Biotechnology application in production of specialized dairy products using probiotic cultures immobilization

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Abstract*: *The aim of the work is to study the process of immobilization of associations of probiotic cultures into gel biopolymers and the development of innovative biotechnologies of specialized dairy products using them. An analytical review of the results of research of foreign and Russian scientists in the field of immobilization of probiotic cultures is presented. In the result of experimental studies, the process of immobilization of associations of probiotic cultures of L. acidophilus, Bifidobacterium, Str. thermophilus gel of biopolymers gelatin - pectin in a ratio of 1: 2 at 20% concentration of solids was developed. The resulting functional biocomponent (membranes) is used in the development of biotechnology of fermented dairy products for specialized nutrition: dietary, prophylactic, gerodietic.

Index Terms*: *probiotic cultures, immobilization, biopolymers, biotechnology, cottage cheese products, specialized nutrition.

I. INTRODUCTION

World experience testifies to the increased interest of the population of all countries to a healthy lifestyle, including not only fitness and sports, but also “healthy eating”, i.e. systemic use of specialized foods enriched with functional and biologically active ingredients that prevent aging of the human body and contribute to the preservation of its efficiency and creative longevity. The Institute of Statistical Studies and Economics of Knowledge of the Higher School of Studies (National Research University Higher School of Economics) as a result of monitoring has established that food biotechnology is a global technological trend, being the field of research and development aimed at obtaining food raw materials, products, ingredients and auxiliary materials with the necessary properties [1] .

Due to the fact that currently the concept of optimal healthy nutrition is being actively developed, aimed at maximally satisfying the individual needs of the body for energy, plastic, regulatory and other biologically active compounds and food components necessary for the normal flow of physiological processes, maintaining health and prevent the possibility of developing acute and chronic diseases. Accordingly, the demand for the development and production of foods enriched with irreplaceable factors and functional dairy-based food products has sharply increased.

The most significant functional ingredients are probiotic microorganisms, which give dairy products special functional properties, regulate their organoleptic, physico-chemical and structural-mechanical properties. Increasing the viability and preservation of probiotics for delivery in an active state in the human gastrointestinal tract is a pressing issue. It should be noted that since most food technologies are based on biocatalytic methods for the conversion of agricultural raw materials, a promising direction for improving production processes in the food industry and, above all, in the dairy industry is to conduct research and develop innovative technologies for specialized functional preventive products for children, athletes, older persons, as well as for the population suffering from certain diseases. Heterogeneous biocatalysts based on immobilized enzymes or bacterial cells can serve as the basis for creating fundamentally new biotechnological processes alternative to traditional production.

An important problem in the production of probiotic-enriched dairy products is the preservation of their population throughout the shelf life of the dairy product. EvaDudrikova with co-authors (Czechoslovakia) conducted a study of assessment of the survival of Lactobacillusdelbrueckiissp. bulgaricus and Bifidobacteriumanimalisssp. lactis BB-12 in yogurt after their manufacturingduring a shelf life of up to 21 days at a temperature of (4 ± 2) °C. It was found that an increase in the number of lactobacilli and bifidobacteria and their survival during storage depended on the type and strains of associative bacteria-yoghurts (only yogurt lactic acid bacteria were controllable, the experimental ones contained, in addition to yogurt lactic acid bacteria, also Bifidobacteriumanimalisssp.

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Lactis BB-12) and packaging material (glass bottle versus plastic cup) [2].

S.V. Andreeva (Russia) also notes the importance of the question of the survival of probiotic microorganisms that make up the fermented milk products of various trade marks in their progress along the digestive tract, which predetermines the need for microbiological research in this direction [3].

The experiments were conducted on a model, in vitro, simulating digestion in the stomach and intestines of humans. It was established that the survival of probiotics in conditions that simulate digestion did not depend on the brand of fermented milk product. There was a significant decrease in the number of viable lactic and bifidobacteria in the fermented milk products of all the brands studied. Thus, based on the obtained experimental data, it can be assumed that under actual conditions, when using the fermented milk products of the studied brands, as a result of passage through the gastrointestinal tract, a reduced amount of probiotic microorganisms enters the human colon [4, 5].

