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## STARTER CULTURES COMPOSITIONS WITH PROBIOTICS FOR FERMENTED MILK PRODUCTS AND COSMETICS

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**Abstract.** The expediency of optimization of starter cultures composition of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium bifidum* BB 01 + *Bifidobacterium longum* BL 01 + *Bifidobacterium breve* BR 01 for the manufacture of fermented milk products and cosmetics for teenagers and people under the age of 40-45 years with probiotics has been substantiated. The value of titratable acidity, number of viable cells of bifidobacteria has been determined, as well as the most probable number of lactobacterium in fermented probiotic clots obtained with different ratios of mixed cultures *Lactococcus sp.* and mixed cultures of *Bifidobacterium sp.* in starter cultures compositions (the initial concentration of the mixed cultures (MC) *Lactococcus sp.* and MC *Bifidobacterium sp.* varied within the range of  $1 \times 10^5 - 1 \times 10^6$  CFU/cm<sup>3</sup> of the inoculated milk, enriched with fructose as a growth factor of bifidobacteria). The high content of probiotics and the lowest values of titratable acidity are typical of the fermented milk clots obtained using starter cultures composition with a ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* 1 : 10. The maximum number of lactococci viable cells is observed in clots obtained using starter cultures composition with the initial ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* 10 : 1. The optimum ratio of MC *Lactococcus sp.* and MC *Bifidobacterium sp.* – 1 : 10 has been established (initial concentration of the cultures at inoculation –  $1 \times 10^5$  and  $1 \times 10^6$  CFU/cm<sup>3</sup>, respectively) for the production of fermented milk products and cosmetics with probiotics, where the maximum value of the quality aggregated factor – 7, 12 is noted. It is shown that a fermented probiotic milk clots obtained using starter cultures composition with an optimum ratio of cultures of lacto- and bifidobacteria (1 : 10) have good sensory characteristics, contain a high number of viable cells of bifidobacteria and lactobacteria –  $(9,15 \pm 0,14) \times 10^9$  and  $(8,50 \pm 0,50) \times 10^8$  CFU/cm<sup>3</sup>, respectively, and a low level of titratable acidity at a specified value of the active acidity and are safe in terms of microbiological characteristics.

**Key words:** milk product, cosmetics, probiotic, *Lactococcus sp.*, *Bifidobacterium sp.*, acidity, quality aggregated index, optimization, surface response.

## ЗАКВАШУВАЛЬНА КОМПОЗИЦІЯ З ПРОБІОТИКАМИ ДЛЯ КИСЛОМОЛОЧНИХ ПРОДУКТІВ ТА КОСМЕТИКИ

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**Анотація.** Обґрунтовано доцільність оптимізації складу заквашувальної композиції зі змішаних культур *Lactococcus sp.* та змішаних культур *Bifidobacterium bifidum* BB 01 + *Bifidobacterium longum* BL 01 + *Bifidobacterium breve* BR 01 для виробництва кисломолочних продуктів та косметики для підлітків і людей віком до 40-45 років з пробіотиками. Визначено значення титрованої кислотності, кількість життєздатних клітин біфідобактерій та найбільш вірогідне число лактобактерій у ферментованих пробіотичних згустках, отриманих за різних співвідношень змішаних культур *Lactococcus sp.* та змішаних культур *Bifidobacterium sp.* у заквашувальних композиціях. Найвищий вміст пробіотиків і найнижчі значення титрованої кислотності мають ферментовані молочні згустки, отримані при використанні заквашувальної композиції зі співвідношенням ЗК *Lactococcus sp.* : ЗК *Bifidobacterium sp.* 1 : 10. Максимальна кількість життєздатних клітин лактококів відзначена у згустках, отриманих при використанні заквашувальної композиції з вихідним співвідношенням ЗК *Lactococcus sp.* : ЗК *Bifidobacterium sp.* 10 : 1. Встановлено оптимальне співвідношення ЗК *Lactococcus sp.* та ЗК *Bifidobacterium sp.* – 1 : 10 для виробництва кисломолочних продуктів та косметики з пробіотиками, при якому відзначається максимальне значення комплексного показника якості – 7,12. Показано, що ферментовані пробіотичні молочні згустки, отримані із застосуванням заквашувальної композиції з оптимальним співвідношенням культур лакто- та біфідобактерій (1 : 10), мають гарні сенсорні характеристики, містять високу кількість життєздатних клітин біфідо- та лактобактерій –  $(9,15 \pm 0,14) \times 10^9$  та  $(8,50 \pm 0,50) \times 10^8$  КУО/см<sup>3</sup> відповідно, невисокий рівень титрованої кислотності при нормованому значенні активної кислотності та є безпечними за мікробіологічними характеристиками.

**Ключові слова:** кисломолочний продукт, косметика, пробіотик, *Lactococcus sp.*, *Bifidobacterium sp.*, кислотність, комплексний показник якості, оптимізація, поверхня відклику.



## Introduction. Formulation of the problem

Over the past three decades probiotics are firmly included in dietary science and healthy nutrition. Due to its high efficiency they have gained a great popularity among consumers worldwide as a safe and effective method of maintaining and/or restoring human health in recent years [1–3]. The milk products enriched with probiotics for child nutrition that carry physiologically significant effect on infant body are of special emphasis [5–8], as well as fermented milk products with probiotics of gerodietetic purpose, with immunomodulation, antagonistic and other special properties [9–10]. The majority of scientific studies related to probiotic products are focused on the gastrointestinal tract of humans [1–10]. However, during the last decades a considerable interest of scientists around the world raise the possibility of applying probiotics topically on the skin, that is a part of personal hygiene items and cosmetics [11–13].

