

STUDY OF KEEPING PROBIOTIC PROPERTIES OF CULTURED BUTTER IN STORAGE

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Abstract

The aim of the work was the study of keeping probiotic properties of cultured butter with inclusion of *Lactobacillus acidophilus* La-5 (La-5) monoculture. *Flora Danica* mesophile culture independently (FD); in combination with La-5 and La-5 independently were used for fermenting cream. The output consistence of culture in cream was 1×10^6 CFU/cm³.

In autumn-winter and spring-summer period of the year four butter groups were prepared, they differed by temperature of cream fermentation: I group – (30±1) °C; II – (37±1) °C; III – stage regimes of combination of fermentation and physical maturing; IV group – introduction of cultures into butter kernel; the output concentration – $1 \cdot 10^8$ CFU/cm³.

As to the features of summer and winter periods, in summer one cream fermentation is more active that is indicated by more number of cells of both microbial cultures. The best parameters of viable cells keeping were typical to the samples at FD+La-5 use and temperature of cream fermentation (30±1) °C. Storage life of sour-cream butter with probiotic properties is 35 days at temperature 0...–5 °C.

Keywords: cream, *Flora Danica*, *L. acidophilus* La-5, cultured butter, probiotic properties.

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1. Introduction

The effective approach to sanitation of the ration of modern people is creation of food products with regulated content – so-called “corrected”, “enriched”, but most often “functional” products [1]. Despite the existing stereotype of incompatibility of flavor with usefulness, functional

products can combine good taste and useful properties and also manifest the treating-prophylactic effect on human organism [2].

Manufacturing of products “for health” with functional properties, which significant part is milk ones (their share on Ukrainian market reaches 67 %), is the one of most important tasks of scientists and practical workers of the milk field [3]. Different additives are often used for formation of such properties. But just naturalness of products occupies the important place in modern trends of food field [4].

Today the preparations and products, created using milk bacteria, are considered as a base of human functional food and favor the prophylaxis of series of diseases [5]. The positive effect is attained both by introduction of lactobacteria viable cells directly in human organism and by using these microorganisms in the composition of ferments at manufacturing of food products including the ones on milk base [6].

Cultured butter is butter, characterized with rich flavor-smell bouquet due to the aromatic substances, created at lactose fermentation by lactate bacteria. The cause of low demand for cultured butter in Ukraine is the contradictions as to the features of production technologies and differences in composition and properties of the Ukrainian raw material [7]. It became just the cause of cultured butter technology restoration. The assortment can be widened due to the additional valuable properties of cultured butter, using probiotic cultures in its production.

The description of the use of fermenting cultures of direct introduction for fermenting cream at sour-cream butter production has not been found in literature. The study of this process is important, because cream is specific medium for the growth and development of lactate bacteria [8].

The process of joint cultivation of *Flora Danica* mixed mesophile cultures and thermophilic acidophilic bacillus also needs a detail study, as far as it is described for fermented beverage [9, 10]. That is why the determination of conditions, at which probiotic cultures keep viability at cultured butter storage, is an actual task.

2. Materials and methods

2.1. Studied materials, used at the research and methodology of experiment realization

The experimental study was carried out in autumn-winter period (end of February – early March) and in spring-summer period (end of August – early September) of 2013. Milk was stored up in PSC “Galicia” (Radehiv city, Lviv region, Ukraine), separated at temperature 40–45 °C. The received cream with mass share of fat 32–33 % was pasteurized at temperature 95 °C without keeping, after pasteurization cream was cooled to the temperature of fermentation. *FD* culture independently, *FD* combination with *La-5* monoculture (1:1) and *La-5* independently were used for fermenting cream. *FD* lyophilisated mesophile mixed culture includes: *Lactococcus lactis ssp. cremoris*, *Lactococcus lactis ssp. lactis*, *Leuconostoc mesenteroides ssp. cremoris*, *Lactococcus lactis ssp. diacetylactis*. The output concentration of *FD* and *La-5* cultures in cream is $1 \cdot 10^6$ CFU/cm³.

