

# Advanced Biotechnology of Specialized Fermented Milk Products



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**Abstract:** *The aim of the study is the analytical substantiation of the perspective trend – the development of specialized fermented foods. According to the stable trend of using a balanced diet for the prevention and treatment of gastrointestinal diseases (for which dairy products are enriched with probiotics), a bioproduct has been developed, the formulation of which includes cottage cheese, cream, starter cultures of probiotic cultures, berry syrup, wheat bran. Cottage cheese is prepared by fermentation of a mixture consisting of buttermilk, wheat bran and biologically active additive "Nash lecitin", a leaven based on the association of starter (probiotic) cultures *Lactobacillus acidophilus*, *B. lactis*, *B. longum*, *Streptococcus thermophilus* in an immobilized form, by nanofiltration. Its chemical composition, nutritional and biological value were studied and microbiological parameters characterizing its functional properties were determined. Cottage cheese bioproduct recommended both for mass catering and for elderly persons nutrition.*

**Index Terms:** *fermented foods, specialized nutrition, biotechnology, probiotic cultures, immobilization, curd product, wheat bran, berry syrup*

## I. INTRODUCTION

In modern conditions, when a new generation, both in Russia and in Western Europe, seeks to maintain a healthy lifestyle, the demand for healthy food is growing [1]. Among

the most important products of healthy food, first of all include milk and products based on it.

In the minds of both foreign and Russian consumers, high-protein dairy products are strongly associated with health and benefit. Their presence in the market is growing. If earlier such products were mainly interested in people engaged in sports and bodybuilding, now they are interesting to ordinary consumers, including the elderly population.

In connection with the above, we need to develop promising technologies for new types of specialized food products based on milk: dietary, preventive, therapeutic and preventive, for nutrition of athletes, for herodietic and diabetic nutrition, enriched with functional ingredients and, first of all, probiotic microflora, contributing to better digestibility of food products and stable function of the human gastrointestinal tract of all age groups [2, 3].

Russian as well as foreign scientists are actively searching for new strains of probiotic cultures, studying their viability in various complex multicomponent media based on milk, as well as developing methods for preserving the activity of probiotic cells in specialized, enriched and functional products throughout their shelf life [4, 5].

V.I. Ganina and I.I. Ionova (Russia) note that in the late 80's – early 90-ies of XX century in Japan there was a new direction – the concept of functional food, which was later supported by scientists from different countries. Of course, our country has also conducted research on the nutritional status and its impact on the health and life expectancy of the population. As a result of which an understanding was formed that a functional food product should not only contain certain nutrients, but also have a beneficial effect on human health, regulate certain processes in the body and reduce the risk of diseases. The positive effect of the product on the body should be confirmed [6]. N. Ah. Tikhomirova et al. (Russia) theoretically and experimentally proved that it is advisable to combine the use of selected strains of probiotic bacteria with certain rational doses of lysozyme and (or) lactoferrin. The implementation of the results will expand the range of dairy products of dietary and preventive orientation [7, 8].

Leading scientists of the Russian Research Institute of Dairy Industry considering the state and prospects of production of functional food products note the importance of the influence of food additives and functional ingredients, as to solve the most important national problems (preservation of public health, ensuring adequate biologically adequate nutrition of all social groups, a variety of functional properties of products). Therefore it is necessary to further enrich dairy products with physiologically functional ingredients.

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Global trends in the production and consumption of food ingredients are associated with a variety of their technological functions. Innovative technologies and developments in the field of food ingredients allow to create a new generation of competitive food products.

It should be noted that the use of food ingredients requires an understanding of the complex transformations that the food system undergoes during the process, taking into account its composition, properties; functional properties of food additives; effects of synergy/antagonism; features of the technology of application; evaluation of the effectiveness of use, including an economical effect [9].

I.S. Polyanskaya and V.F. Semenikhina (Russian Research Institute of Dairy Industry) developing the principles of food enrichment, proposed their classification by functional food ingredients [10].