E.S. Shigina (Russia) with coauthors studied the acid resistance of probiotic strains included in fermented products in order to determine the degree of significance of various survival factors for probiotics, both in fermented products and in gastrointestinal conditions. [6].

A. Das, S. Ray (USA) studied the process of microcapsulation of probiotic bacteria and their use in food biotechnology [7].

Maryam Yari with coauthors (Tehran, Iran) presented the results of a study of the microencapsulation and fermentation process of Lactobacillus acidophilus La-5 and Bifidobacterium BB-12. Immobilization was carried out in calcium alginate by extrusion in the laboratory conditions with a syringe. Sodium alginate was obtained from Sigma Aldrich (USA). Calcium chloride, L-cysteine-HCl, MRS (broth and agar), glucose, yeast extract and other materials were supplied by Merck (Germany). B. animalissubsp.lactis strain BB-12 and L. acidophilus strain La-5 were obtained from the company Christian Hansen (Denmark). The encapsulation process led to the formation of spherical granules containing bacteria (alginate beads) [8].

N.B. Gavrilova, N.L. Chernopolskaya (Russia) presented a scientific substantiation of the relevance of the study of the process of probiotic immobilization [9, 10].

Immobilization of microorganisms, including probiotic ones, is carried out, in contrast to immobilization of enzymes, for their protection, improvement of viability and preservation in aggressive conditions of technological processes, as well as the human gastrointestinal tract. This postulate is to some extent investigated by scientists - biotechnologists, geneticists, physicians, microbiologists, the results of which will be presented later.

P. Rattanapitigorn, P. Raviyan (Thailand) and co-authors studied the degree of viability of Bifidobacterium strains in the process of freeze-drying. Alginate gel (Ki-Yong and Tae-Ryeon, 2000) and tapioca starch (TSB) were used as carriers. The effectiveness of the method was tested in simulated conditions of the gastrointestinal fluid [11].

K. Azuma, T. Osaki, Al - Sisi and other scientists after studying the characteristics of chitosan in dynamics, recommend using it in medicine to improve the quality of life of the population [12, 13, 14, 15, 16, 17, 18, 19].

A.V. Bannikova, together with foreign scientists, investigated the kinetics of the release of proteins and vitamins from multilayer microcapsules containing alginate and carboxymethylcellulose (CMC) undergoing a model process of degradation in the gastric and intestinal juice. The mechanical characteristics of the capsules were also studied, which allows confirming the controlled delivery of model bioactive compounds [20, 21, 22, 23, 24, 25, 26].

In Korea, scientists studied the viability of Bifidobacteriumlongum, strains ATCC 15707 and HLC 3742, immobilized in a CaCO3 carrier — alginate in the form of granules (or beads). The following method of preserving the bacterial suspension was used. Bacterial cells were cultured in medium consisting of 10% skimmed milk, 2% glucose and 1% yeast extract for 20 hours. The resulting suspension was immobilized into a calcium-carbonate-alginate (CaCO3-alginate) system in the form of granules. Studies have shown that the granules contain a large number of viable cells of bifidobacteria: B. longum ATCC 15707 2.80 • 1010 CFU / ml and B. longum HLC 3742 6.28 • 1010 CFU / ml. Then, the granules were stored for 180 days at different temperatures: 4 ° C and minus 20 ° C. At the expiration of the storage period at a temperature of 4 ° C, the granules contained B. longum ATCC 15707 2.24 • 108 CFU / ml and B. longum HLC 3742 2.97 • 108 CFU / ml, and at a minus temperature of 20 ° C - B longum ATCC 15707 1.67 • 109 CFU / ml and B. longum HLC 3742 2.58 • 109 CFU / ml [27].