Taking into account the features of start cultures development at different temperatures and technologies of sour-cream butter production in autumn-winter and spring-summer period of the year, four groups of butter were separated for the study:

I group (Z1, Z2, Z3 and L1, L2, L3 at fermenting *FD*, *FD + La-5*, *La-5* cream in winter and summer periods respectively). In this group fermentation took place at temperature (30±1) °C and physical maturing at temperature (7±1) °C.

II group (Z4, Z5, Z6 and L5, L6, L7 at fermenting *FD*, *FD + La-5*; *La-5* cream in winter and summer periods respectively). Cream fermentation in this group took place at temperature (37±1) °C and physical maturing at temperature (7±1) °C.

III group differed by the period of year. In autumn-winter period of year Z7, Z8, Z9 samples were formed at fermenting *FD*, *FD + La-5*, *La-5* cream respectively. Cream fermentation took place after physical maturing. Fermentation temperature was (20±1) °C. In spring-summer period L7, L8, L9 samples were formed at fermenting *FD*, *FD + La-5*, *La-5* cream respectively. In this group cream fermentation at temperature (20±1) °C took place before physical fermentation.

IV group (Z10, Z11, Z12 and L10, L11, L12 at fermenting *FD*, *FD + La-5*, *La-5* cream in winter and summer periods respectively) – introduction of fermenting cultures in butter kernel. The output concentration of cells at inoculations – $1 \cdot 10^8$ CFU/cm³. Z10–Z12 samples were kept at temperature (9 ± 1) °C during 3 days for the increase of acid-creating activity.

Acid-creating activity was controlled in fermented cream by the change of titrated and active acidity and plasma acidity. The study was thrice repeated. Sour-cream butter was prepared by the method of periodic beating of cream and packaged in polystyrene glasses with capacity 200 ml. Butter was stored at temperature $0 \dots -5$ °C during 42 days. Organoleptic and microbiological parameters were determined in sour-cream butter samples. The number of viable *FD* and *La-5* cells was analyzed in ready product on 1-st day and at storage on 7-th, 14-th 21-th, 28-th, 35-th and 42-th days.

2. 2. Methods of experimental studies, used at the research

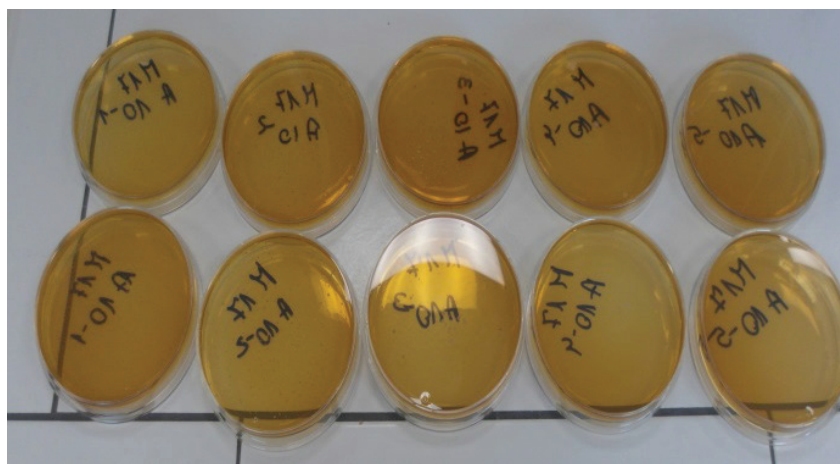
At the study the determination of titration acidity in cream was realized according to SST 3624-92 “Milk and milk products. Titrimetric methods of acidity determination”. The determination of active acidity was carried out by potentiometric method according to SST 19881-74 “Potentiometric analyzers for control of pH in milk and milk products. General technical conditions”.