N.Ah. Tikhomirova (Russia) considering the socio-economic aspects of nutrition and public health, the existing theories of nutrition highlighted the special role of functional food technology. She has joint development with M. E. Titova about protein module with functional ingredients containing the following components: vitamin-mineral premix, complex of bioactive milk proteins "Milkang", the drug of blood of slaughter animals, the drug of chicken lysozyme, iodine, whey protein concentrate and dry milk [11].

N.B. Gavrilova with co-authors (Omsk SAU, Russia) theoretically substantiated and experimentally proved the prospects of the principles of designing multicomponent starter cultures based on the joint use of lactic acid cultures and bifidobacteria for their use as functional ingredients in the technology of fermented dairy products in immobilized form [12].

I.A. Evdokimov (North Caucasus Federal University, Russia) has developed the concept of intensive technology of milk sugar and the use of lactulose in the technology of functional foods as an important ingredient [13, 14].

Z.S. Zobkova et al. (Russia) based on the accumulated data in the world and domestic literature on the important role of antioxidants in the prevention of cancer and cardiovascular diseases, oxidative stress, along with information about the insufficient intake of the diet indicate the feasibility of their widespread use as an additive that enriches food products, proposed to use the antioxidant effect of flavonoids (biologically active substances) for functional dairy products [15].

Dan C Vodnar and Carmen Socaciu (Romania) studied the stimulating effect of green tea extract on the viability and stability of *B. infantis* ATCC 15697 and *B. breve* ATCC 15700 microencapsulated in chitosan. After that, the microcapsules were covered with alginate and stored in the refrigerator. The conditions of the gastrointestinal tract were simulated and the degree of survival of probiotic bacteria was determined. Samples without addition of green tea extract served as control [16, 17, 18, 19].

As noted by Valeria Mozzetti with co-authors (New Zealand) when used in the process of immobilization of bifidobacteria, it is necessary to take into account that they are strict anaerobes and oxidative stress can seriously reduce their viability. A possible positive factor in the stabilization of this process may be the use of hydrogen peroxide, but it is necessary to carry out an experimental selection of cells adapted to hydrogen peroxide. Continuous cultivation with cell immobilization is an effective approach to the selection of

cells adapted to hydrogen peroxide. Elucidation of mechanisms of adaptation of probiotics to H<sub>2</sub>O<sub>2</sub> may be useful for the development of acid-resistant bifidobacteria [20, 21].

Carlos Pasqualin Cavalheiro with co-authors (Brazil) theoretically proved the importance of the use of probiotic microorganisms in the technology of dairy products subjected to heat treatment. Among the main methods of encapsulation are extrusion, spray drying and emulsification, which have their advantages and disadvantages. Currently, different materials can be used to encapsulate bacteria, however, alginate is the most commonly used. To obtain capsules with greater viability even after heat treatment it is necessary to bind thermally resistant probiotic strains and suitable materials using encapsulation methods [22]. After conducting analytical studies comparing different methods and conditions of encapsulation of probiotics, the authors came to the conclusion that the choice of probiotics and substrate material requires additional research.

Gauri Aeron and Shiwangi Morya (India) discussed the results of studies presented by scientists and specialists from different countries on the immobilization and microencapsulation of probiotic cultures [23, 24, 25, 26, 27, 28].

It was found that probiotics are an essential functional ingredient of commercial dairy products, as they prevent colonization, cell adhesion and invasion by pathogenic microorganisms, in addition, they have direct antimicrobial activity and modulate the immune response, it is necessary to use new methods that increase their viability [29].

A.V. Bannikova substantiating the scientific and practical aspects of the creation of technologies of products with high protein content, studied the process of encapsulation of protein and antioxidants, as well as their release under enzymatic hydrolysis in vitro [30, 31].

The aim of the research is to develop a promising biotechnology of fermented milk-based product for herodietic nutrition.