Therefore, an urgent task today is the development of starter cultures composition with probiotic with an optimum composition of microbiota, which could be used in the manufacture of the fermented milk products and cosmetics with the given special properties.

## Analysis of recent research and publications

Probiotics are live microorganisms which, when used in a certain amount, provide a healthy action in addition to action typical of main nutrition [14]. With the application of probiotics as components of foods the health-improving effect is aimed at the normalization of the intestinal microflora [1,2].

Development of technologies of probiotic foods for healthy nutrition in recent decades is a subject of researches of foreign and domestic scientists. The fermented milk products take the first position within the range of probiotic products for a healthy nutrition [5–10]. Microorganisms-probiotics are selected according to certain criteria, taking into account safety and with the mandatory clinical studies. They must meet the basic requirements: to be normal representatives of the gastrointestinal tract, to be non-pathogenic and non-toxicogenic, to be metabolically active, have the ability to adhesion, to synthesize antibacterial agents, to prevent the development of pathogenic microorganisms, to be safe when using the products and the clinic, to implement a clearly defined and confirmed in clinical studies positive effect on the health of humans or animals [3,15–17].

The functions of probiotic bacteria in the human body that are already used when creating a probiotic food and can be used to develop "live" and "probiotic" cosmetics are [18] analyzed in study. One of the important steps in the creation of food and cosmetic products with probiotics is the selection of bacteria

cultures with the given properties and optimization of starter cultures composition with their application [3,19]. There is a large number of scientific studies regarding the selection of probiotics for the manufacture of special and functional milk products [17,19–21], the rationale for the composition of starter cultures composition of probiotic cultures of lactobacteria and bifidobacteria for the manufacture of fermented milk products of child nutrition [22–24] and functional fermented milk products [19,25–27]. Studies of impact of probiotics on a human mood are even known [28]. However, the number of studies regarding optimization of starter cultures composition of lacto- and bifidobacteria for manufacture of probiotic cosmetic products is rather limited [29–30], although there is a significant number of studies regarding the positive effects of probiotics on the skin [11–13,18].

The basis for the manufacture of a wide range of fermented milk products are mixed cultures *Lactococcus sp.* [19]. Cheese (including lactic cheese), sour cream, yogurt, kefir are manufactured using these cultures. The combination of mixed cultures *Lactococcus sp.* with the monocultures/mixed cultures *Bifidobacterium* enables to obtain fermented milk products with high content of viable cells of bifidobacteria and lactococci, as these cultures are in symbiosis with the use of different methods of growth stimulation of bifidobacteria in milk [19,25,26]. Moreover, bacteria of *Lactococcus sp.* and their metabolites are used to inhibit the development of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Propionibacterium acnes*, which can cause inflammatory skin processes and the formation of rash (acne) [11]. Therefore, starter cultures composition for manufacture of fermented milk products and cosmetics should consist of the mixed cultures *Lactococcus sp.* As a probiotic component, it is advisable to use mixed cultures *Bifidobacterium sp.*, as each individual culture inhibits the development of certain types (or strains) of pathogenic or opportunistic pathogenic bacteria and a complex of bifidobacteria has a much wider range of action [3,19]. The consumer market of Ukraine today comprises two bacterial concentrates of direct application of mixed cultures *Bifidobacterium sp.* manufactured by ALCE MOFIN GRUPPO (Italy) – *Liobac 3 BIFIDI* that contains mixed cultures of *Bifidobacterium bifidum BB 01* + *Bifidobacterium longum BL 01* + *Bifidobacterium adolescentis BA 01* and *Liobac BIFI* that contains mixed cultures of *Bifidobacterium bifidum BB 01* + *Bifidobacterium longum BL 01* + *Bifidobacterium breve BR 01*.

Cultures of *Bifidobacterium bifidum BB 01* and *Bifidobacterium longum BL 01* colonize the human intestine throughout the life, cultures *Bifidobacterium adolescentis BA 01* are typical of the human gastrointestinal tract in old age and cultures *Bifidobacterium breve BR 01* in adolescence and young age [3,19]. It is therefore advisable to use a

bacterial concentrate Liobac BIFI as a probiotic component in starter cultures composition for manufacture of fermented milk products and cosmetics with probiotics designed for teenagers and people under the age of 40-45 years.

**Objective** of the study – optimization of starter cultures composition of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* for manufacture of fermented milk products and cosmetics with probiotic properties.

**Tasks** of study:

- to determine a titratable acidity and number of viable cells of lacto - and bifidobacteria in fermented probiotic clots obtained with different ratios of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* in starter cultures composition;
- to establish the optimum ratio of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* in starter cultures composition for manufacture of fermented milk products and cosmetics with probiotics;
- to determine the quality parameters of probiotic clot obtained by fermentation of milk enriched with fructose, starter cultures composition of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* at the optimum ratio.

#### Research Materials and Methods

For studies the following raw ingredients and materials were used: whole cow milk grade A according to DSTU 3662-97 received in the raw area of Gormolzavod No.1 LLC (Odessa, Ukraine); fructose purchased in Cemargl Trading House (Kiev, Ukraine); starter cultures bacterial concentrate of direct application *FD DVS CH N 19* which is composed of mixed cultures *Lactococcus lactis ssp. lactis + Lactococcus lactis ssp. cremoris + Lactococcus lactis ssp. diacetylacti* provided *Chr. Hansen* Company (Denmark) and starter cultures bacterial concentrate of direct application *Liobac BIFI* that contains mixed cultures *Bifidobacterium bifidum BB 01 + Bifidobacterium longum BL 01 + Bifidobacterium breve BR 01* provided by *ALCE MOFIN GRUPPO* Company (Italy).

