml of protein component; the radical scavenging activity in u. TEAC was 0.173 ± 0.005 μmol/mg.

A study of cation radical reduction kinetics after introduction of enzymatic hydrolysate experimental sample into the test system was performed. As in case of native WPC, rapid $ABTS^{\bullet+}$ decrease stage during the first minute of reaction was observed. One should also note process slowdown at the 4th min and gradual fading of radical reduction at the 30th min of reaction. To determine the total content of antioxidants (compounds with various efficiency of interaction with $ABTS^{\bullet+}$) key AOA indicators for 30 min of reaction time were calculated. The increase in free radical scavenging activity caused by enzymatic cleavage of the protein component in comparison with the original WPC was obvious. So, IC_{50} value for the experimental hydrolysate sample had been achieved at concentration 29.02 ± 1.92 μg/ml of protein component as shown in Fig. 1; AOA was 0.551 ± 0.035 μmol of trolox/mg of protein. $ABTS^{\bullet+}$ reduction ability after introduction of hydrolyzed substrate increased 2.98–3.39 times in comparison with native WPC.

According to literature, a significant increase in DPPH radical reducing activity of whey appeared after Flavourzyme 500L® hydrolysis [8]. Another study [21] mentions increased free radical scavenging potential of WPC hydrolysate ultrafiltrates (5 kDa) in comparison with the original substrate (ORAC-method). These ultrafiltrates were obtained using alcalase, neutrase, flavourzyme and Corolase PP. Increasing of hydrolysate AOA compared to native proteins is associated with cleavage of protein macromolecules into peptides, accompanied by additional exposure of amino acid radicals. Expressed antioxidant effect is associated with proton donor properties of indole and phenol groups of tryptophan and tyrosine respectively, as well as with the formation of methionine sulfoxide and oxidation of cysteine sulfhydryl group (−SH) [7].

Next, we studied antioxidant and antigenic properties, peptide profile of whey protein partial enzymatic hydrolysates of foreign manufacture: PRODIET GF 006 (Ingredia, France), Hilmar 8350 (Hilmar, United States). Table 3 presents the comparison between the obtained hydrolysate sample and foreign analogues.

We obtained dependences between $ABTS^{\bullet+}$ degree of reduction by enzymatic hydrolysates and reaction time (30 min). The process is characterized by rapid decrease of $ABTS^{\bullet+}$ amount during the first minute of reaction followed by less intensive increase of reduced radical fraction at 4th−6th minute. This is followed by a slow reaction stage (up to 30th min). The purpose of experiment was to assess antiradical properties of hydrolysate as a set of different antioxidants, that is why AOA indicators were calculated for 30 min of reaction time.

Study on hydrolysates of foreign manufacture and experimental sample allowed to obtain correlation between depth of whey protein cleavage and the level of AOA. Thus, when the degree of hydrolysis is equal to 12.5% (Hilmar 8350) and 15.5 ± 0.6% (experimental sample), free radical scavenging activity of the studied protein components reaches 0.559 ± 0.022 and 0.551 ± 0.035 μmol of trolox/mg of protein, respectively. Along with this, degree of hydrolysis of protein component PRODIET GF 006 is 20–25%; radical scavenging activity raises to 0.982 ± 0.014 μmol of trolox/mg of protein. It is obvious that increase of low molecular peptide fraction yield causes increase of antioxidant capacity.

According to the results of competitive ELISA, residual AG of “PRODIET GF 006” sample is $(0.84 \pm 0.04) \times 10^{-3}$ rel. u. and the free radical scavenging activity level reaches 0.982 ± 0.014 μmol of trolox /mg of protein, whereas the same indicators for "Hilmar 8350" are equal to $(52.9 \pm 2.0) \times 10^{-3}$ rel. u. and 0.559 ± 0.022 μmol of trolox/mg of protein, respectively. The obtained data confirm that increasing degree of substrate cleavage is accompanied by increase in free radical scavenging activity and decrease of hydrolysate allergenic potential.

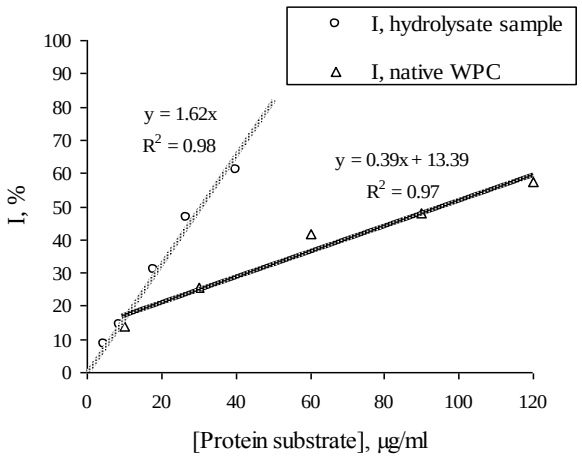

Fig. 1. Dependence of absorption inhibition (I, %) from concentration of WPC and hydrolysate sample.

Table 3. Characteristic of partial whey protein hydrolysates of foreign manufacture

Name of hydrolysate	Peptide profile	Ratio between α-amino and general nitrogen *, AN/TN, %	IC50, μg/ml	TEAC, μmol of trolox / mg of protein	TEAC (h-t) / TEAC (WPC)	Residual AG, 10^{-3} rel. u.
Experimental hydrolysate sample	≤ 10 kDa 98%	15.5 ± 0.6	29.88 ± 1.92	0.551 ± 0.035	3.19 ± 0.20	1.22 ± 0.07
Hilmar 8350 (Hilmar, United States)	< 20 kDa 83.0% *	12.5 *	29.46 ± 1.18	0.559 ± 0.022	3.23 ± 0.13	52.9 ± 2.0
PRODIET GF 006 (Ingredia, France)	< 5 kDa 97.2% *	22.5 ± 2.5 *	16.75 ± 0.24	0.982 ± 0.014	5.68 ± 0.08	0.84 ± 0.04

Note. * indicators are presented according to the data from manufacturers.

