Досліджено кількість життєздатних клітин L. acidophilus пробіотичного штаму La-5 за використання її самостійно і у поєднанні із мезофільною культурою Flora Danica при зберіганні кисловершкового масла, використовуючи різні температурні режими ферментації вершків і різні технології. Встановлено, що в обидва сезони року найкращі пробіотичні властивості кисловершкового масла проявляються при поєднанні Flora Danica + L. acidophilus La-5 за температури ферментації вершків (30±1) °С

Ключові слова: кисловершкове масло, Flora Danica, L. acidophilus La-5, життєздатні клітини, пробіотичні властивості

Исследовано количество жизнеспособных клеток L. acidophilus пробиотического штамма La-5 при использовании ее самостоятельно и при сочетании со мезофильной культурой Flora Danica при хранении кислосливочного масла, используя различные температурные режимы ферментации сливок и различные технологии. Установлено, что в оба сезона года лучшие пробиотические свойства кислосливочного масла проявляются при сочетании Flora Danica + L. acidophilus La-5 при температуре ферментации сливок (30±1) °С

Ключевые слова: кислосливочное масло, Flora Danica, L. acidophilus La-5, жизнеспособные клетки, пробиотические свойства

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RESEARCH INTO PROBIOTIC PROPERTIES OF CULTURED BUTTER DURING STORING

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1. Introduction

Among a wide range of cultured milk foods, special attention should be paid to products whose manufacturing employs the use of physiologically active natural symbiotic microflora [1]. Production of such foodstuffs is based on the improvement of technology and application of microorganisms that exhibit probiotic properties [2].

Prominent place in the modern trends in food industry occupies product naturalness [3]. Providing the naturalness of a product and rendering it functional properties can be achieved through the use of probiotic microbial cultures. They create special relations with the macro-organism, which improves human health [4]. Scientific approaches to the improvement of the human organism, which are based on the massive use of dairy products with probiotic properties is a new promising area in medicine. These issues have become the strategy of many foreign researchers and companies, which will greatly improve the health status of the population [5]. According to data from Japanese researchers, the use of lactic acid bacteria in functional foods would reduce the existing market of chemical medicines by half [6].

Cultured butter is very popular product in the European countries, in contrast to Ukraine. The cause of low demand for cultured butter in Ukraine is not only differences in the tastes of consumers, but also certain controversy regarding the peculiarities of production technology, but the inadaptability of technological modes to differences in the composition and properties of Ukrainian raw materials [7]. This in turn has caused a revival of interest in the technology of cultured butter. It is possible to extend the range and motivate increased interest of consumers through additional valuable properties of the butter by using probiotic cultures in the production of its cultured types.

Cream is a special medium for the cultivation of lactic acid bacteria. Formation of microbial composition for the production of cultured butter is an important issue, because its taste and flavor is formed exclusively due to the activity of bacteria [8]. As far as the possibility of employing the probiotic cultures into the fermentation of cream in the production of cultured butter is concerned, there are limited data in the scientific literature. This issue requires special attention from the point of view of temperature mode selection and combination of processes of biological and physical maturing of cream. For the fermented cultured milk products with probiotic properties, the key feature is the viability of probiotic cultures and preserving them in the amount required to provide functional properties in a product [9]. Therefore, determining the conditions under which the probiotic cultures retain viability, simultaneously with the formation of excellent organoleptic properties and standard physical-chemical parameters, is a relevant task.

2. Literature review and problem statement

One of the outstanding achievements of the early 21st century is the development of the concept "Probiotics and functional nutrition" followed by the start of its practical implementation [10]. An important constituent of the market for products of functional purpose is dairy products, which are in Ukraine and the European countries make up 67 % of their total volume. Larger than 80 % of the market for dairy products of functional purpose is represented by products from and/or prebiotics, 8 % by products with biologically active substances, about 12 % are other food products [1].

The role of functional foods is growing around the world. There is a large amount of information regarding the necessity of special diets for the prevention and treatment of certain diseases, which allows the food industry to develop new functional food products [6].

A significant contribution to the development of theoretical and practical bases for the production of cultured butter is the result of work of many scientists [11, 12]. However, the technology of cultured butter with the probiotic properties is not developed and it requires the scientific substantiation.

When selecting cultures for the formulation of souring compositions for fermented milk products, one considers biochemical, microbiological and functional-technological indicators of strains. For this purpose, they select strains with antagonistic activity against conditionally pathogenic and pathogenic microflora, resistant to bacteriophages. Special attention is paid to the compatibility of strains during combined cultivation [13].