To optimize starter composition of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* for the manufacture of fermented milk products and cosmetics with probiotic properties the methodology of response surface was used [31]. The above method is a collection of mathematical and statistical techniques that are aimed at modeling of technological processes and determination of ratios of the experimental series of predictors to optimize the response function  $\hat{y}(x, b)$  which in general form is described by the following polynomial:

$$\hat{y}(x, b) = b_0 + \sum_{i=1}^n b_i x_i + \sum_{k=1}^n b_k x_k^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n b_{ij} x_i x_j, \quad (1)$$

where  $x \in R^n$  – change vector,  $b$  – parameter vector.

Modeling and processing of experimental data were performed within *Statistica 10* (*StatSoft, Inc., USA*) software package.

The initial concentration of mixed cultures *Lactococcus sp.* was varied as a part of starter cultures composition within  $1 \times 10^5 - 1 \times 10^6$  CFU/cm<sup>3</sup> of infant formula milk and of mixed cultures *Bifidobacterium sp.* within the same limits.

To obtain the fermented clots using composed starter cultures composition in milk with mass fraction of fat of 1.0% fructose was added as a growth factor of bifidobacteria in the amount of 0.1% [19], the mixture was heated to a temperature of 40–45°C, the purified mixture was cleaned and warmed to a temperature of 60–65°C and homogenized at a pressure of 12 MPa, sterilized at a temperature of (120±1)°C for 19–21 minutes. Mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* were cooled to the temperature of (37±1)°C and added in the required quantities in order to exclude the impact of residual microflora on the development of starter cultures composition. Fermentation of inoculated mixtures was carried out at a temperature of (37±1)°C that is the optimum temperature for the development of bifidobacteria to achieve the isoelectric state of the protein of milk (pH=4,6). A titratable acidity (TA, °T), the number of viable cells of bifidobacteria, the most probable number of lactobacteria were determined in fermented clots.

The initial concentrations of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* were considered as optimum, at which the clots had a maximum number of bifido- and lactobacteria and the minimum value of titratable acidity.

Based on the developed recommendations the probiotic clots were produced using starter cultures composition with an optimum ratio of bifidobacteria and lactococci and their key quality parameters were determined. According to the results of these studies the conclusions about the possibility of manufacture of fermented milk products and cosmetics with the probiotics using the developed starter cultures composition were made.

During study a titratable acidity of the fermented clots was determined by titrimetric method according to GOST 3624-92, the preparation of samples of clots and their dilutions for microbiological studies were made according to DSTU IDF 122C:2003; number of viable cells of bifidobacteria – by inoculation into thioglycollate medium divided into test tubes with a high column and by temperature control at 37°C without access of oxygen for 48–72 hours. was determined according to GOST 7355:2013, the most probable number of viable cells of lactic acid bacteria – by inoculation into sterilized skim milk with a high column and by temperature control within 72 hours. was determined according to GOST 10444.11-89, counting the number of coliforms – by inoculation on Kessler media according to DSTU IDF 73A:2003, determination of *Salmonella* – by inoculation on Ploskerev me-

dia according to DSTU IDF 93A:2003, number of yeasts and molds – by inoculation on Saburo media according to GOST 10444.12-88.

### Results of the research and their discussion

A decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of the clot ( $LgCbb$ ), the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of the clot ( $LgClb$ ), titratable acidity clot (TA, °T) and the aggregated quality index (AQI – the index, which takes into account the cumulative impact of the common logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of the clot of the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of clot, titratable acidity of the clot and the weight coefficients (WC) of the individual indicators) were chosen as criteria of optimization of the starter cultures composition of mixed cultures *Lactococcus lactis* ssp. *lac-*

*tis* + *Lactococcus lactis* ssp. *cremoris* + *Lactococcus lactis* ssp. *diacetylactis* (MC *Lactococcus* sp.) and mixed cultures *Bifidobacterium bifidum* BB 01 + *Bifidobacterium longum* BL 01 + *Bifidobacterium breve* BR 01 (MC *Bifidobacterium* sp.). The decimal logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of inoculated milk formula ( $LgCbb_{init}$ ) and the logarithm of the initial concentration of viable cells of lactobacteria in 1 cm<sup>3</sup> of inoculated milk formula ( $LgClb_{init}$ ) were chosen as independent factors that varied in the experiment.

The response function was chosen for modeling and optimization of the decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of clot ( $LgCbb$ ), the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of clot ( $LgClb$ ), titratable acidity of clot (TA, °T) and the quality aggregated index (QAI); the function has the form of a second order polynomial:

$$LgCbb = b_0 + b_1 \cdot LgCbb_{init} + b_{11} \cdot LgCbb_{init}^2 + b_2 \cdot LgClb_{init} + b_{22} \cdot LgClb_{init}^2 + b_{12} \cdot LgCbb_{init} \cdot LgClb_{init}, \quad (2)$$

$$LgClb = b_0 + b_1 \cdot LgCbb_{init} + b_{11} \cdot LgCbb_{init}^2 + b_2 \cdot LgClb_{init} + b_{22} \cdot LgClb_{init}^2 + b_{12} \cdot LgCbb_{init} \cdot LgClb_{init}, \quad (3)$$

$$TA = b_0 + b_1 \cdot LgCbb_{init} + b_{11} \cdot LgCbb_{init}^2 + b_2 \cdot LgClb_{init} + b_{22} \cdot LgClb_{init}^2 + b_{12} \cdot LgCbb_{init} \cdot LgClb_{init}, \quad (4)$$

$$QAI = b_0 + b_1 \cdot LgCbb_{init} + b_{11} \cdot LgCbb_{init}^2 + b_2 \cdot LgClb_{init} + b_{22} \cdot LgClb_{init}^2 + b_{12} \cdot LgCbb_{init} \cdot LgClb_{init}, \quad (5)$$

where  $b_0$  – a constant;  $LgCbb_{init}$  – a decimal logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of inoculated milk formula;  $LgClb_{init}$  – a decimal logarithm of the initial concentration of viable cells of lactobacteria in 1 cm<sup>3</sup> of inoculated milk formula;  $b_1$ ,  $b_{11}$ ,  $b_2$ ,  $b_{22}$ ,  $b_{12}$  – coefficients for each element of the polynomial.