TulayOzcan with co-authors (Turkey) investigated the survival rate of two probiotic microorganisms Lactobacillus acidophilus La-5 and Bifidobacteriumbifidum BB-12 in rice pudding. The study period was 21 days. The survival rate of L. acidophilus La-5 and B. bifidum BB-12 in immobilized form in rice pudding at the end of the study period (21 days) was 89.44% and 87.40%, respectively [28].

Michael T. Cook with co-authors (United Kingdom) suppose that the main task of micro-encapsulation of probiotics is the delivery to the gastrointestinal tract. The viability of two strains of Bifidobacteria (Bifidobacteriumlongum and Bifidobacteriumbreve) in simulated gastric juice (2% NaCl was adjusted to pH 2 with 1 M HCl) was studied. Cell counting was performed using a plate on Wilkins-Chalgren agar. In this case, Bifidobacteriumbreve was encapsulated in alginate microcapsules and subjected to the action of simulated gastric juice. Cell lysis was studied at the stages of exposure to juice during 0, 30, 60 min. Bifidobacteriumbreve was stained with succinimidyl ether and examined by confocal microscopy.

It has been established that the appearance of damage to the cell membrane and the possible destruction of the cell can be reduced by microencapsulating bacteria in a polymer matrix. It is important that the microencapsulation matrix provides good protection against acid, and that the preparation procedure should be performed gently enough so as not to harm the cell.

The analysis of the above results of modern research allows identifying the main directions of development of food biotechnology, such as:

- the use of membrane methods for processing raw milk for concentrating and isolating the most significant components of raw milk;
- increase the viability of probiotic microorganisms in various milk-based products;
- research and creation of new functional ingredients for specialized food products.

The purpose of the research is to present a scientific and experimental substantiation of the effectiveness of the use of probiotics of biopolymers immobilized in a gel in biotechnology of specialized dairy products.

II. MATERIALS AND METHODS

The following research objects were used in the work:

- associations of cultures Lactobacillus acidophilus, Bifidobacteriumbifidum, Bifidobacteriumlongum, Bifidobacteriumadolescentis, Streptococcus thermophilus; Lactobacillus acidophilus, bi-fidobacteria (B. bifidum, B. longum, B. adolescentis), Streptococcus thermophilus; Debaryomy-ceshansenii, Kluyveromycesmarxianussubsp. marxianus, bifidobacteria (B. longum, B. lactis, B. adolescentis), Lactococcuslactissubsp. cremoris, Lactococcuslactissubsp. lactisStreptococ-custhermophilus; Propionibacteriumfreudenreichiisubsp. shermanii, bifidobacteria (B. lactis, B. bifidum, B. longum and / or B. adolescentis), Streptococcusthermophilus;
- gelatin, pectin, carrageenan biopolymers.

In the process of dissolution, the quantitative ratio of biopolymers was 1: 2, with their total concentration in 20% solution.

For microbiological studies, certified methods of performing measurements were used.

The determination of the number of active cells of the studied cultures was carried out using the following methods:

- limiting dilutions using nutrient media - sterile skimmed milk and agar with hydrolyzed skimmed milk;
- limiting dilutions using the Blourock environment.

A microbiological box with a TENCAN purification system (manufactured in China) was used for research.

The study of the morphology of microorganisms was carried out by the method of microscopy of fixed and fuchsin-stained preparations on an Axioskop 40 microscope (made in Germany) at a magnification of 10 × 63.

Studies were conducted in a special box in the following sequence:

- activation of the biomass of cells of probiotic cultures on sterilized and cooled in skim milk to a temperature of (38 ± 1) ° C, as the optimum temperature of life of monocultures included in the association is (38 ± 1) ° C;
- preparation of a mixture of biopolymers was carried out at a temperature of 20 ° C;
- in the reactor, the association of probiotic cultures in an activated form at a temperature of (33 ± 1) ° C was combined with biopolymer gel, stirred for (15 ± 5) minutes;
- then dosing the mixture into sterile forms was carried out;
- the time for holding the molds under the conditions of a special box is 15-20 minutes. As a result, in the forms thin films (membranes) were formed. Membrane storage temperature is (4 ± 2) ° C.