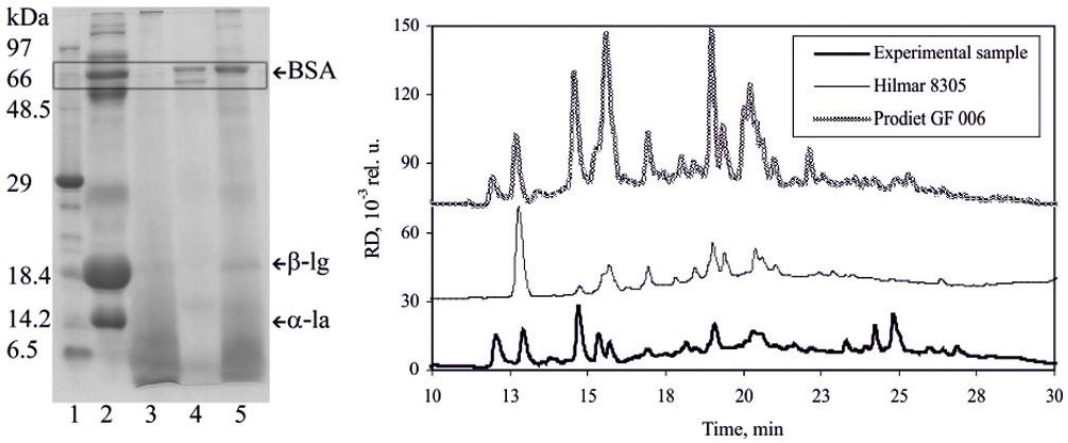

Fig. 2. SDS electrophoregram (a) and HPLC profiles (b) of whey protein hydrolysates: 1 – marker; 2 – WPC control; 3 – experimental hydrolysate sample; 4 – "PRODIET GF 006"; 5 – "Hilmar 8350"; β-lg – β-lactoglobulin, α-la – α-lactalbumin.

Comparable degree of hydrolysis and AOA level were indicated for experimental hydrolysate sample and "Hilmar 8350" whereas decrease of antigenic potential of hydrolyzed WPC to 1.22 ± 0.07 rel. units (similar to "PRODIET GF 006") are probably associated with prior thermal treatment of WPC [22]. Heating of whey protein solution in optimal conditions and subsequent hydrolysis by alcalase resulted in cleavage of all substrates into intermediate peptides. According to SDS electrophoretic analysis (Fig. 2a, framed) PRODIET GF 006 and Hilmar 8350 hydrolysates have native bovine serum albumin (BSA), which possesses allergenic potential. Thus, advantage of the experimental sample is the total cleavage of all allergenic whey proteins. Moreover, comparison between peptide profiles of experimental sample and analogues (Fig. 2b) indicates the use of proteolytic enzymes with different catalytic activity mechanisms and substrate specificity that ensures obtaining of hydrolysates with specific composition.

According to experimental data, the enzymatic hydrolysis of whey proteins (WPC) resulted in increase of free radical scavenging activity of obtained peptide fraction by 2.98–3.39 times. It was established that increasing degree of substrate cleavage is accompanied by increasing radical scavenging activity and decreasing allergenic potential of hydrolysates.

Toxicological and hygienic assessment of milk whey protein concentrate and its enzymatic hydrolysate on *T. pyriformis* model

For toxicity study in the acute experiment 100 000 infusoria in stationary growth phase were added to suspensions containing 30, 90, 120 and 150 mg/ml of WPC and hydrolysate (24, 72, 96, and 120 mg/ml of protein respectively). After 30, 60 and 120 min of exposure there was no dead infusoria in studied samples, so the exposure time for acute toxicity determination was increased up to 240 min. One could observe decrease in number of organisms by 10% compared to control in samples containing 90, 120 and 150 mg/ml of enzymatic hydrolysates of milk whey proteins. Single dead infusoria were found in sample with concentration 90 mg/ml. Along with this, a sample of milk whey protein concentrate had no visible impact on the organisms and did not cause their death.

For toxicity study in subacute experiment 100 000 infusoria in stationary growth phase were added to suspensions containing 30, 90, 120 and 150 mg/ml of WPC and hydrolysate (24, 72, 96, and 120 mg/ml of protein respectively). Exposure time was 24 hours. The number of infusoria in samples with concentration 30, 60 and 90 mg/ml increased compared to the control by 152, 171 and 145%,

respectively. Population size increased by 34 and 24% at concentrations 120 and 150 mg/ml. However, these samples contained dead organisms: lethality 10 and 19%, respectively. Infusoria in all samples were large, they acquired rounded shape and became darker in comparison with the control. But WPC sample had no visible impact on the organisms. Population size increased 3 times compared to the control in samples with concentration 90, 120, 150 mg/ml and 2.7 times – in samples with concentration 30, 60 mg/ml.

Noncarbohydrate medium with 4.0 mg/ml of peptone, 1.0 mg/ml of NaCl, 1.0 mg/ml of yeast extract was prepared for chronic toxicity experiment. For chronic toxicity study of whey protein hydrolysates, *T. pyriformis* population was cultivated throughout life cycle in medium with 10, 30, 60, 90, 120 and 150 mg/ml of experimental sample (Table 4). The chronic toxicity of whey protein concentrate was studied at concentrations 10, 30 and 60 mg/ml, because the protein in samples with concentration 90, 120 and 150 mg/ml has folded on sample preparation stage (during tyndalization).

Cultivation medium of *T. pyriformis* with previously added enzymatic hydrolysate of milk whey protein at concentration 10 mg/ml stimulated infusoria growth during 48–96 h of population life cycle in comparison with the control level ($p > 0.05$). Increasing of sample content up to 30 mg/ml lead to decrease in population size in lag-phase and logarithmic growth phase by 30% and to subsequent stimulation in slow growth and stationary phases by 11 and 131%, respectively, relative to the control level ($p > 0.05$). At concentrations 60 and 90 mg/ml population growth was inhibited during 24–48–72 h of life cycle. In addition, with increasing cultivation time the population was gradually approaching the control level and reached it after 96 h, or even exceeded it by 51% ($r > 0.05$) at sample concentration 60 mg/ml. Increase of hydrolysate content in medium up to 120 and 150 mg/ml caused infusoria growth inhibition to increase and to reach 77 and 88% in logarithmic growth phase, respectively, relative to the control level ($p > 0.05$) (Table 4).

Table 4. Changes in size of *T. pyriformis* population cultivated in medium with enzymatic hydrolysate of milk whey proteins and whey protein concentrate