A central composite rotatable design is used in studies [31]. The selection of levels and intervals of factor variation was performed according to the results of previous experiments [19]; a decimal logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of inoculated milk formula varied within the range of 5.0–6.0 (the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> at inoculation was  $1 \times 10^5$ – $1 \times 10^6$  CFU/cm<sup>3</sup> of milk formula); the decimal logarithm of the initial concentration of viable cells of lactobacteria in 1 cm<sup>3</sup> of inoculated milk formula varied within the range of 5.0–6.0 (the concentration of viable cells of lactobacteria in 1 cm<sup>3</sup> at inoculation was  $1 \times 10^5$ – $1 \times 10^6$  CFU/cm<sup>3</sup> of milk formula).

The design matrix and experimental values of response functions are shown in Table 1. The sequence of experiments was randomized to reduce the influence of systematic errors caused by external conditions.

The above Pareto charts (Fig. 1) show standardized coefficients which are sorted by absolute values. Data analysis, Fig. 1 b, shows that the decimal logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of inoculated milk formula ( $LgCbb_{init}$ ) is quadratic and product of the initial regression parameters (3) is insignificant, since the columns of marks of these effects do not cross the vertical line, which is the 95% confidence probability. Taking into account this fact, the above regression terms were eliminated from the model (3). All the coefficients are significant for regressions (2) and (4) according to the data given in Fig. 1 a and Fig. 1 c, respectively. The obtained equations with the calculated coefficients have the following form:

$$LgCbb = -43,83 + 9,67 \cdot LgCbb_{init} - 0,37 \cdot LgCbb_{init}^2 + 9,72 \cdot LgClb_{init} - 0,47 \cdot LgClb_{init}^2 - 0,02 \cdot LgCbb_{init} \cdot LgClb_{init}, \quad (6)$$

$$LgClb = -11,15 - 0,44 \cdot LgCbb_{init} + 7,80 \cdot LgClb_{init} - 0,65 \cdot LgClb_{init}^2, \quad (7)$$

$$TA = -274,31 + 81,67 \cdot LgCbb_{init} - 12,16 \cdot LgCbb_{init}^2 + 38,88 \cdot LgClb_{init} - 8,16 \cdot LgClb_{init}^2 + 10,20 \cdot LgCbb_{init} \cdot LgClb_{init}, \quad (8)$$

Table 1 – Design matrix and response functions

Experiment number	<i>LgCbb<sub>init</sub></i>		<i>LgClb<sub>init</sub></i>		<i>LgCbb</i>	<i>LgClb</i>	TA, °T
	Coded level	–	Coded level	–			
1	0	5.50	0	5.50	9.40	9.50	82.50
2	0	5.50	0	5.50	9.42	9.50	83.00
3	0	5.50	0	5.50	9.37	9.60	83.00
4	0	5.50	0	5.50	9.38	9.60	82.50
5	–√2	5.00	0	5.50	9.10	9.80	77.00
6	+√2	6.00	0	5.50	9.50	9.40	81.50
7	0	5.50	–√2	5.00	9.55	9.11	77.50
8	0	5.50	+√2	6.00	9.00	9.70	83.00
9	–1	5.15	–1	5.15	9.15	9.40	79.00
10	–1	5.15	+1	5.85	9.00	9.90	80.00
11	+1	5.85	–1	5.15	9.80	9.13	79.00
1	0	5.50	0	5.50	9.40	9.50	82.50

Pareto charts shown in Fig. 1 were constructed to verify regression coefficients (2), (3) and (4) (L – linear effect, Q – quadratic effect).

The adequacy of the developed models (6), (7) and (8) was verified by analysis of variance. Its results, in particular, the values of coefficients of determination (model (6)  $R^2=0.981$  i  $R^2_{adj}=0.965$ ; for model (7)  $R^2=0.979$  i  $R^2_{adj}=0.962$ ; for model (8)  $R^2=0.967$  i  $R^2_{adj}=0.939$ ) and absence of loss of coherence (for all models the significance level of this indicator  $p>0.05$ ) indicate that the models describe the experiment adequately.

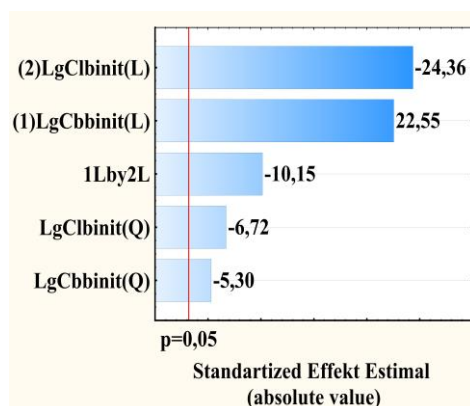
Described by the polynomials (6), (7) and (8) the cumulative impact of the decimal logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of inoculated milk formula (*LgCbb<sub>init</sub>*) and the decimal logarithm of the initial concentration of viable cells of lactobacteria in 1 cm<sup>3</sup> of inoculated milk formula (*LgClb<sub>init</sub>*) on the decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> (*LgCbb*), the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> (*LgClb*) and titratable acidity (TA, °T) of fermented milk clots are graphically represented in Fig. 2 a, b, c respectively.