The main group of microorganisms that are used in the composition of modern probiotic preparations and products are bacteria of the genera *Lactobacillus* and *Bifidobacterium*. This is due to the fact that they are constantly present in the composition of human standard biocenose and play a big role in the functioning of microecological system of healthy people.

Lactobacillus acidophilus is called a classical probiotic as it is the main representative of the microflora of the intestine and performs regulatory functions within the population of intestinal bacteria. Many of its strains possess a pronounced virucidal action relative to the human immunodeficiency virus through the production of highly active hydrogen peroxide. Representatives of *L. acidophilus* are also exploited as antioxidants and stimulants to develop other lactobacilli. These microorganisms demonstrate antitumor and immunomodulation effect [14].

In different countries of the world, *L. acidophilus* is introduced to the monocultures, or in combination with various types of lactic acid bacteria in the composition of cultured milk products. [15] proved the possibility and feasibility of combined cultivation of mesophilic lactic acid lactococci and acidophilus bacillus, which enables obtaining a product with fairly high concentration of viable cells of the both groups of microorganisms.

Cultured butter is the butter characterized by a rich, aromatic bouquet due to lactic acid and aromatic substances (diacetyl and volatile organic acids) [16]. The presence of these metabolites of lactic acid bacteria in the cultured butter is essential for improving its functional value compared with other types of butter, as well as to increase its shelf life. Lactic acid and diacetyl exhibit antibacterial action against extraneous microflora – they inhibit the development of putrefactive bacteria, the growth of *Escherichia coli, Staphylococcus aureus, Salmonella*. At the same time, cultured butter is a source of a number of nutrients due to the high content of milk fat [17].

To produce cultured butter, souring cultures of specially selected type of composition, mostly of mesophilicof lactic acid bacteria, are employed, such *L. lactis, L. cremoris, L. diacetilactis, Leuconostoc*. Such cultures are capable of forming a considerable amount of aromatic substances and they are moderate acid-forming agents.

Given the organoleptic indicators of cultured butter, key criteria for the estimation of prospects of using the strain is its production of lactic acid and active synthesis of taste-aromatic compounds [16].

At present, cultured butter is made in two ways – conversion of highly fat cream and cream whipping using butter makers of periodic and continuous action. A standard for cultured butter is the butter of classic composition, manufactured by the method of whipping the cream, which was preliminary exposed to the fermentation by introducing a souring composition under certain temperature conditions. Favorable conditions are thus created for the formation of characteristic taste bouquet that combines distinct creamy and fermented milk taste and flavor [18].

In order to ensure a good consistency of butter, it is recommended to use differentiated modes of physical maturing and biological fermentation of cream depending on the season and chemical composition of milk fat at medium temperatures. Differentiated multistage temperature regimes contribute to the strengthening of butter structure in spring and summer and to the reduction of mechanical strength in autumn and winter. These are the Alnarp and Danish modes of maturing [16].

The scientific literature data on the cultured butter as a functional product are very limited. We could not find in the scientific literature a description of using the starting cultures of direct application for the souring of cream in the production of cultured butter. Studying this process is important because cream is a specific medium for the growth and development of lactic acid bacteria [19].

It is also required to examine in detail the process of combined cultivation of mixed mesophilic cultures *Flora Danica* and thermophilic *Lactobacillus acidophilus* in the production of cultured butter.

3. Research goal and objectives

The goal of present work is to examine probiotic properties of cultured butter during storing with the application of mesophilic cultures made by firm Chr. Hansen, Denmark – *Flora Danica* (*FD*) and probiotic monoculture *L. acidophilus La-5* (*La-5*).

To accomplish the set goal, the following tasks have to be solved:

– to establish a possibility of combining *FD* and *La-5* for the fermentation of cream;

– to determine the effect of different technological parameters in the production of cultured butter on microbiological indicators in the finished product;

– to analyze preservation of probiotic properties of cultured butter during storing;

– to provide recommendations on the scientific substantiation of technologies for cultured butter with probiotic properties.

4. Materials and methods for examining the preservation of probiotic properties of cultured butter during storing

Research was conducted in the laboratory of the Department of Technology of Milk and Dairy Products and in the laboratory of TsSK FUD Enrichment-Ukraine. Dairy raw materials were collected at PrAT "Galychyna" (Radekhiv, Lviv oblast, Ukraine), which were exposed to the separation at temperature 40–45 °C. The obtained cream with fat mass fraction of 32–33 % was pasteurized at temperature 95 °C without curing, after pasteurization the cream was cooled to the temperature of fermentation. Cultured butter was made by whipping the cream in a butter maker of continuous action.