Content of hydrolysate/ WPC, mg/ml	Exposure time, h			
	24	48	72	96
Population				
0 (control)	12 500 ± 928	100 500 ± 577	251 000 ± 6 360	289 000 ± 2 404
Population to control,%				
0 (control)	100 ± 7.4	100 ± 0.6	100 ± 2.5	100 ± 0.8
Milk whey protein hydrolysate				
10.0	11 000 ± 0	121 500 ± 167 *	394 500 ± 19 641 *	559 000 ± 577 *
30.0	10 000 ± 167	70 000 ± 2 309 *	278 000 ± 4 619 *	668 000 ± 8 083 *
60.0	6 000 ± 0 *	56 000 ± 1 443 *	243 000 ± 6 351	436 000 ± 1 155 *
90.0	6 500 ± 441 *	37 000 ± 2 179 *	128 000 ± 2 646 *	298 000 ± 8 083
120.0	3 500 ± 167 *	23 000 ± 1 014 *	60 500 ± 2 309 *	182 000 ± 1 155 *
150.0	4 500 ± 0 *	12 000 ± 167 *	47 000 ± 289 *	131 000 ± 1 732 *
Population to control,%				
10.0	88 ± 0	121 ± 0.2 *	157 ± 7.8 *	193 ± 0.2 *
30.0	80 ± 1.3	70 ± 2.3 *	111 ± 1.8 *	231 ± 2.8 *
60.0	48 ± 0 *	56 ± 1.4 *	97 ± 2.5	151 ± 0.4 *
90.0	52 ± 3.5 *	37 ± 2.2 *	51 ± 1.1 *	103 ± 2.8
120.0	28 ± 1.3 *	23 ± 1.0 *	24 ± 0.9 *	63 ± 0.4 *
150.0	36 ± 0 *	12 ± 0.2 *	19 ± 0.1 *	45 ± 0.4 *
Milk whey protein concentrate				
10.0	11 000 ± 601	69 500 ± 1 302 *	322 500 ± 26 238	569 000 ± 4 041 *
30.0	12 000 ± 289	56 000 ± 2 309 *	191 000 ± 8 743 *	418 000 ± 8 083 *
60.0	9 000 ± 577 *	77 000 ± 0 *	307 000 ± 68 537	439 000 ± 2 887 *
Population to control,%				
10.0	88 ± 4.8	69 ± 1.3 *	128 ± 10.5	197 ± 1.4 *
30.0	96 ± 2.3	56 ± 2.3 *	76 ± 3.5 *	145 ± 2.8 *
60.0	72 ± 4.6 *	77 ± 0 *	122 ± 27.3	152 ± 1.0 *

Note. * statistically significant differences in relation to control level ($p < 0.05$).

One could observe following processes in *T. pyriformis* cultivation medium with 10 and 60 mg/ml of WPC: decrease of *T. pyriformis* population size in lag-phase and logarithmic growth phase up to 31%; subsequent stimulation by 97 and 52% in slow growth and stationary phases, respectively ($p > 0.05$). Decrease in population size by 14% in lag-phase, by 44% in logarithmic growth phase and by 24% in slow growth phase was observed at WPC concentration 30 mg/ml. The 45% increase relative to the control level was reached after 96 h.

Probit analysis was used to calculate parameters of acute, subacute and chronic toxicity of milk whey protein concentrate and its enzymatic hydrolysate in acute, subacute (infusoria lethality) and chronic (inhibition of generative function under the influence of investigated samples) experiments.

According to experimental data of toxicological and hygienic assessment on *T. pyriformis* model presented in Tables 5 and 6, whey protein concentrate and its enzymatic hydrolysate belong to hazard class 4 with absence of cumulative properties.

Thus, the presented samples of milk and whey protein concentrate and partial enzymatic hydrolysate of milk whey proteins are non-toxic and do not possess any cumulative properties.

Table 5. Toxicity parameters of whey protein enzymatic hydrolysate according to assessment results obtained on *T. pyriformis* model

Toxicity index	Toxicity value	Hazard class
Acute toxicity		
LD_{16}, mg/ml	–	–
LD_{50}, mg/ml	more than 100	5
LD_{84}, mg/ml	–	–
Subacute toxicity		
LD_{16}, mg/ml	–	–
LD_{50}, mg/ml	more than 150	–
LD_{84}, mg/ml	more than 200	–
K_{CUMac}	more than 1	5
Chronic toxicity in logarithmic phase (48 h)		
ED_{16}, mg/ml	8.13	–
ED_{50}, mg/ml	70.0 ± 0.12	–
ED_{84}, mg/ml	131.88	–
Chronic toxicity in stationary growth phase (96 h)		
ED_{16}, mg/ml	105.18	–
ED_{50}, mg/ml	140.96 ± 0.09	–
ED_{84}, mg/ml	176.75	–
K_{CUMchr}	2.01	5
Z_{chr}	less than 2.5	4

Table 6. Toxicity parameters of milk whey proteins according to the results obtained on *T. pyriformis* model

Toxicity index	Toxicity value	Hazard class
Acute toxicity		
LD_{16}, mg/ml	–	–
LD_{50}, mg/ml	more than 100	5
LD_{84}, mg/ml	–	–
Subacute toxicity		
LD_{16}, mg/ml	–	–
LD_{50}, mg/ml	more than 150	–
LD_{84}, mg/ml	more than 200	–
K_{CUMac}	more than 1	5
Chronic toxicity		
ED_{50}, mg/ml in logarithmic phase (48 h)	more than 50	–
ED_{50}, mg/ml in stationary growth phase (96 h)	more than 50	–
K_{CUMchr}	more than 1	5
Z_{chr}	less than 2.5	4

CONCLUSION

We performed integrated analysis of physico-chemical and bioactive properties (antigenic and free radical scavenging activity) of obtained enzymatic hydrolysates of milk whey proteins. It has been established that the experimental hydrolysate sample possesses acceptable organoleptic properties and low antigenicity due to absence of high molecular fraction.

Antioxidant properties of whey protein concentrate from cow's milk and its enzymatic hydrolysate were studied using TEAC (Trolox equivalent antioxidant capacity) technique. It has been established that enzymatic hydrolysis of whey proteins leads to the increase in free radical scavenging activity of obtained peptide fraction by 2.98–3.39 times. According to the comparative characteristics of experimental hydrolysate sample and foreign analogues, increasing degree of protein substrate cleavage is accompanied by rising of radical scavenging activity and decreasing of allergenic potential of hydrolysates.

Higher antiradical properties of hydrolysates (compared to native whey protein) are associated with splitting of protein macromolecules into peptides. This process is accompanied by exhibiting of additional amino acid radicals with proton donor properties of indole and phenol groups of tryptophan and tyrosine, respectively, and the formation of methionine sulfoxide and oxidation of cysteine sulfhydryl groups (−SH). Enzymatic hydrolysis of substrates also leads to splitting of antigenic determinant areas, and hence provides the protein component with low antigenic potential.

Enzymatic hydrolysate experimental sample obtained using bacterial endopeptidase (alcalase) is comparable by physicochemical parameters, antigenic and antioxidant activity with foreign analogues: PRODIET GF 006 (Ingredia, France) and Hilmar 8350 (Hilmar, United States), which are used in the manufacture of functional products. Advantage of the experimental hydrolysate sample is the total cleavage of all whey protein allergens.

According to the results of toxicological and hygienic assessment in acute and subacute experiments on *T. pyriformis*, whey protein concentrate and its enzymatic hydrolysate belong to hazard class 5 with absence of cumulative properties and to hazard class 4 according to zone of chronic action. Investigated agents do not possess toxic effects that lead to vital activity disruption of individual organs, systems or the whole organism. This indicates the absence of cumulative properties of these agents.