All fermented clots obtained in experimental studies have a high content of viable cells of probiotic cultures of bifidobacteria used in starter cultures compositions of  $8.8 \times 10^8$  CFU/cm<sup>3</sup> to  $9.3 \times 10^9$  CFU/cm<sup>3</sup> (Fig. 2, a) resulting in a high probiotic properties [3, 19]. The highest content of probiotics and, accordingly, the highest probiotic properties are typical of fermented milk clots obtained using starter cultures composition at a ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* 1 : 10 at initial concentrations of the respective cultures in starter cultures milk formula  $1 \times 10^5$  and  $1 \times 10^6$  CFU/cm<sup>3</sup>. This is due to the fact that the growth of titratable and reduced active acidity of infant formula milk is slower than the maximum content at the minimum concentration of lactobacteria in starter cultures composition during the first two hours of fermentation. Due to this fact bifidobacteria, for growth which a neu-

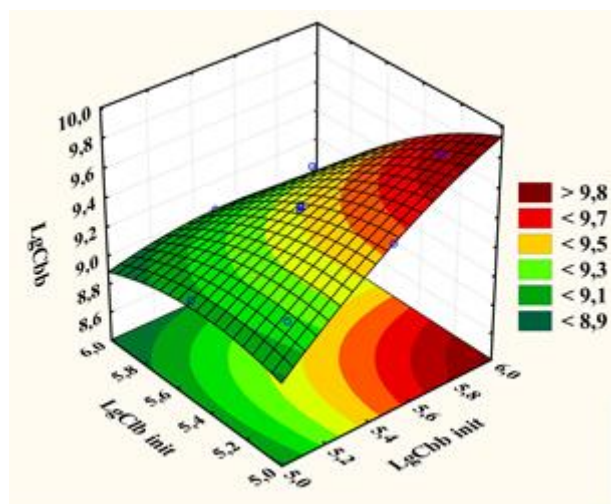
tral or weakly alkaline medium is optimum for the first 2 hours of fermentation and whose number is 10 times higher than that for lactobacteria at an inoculation, have in 2.2–2.4 times higher specific rate of cell growth from that of lactobacteria. The clots obtained with the use of starter cultures compositions at a ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* 10 : 1 at the initial concentration of the respective cultures in starter cultures formula of  $1 \times 10^6$  and  $1 \times 10^5$  CFU/cm<sup>3</sup> have the minimum concentration of probiotic viable cells of bifidobacteria  $8.8 \cdot 10^8$  CFU/cm<sup>3</sup> (Fig. 2, a). At the same time these clots have the highest content of viable cells of Lactobacteria –  $9.0 \times 10^9$  CFU/cm<sup>3</sup> (Fig. 2, b) which provide high sensory characteristics (taste and smell, consistency and appearance), the required rheological (viscosity), synergetic (separation of serum) and physicochemical (titratable and active acidity) parameters of the fermented clots in the manufacture of fermented milk products, as well as contribute to the proteolysis of milk protein fractions with obtaining short-chain peptides, which can be fibroblast stimulants in the dermis when applying them in beauty products [3,11,18,19].

Titrated acidity obtained in the experiment of fermented probiotic clots ranges from 74.5 to 85.0 °T (Fig. 2). The lowest titratable acidity have clots obtained by fermentation of milk enriched with fructose, starter cultures composition with a ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* 1 : 10 (concentration of the respective cultures when inoculated to  $1 \times 10^5$  and  $1 \times 10^6$  CFU/cm<sup>3</sup>). This is due to the fact that these clots have a maximum cell concentration of bifidobacteria (Fig. 2, a) and a minimum cell concentration of lactobacteria (Fig. 2, b). Bifidobacteria during the fermentation of lactose in addition to lactic acid form acetic acid in the process of milk fermentation, in contrast to homofermentative lactobacteria, which produce 90% of lactic acid [3,19]. Acetic acid is a strong electrolyte in comparison with milk, therefore, it stipulates obtaining the fermented clots with a low level of titratable acidity at active acidity of 4.6 pH.