The amount of viable cells of *FD* and *La-5* was analyzed in the finished product on the first day and during storing on day 7, 14, 21, 28, 35 and 42.

The methodology of conducting the given research is described in detail in article [20].

5. Results of research into the preservation of probiotic properties of cultured butter during storing

5. 1. A change in the organoleptic indicators when storing the samples of cultured butter

The character and intensity of microbiological processes during storing, which depend on the composition of the sour and preservation temperature, affect the organoleptic indicators of the finished product at storing. When storing butter, especially at elevated temperatures, there may appear flavor caused by the products of decomposition of butter components. Biochemical transformations in cultured butter take place with the participation of cultures of the souring compositions, whereas in sweet cream butter the changes mainly occur as a result of life activity of residual microflora.

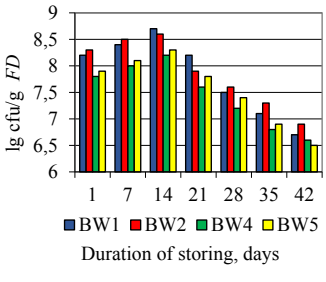
In the spring-summer and autumn-winter seasons, experimental samples BW (Butter Winter)1, BW2 and BS (Butter Summer)1, BS2 maintained over 35 days a pure, pronounced pleasant fermented milk taste and flavor. These samples exhibited the highest number of points; they also had the best physical appearance due to saturated yellow color. The rest of the samples as early as on day 26 of storing had insufficiently pure taste and flavor with a pronounced sour aftertaste. All experimental samples of cultured butter after 35 days of storing were characterized by sour taste and flavor. Color of the samples of butter varied from light yellow to yellow, uniform over the entire mass. Consistency of the butter samples during storing was homogeneous, elastic, thick, the butter surface on the cut was slightly shiny and dry in appearance with the presence of the tiniest drops of moisture.

5. 2. A change in the microbiological indicators of the samples of cultured butter, produced in the autumn-winter season

When storing the cultured butter, we determined at intervals of 7 days the amount of viable cells of *FD* and *La-5* per 1 gram of product.

Despite the temperature conditions of storing, lactic acid lactococci in all samples continued, albeit slowly, to develop (Fig. 1). At the beginning of storing, the amount of viable cells of *FD* was 7.8–8.3 lg cfu/g. Over 14 days of storing, their number increased to 8.1–8.7 lg cfu/g, which is caused by an increase in the biomass of *La-5*. In the experimental samples BW1 and BW2, over 14 days of storing, the amount of viable cells of *FD* was 9.4–9.5 % larger than in the samples BW4 and BW5. In the samples BW7, BW8, BW10 and BW11, the number of viable cells of mesophilic lactococci was within the limit of 8.1–8.5 lg cfu/g on day 14 day of storing. However, over the subsequent period of storing, we observed a gradual decrease in the amount of viable cells of *FD* to the level of 6.5–6.9 lg cfu/g (on day 42). In particular, significant cultures' dying off was observed for the samples BW4, BW5 and BW7 to the level of 6.5–6.6 lg cfu/g.

The best viability over 42 days of storing was demonstrated by the samples BW1 and BW2, in the production of which we exploited optimal conditions for the fermentation of cream for the mixed mesophilic cultures *Flora Danica* – (30±1) °C.



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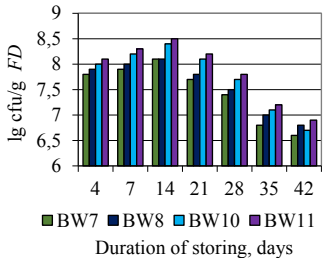

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Fig. 1. A change in the number of viable cells of *FD* when storing the cultured butter at temperature 0...– 5 °C in the autumn-winter period: *a* – groups I and II; *b* – groups III and IV