The obtained partial whey proteins hydrolysate can serve as physiologically active component in the development of new specialized food including functional products.

REFERENCES

1. Hurley W.L. *Milk protein*. Vol. 1. Rijeka, Croatia: InTech, 2012. 352 p.
2. Wal J.M. Bovine milk allergenicity. *Ann. Allergy Asthma Immunol*., 2004, vol. 93, pp. 2–11.
3. Raikos V. and Dassios T. Health-promoting properties of bioactive peptides derived from milk proteins in infant food: a review. *Dairy Sci. & Technol*., 2014, vol. 94, pp. 91–101.
4. Mahmoud M.I. The physicochemical and functional properties of protein hydrolysates in nutritional products. *Food Technol*., 1994, vol. 48, no. 10, pp. 89–95.
5. Maldonado J., Gil A., Narbona E. and Molina J.A. Special formulas in infant nutrition: a review. *Early Hum. Dev*., 1998, vol. 53, no. 1, pp. 23–32.
6. Zulueta A., Maurizi A., Frígola A., Esteve M.J., Coli, R. and Burini G. Antioxidant capacity of cow milk, whey and milk deproteinized. *Int. Dairy J*., 2009, vol. 19, no. 6–7, pp. 380–385.
7. Hernández-Ledesma B., Dávalos A., Bartolomé B. and Amigo L. Preparation of antioxidant enzymatic hydrolysates from alpha- lactalbumin and beta-lactoglobulin. Identification of active peptides by HPLC-MS/MS. *J. Agric. Food Chem*., 2005, vol. 53, no. 3, pp. 588–593.
8. De Castro R.J.S. and Sato H.H. Comparison and synergistic effects of intact proteins and their hydrolysates on the functional properties and antioxidant activities in a simultaneous process of enzymatic hydrolysis. *Food and Bioproducts Proc*., 2014, vol. 92, no. 1, pp. 80–88.
9. Hernández-Ledesma B., Quirós A., Amigo L. and Recio I. Identification of bioactive peptides after digestion of human milk and infant formula with pepsin and pancreatin. *Int. Dairy J*., 2007, vol. 17, no. 1, pp. 42–49.
10. Sinks G. and Schultz T. Correlation of Tetrahymena periformis and Pimephales toxicity: evaluation of 100 additional compounds. *Environ. Toxicol. Chem*., 2001, vol. 20, no. 4, pp. 917–921.
11. Zhu H., Tropsha A., Fourches D., Varnek A., Papa E., Gramatica P., Oberg T., Dao P., Cherkasov A. and Tetko I.V. Combinatorial QSAR modeling of chemical toxicants tested against Tetrahymena periformis. *J. Chem. Inf. Model*., 2008, vol. 48, no. 4, pp. 766–784.
12. Kurchenko V.P., Kapustin M.A., Butkevich T.V., Rizevskiy S.V., Halavach T.N. and Strogiy V.N. Integrated technology of whey processing. *Molochnaya Industriya*, 2013, no. 2, pp. 38–41. (In Russian).
13. Osterman L.A. *Research methods of proteins and nucleic acids: Electrophoresis and ultracentrifugation (practice note)*. Moscow: Nauka Publ., 1981. 288 p. (In Russian).
14. Kim S.B., Seo I.S., Khan A., Ki K.S., Lee W.S. and Lee H.J. Enzymatic hydrolysis of whey is heated: iron-binding ability of peptides and antigenic protein fractions. *J. Dairy Sci*., 2007, vol. 90, pp. 4033–4042.
15. Kim S.B., Ki K.S., Khan A., Lee W.S., Lee H.J. and Ahn B.S. Peptic and tryptic hydrolysis of native and heated whey protein to reduce its antigenicity. *J. Dairy Sci*., 2007, vol. 90, pp. 4043–4050.

16. Kruglik V.I., Zorin S.N., Gmoshinskiy I.V., Ponomarev D.V., Nikitina N.E., Abramova A.A., Volkova I.N. and Revyakina N.V. *Sposob polucheniya fermentativnogo gidrolizata syvorotochnykh belkov so sredney stepen'yu gidroliza* [Obtaining method of whey protein enzymatic hydrolysate with moderate degree of hydrolysis]. Patent RF, no. 2375910, 2009.
17. Kiselev P.A., Oreshko N.A., Bovdey N.A., Le Min Kha, Fam Kuok Long, Baranovskiy A.V., Khripach N.B., Khlebnikova T.S. and Lakhvich F.A. *Obtaining and characterization of antioxidant activity of Iris Domestica extracts and their individual components. Proceedings of the Belarusian State University*, 2012, vol. 7, pp. 228–237. (In Russian).
18. Kutsenko S.A. *Toxicology basics: method. pub*. St. Petersburg: Foliant Publ., 2004. 720 p. (In Russian).
19. Podunova L.G., Menshikova, T.A. and Dvoskin Ya.G. *Alternative research methods (express methods) for toxicological-hygienic assessment of materials, products and environmental objects: method. pub*. Moscow, 1999. 108 p. (In Russian).
20. Prozorovsky V.B. Least square method for probit analysis of mortality curves. *Farmakologiya i toxikologiya*, 1962, no. 1, pp. 41–63. (In Russian).
21. O'Keeffe M.B. and FitzGerald, J.R. Antioxidant effects of enzymatic hydrolysates of whey protein concentrate on cultured human endothelial cells. *Int. Dairy J*., 2014, vol. 36, no. 2, pp. 128–135.
22. Halavach T.M., Kurchenko V.P. and Albulov A.I. Enzymatic hydrolysis of milk proteins as a basis of specialized food products biotechnology. *Ural Scientific Bulletin*, 2014, vol. 104, no. 25, pp. 69–79.

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PRECLINICAL STUDIES OF KEFIR PRODUCT WITH REDUCED ALLERGENICITY OF β-LACTOGLOBULIN

R. P. Korzhov, A. N. Ponomarev, E. I. Melnikova, E. V. Bogdanova*

Voronezh State University of Engineering Technologies,
Revolution Avenue 19, Voronezh, 394036, Russian Federation

* e-mail: ek-v-b@yandex.ru

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Abstract: The most promising approach for reducing the allergenicity of milk products with high biological value is biocatalytic conversion of whey proteins, producing hydrolysates with the specified molecular-mass distribution and residual allergenicity. The purpose of this study was to evaluate the biological effect of kefir product produced with the use of whey protein hydrolysate. The experimental works were conducted at the A.N. Bach Institute of Biochemistry of Russian Academy of Sciences. The objects of the studies were males of Brown Norway rats of BN/SsNolaHsd line with RT1n haplotype with initial mass of about 150 g, which had been obtained from the centre for Laboratory Animal Breeding of Harlan Laboratories, Inc. Company and had passed quarantine for at least 3 weeks after delivery. A model for testing the bio-functional properties of fermented milk products on the basis of enzymatic whey protein hydrolysates was created. Its essence lies in the induction of IgE-mediated allergic reaction to antigens of dairy products by oral administration with adjuvant – chlorea toxin that increases the permeability of the intestinal wall. Multiple investigations on laboratory animals found that the developed kefir product, produced with the use of whey protein hydrolysate, is characterized by hypotensive, hypolipidemic and hypocholesterolemic effect compared to kefir produced by traditional technology, as well as by reduced allergenicity of β-lactoglobulin. According to the opinion of the Scientific Research Institute of Nutrition of the Russian Academy of Medical Sciences a status of diet (preventive) food product for adults with symptoms of food allergy to milk proteins was assigned to the fermented milk drink.