The experiments were conducted in five replications. The results were processed using the methods of mathematical statistics using standard software packages “MathCAD - 14 Professional”.

III. RESULTS AND DISCUSSION

Pectin and gelatin are particularly effective and are recommended for the immobilization of probiotic cultures of microorganisms, since both biopolymers represent an open pore system with good conditions for gas exchange, the gels of these biopolymers have good diffuse qualities, and are capable of forming structures with optimal size of pores. Gelation occurs at pH = 4.0-4.5 units, which is the determining condition for the viability of probiotic microflora. In addition, latin is a source of glutamic acid and arginine, pectin contains dietary fibers that stimulate the growth of viable cells of bifidobacteria, i.e. possesses properties of prebiotic. To select the quantitative ratio of the studied biopolymers, experimental studies were performed, the results of which are presented in Tables 1 and 2.

Table 2 - Organoleptic indicators of membranes

Appearance	Consistency	Taste and smell	Color
Regular shape, has standard weight and sizes	Elastic, preserving structure	Without taste and smell	White with cream shade

Microbiological studies were performed in the following samples:

- control - the association of probiotic microorganisms in activated form: L. acidophilus: Bifidobacterium: S. thermophilus in a ratio of 1: 1: 1;
- experiment - the association of probiotic microorganisms in immobilized form (membrane).

Table 2 - The number of viable cells of probiotic microorganisms in membranes

Variant	Total amount of lactic acid microorganisms, CFU / cm^3	Number of bifidobacteria, CFU / cm^3	Number of acidophilus bacterium, CFU / cm^3
Control	$9{,}2 \cdot 10^{10}$	$8{,}6 \cdot 10^{9}$	$8{,}9 \cdot 10^{10}$
Experiment 1	$5{,}2 \cdot 10^{10}$	$2{,}0 \cdot 10^{9}$	$1{,}7 \cdot 10^{10}$

Analyzing the data presented in table 2, one can say that the degree of concentration of viable cells of probiotic microorganisms in the studied membranes is within the established requirements. The logarithm of the number of viable cells of immobilized microorganisms in the gel of biopolymers is presented in Figure 1.

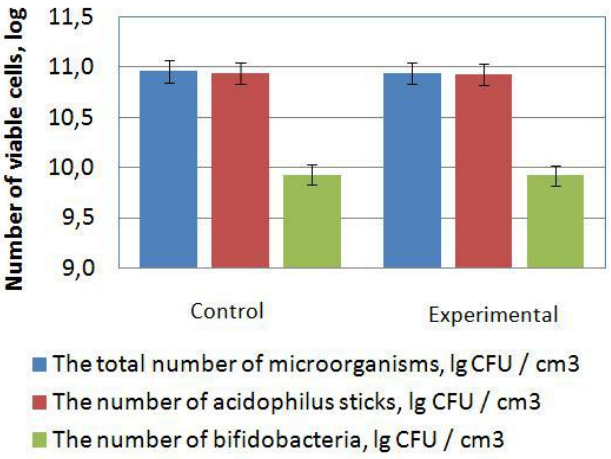

Figure 1: Logarithm of the number of viable cells of microorganisms immobilized in gel biopolymers gelatin - pectin

Thus, the analysis of the presented data indicates a slight decrease in the degree of concentration of viable cells of microorganisms during immobilization compared with the control - the biomass of microorganisms in the activated form.

In addition to microbiological indicators, the degree of stability of the association of probiotic cultures in immobilized form (experiment) with respect to the control sample and the alkaline reaction of the medium (pH unit) was determined. The following data were obtained: control sample 8.3-9.6; test sample 8.3-9.2. It was hypothetically suggested that the test sample is more resistant to the aggressive conditions of the gastrointestinal tract due to the combination of components - proteins and polysaccharides concentrated in the cell walls of microorganisms containing pectins, which are complementary to the corresponding receptors located on the membranes of epithelial cells. Pectins, in this case, are adhesion mediators due to their protein or glycoprotein nature. Thus, they contribute to an increase in the number of viable cells of the association of probiotic cultures.