To determine the probiotic properties of cultured butter when storing, we explored a change in the number of viable cells of *La-5*. Similarly to the change in the amount of viable cells of *FD* over 14 days of storing, the number of *La-5* increased (Fig. 2), they showed resistance to acidic environment during storing. The number of viable cells of *La-5* increased over 14 days of storing in the sample BW2, fermented at temperature (30±1) °C, from 8.2 to 8.9 lg cfu/g. After 14 days, we observed a decrease in the amount of cells and on day 42 day it was 6.9 lg cfu/g. Such amount does not enable probiotic properties in the product, so it is advisable to store it no longer than 35 days at temperature 0...–5 °C. On day 35 of storing, the number of viable cells of *La-5* in the indicated sample amounted to 7.5 lg cfu/g. An analysis of dynamics of changes in the number of viable cells of *La-5* in the rest of the samples shows its similarity. In the samples BW3, BW5-BW6, the cells of *La-5* actively developed: from the beginning of storing up to day 14, their number increased from 7.8–8.1 lg cfu/g to 8.4–8.6 lg cfu/g. After day 14 of storing, the amount of cells of *La-5* in these samples decreased and made up 7.1–7.3 lg cfu/g on day 35

and 6.5–6.6 lg cfu/g – on day 42 of storing. In the samples BW8–BW9, the number of viable cells of *La-5* at the beginning of storing amounted to 7.6–7.8 lg cfu/g. Over the next 14 days, the number of cells increased slightly and amounted to 8.1–8.2 lg cfu/g. After 14 days, we noted a sharp dying off and, on day 35, their number decreased to 7.1–7.2 lg cfu/g. In the samples BW11– BW12, at original concentration of culture of *La-5* in butter grain $1{\cdot}10^8$ cfu/cm^3, after 4 days of storing, the quantity of cells amounted to 7.5–7.6 lg cfu/g. Such number of viable cells of *La-5* corresponds to low acidity of the plasma. In the process of storing over the next 10 days, the number of *La-5* slightly increased and amounted to 8.1–8.2 lg cfu/g. Following that, we noted a sharp decrease in cells to 6.8–6.9 lg cfu/g on day 35. The number of viable cells of *L. acidophilus La-5* does not provide the product with probiotic properties.

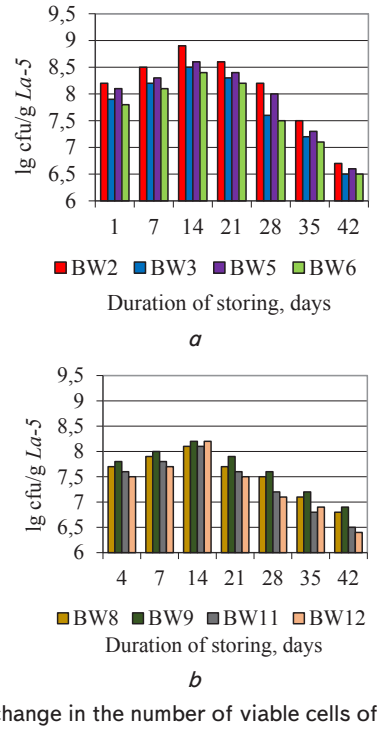


Fig. 2. A change in the number of viable cells of *La-5* when storing the cultured butter at temperature 0...– 5 °C in the autumn-winter period: *a* – groups I and II; *b* – groups III and IV

By summing up, it can be noted that the sample BW2 over 35 days of storing demonstrated a large amount of viable cells of both cultures – *FD* and *La-5*. Thus, the souring composition composed of *FD* and *La-5* at temperature of the cream fermentation (30±1) °C enables obtaining the cultured butter with high probiotic properties.

5. 3. A change in the microbiological indicators of the samples of cultured butter, produced during spring-summer season

During spring-summer season, at the beginning of storing, the number of viable cells of *FD* was 8.2 and 8.3 lg cfu/g for BS1 and BS2, respectively. While storing at temperature 0...–5 °C, in the samples of butter on day 14 we observed the maximal amount of lactic acid lactococci – 8.7 and 8.6 lg cfu/g for BS1 and BS2, respectively. Next, the number of viable cells of *FD* in the interval of 14–42 days of storing decreased to 6.9 and 6.7 lg cfu/g (Fig. 3).

In the course of fermentation of cream by the souring culture *FD* at temperature (37±1) °C, the number of cells in the samples BS4 and BS5 at the beginning of storing was 7.9–8.0 lg cfu/g. Over the first 14 days of storing, their number increased to 8.3–8.4 lg cfu/g. At low temperatures enzymes are inactivated, in addition, viscosity of cytoplasm changes, as well as the properties of protein-lipid membranes that leads eventually to the irreversible processes in the cell and its destruction. After 14 days of storing, cells of *FD* in the samples BS4 and BS5 died. On day 21, 28, 35 and 42 of storing, their number made up 7.9–8.1, 7.4–7.5, 7.0–7.1 and 6.4–6.5 lg cfu/g, respectively.