Keywords: β-lactoglobulin, allergenicity reduction, hydrolysate, kefir product, in vivo biological effects

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INTRODUCTION

The production of protein products with high content of milk (cheese, curd, technical casein) provides getting whey, which causes a significant economic damage and irreparable harm to the environment [1]. The volumes of the obtained whey reach 90% of the volume of processed milk: almost they are somewhat less because of incomplete gathering and process losses. About 50% of milk solids go into the whey. According to the International Dairy Federation data of 120 million tons of whey, produced in the world (in Russia – more than 15 million tons), up to 15% is discharged into the sewer which leads to the waste of about 400 thousand tons of milk protein and other components of raw milk [2, 3].

The most valuable raw material resource for the dairy industry is cheese whey – a complex biological system. Annually about 20 million tons of cheese are produced in the world, the whey production amounts to more than 160 million tons. In developed countries (USA, Canada, Germany, France, Sweden) the dairy industry processes from 60 to 95% of the whey resources. Russia is characterized by a low level of industrial processing – less than 40% [4, 5].

The most valuable part of this raw product is whey proteins with a high biological value and digestibility. The increase in volumes of whey extraction and large-scale implementation of membrane technologies in fractionation of raw milk components have caused great interest in the use of whey proteins, have led to the study of their functional properties.

The main whey protein fractions, represented by β-lactoglobulin, α-lactalbumin, immunoglobulin are of a globular shape and have a compact structure due to the low content of proline and the disulfide bridges between molecules of cysteine [6].

The widespread use of whey proteins in food technologies is constrained by their residual antigenicity. According to the data of International Union of Immunological Societies, the known food-borne allergens in dairy products include: caseins, immunoglobulins, β-lactoglobulins, α-lactalbumins, bovine serum albumin [7].

Entering the body, these proteins are recognized by the immune system which leads to its sensibilization, or sensitization to a specific allergen containing the antigen. Allergen's re-entering the body leads to the development

of allergic reactions with specific symptoms [8, 9].

According to the results of experimental evaluations of the European Academy of Allergy and Clinical Immunology (EAACI), there has been significant spread of allergic diseases in the last decade [10]. According to the data of the Institute of Immunology of Russia's Federal Medico-Biological Agency, every third inhabitant of our country is affected by allergy [11].

One of the most common forms of allergic diseases (about 80%) is food allergy, in which nutrition, providing for the correction of the patient's diet through the use of specialized products, including ones with reduced content of major allergen milk proteins, is of great importance [12].

Thus, modification of the composition and properties of cheese whey to reduce the residual antigenicity, its protein fractions, in particular, β-lactoglobulin, is particularly relevant. The most promising for this purpose is to apply the bioconversion of whey proteins with high residual antigenicity [13].

A method for producing hydrolysate of whey proteins with reduced allergenicity of β-lactoglobulin, providing for the bioconversion of whey proteins of cheese whey ultrafiltration concentrate, is well-known [14]. The purpose of these studies is to evaluate the biological effect of kefir product produced with the use of whey protein hydrolysate.

OBJECTS AND METHODS OF STUDY

The experimental works were conducted at the A.N. Bach Institute of Biochemistry of Russian Academy of Sciences.

The objects of the studies were males of Brown Norway rats of BN/SsNolaHsd line with RT1n haplotype with initial mass of about 150 g, which had been obtained from the centre for Laboratory Animal Breeding of Harlan Laboratories, Inc. Company and had passed quarantine for at least 3 weeks after delivery. During quarantine and follow-up experiment the animals were kept by 4 in standard cages with an upper wire frame and a plastic drinker at (20 ± 2)°C temperature, 60% relative humidity and 12 h/day lighting cycle duration. The change of bedding and drinking water was carried out daily. Feed and animal drinking water were provided ad libitum. During quarantine and follow-up experiment the animals were kept on complete granular feed (recipe PK-120 for breeding laboratory animals (LLC Laboratorkorm)) that did not include components and ingredients derived from milk (Table 1).

The test results of the physical and chemical parameters of feed ration are presented in Table 2.

Before the experiment, all the animals underwent blood sampling from vena saphenus lateralis to control the absence of circulating antibodies, specific to milk proteins, in blood serum. Then the animals were randomly divided into 5 groups of at least 10 animals each.

The animals of group 1 served as the positive control. On the first day of the experiment the animals were injected with a mixture of 0.2 cm^3 colloidal suspension of 40 mg/cm^3 aluminum hydroxide (Thermo Fisher Scientific, USA) with 0.5 cm^3 solution of skimmed milk powder (0.2 mg/cm^3 in sterile isotonic solution of sodium chloride) intraperitoneally. Then on the 2nd, 4th, 6th, 8th, 10th and 12th days the animals were reimmunized, injected with 0.5 cm^3 solution of skimmed milk powder (0.2 mg/cm^3 in sterile isotonic solution of sodium chloride) intraperitoneally. On the 28th day of the experiment the animals were euthanized by a method of carbon dioxide euthanasia. The flow rate of carbon dioxide was determined by a rotameter at the level of 3.5 dm^3/min. The end of exposure time of the animal in the chamber for carbon dioxide euthanasia was determined visually by the cessation of respiratory movements of animals. Blood samples were collected with a sterile syringe from the heart cavity and were incubated for 1 h at indoor temperature. Blood serum was separated by centrifugation for 10 min in CM6 centrifuge (Elmi, Latvia) (at 3 500 rpm). From the obtained samples of blood serum a combined sample, which was poured into portions of 150 μl, was frozen in liquid nitrogen and stored in low temperature (–80°C) freezer, was prepared. The combined sample of serum was used as the positive control in the analysis of specific antibody titers in blood serum of experimental animals.