## II. MATERIALS AND METHODS

The following objects of research were used in this study:

- biopolymers – gelatin, pectin;
- association of probiotic cultures *Lactobacillus acidophilus*, *B. lactis*, *B. longum*, *Str. thermophilus*;
- wheat bran;
- buttermilk according to GOST 34354-2017;
- biologically active food supplement "Nash lecitin" according to the current regulatory documentation;
- berry syrup according to the current regulatory documentation;
- cream according to GOST 31355-2017;
- antioxidant Astaksantin".

In experimental studies, standard and generally accepted methods for determining chemical parameters of products were used.

For microbiological studies were used certified in accordance with the established procedure methods of measurement and microbiological box with cleaning system TENCAN (made in China).

Immobilization experiments were carried out in a special box in the following sequence:

- activation of cell biomass of probiotic cultures on sterilized and cooled to a temperature of  $(38\pm 1)^{\circ}\text{C}$  skim milk, as the optimal temperature of life of monocultures included in the association is  $(38\pm 1)^{\circ}\text{C}$ ;
- preparation of a mixture of biopolymers was carried out at a temperature of  $20^{\circ}\text{C}$ ;
- in the reactor, the association of probiotic cultures in an activated form at a temperature of  $(33\pm 1)^{\circ}\text{C}$  was combined with a gel of biopolymers, mixed for  $(15\pm 5)$  min;
  - then there was the dosing of the mixture in a sterile form;
  - exposure time forms in the special box is 15-20 min. as a result, in the shapes formed by a thin film (membrane). Membrane storage temperature  $(4\pm 2)^{\circ}\text{C}$ .

The experiments were carried out in five-fold repetition. The results were processed using mathematical statistics methods using standard software packages "Math CAD – 14 Professional".

### III. RESULTS AND DISCUSSION

Based on the findings of the Institute of gerontology (Russia) found that properly organized nutrition is a powerful tool to influence the aging process and prevent premature development of changes and disorders in the human body. In the elderly, there is a high risk of food deficiency, primarily in proteins, minerals and vitamins, insufficient consumption of which causes various pathologies: greater sensitivity to infections, slow healing of wounds, immunological disorders, cancer, etc. In recent years, there has been a steady trend to use a balanced diet for the prevention and treatment of gastrointestinal diseases for which dairy products are enriched with probiotics.

According to the given above, the recipe for cottage cheese bioproduct included cottage cheese, cream, starter probiotic cultures, berry syrup, wheat bran. Cottage cheese is prepared by fermentation of a mixture consisting of buttermilk, wheat bran and biologically active food supplement "Nash lecithin", a leaven based on the association of starter (probiotic) cultures *Lactobacillus acidophilus*, *B. lactis*, *B. longum*, *Streptococcus thermophilus* in an immobilized form, by nanofiltration.

The introduction of buttermilk in the formulation for cottage cheese bioproduct, rich in vitamins A, E, K, B1, B2, B6, C, H, protein, lecithin, allows to obtain a product with high nutritional and biological value.

Biologically active food supplement "Nash lecithin" increases the biological value of the bioproduct, as lecithin promotes the absorption of fat-soluble vitamins A, D, E and K, necessary for the nutrition of all cells of the body, reduces cholesterol and the concentration of fatty acids in the blood, clears the walls of blood vessels from cholesterol plaques and has a positive effect on the gastrointestinal tract.

Probiotic cultures were used in an immobilized form, since the immobilized ferment enriches the product with a viable probiotic microflora that stimulates the growth and activity of its own (indigenous) protective microflora, which increases the functional properties of the product.