The determination of organoleptic parameters of butter samples were carried out at temperature (12 ± 2) °C according to the requirements of SSTC 4399:2005 “Cream butter. Technical conditions” and TC U 10.5-00492990-006:2014 “Sour-cream butter with probiotic properties”.

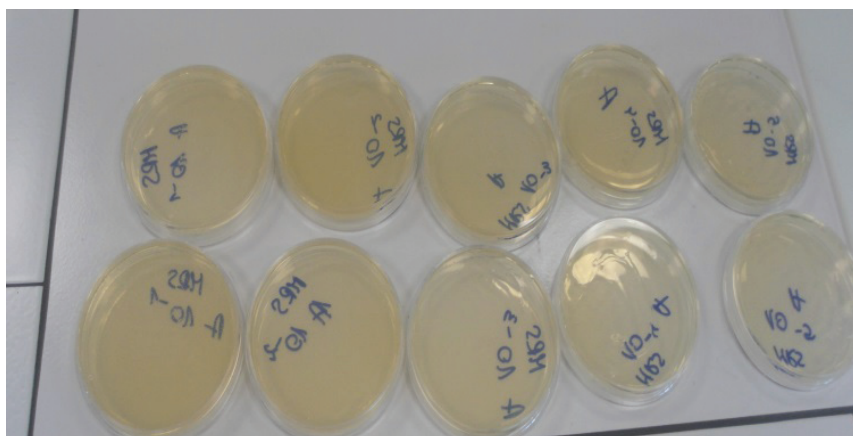
The preparation of butter samples for microbiological analyses was carried out according to the following method: Milk and milk products. Preparation of samples and solutions for microbiological studies (IDF 122C:1996, IDT): SSTC IDF 122C:2003). The cultivation of microorganisms was realized according to the normative documents “Milk and milk products. Determination of microorganism number. Method of colonies calculation at temperature $30 < \text{degrees} > \text{C}$ ” (IDF 100B:1991, IDT): SSTC IDF 100B:2003. The sample with mass 10 g was taken by the sterile palette knife, melted on water bath at temperature 45 °C and mixed to the homogenous emulsion. For calculating the viable cells the preparation of decimal solutions was carried out in 0,1 % peptone water. *FD* cultures were calculated by inoculation (in two parallels) using M17 Agar CM 0785 medium (Himedia, India) (Fig. 2, a). The cups were incubated in anaerobic conditions during 72 hours at temperature 30 °C (Fig. 1). *L. acidophilus La-5* culture was calculated by inoculation (in two parallels) using Lactobacillus MRS Agar M 641-500G medium (Himedia, India) (Fig. 2, b). The cups were incubated in anaerobic conditions during 72 hours at temperature 37 °C. The results were registered as a number colony forming units (CFU) in g of product.



Fig. 1. Thermostat by Memmert IPP-200 (Germany)



a



b

Fig. 2. Petri cups at using mediums: *a* – M17 Agar; *b* – Lactobacillus MRS Agar

Mathematical processing of experimental data was used using the programs of statistical processing Microsoft Excel 2003 and SC4 (Ukraine).

3. Results

In spring-summer and autumn-winter periods of year the samples Z1, Z2 and L1, L2 kept the pure, expressed pleasant sour-milk flavor and smell during 35 days. Other samples had insufficiently pure flavor and smell with expressed sour smack already on 26 day of storage.

During the storage of sour-cream butter the number of *FD* and *La-5* viable cells in 1 g of product was determined with 7 day periodicity.

At the beginning of storage the number of *FD* viable cells was 7,8–8,3 lg CFU/g. During 14 days of storage their quantity increased to 8,1–8,7 lg CFU/g that was caused by *La-5* biomass increase (**Fig. 3, a**). But in next period of storage there was observed the gradual decrease of the number of *FD* viable cells to the level 6,5–6,9 lg CFU/g (on 42 day).