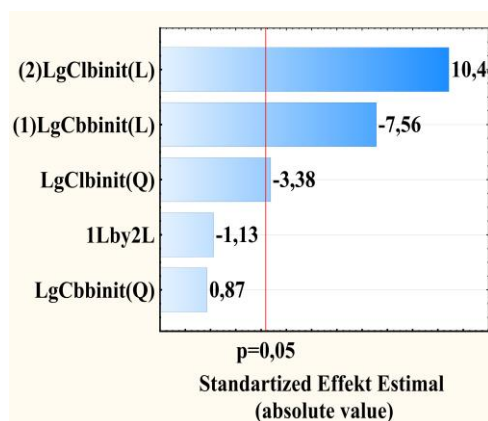




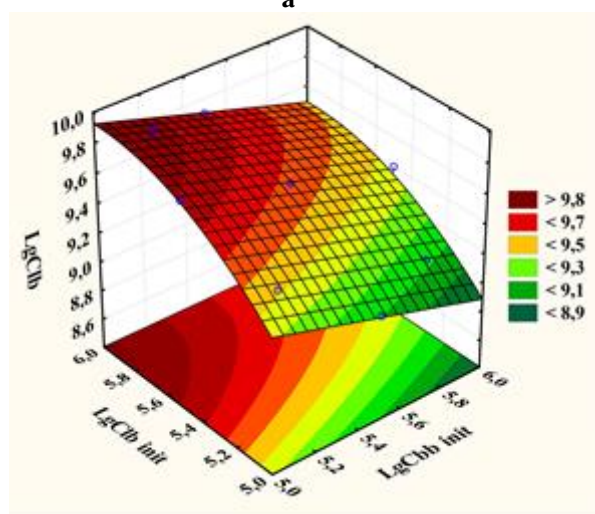
a



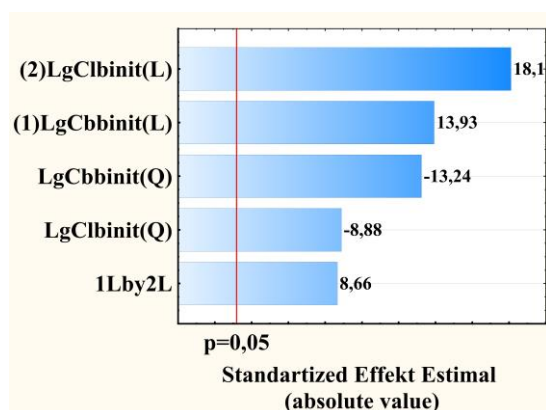
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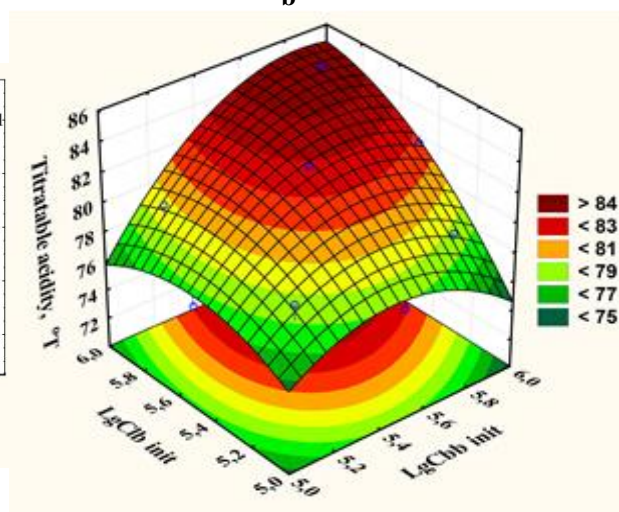
b



b



c



c

Fig. 1. Pareto chart to verify the significance of coefficients:  
a – regression (2); b – regression (3); c – regression (4)

Fig. 2. Dependence: a – of decimale logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of clot (*LgCbb*), b – decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of clot (*LgClb*), c – titratable acidity of clot (*TK*, °T) – from decimale logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of inoculated

Fermented clots with maximum number of viable cells of probiotic cultures of bifidobacteria, a high cell concentration of lactobacteria and a minimum level of titratable acidity must be obtained for manufacture of probiotic fermented milk and cosmetic products. The obtained results, shown in Fig. 2, do not allow determining the optimum initial concentration of bifidobacteria and lactobacteria in starter cultures formula, therefore, to optimize starter cultures composition of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* for manufacture of fermented milk and cosmetic products with probiotic properties the quality aggregated index (QAI) was used, which was determined as a function of estimates of individual quality indices – decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of clot (*LgCbb*), the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of clot (*LgClb*) and titratable acidity of clot (TA, °T) (Table. 1) converted into scaled values, taking into account the weight coefficients of individual indices ( $M_i$ ) [32, 33]:

$$QAI = M_1 \cdot LgCbb + M_2 \cdot LgClb + M_3 \cdot TA \quad (9)$$

where –  $M_1$ ,  $M_2$ ,  $M_3$  – the weight coefficients of individual indices – the decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of clot of the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of clot and titratable acidity of clot, respectively. Moreover

$$\sum_{i=1}^n M_i = 1,0 \quad (10)$$

To convert the decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of clot and the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of clot within the range of [1-10] (that is, to maximize the maximum values of individual indices) the initial data, given in Table 1, were scaled by the following formula (11):

$$y = \frac{(y_{\max} - y_{\min}) \cdot (x - x_{\min})}{x_{\max} - x_{\min}} + y_{\min} \quad (11)$$

where  $y$  – scaled data;  $x$  – the initial data given in Table 1;  $x_{\min}$  and  $x_{\max}$  – minimum and maximum value of initial data (decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of the clot and the decimal logarithm of the most probable number of cells of lactobacteria in 1 cm<sup>3</sup> of clot  $x_{\min}$  та  $x_{\max}$  were calculated by models (6) and (7) respectively);  $y_{\min}$  and  $y_{\max}$  – the minimum and maximum values of the new range (1 and 10 respectively).

To convert a titratable acidity of the clot in the range of [1-10] (that is, to maximize the minimum values of individual indices) the initial data, given in Table 1, were scales by the following formula (12):

$$y = y_{\max} - \frac{(y_{\max} - y_{\min}) \cdot (x - x_{\min})}{x_{\max} - x_{\min}} \quad (12)$$

$$QAI = -274,94 + 33,51 \cdot LgCbb_{vit} + 68,98 \cdot LgClb_{vit} - 3,38 \cdot LgClb_{vit}^2 - 5,98 \cdot LgCbb_{vit} \cdot LgClb_{vit} \quad (13)$$

where  $y$  – scaled data;  $x$  – initial data given in Table 1;  $x_{\min}$  та  $x_{\max}$  – the minimum and maximum value of initial data (for titratable acidity of clot  $x_{\min}$  and  $x_{\max}$  were calculated by model (8));  $y_{\min}$  та  $y_{\max}$  – minimum and maximum value of the new range (1 and 10 respectively).