The process of production of cottage cheese bioproduct is as follows. Buttermilk is heated to a temperature of  $(50\pm 5)^{\circ}\text{C}$ . Then wheat bran is introduced in a crushed form, the biologically active additive "Nash lecithin", an antioxidant and

stirred for 5-10 minutes. The resulting mixture is pasteurized at a temperature of  $(80\pm 5)^{\circ}\text{C}$  with an exposure of 10-15 minutes, cooled to a fermentation temperature  $(30\pm 2)^{\circ}\text{C}$ . Leaven of probiotic cultures consists of the association of cultures *Lactobacillus acidophilus*, *B. lactis*, *B. longum*, *Streptococcus thermophilus* immobilized by layering. Make the starter in a solution prepared of biopolymers – a mixture of gelatine and pectin, with subsequent overlaying. Cooled to the temperature of the fermentation mixture is fermented with immobilized yeast for 4.5-6.0 hours to obtain the bundle pH 4.24-4.26. The resulting clot is mixed, cooled to a temperature of  $10^{\circ}\text{C}$  and fed to a nanofiltration plant. The cream and syrup of honeysuckle berries are added to the resulting curd, thoroughly mixed, thermized at a temperature of  $(65\pm 5)^{\circ}\text{C}$  with an extract of 15-20 s, packed and stored at a temperature of  $(4\pm 2)^{\circ}\text{C}$ . The block diagram of the production of cottage cheese bioproduct is shown in figure 1.

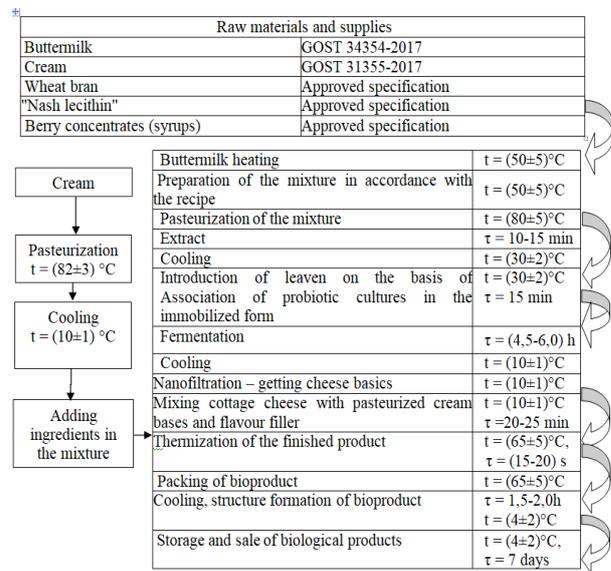


Figure 1 - Block diagram of cheese bioproduct production for gerodietic nutrition

Production of cottage cheese by nanofiltration contributes to the production of a product with high nutritional and biological value, functional properties due to the concentration of whey proteins and milk phospholipids. To increase the antioxidant properties of the product, astaxanthin is introduced into the mixture.

The expansion of the range of curd products is achieved by the addition of berry syrup honeysuckle berries, rich in vitamins A, C and B, macro- and microelements (calcium, phosphorus, copper, potassium, iodine and magnesium), pectin and tannins, which improves organoleptic characteristics, nutritional and biological value of the product (see Table 1).

**Table 1 - Assortment range of cottage cheese bioproduct**

Name of components	Recipe per 1000 kg finished product, kg		
	1	2	3
Butermilk	3698,5	3833,5	3918,5
Wheat bran	15	20	25
Biologically active food supplement "Nash lecitin"	30	40	50
Ferment on the basis of the association of probiotic cultures Lactobacillus acidophilus, B. lactis, B. longum, Streptococcus thermophilus in immobilized form	1,5	1,5	1,5
The curd is obtained by nanofiltration	750	780	800
Cream with fat mass fraction 10 %	150	-	-
Cream with fat mass fraction 15 %	-	100	-
Cream with fat mass fraction 20 %	-	-	50
Antioxidant "Astaxanthin»	5	5	5
Honeysuckle berry syrup	100	120	150
Total:	1000	1000	1000

Table 2 shows the organoleptic characteristics of the curd product.