Another aspect of the research was the preservation of viable cells in the experimental association of probiotic cultures in the stomach, where the number of microbes is insignificant due to its acidic environment, and in the intestine, in which the growth of microorganisms creates difficulties due to the presence of aggressive digestive enzymes.

To clarify the quality of the above properties, experimental studies (Figure 2) of the viability of cells of probiotic cultures entering the GI tract in the form of micromembranes were carried out.

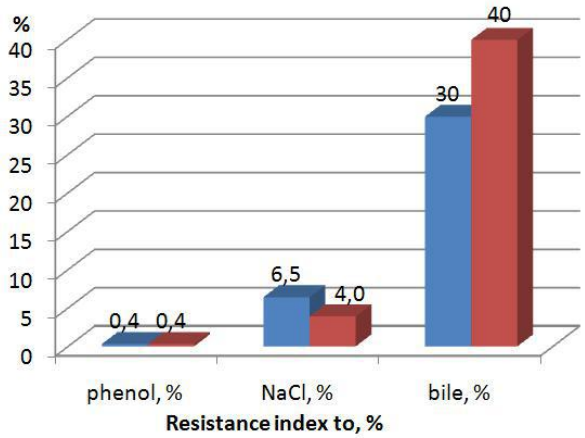

Figure 2: The value of indicators characterizing viability of probiotic cultures in the gastrointestinal tract

As a result, it was found that the immobilized microorganisms showed resistance to the tested concentrations of the test substances, which can be considered as an indirect indicator of their better ability to survive in the human gastrointestinal tract. Based on the results of the study of the immobilization of the association of L. acidophilus, Bifidobacterium, S. thermophilus, it can be recommended for use in biotechnology of fermented (sour milk) products, which by the nature of fermentation of milk sugar belong to the first group, based on the production of lactic fermentation with the formation of lactic acid.

During the development of biotechnology of new cottage cheese products for specialized nutrition, the process of fermentation of the milk base was studied using the developed biocomponent (immobilized starter cultures) and the technological parameters were established: the amount - 0.05% by weight of the normalized mixture, fermentation time - (4.5 ± 0.5) h, temperature - (38 ± 2) °C, titratable acidity - (75.0 ± 5.0) ° T, pH - (4.45 ± 0.05). To increase the concentration of dry substances, including protein, the fermented mixture was sent to the "TetraAlcross UC" ultrafiltration unit with the following parameters: concentration ratio 3.4-3.5, ultrafiltration temperature (48 ± 2) °C, mass fraction of dry substances in retentate (17.71 ± 0.22)%, including proteins (11.36 ± 0.14)%. General technological operations for the production of cottage cheese pudding and cottage cheese bioproduct are presented in Figure 3. Production of pudding is different in that the stabilizer "Stabisol JTL" is used to form its consistency (according to the current regulatory documentation). The technology of cottage cheese cream is characterized by the fact that in the process of normalization a vegetable ingredient is introduced into milk - mustard oil for correcting the fatty acid composition of the product and for forming a delicate consistency by beating the cottage cheese mass before packaging it. In order to improve the taste and expand the product rangethere has been studied the possibility of enriching berry syrups with biologically active substances, in particular from honeysuckle and cranberry berries [29]. Characteristics of cottage cheese products are presented in tables 3, 4, 5.

The novelty of technological solutions is reflected in the patent of the Russian Federation No. 2543153 [30].

