PREPARATION AND USE OF WHEY PROTEIN MICROPARTICULATE IN SYNBIOTIC DRINK TECHNOLOGY

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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 microparticulation 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,

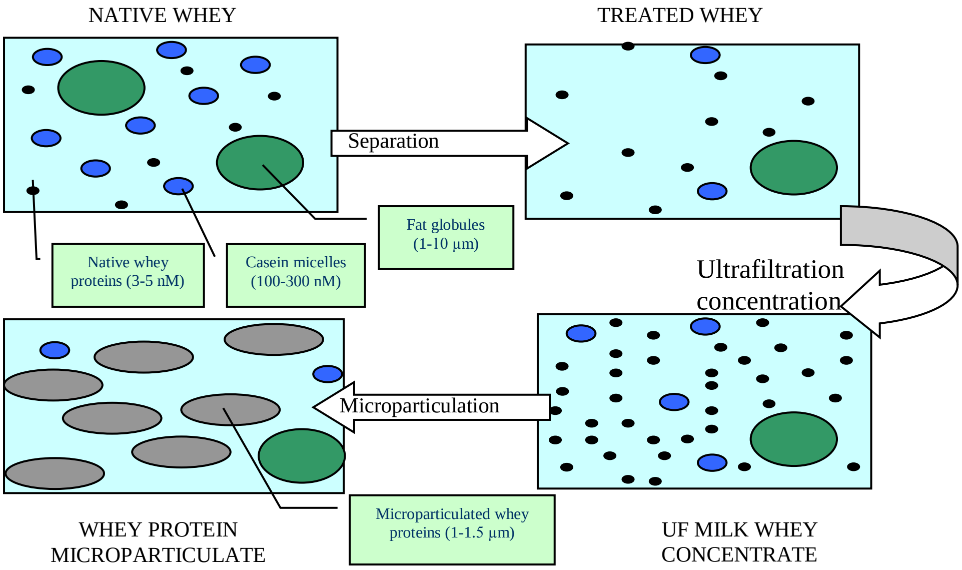


Fig. 1. Diagram of modification of whey cheese composition and properties.

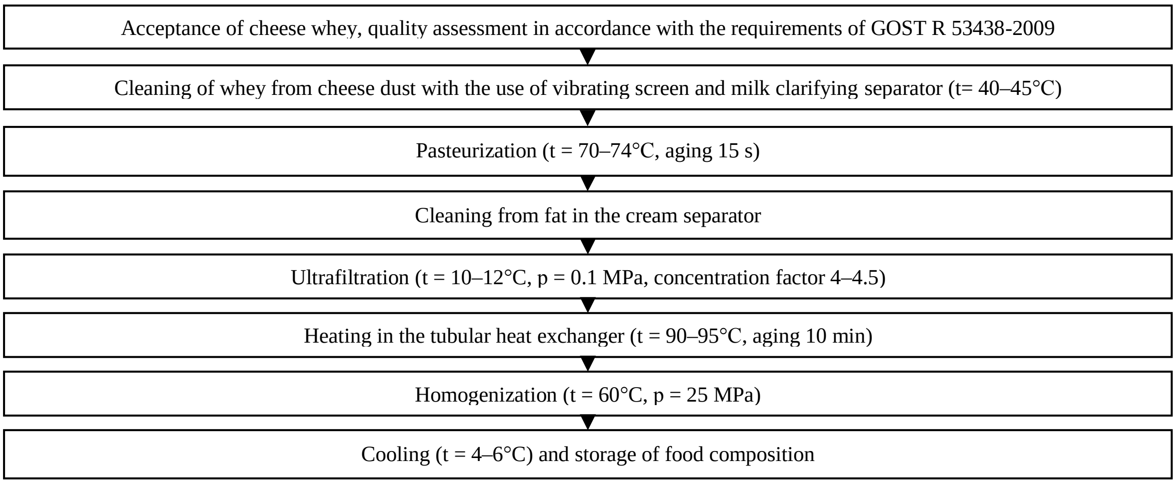


Fig. 2. Process flow diagram of obtaining whey protein microparticulate.

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.