The amount of viable cells of *FD* in the samples BS7 and BS8 at the beginning of storing was 7.8–7.9 lg cfu/g. Such a low number of viable cells correlate with low titrated acidity of the butter plasma. Over 14 days of storing at temperature 0...–5 °C, their number increased to 8.1–8.2 lg cfu/g, over the next 28 days of storing their number decreased to 6.5–6.7 lg cfu/g.

The concentration of viable cells of *FD* in the samples BS10 and BS11 after 4 days of storing was 7.4–7.6 lg cfu/g, which led to low acidity of the plasma. On day 14 of storing, their number in these samples increased slightly by 5.1–5.3 %, after which they passed to the stage of dying, and on day 42, 17.5–17.9 % were lost for BS10 and BS11, respectively (Fig. 3, *b*).

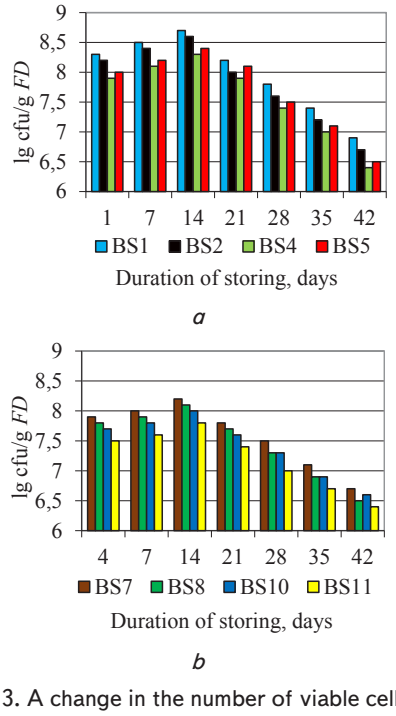


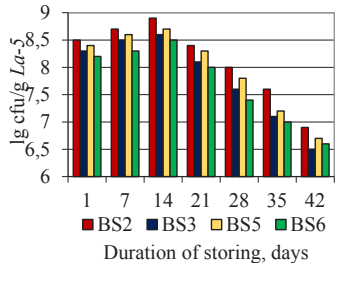
Fig. 3. A change in the number of viable cells of *FD* when storing the cultured butter at temperature 0...–5 °C during spring-summer season: *a* – groups I and II; *b* – groups III and IV

The most stable when storing were the cells of *La-5* (Fig. 4, *a*). The phase of active growth in the cultured butter for *La-5* lasted 14 days. During this period, in the sample BS2, the number of viable cells increased from 8.5 to 8.9 lg cfu/g. Next, the amount of cells gradually died and on day 42 decreased to 6.9 lg cfu/g. Such number of viable cells does not make it possible to relate the product to the probiotic ones, which is why it is advisable to store it no longer than 35 days at a temperature of 0...–5 °C. On day 35 of storing, the number of cells in BS2 amounted to 7.6 lg cfu/g.

Over 35 days of storing, the sample BS2 demonstrated high concentration of the cultures *FD* and *La-5*. When using *La-5* separately at temperature of fermentation (30±1) °C, the number of viable cells at the beginning of storing was to 8.3 lg cfu/g. Their number after 42 days of storing at a temperature of 0...– 5 °C was 6.6 lg cfu/g.

When souring the cream at temperature (37±1) °C, in the samples BS5 and BS6, over 14 days, we observed a growth in the number of cells of *La-5* by 3.6–3.7 %, after which they began to die. In the samples BS8 and BS9, the viable cells of *La-5* actively developed over 14 days, similar to the previous samples. Starting from day 1 to day 14 of storing, their number increased from 7.6–7.8 to 8.1–8.3 lg cfu/g. After 14 days, a small amount of them died and on day 35 made up 6.8–7, on day 42 – 6.3–6.5 lg cfu/g.

In the samples BS11 and BS12 at the original concentration of culture of *La-5* in a butter grain of $1{\cdot}10^8$ cfu/cm^3, after 4 days of storing, their number was 7.4–7.5 lg cfu/g (Fig. 4, *b*). This number of cells correlates with low acidity of the plasma. Over the next 10 days, their number grew slightly and reached 7.6–7.8 lg cfu/g. After this, we noted a sharp dying of cells; on day 35, their number was 6.5–6.8 lg cfu/g, which is not sufficient for the probiotic properties.