The animals of group 2 served as the negative control. During 56 days they were kept on complete granular feed (recipe PK-120 for breeding laboratory animals) with periodic sampling of 0.5 cm^3 blood from vena saphenus lateralis on the 20th, 32nd, 44th and 56th days of the experiment. Blood serum was separated by centrifugation for 10 min in Mini spin centrifuge (Eppendorf, Germany) (at 3 500 rpm). From the obtained samples of blood serum a combined sample, which was poured into portions of 150 μl, was frozen in liquid nitrogen and stored in low temperature (–80°C) freezer, was prepared. The combined sample of serum was used as the negative control in the analysis of specific antibody titers in blood serum of experimental animals.

The animals of group 3 during 49 days were injected with chlorea toxin at a dosage of 1 μg/day as an adjuvant using probes with diameter of 1.02 mm (18 Gauge, Kent Scientific, USA) intragastrically. On the 8th-49th days of the experiment, together with chlorea toxin each animal was injected with 1.0 cm^3 ultrafiltration concentrate of whey milk proteins intragastrically. On the 21st, 35th and 49th days of the experiment each animal underwent 0.5 cm^3 sampling of blood from vena saphenus lateralis. Blood serum was separated by centrifugation for 10 min in Mini spin centrifuge (Eppendorf, Germany) (at 3 500 rpm). In the obtained blood serum samples the total content of IgE, IgG1 titer, IgE, IgG2α, specific to β-lactoglobulin, α-lactalbumin and caseines, as well as the activity of mast cell-specific protease were determined. On the 56th day of the experiment the animals were injected with 2 cm^3 of 14% aqueous solution of skimmed milk powder intragastrically. After 30 min under

Table 1. Chemical composition of feed ration of rats

Component/parameter	Measurement units	Content
moisture	%	10.00
crude protein	%	22.00
crude fiber	%	4.00
crude fat	%	5.00
crude ash	%	4.36
lysine	%	1.09
methionine+cystine	%	0.70
calcium	%	1.80
phosphorus	%	1.10
sodium	%	0.10
metabolizable energy	kcal/100 g	310
vitamin A	thousand IU/kg	5.00
vitamin D3	thousand IU/kg	0.50
vitamin K3	mg/kg	0.30
vitamin E	mg/kg	30.00
vitamin B1	mg/kg	2.24
vitamin B2	mg/kg	1.20
vitamin B3	mg/kg	6.00
vitamin B5	mg/kg	22.15
vitamin B6	mg/kg	2.33
vitamin B9	mg/kg	6.60
vitamin B12	mg/kg	0.05
vitamin H	mg/kg	0.04
ferrum	mg/kg	18.00
manganese	mg/kg	9.00
zinc	mg/kg	22.50
copper	mg/kg	2.40
cobalt	mg/kg	0.18
iodine	mg/kg	0.24
selenium	mg/kg	0.06

Table 2. Physical and chemical parameters of feed ration of rats

Parameter	Measurement units	Value (M ± SD)	Method of analysis
Mass fraction of moisture	%	5.89 ± 0.26	GOST 13496.3
Mass fraction of total ash on a dry weight basis	%	8.32 ± 0.01	GOST 26226
Mass fraction of protein on a dry weight basis	%	23.16 ± 0.02	GOST 25011
Mass fraction of fat on a dry weight basis	%	5.08 ± 0.04	GOST 17681
Mass fraction of soluble dietary fibers on a dry weight basis	%	7.57 ± 0.32	GOST R 54014
Mass fraction of insoluble dietary fibers on a dry weight basis	%	17.65 ± 0.14	GOST R 54014

aseptic conditions blood sampling from the cavity of the heart was performed. Besides, under aseptic conditions spleen sampling for subsequent ex vivo experiments was carried out. The spleen was placed in a sterile falcon of 15 cm^3 volume of 5 cm^3 RPMI 1640 medium (PanEco, Russia) with 25 mM Hepes, L-glutamine, 1% penicillin-streptomycin (PanEco, Russia), 5% heat-inactivated fetal calf serum and 75 mM mercaptoethanol.

The animals of group 4 during 49 days were injected with chlorea toxin at a dosage of 1 μg/day as an adjuvant using probes with diameter of 1.02 mm (18 Gauge, Kent Scientific, USA) intragastrically. On the 8th-49th days of the experiment, together with chlorea toxin each animal was injected with 1.0 cm^3 enzymatic whey protein hydrolysate intragastrically. The further course of the experiment is similar to the one described previously for the animals of group 3.

The animals of group 5 during 49 days were injected with chlorea toxin at a dosage of 1 μg/day as an adjuvant using probes with diameter of 1.02 mm (18 Gauge, Kent Scientific, USA) intragastrically. On

the 8th–49th days of the experiment, together with chlorea toxin each animal was injected with 1.0 cm^3 kefir product with 1% mass fraction of fat produced with the use of enzymatic whey protein hydrolysate intragastrically. The further course of the experiment is similar to the one described previously for the animals of group 3.

Determination of the activity of mast cell-specific protease, the total IgE content and the specific antibody titers in blood serum of experimental animals

To determine the activity of mast cell-specific protease in blood serum of laboratory animals Mast cell protease II ELISA kit (Antibodies-online Gmbh, UK) was used. To determine the total IgE content in blood serum of laboratory animals total rat IgE ELISA KIT (Innovative research, USA) was applied.

Determination of the specific antibody titers in blood serum was performed by the method of noncompetitive enzyme-linked immunosorbent assay (ELISA). Sodium caseinate, α-lactalbumin, β-lactoglobulin (Sigma, USA) were used as the antigens. The antigens – (2 μg/cm^3 by protein, in 50 mM phosphate-buffered saline, pH 7.4) were sorbed in the microplate wells of 100 μl volume for 16 hours at 4°C. After four times of washing with 50 mM phosphate-saline buffer, pH 7.4 in the plate wells 100 μl of 0.1% gelatin solution were administered to prevent nonspecific sorption and were thermostated for 1 hour at 37°C, followed by 4 times of washing with 50 mM phosphate-saline buffer, pH 7.4 with 0.05% Triton X-100. Next, the wells were added with 100 μl dilution of biotinylated mouse antibodies to IgE, IgG1 or IgG2α (BD Biosciences, USA) rat antibodies and were incubated for 1 hour at 37°C. Then the plates were re-washed and were added with 100 μl solution of streptavidin conjugate with horseradish peroxidase (BD Biosciences, USA). After 1 hour incubation at 37°C the plate was washed first with 50 mM phosphate-buffered saline, pH 7.4 with 0.05% Triton X-100 (three times), then with distilled water, and the enzymatic activity, conjugated to a peroxidase label bearer, was detected. In the wells 100 μl of tetramethylbenzidine solution (TMB substrate reagent set, BD Biosciences, USA) were added as the substrate. After 15 min incubation at indoor temperature the reaction was terminated by adding 100 μl of 2M sulfuric acid solution. The optical density of solutions at 450 nm was determined with Synergy 2 photometer-fluorimeter (BioTek, USA). Based on the measurement results a curve for the antibody titration – dependence of the optical density of tetramethylbenzidine oxidation products of blood serum dilution – was plotted.