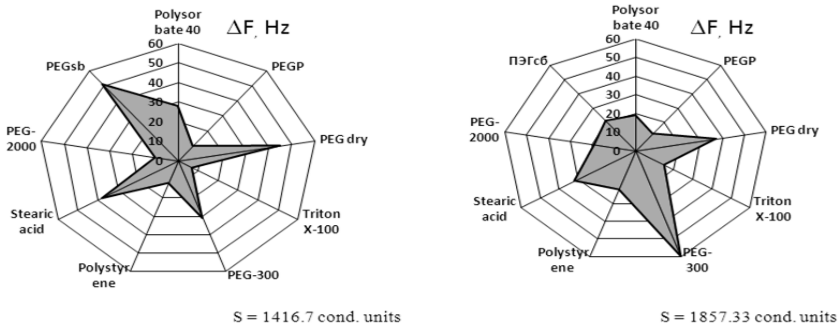
This determines the feasibility of replacing skimmed milk by the new food composition in technology of a wide range of low-calorie food products with high biological value due to the change in the ratio of casein to whey proteins.

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



(a) (b)

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

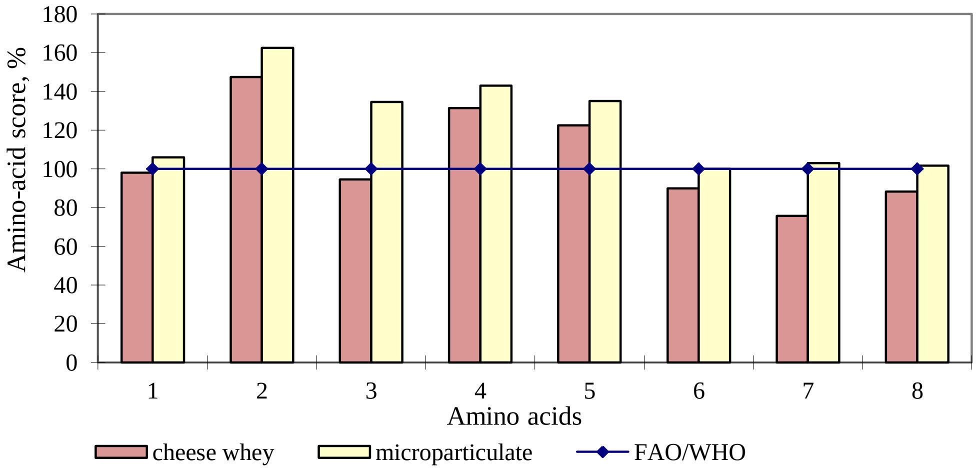


Fig. 4. Amino-acid score of whey protein microparticulate and cheese whey: 1 – valine; 2 – isoleucine; 3 – lysine; 4 – methionine + cysteine; 5 – threonine; 6 – tryptophan; 7 – leucine; 8 – phenylalanine + tyrosine.

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	$7\cdot10^5$	$6\cdot10^8$	$9\cdot10^9$
24	$1\cdot10^6$	$8\cdot10^9$	$1\cdot10^{10}$
48	$6\cdot10^7$	$7\cdot10^{10}$	$7\cdot10^{11}$

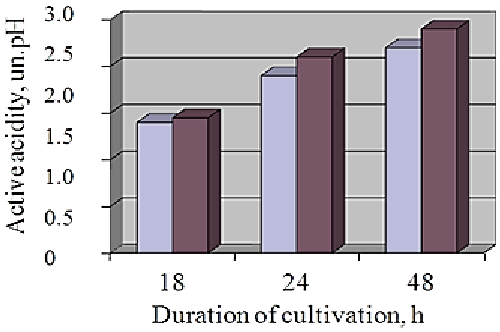


Fig. 5. Dynamics of changes in active acidity of culture medium.

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

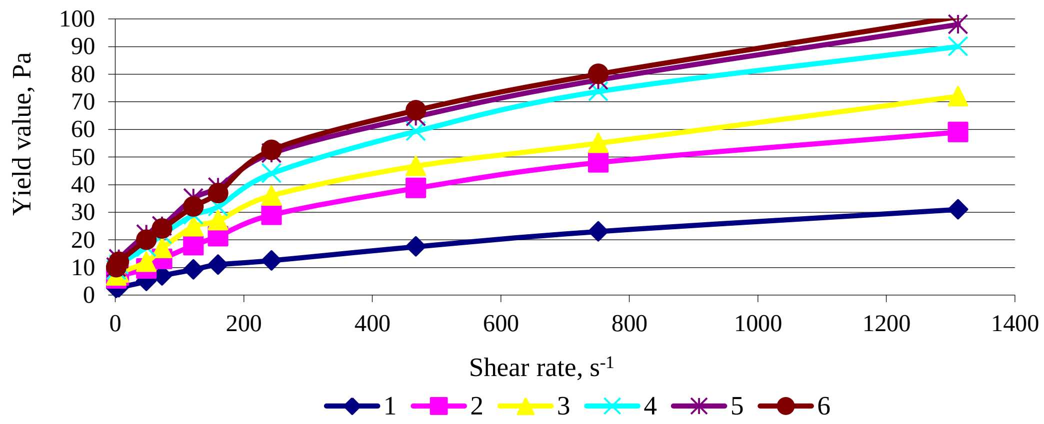


Fig. 6. The dependence of the yield value of the shear rate of drink samples.