Table 2 - Organoleptic indicators of cottage cheese products

Indicator	Cottage cheese pudding	Cottage cheese bioproduct	Cottage cheese cream
Appearance, consistency	Soft, spreadable		Fine texture
Taste and flavor	Clean flavor, fermented milk taste. Moderately sweet, with a taste and smell of filler		Clean flavor, fermented milk taste with a touch of plant filler
Color	Due to the color of the filler, uniform throughout the mass		Creamy

Table 4 – Physical and chemical indicators of cottage cheese products

Indicator	Cottage cheese pudding	Cottage cheese bioproduct	Cottage cheese cream
Dry solids weight ratio, %	28.5±1.0	33.0±1.0	28.5±1.0
including:			
- fat	1.8±0.5	3.0±0.5	5.0±0.5
- protein	18.3±0.5	18.3±0.2	18.3±0.5
- carbohydrate	10.2±0.5	11.7±0.2	5.2±0.2
Active acidity, pH	4.25±0.05	4.26±0.05	4.25±0.05

Table 5 – Microbiological indicators of cottage cheese products

Indicator	Cottage cheese pudding	Cottage cheese bioproduct	Cottage cheese cream
Escherichia coli group bacteria – in 0.01 g	Not allowed		Not allowed
Pathogens, including salmonella in 25 g of product	Not allowed		Not allowed
Staphylococcus S. aureus in 0.1 g of product	Not allowed		Not allowed
Lactic acid microorganisms. CFU/g, not less	$1 \cdot 10^8$		$1 \cdot 10^8$
Including bifidobacteria. CFU/g, not less	$1 \cdot 10^6$		$1 \cdot 10^7$
Propionic acid bacteria, CFU/g, not less	$1 \cdot 10^7$	-	$1 \cdot 10^7$

In the development of soft cottage cheese biotechnology with the use of functional ingredients: probiotic cultures and dietary fiber, which makes it possible to attribute it to functional food products and recommend for healthy nutrition of the population of different age groups.

Separate method was chosen as the main method of cottage cheese production. Acid coagulation method was chosen. The peculiarity of the preparation of dairy raw materials (skimmed milk) is high-temperature pasteurization of 92-95 · C with an aging (soaking) during 4 hours. The coagulation process of skimmed milk was studied using the DVS culture of CHN-22, which contains a mixture of multiple strains of Lactococcuslactissubsp. cremoris, Lactococcuslactissubsp. lactis, Leuconostocmesenteroidessubsp. cremoris and Lactococcuslactissubsp. diacetylactis. Quality indicators of skimmed milk and whey were defined. For normalization of low-fat cottage cheese and enriching it with functional ingredients, cream with fat content 20% was pasteurized at a temperature of (92 ± 2) ° C, homogenized at a pressure of 10-15 MPa, cooled to the temperature of innoculation of the starter.

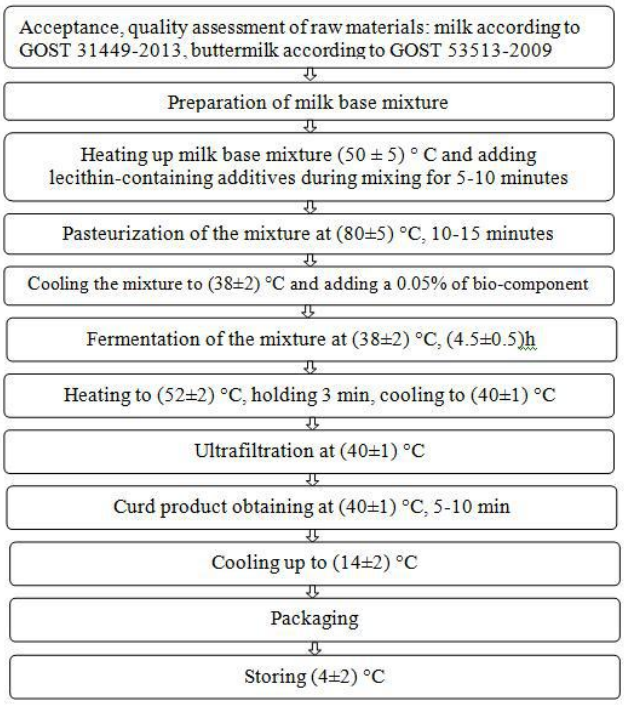

Figure 3: The technological process of production of cottage cheese products

The composition of the starter includes the association of probiotic cultures Propioni bacterium freudenreichiisubsp. shermanii, bifidobacteria (B. lactis, B. bifidum, B. longum and / or B. ado-lescentis), Streptococcus thermophilus immobilized in biopolymergelatin gels - carrageenan.