Determination of the differential blood count in blood of experimental animals

For this purpose 0.5 cm^3 sampled blood were transferred into the microtubes with K3-EDTA (Greiner Bio One, Germany). The smears of blood by the standard method no later than 1 hour after sampling were prepared. The air dried smears were stained by the Romanowsky method. The microscopic examination of blood smears (increase of 100, under immersion) using microscope (Motic BA300, Canada) and differential blood count of 100 cells were performed.

Determination of cytokine production by splenocytes

To obtain splenocyte suspension under aseptic conditions the spleen was rubbed by the sterile syringe plunger of 10 cm^3 volume through 70 μm nylon cellular extractor. Cells were washed 2 times with 5 cm^3 RPMI 1640 medium (PanEco, Russia) with 25 mM Hepes, L-glutamine, 1% penicillin-streptomycin, 10% fetal calf serum and 75 mM mercaptoethanol. Cell mass was separated by centrifugation at 3 500 rpm in 5702R centrifuge (Eppendorf, Germany). After washing the splenocytes were resuspended in 5 cm^3 of the above mentioned medium, and calculation of the number of cells was made in the Gorjaev's chamber. Then 100 μl splenocyte suspension with 4×10^6 cytosis was introduced into the wells of sterile 96-well microplates with high sorption capacity (Greiner Bio One, Germany). In the cultivation medium the sterile solutions of antigens (sodium caseinate, α-lactalbumin, β-lactoglobulin) were added into the wells so that their final concentrations should be 0 (control wells), 0.5 and 1.0 mg/cm^3. Cells were incubated in CO_2 incubator for 96 hours. The similar samples, in which concavalin A in the final concentration of 3.33 mg/cm^3 followed by incubation was added as mitogen for 48 hours additionally, served as the positive control. Upon completion of the incubation the contents of the wells were aspirated and centrifuged for 10 min at 5000 rpm in Mini spin centrifuge (Eppendorf, Germany), and in the supernatant IL-2, IL-12, IL-4, TNFα, IFNγ concentrations using IL-2 Rat ELISA Kit (Life Technologies, USA), IL-12 Rat ELISA Kit (Life Technologies, USA), Rat IL-4 BDOpt EIA ELISA Set (BD Biosciences, USA), Rat TNFα BDOpt EIA ELISA Kit (BD Biosciences, USA), Rat IFNγ BDOpt EIA ELISA Set (BD Biosciences, USA) were determined respectively.

RESULTS AND DISCUSSION

A model for testing the bio-functional properties of fermented milk products on the basis of enzymatic whey protein hydrolysates has been created. Its essence lies in the induction of IgE-mediated allergic reaction to antigens of dairy products by oral administration with adjuvant – chlorea toxin that increases the permeability of the intestinal wall. The list of monitored parameters included the total contents of IgE in blood serum, serum antibody titers of IgE, IgG1 and IgG2α-isotypes specific to the allergenic proteins of dairy products, the activity of mast cell-specific protease in blood serum, which is a specific marker of mast cell degranulation after IgE- mediated allergic reaction, the determination of the products of pro- and anti-inflammatory cytokines

by splenocytes in *ex vivo* culture after exposure of mitogen and allergenic proteins of dairy products.

Multiple investigations on laboratory animals found that the developed kefir product, produced with the use of whey protein hydrolysate, is characterized by hypotensive, hypolipidemic and hypocholesterolemic effect compared to kefir produced by traditional technology (Table 3).

The developed technological scheme of production of kefir product with reduced allergenicity of β-lactoglobulin (Fig. 1) provides modification to the traditional technology, which consists in the introduction of additional operations for getting whey protein hydrolysate and administrating it to the normalized mixture.

TOR 9222-512-00419785-13 technical documentation "Fermented milk products for dietary preventive nutrition", which has passed experimental-industrial testing in conditions of OJSC Dairy industrial complex "Voronezh" has been developed and approved.

The finished kefir product is characterized by qualitatively new consumer properties and reduced allergenicity of β-lactoglobulin. According to the opinion of the Scientific Research Institute of Nutrition of the Russian Academy of Medical Sciences a status of diet (preventive) food product for adults with symptoms of food allergy to milk proteins has been assigned to the fermented milk drink.

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Table 3. Biofunctional properties of the investigated products

Product name	*In vivo* biological effects		
	Hypocholesteremic effect	Hypotensive effect	Hypolipidemic and hypocholesterolemic effect
Biokefir with 1% mass fraction of fat produced by traditional technology	—	—	In the use of the product in amounts of 1 cm^3/day on the background of consumption of feed ration with high lipid loading (the content of low molecular weight fatty acids is 6.2%) the hypocholesterolemic effect, expressed as decrease in serum concentration of total cholesterol by 15.2% ($p < 0.01$), high- density-lipoprotein-non-atherogenic fraction by 16.9% ($p < 0.001$) and low-density- lipoprotein-atherogenic fraction by 27.9% ($p < 0.05$) compared to the control with high alimentary lipid loading, was shown during 42 days. For the animals a statistically significant decrease in serum concentration of triglycerides by 62.5% ($p < 0.01$) compared to the value of this parameter in no. 2 control group, which indicates the presence of hypolipidemic properties in bio-kefir, was shown.
Biokefir with 1% mass fraction of fat with the introduction of 30% whey protein hydrolysate	The hypocholesteremic effect in exogenous induction of free- radical pathology of liver intoxicated with carbon tetrachloride.The decrease in serum concentration of total cholesterol and high density lipoproteins by 9.1% ($p < 0.07$) and 5.7% ($p < 0.05$) compared to the control respectively.	Hypotensive effect in conditions of high alimentary lipid loading. On normotensive Wistar rats in the use of biokefir functionnal product in amounts of 1 cm^3/day on the background of consumption of feed ration with high lipid loading (the content of low molecular weight fatty acids is 6.2%) the hypotensive effect, expressed as decrease in systolic and mean arterial pressure by –19.47 and –13.42 mmHg compared to the control, was shown during 42 days.	Hypolipidemic and hypocholesterolemic effect in conditions of high alimentary lipid loading. In the use of biokefir in amounts of 1 cm^3/day on the background of consumption of feed ration with high lipid loading (the content of low molecular weight fatty acids is 6.2%) the hypocholesteremic effect, expressed as decrease in serum concentration of total cholesterol by 15.6% ($p < 0.01$), high- density-lipoprotein-non-atherogenic fraction by 24.8% ($p < 0.001$) and low-density-lipoprotein-atherogenic fraction by 22.2% ($p < 0.01$) compared to the control with high alimentary lipid loading, was shown during 42 days. For the animals a statistically significant decrease in serum concentration of triglycerides by 68.1% ($p < 0.001$) compared to the value of this parameter in no. 2 control group, which indicates the presence of hypolipidemic properties in bio-kefir, was shown.