The values of the specific indices scaled by formulas (11) and (12) and values of the quality aggregated index (QAI) calculated by formula (9) are shown in Table 2 (the following values of the weight coefficients were accepted on calculation of QAI – in accordance with the recommendations of the Expert Committee:  $M_1=0.54$ ,  $M_2=0.36$ ,  $M_3=0.10$ , as the decimal logarithm of the concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of clot is the most important specific index). To certify the significance of regression coefficients (5) the Pareto charts was built which is shown in Fig. 3 (L – linear effect, K – quadratic effect). Data analysis shown in Fig. 3 indicates that the decimal logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of starter cultures formula ( $LgCbb_{init}$ ) for the quadratic regression (4) is insignificant because the column of evaluation of this effect does not cross the vertical line that is the 95% confidence probability. Taking into account this fact the given regression term was eliminated from the model (5).

The resulting equation with the calculated regression coefficients has the following form:

Adequacy of the developed model (12) was verified by variance analysis, the results of which are shown in Table 3.

The data given in Table 3, in particular absence of loss of coherence (the level of significance  $p > 0.05$ ) and value of coefficients of determination ( $R^2$  і  $R^2_{adj}$ ), close to one, allow us concluding that the obtained model (13) describes adequately the response.

The cumulative impact of the common logarithm of the initial concentration of viable cells of bifidobacteria in 1 cm<sup>3</sup> of starter cultures formula ( $LgCbb_{init}$ ) described by the polynomial (13) and decimal logarithm of the initial concentration of viable cells of lactobacteria in 1 cm<sup>3</sup> of starter cultures formula ( $LgClb_{init}$ ) on the quality aggregated index (QAI) of the fermented probiotic milk clots is shown graphically in Fig. 4.

The minimum content of lacto - and bifidobacteria in starter cultures compositions and their maximum content, cause low values of QAI – 3.51 and 3.04 respectively (Fig. 4), while minimum content in the composition of bifidobacteria and the maximum content of lactobacteria in it the quality aggregated index is 5.41. The highest values of QAI – 6.85–7.12 (Fig. 4) – note with a minimum content in starter cultures composition in mixed cultures *Lactococcus sp.* and the maximum content of mixed cultures *Bifidobacterium sp.*

Table 2 – Scaled values of specific indices and calculated values of quality aggregated index

Experiment number	$LgCbb_{\text{am}}$	$LgClb_{\text{sc}}$	$TA_{\text{sc}}$	$QA_I$
1	5.42	6.34	3.07	5.51
2	5.59	6.34	2.63	5.56
3	5.16	7.20	2.63	5.64
4	5.24	7.20	3.07	5.73
5	2.83	8.92	7.85	5.53
6	6.28	5.47	3.94	5.75
7	6.71	2.98	7.41	5.44
8	1.97	8.06	2.63	4.23
9	3.26	5.47	6.11	4.34
10	1.97	9.78	5.24	5.11
11	8.86	3.15	6.11	6.53
12	3.69	6.34	0.89	4.37

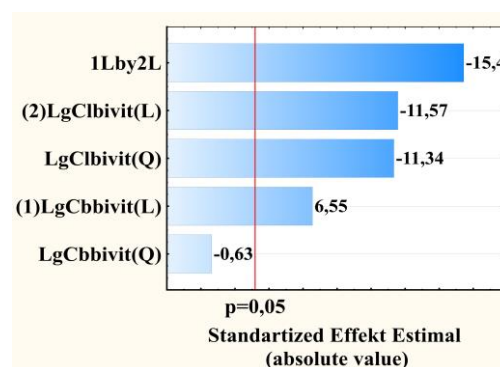


Fig. 3. Pareto charts for verification of significance of regression coefficients (5)

Table 3 – Variance analysis of model (13)

Factor	SS	df	MS	F-criterion	p
(1) $LgCbb_{\text{lux}}$ (L)	0,385774	1	0,385774	42,8754	0,007242
$LgCbb_{\text{lux}}$ (K)	0,003552	1	0,003552	0,3948	0,574359
(2) $LgClb_{\text{lux}}$ (L)	1,204302	1	1,204302	133,8474	0,001387
$LgClb_{\text{lux}}$ (K)	1,156478	1	1,156478	128,5321	0,001472
1L на 2L	2,146129	1	2,146129	238,5229	0,000590
Loss of coherence	0,218997	3	0,072999	8,1132	0,059635
Net error	0,026993	3	0,008998		
The total sum of squares	5,167381	11			
Coefficient of determination $R^2 = 0,952$					
Adjusted coefficient of determination $R^2_{\text{adj}} = 0,913$					

Processing of the polynomial (13) in *Statistica 10* medium allowed to establish the optimum values of the decimal logarithm of the initial concentration of viable cells of bifidobacteria ( $LgCbb_{\text{init}}$ ) and decimal logarithm of the initial concentration of viable cells of lactobacteria ( $LgClb_{\text{init}}$ ) in 1 cm<sup>3</sup> of starter cultures milk formula is 6.0 and 5.0 respectively, where the maximum value of QAI is reached (7,12). Under these conditions the initial concentration of viable cells in

mixed cultures *B. bifidum* BB 01 + *B. longum* BL 01 + *B. breve* BR 01 and mixed cultures *Lactococcus* sp. when inoculated is  $1 \times 10^6$  and  $1 \times 10^5$  CFU/cm<sup>3</sup>.

Sensory characteristics and key quality parameters of fermented milk clots obtained using starter cultures composition with the optimum ratio of MC *Lactococcus* sp. : MC *Bifidobacterium* sp. (1 : 10) are given in Table 4.