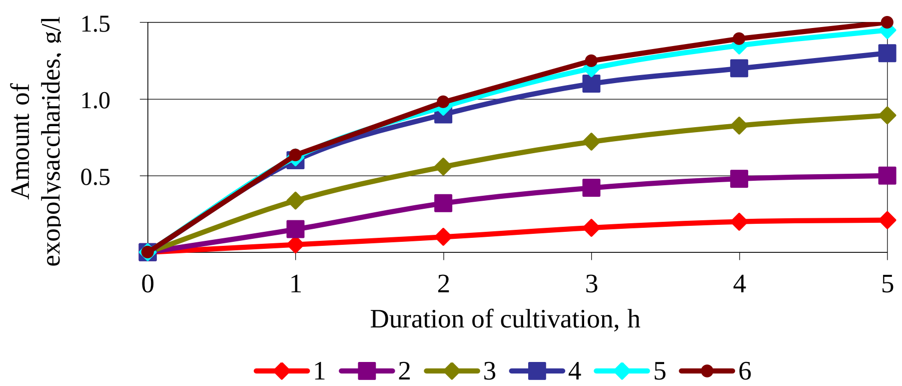


Fig. 7. The influence of normalized mixture composition on EPS synthesis.

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		

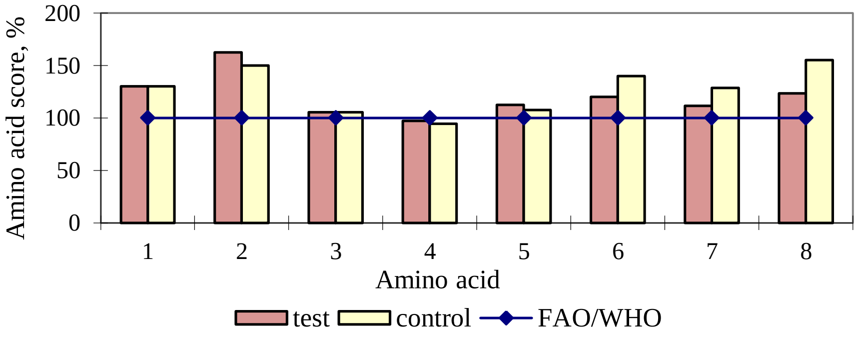


Fig. 8. Amino acid score of the test and control sample of yogurt: 1 – valine; 2 – isoleucine; 3 – lysine; 4 – methionine + cysteine; 5 – threonine; 6 – tryptophan; 7 – leucine; 8 – phenylalanine + tyrosine.

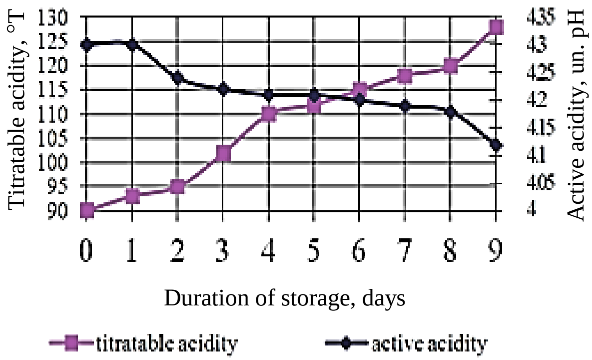


Fig. 9. Change of titratable and active acidity of the drink during storage.

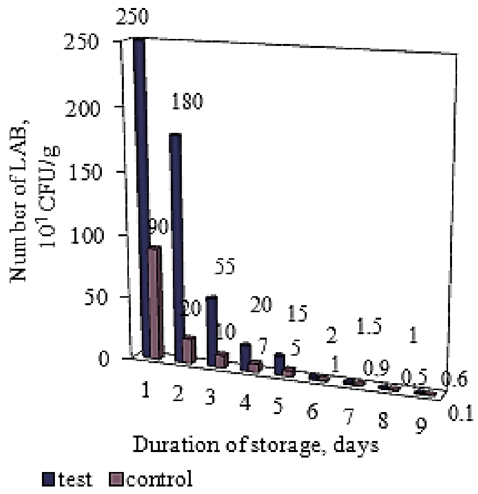


Fig. 10. Change of the number of lactic-acid bacteria (LAB) of yogurt during storage.