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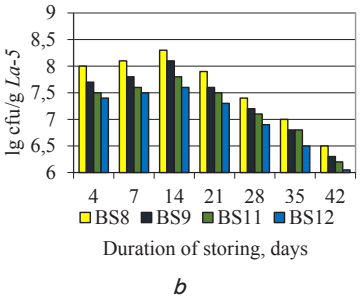

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Fig. 4. A change in the number of viable cells of *La-5* when storing the cultured butter at temperature 0...– 5 °C during spring and summer: *a* – groups I and II; *b* – groups III and IV

Therefore, given the need to ensure high probiotic status of the cultured butter, it is advisable to store it no longer than 35 days at a temperature of 0...–5 °C. The cultured butter that was made using the souring composition *FD* and *La-5* at the fermentation temperature (30±1) °C was characterized by a large amount of representatives of both cultures.

6. Discussion of results concerning the impact of starting cultures and technologies on the probiotic properties of cultured butter

Results confirm that the microflora of the starting culture *FD* blends well with *La-5*. It is important to note that at combined cultivation the cell survival of *La-5* is larger, indicating their synergy. This tendency is registered for all samples exposed to combined cultivation, except for the Alnarp technology in autumn–winter. This technology implies the use of a stepwise mode of biological and physical maturing (8±1) °C→(20±1) °C→(12±1) °C; fermentation takes place at a temperature of (20±1) °C after the stage of physical maturing. It is obvious that such a regime, especially at the first stage, is unfavorable for thermophilic acidophilic bacillus at combined cultivation. In summer, when using a stepwise mode of the combination of physical and biological maturing: (20±1) °C→(6±1) °C→(10±1) °C, the combined cultivation enhances the survival of *La-5*.

While analyzing different temperature modes in the fermentation of cream, it is worth noting the possibility of compromised temperature for mesophilic lactococci and thermophilic acidophilic bacillus – (30±1) °C. Under such conditions, high activity of *Leuconostoc mesenteroides ssp. cremoris* and *Lactococcus lactis ssp. diacetylactis is achieved*, which is indicated by the accumulation of aromatic compounds [18]. In addition, at the fermentation temperature of cream (30±1) °C, we noted a high level of survival of acidophilic bacillus. Such pattern is typical for both spring–summer and autumn–winter periods.

A signature of the technology is the combination of lyophilized starting cultures of direct application *FD* + *La-5* in the ratio of 1:1. We determined their original concentration in cream – $1{\cdot}10^6$ cfu/cm^3. Such concentration provides the required amount of probiotic culture for providing the product with functional properties.

It is necessary to take into account the fact that until day 14 of storing the cultured butter, the number of viable cells grows, that is, its probiotic properties strengthen. However, the shelf life is limited to only 35 days because after the specified time the number of cells is dramatically reduced. This pattern is characteristic for all the variants.

Regarding the features of summer and winter periods, then in summer the fermentation of cream is more active, indicated by a larger number of cells of both microbial cultures, which is quite natural due to the higher content of growth factors in milk of summer period. In spring and summer, the number of viable cells of *La-5* in the finished product in the sample BS2 was 10.4 % larger compared to that over autumn-winter.

Based on the experimental research, we developed a technology of cultured butter with the probiotic properties. We determined the basic technological parameters of fermentation, physical maturing of cream and the duration of storing. Compliance with all technological parameters makes it possible to manufacture a product with functional properties in both periods of the year.

7. Conclusions

1. We established a possibility of the combination of mixed mesophilic cultures *Flora Danica* with thermophilic monoculture Lactobacillus *acidophilus La-5* at fermentation of cream in the technology of cultured butter with the probiotic properties. The dose of inoculation and rational ratio between *FD* and *La-5* was experimentally determined in the formulation of a souring composition of direct application – 1:1 at original concentration of each culture in cream of $1{\cdot}10^6$ cfu/cm^3.

2. We determined optimal temperature for the fermentation of cream (30±1) °C at the combination of starting cultures *FD*

and *La-5* for the largest number of viable cells of La-5 in the finished product.

3. It was found that the number of viable cells of *La-5* on day 35 of storing for all the samples of cultured butter was over $1 \cdot 10^7$ cfu/cm^3. The largest amount of viable cells of *La-5* was registered for the sample, fermented at temperature (30±1) °C when *FD* and *La-5* were combined. The duration of storing the cultured butter with the probiotic properties is 35 days at a temperature of 0...–5 °C.

4. It is proposed to use in the technology of cultured butter a souring composition, composed of the mixed mesophilic cultures *FD* and thermophilic monoculture *La-5*. We determined the temperature of cream fermentation when the souring cultures *FD* and *La-5* are combined – (30±1) °C.

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