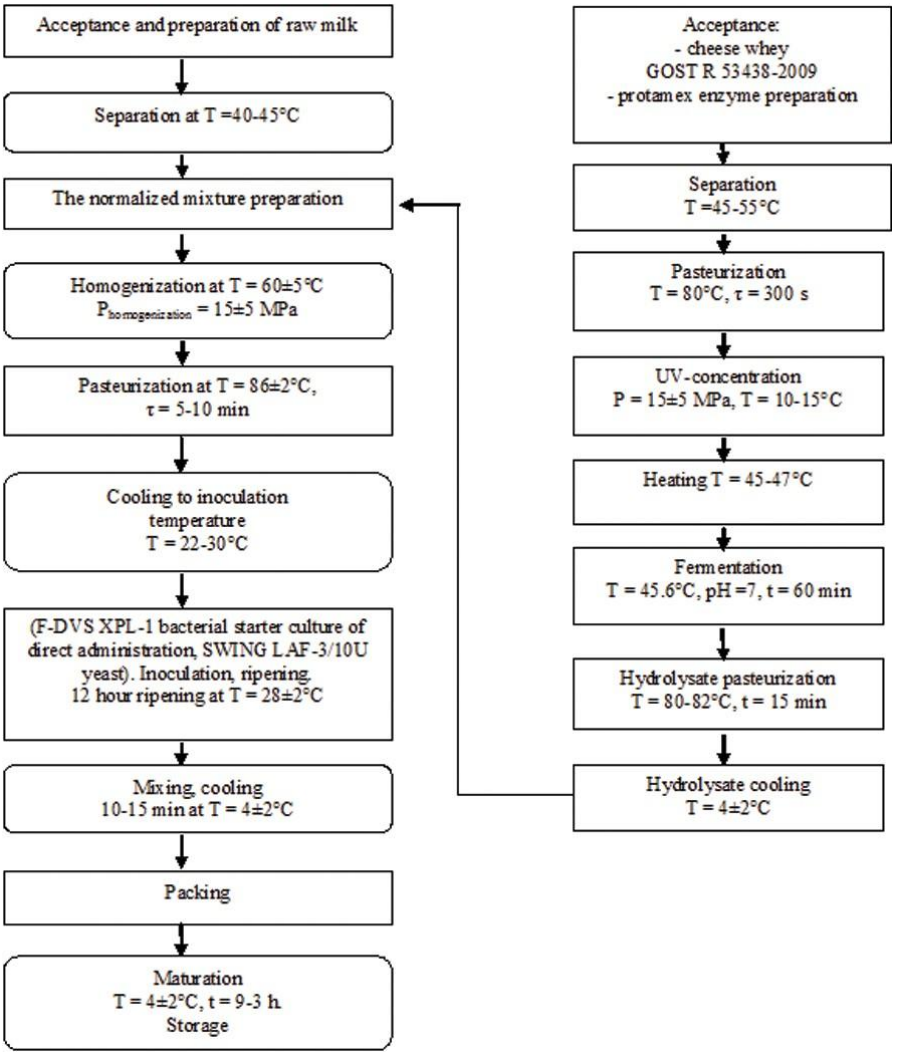

Fig. 1. Technological scheme of production of kefir product.

REFERENCES

1. Musina O.N. The modern state of combined dairy products' biotechnology. *Storage and processing of farm products*, 2010, no. 3, pp. 59–63. (In Russian).
2. Golovach G.N. and Kurchenko V.P. Allergenicity of milk proteins and ways to reduce. *Proceedings of the Belarusian State University*, 2010, vol. 5, no. 1, pp. 9–11. (In Russian).
3. Gavrilov G.B. and Kravchenko E.F. Rational use of whey. *Dairy Industry*, 2012, no. 7, pp. 32–33. (In Russian).
4. Ozhgikhina N.N. and Volkova T.A. Rational processing of whey. *Milk Processing*, 2012, no. 9, pp. 44. (In Russian).
5. Khramtsov A.G., Ryabtseva S.A. and Evdokimov I.A. World trends in whey processing. *Milk Processing*, 2009, no. 5, pp. 18. (In Russian).
6. Tepel A. *Chemistry and physics of milk*. St. Petersburg: Profession Publ., 2012. 832 p. (In Russian).
7. Koroleva O.V., Agarkova E.Yu., Botina S.G., Nikolaev I.V., Ponomareva N.V., Mel'nikova E.I., Kharitonov V.D., Prosekov A.Yu., Krokhmal' M.V. and Rozhkova I.V. Prospects for the use of whey protein hydrolysate in the technology of fermented milk products. *Dairy Industry*, 2013, no. 7, pp. 66–67. (In Russian).
8. Khaitov R.M. and Il'ina N.I. *Allergy*. Moscow: GEOTAR-Media Publ., 2010. 228 p. (In Russian).
9. Frenhani P.B. and Burini R.C. Mechanisms of absorption of amino acids and oligopeptides. Control and implications in human diet therapy. *Arq. Gastroenterol*, 1999, vol. 36, no. 4, pp. 227–237.
10. Johansson S.G., Hourihane J.O., Bousquet J., Bruijnzeel-Koomen C., Dreborg S., Haahtela T., Kowalski M.L., Mygind N., Ring J., van Cauwenberge P., van Hage-Hamsten M. and Wüthrich B. A revised nomenclature for allergy. An EAACI position statement from EAACI nomenclature task force. *Allergy*, 2001, no. 56, pp. 813–824.
11. Novoselova M.V., Borisova G.V., Bondarchuk O.N. and Malova Yu.S. Selection of parameters of enzymatic hydrolysis of casein. *Modern Problems of Science and Education*, 2012, no. 6. (In Russian).
12. Kharitonov V.D., Budrik V.G., Agarkova E.Yu., Botina S.G., Berezkina K.A., Kruchinin A.G., Ponomarev A.N. and Mel'nikova E.I. Perspective directions of struggle with allergy. *Food Processing: Techniques and Technology*, 2012, vol. 27, no. 4, pp. 3–6. (In Russian).

13. Maksimyuk N.N. and Mar'yanovskaya Yu.V. About the advantages of the enzymatic method of obtaining protein hydrolysates. *Fundamental Research*, 2009, no. 1, pp. 34–35. (In Russian).
14. Prosekov A.Yu., Ul'rikh E.V., Mel'nikova E.I., Noskova S.Yu., Budrik V.G., Botina S.G. and Agarkova E.Yu. The getting enzymatic whey protein hydrolyzate using proteolytic enzyme. *Fundamental Research*, 2013, no. 6, part 5, pp. 1089–1093. (In Russian).

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