**Table 2 - Organoleptic characteristics of cottage cheese bioproduct**

Name of indicators	Characteristics		
	Recipe 1	Recipe 2	Recipe 3
Appearance, consistency	Soft, spotting, with the inclusion of wheat bran		
Taste and smell	Pure, fermented milk. Moderately sweet, with a taste and smell of honeysuckle		
Color	Due to the color of the syrup of honeysuckle berries, uniform throughout the mass		

Analysis of the data presented in table 2 indicates that the curd bioproduct has high organoleptic characteristics. Table 3 shows the chemical composition and indicators of cottage cheese bioproduct.

**Table 3 - Chemical composition and indicators of cottage cheese bioproduct**

Name of indicators	Characteristic and norm		
	Recipe 1	Recipe 2	Recipe 3
Mass fraction of solids. %	33.0±1.00	34.50±1.00	36.10±1.00
Mass fraction of fat. %	3.00±0.50	3.10 ±0.50	2.60±0.50
Mass fraction of protein. %	18.30±0.50	18.50±0.50	18.70±0.50
Mass fraction of carbohydrates. %	11.70±0.50	12.90±0.50	14.80±0.50
Active acidity. pH	4.26±0.05	4.24±0.05	4.25±0.05

Table 4 shows the microbiological parameters of the curd bioproduct.

**Table 4 - Microbiological parameters of cottage cheese bioproduct**

Name of indicators	Characteristics
Coliforms in 0.01 g	Not allowed
Pathogenic microorganisms, including Salmonella in 25 g of product	Not allowed
S. aureus staphylococci in 0.1 g of product	Not allowed
Probiotic microorganisms, CFU/g, not less	1·10 <sup>8</sup> lactic acid microorganisms. 1·10 <sup>7</sup> bifododacteria
Yeast. CFU/g. no more	50
Mold. CFU/g. no more	50

Analysis of microbiological indicators shows that the cottage cheese bioproduct has high functional properties that meets the requirements of GOST R 52349-2025.

The energy and biological value of proteins of cottage cheese bioproduct, which is characterized by qualitative and quantitative composition of free amino acids, is determined in the new bioproduct (Table 5).

**Table 5 - Amino acid composition of proteins of cottage cheese bioproduct**

Name of amino acid	Mass fraction of mg/100 g of product		
	Recipe 1	Recipe 2	Recipe 3
Indispensable, including	5679.60	5731.1	5738.63
Valine	739.10	746.4	745.35
Isoleucine	736.80	745.8	745.40
Leucine	1358.25	1374.8	1376.65
Lysine	1061.70	1075.0	1076.15
Methionine	352.80	357.3	357.25
Threonine	592.05	598.0	598.35
Tryptophan	137.55	139.2	139.30
Phenylalanine	686.4	693.6	695.45
Interchangeable, including	7655.55	7755.8	7768.50
Alanine	336.60	341.2	342.60
Arginine	603.75	615.0	619.40
Aspartic acid	752.55	761.6	762.65
Histidine	413.10	418.8	420.15
Glycine	205.50	210.3	213.50
Glutamic acid	2460.30	2493.4	2494.10
Proline	1504.80	1522.0	1522.40
Glutamic acid	614.25	619.6	619.10
Tyrosine	685.50	691.9	691.90
Cystine	80.10	82.0	83.0
The total number of amino acids	13320.15	13486.9	13502.15

The nutritional and energy value of the curd bioproduct is presented in table 6.

Table 6 - Food and energy value of cottage cheese bioproduct

Name of product	Mass fraction, %			Power value	
	Fat	Protein	Carbohydrates	Kcal	Kilojoules
Recipe 1	3.0	18.3	11.7	147.0	615.05
Recipe 2	3.1	18.5	12.9	153.5	642.24
Recipe 3	2.6	18.7	14.8	157.4	658.58

#### IV. CONCLUSION

As a result of analytical and experimental studies, biotechnology of a new type of cottage cheese bioproduct has been developed. Its chemical composition, nutritional and biological value were studied, microbiological parameters characterizing its functional properties were determined. Cottage cheese bioproduct recommended, both for mass catering and for elderly persons nutrition.

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