The starter was inoculated in activated form. The amount of dietary fibers “Citri-Fi” 1.5-2.0% by weight of cream with fat content 20 % was experimentally chosen. Then fat-free cottage cheese was mixed with fermented cream, enriched with probiotic cultures and dietary fiber, and packaged. The technological process of producing soft cottage cheese is presented in Figure 4 [31, 32].

Organoleptic characteristics of the new product are shown in Table 6.

Table 6 - Organoleptic indicators of soft cottage cheese

Name of the indicator	Characteristic
Appearance and consistency	Gentle, soft curd mass, slightly smearing consistency with the presence of light graininess
Taste and flavor	Pure taste, of fermented milk, with a strong pasteurization flavor
Colour	Color is light cream, cream, uniform throughout the mass

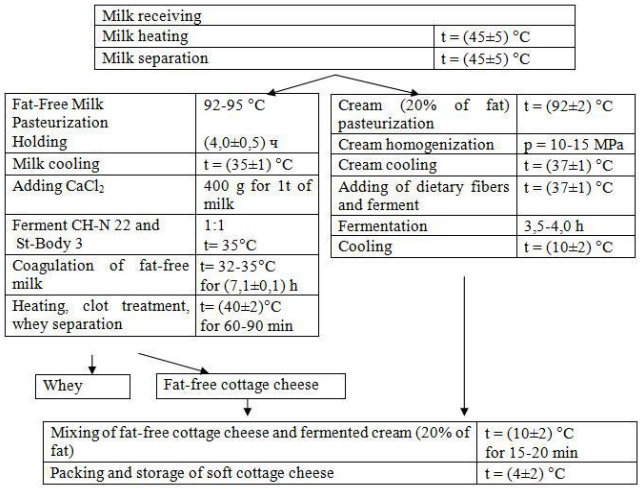

Figure 4: The technological process of production of soft cottage cheese

Physico-chemical and microbiological indicators of soft cottage cheese are characterized by the following data presented in tables 7, 8.

Table 7 - Physical and chemical indicators of soft cottage cheese

Indicator	Value
Fat, %, not less	5,0
Moisture, %, no more	68,0
Protein, %, not less	21,0
Acidity, °T, no more	175-230
Phosphatase	absent

Table 8 - Microbiological indicators of soft cottage cheese

Indicator	Value
Escherichia coli group bacteria – in 0.01 g	Not detected
Staphylococcus S. aureus in 0.1 g of product	0,01
Pathogens, including salmonella in 25 g of product	Not detected
Yeast, CFU/ g	Not detected
Mold, CFU/g	Not detected
Probiotic microorganisms:	
Bifidobacteria, CFU/g, not less	$1 \cdot 10^6$
lactic, CFU/g, not less	$1 \cdot 10^7$

IV. CONCLUSION

Experimental studies and their analysis allow drawing the following conclusions:

- the use of functional biocomponent (membranes) for the production of new cottage cheese products provides a high level of probiotic microflora cells in the finished product. Immobilization of microorganisms helps to preserve the number of viable cells at all stages of the production of the product and to overcome the acid barrier of the human gastrointestinal tract;
- production of cottage cheese and cottage cheese products using ultrafiltration increases the nutritional and biological value due to the concentration of whey proteins, increases the yield of the product. The systematic use of such products contributes to the maintenance of physiological functions that enhance human health and its resistance to the effects of external environmental factors.

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