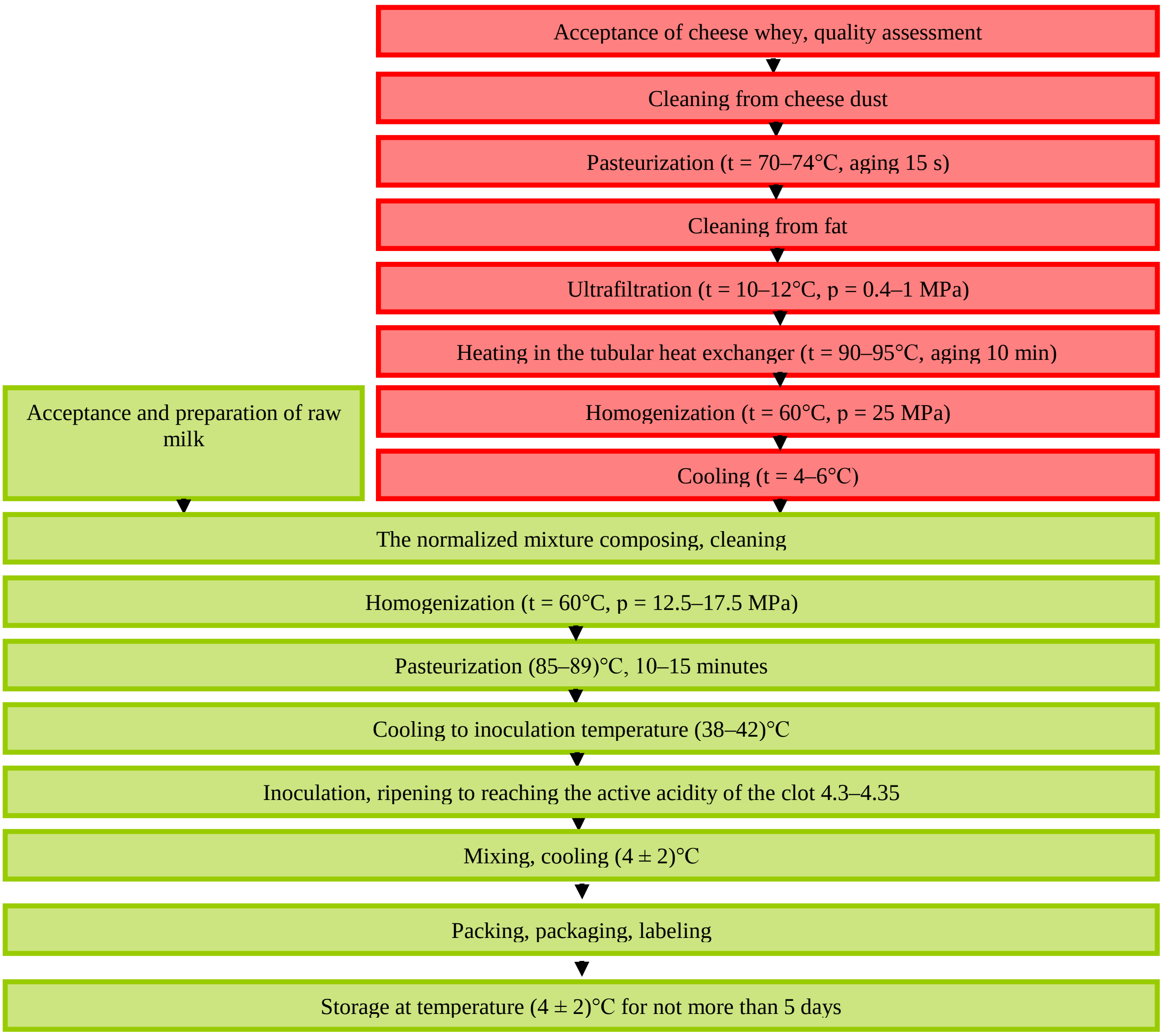


Fig. 11. Process flow diagram of yogurt production.

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.

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