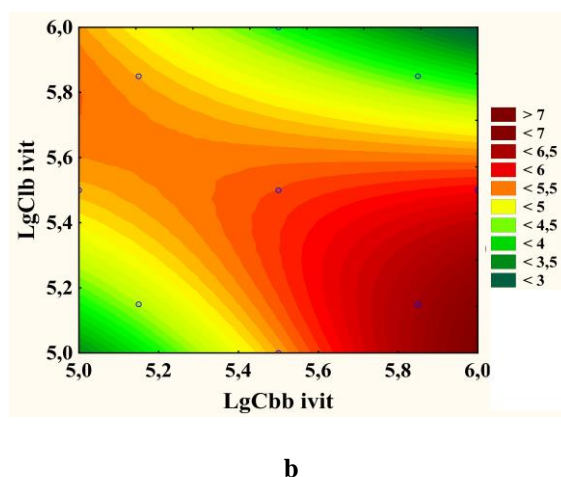
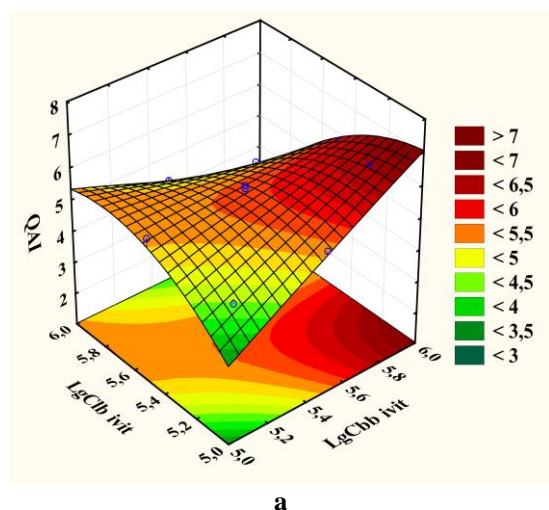


Fig. 4. QAI dependence on  $LgCbb_{\text{init}}$  and ( $LgClb_{\text{init}}$ ): a – response surface; b – counter plot



Table 4 – Sensory characteristics, physical/chemical, rheological and microbiological indices of quality of fermented probiotic milk clots (n = 5, p ≤ 0,05)

Criterion	Criterion characteristic for probiotic fermented milk clot
<i>Sensory characteristics</i>	
Taste and odour	Clear fermented, Pure milk, without foreign tastes and odors – 10 points
Consistency and appearance	Homogenous consistency, viscous, without whey sediment – 5 points
Colour	White with a cream tint, homogenous over the whole clot mass – 5 points
<i>Physical/chemical and rheological criteria</i>	
Active acidity, pH	4.60 ± 0.03
Titrated acidity, °T	77.5 ± 0.5
Moisture retaining capacity, %	72.5 ± 3.5
Peroxidase	Absent
<i>Microbiological criteria</i>	
Number of viable cells of MC <i>Bifidobacterium sp.</i> in 1 cm <sup>3</sup> of clot, CFU	(9.15 ± 0.14) × 10 <sup>9</sup>
The most probable number of viable cells of MC <i>Lactococcus sp.</i> in 1 cm <sup>3</sup> of clot, CFU	(8.50 ± 0.50) × 10 <sup>8</sup>
Coliforms 1 cm <sup>3</sup> of clot, CFU	absent
Pathogenic bacteria; including <i>Salmonella</i> , in 25 cm <sup>3</sup> of clot, CFU	absent
Number of viable cells of yeasts and molds in 1 cm <sup>3</sup> of clot, CFU	< 10

Fermented probiotic milk clots obtained using starter cultures composition with an optimum ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* (1: 10), have good sensory characteristics, contain a high number of viable cells of bifidobacteria and lactobacteria are (9.15±0.14)×10<sup>9</sup> and (8.50±0.50)×10<sup>8</sup> CFU/cm<sup>3</sup>, respectively, and a low level of titratable acidity at a specified value of the active acidity and are safe that is proven by indices shown in Table. 4.

### Conclusions

1. The value of a titratable acidity, number of viable cells of bifidobacteria and the most probable number of lactobacteria in fermented probiotic clots obtained with different ratios of mixed cultures *Lactococcus lactis ssp. lactis* + *Lactococcus lactis ssp. cremoris* + *Lactococcus lactis ssp. diacetylactis* and mixed cultures *Bifidobacterium bifidum BB 01* + *Bifidobacterium longum BL 01* + *Bifidobacterium breve BR 01* is determined in starter cultures composition. The highest content of probiotics and the lowest values of a titratable acidity are typical of the fermented milk clots obtained using starter cultures composition at a ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* 1 : 10 at initial concentrations of

the respective cultures in inoculated milk formulas 1×10<sup>5</sup> and 1×10<sup>6</sup> CFU/cm<sup>3</sup>. The maximum number of viable cells of lactobacteria is observed in clots obtained using starter cultures composition with the initial ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* 10 : 1 (concentration of the respective cultures when inoculated to 1×10<sup>6</sup> and 1×10<sup>5</sup> CFU/cm<sup>3</sup>).

2. The optimum ratio of mixed cultures *Lactococcus sp.* and mixed cultures *Bifidobacterium sp.* – 1 : 10 is determined at the initial concentration of cultures in starter cultures composition of 1×10<sup>5</sup> and 1×10<sup>6</sup> CFU/cm<sup>3</sup>, respectively, for the manufacture of fermented milk products and cosmetics with probiotics, at which a maximum value of the quality aggregated index – 7.12.

3. It is shown that fermented probiotic milk clots obtained using starter cultures composition with an optimum ratio of MC *Lactococcus sp.* : MC *Bifidobacterium sp.* (1 : 10) have good sensory characteristics, contain a high number of viable cells of bifidobacteria and lactobacteria – (9,15±0,14)×10<sup>9</sup> and (8,50±0,50)×10<sup>8</sup> CFU/cm<sup>3</sup>, respectively, and a low level of titratable acidity at a specified value of active acidity and are safe in terms of microbiological characteristics.

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