The best viability during 42 days of storage was inherent to the samples Z1 and Z2, at which production the optimal conditions of cream fermentation for *Flora Danica* mixed mesophile cultures were used – (30±1) °C.

For determining the probiotic properties of sour-cream butter at storage, the change of the number of *La-5* viable cells was studied. During 14 days of storage the number of *La-5* increased (**Fig. 4**), so they were resistant to acid medium at storage.

After 14 days the decrease of cells number was registered and on 42 day it was 6,9 lg CFU/g. Such number doesn't provide the probiotic features of product that is why it must be stored no more than 35 days at temperature 0...-5 °C. The analysis of dynamics of *La-5* viable cells number in other samples testifies to its similarity.

The sample Z2 during 35 days of storage was characterized with great number of viable cells of both cultures – *FD* and *La-5*.

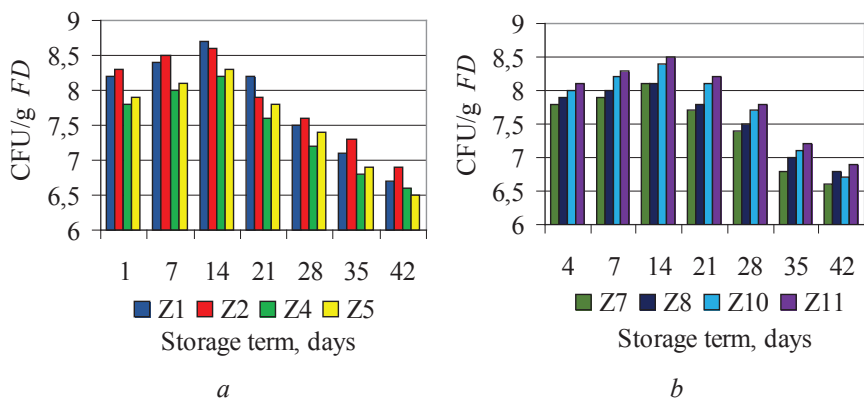


Fig. 3. Change of the number of *FD* viable cells at storage of sour-cream butter at temperature 0...-5 °C in autumn-winter period of year: *a* – in I and II groups; *b* – in III and IV group

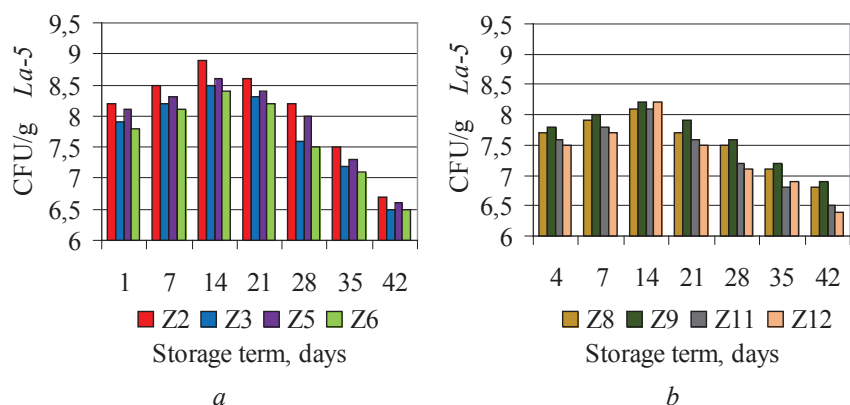


Fig. 4. Change of the number of *La-5* viable cells at storage of sour-cream butter at temperature 0...-5 °C in autumn-winter period of year: *a* – in I and II group; *b* – in III and IV group

In spring-summer period of year at the beginning of storage the number of *FD* viable cells was 8,3 and 8,2 lg CFU/g for L1 and L2 respectively. At storage at temperature 0...-5 °C in the butter samples on 14 day was observed the maximal number of sour-milk lactococci – 8,7 and 8,6 lg CFU/g for L1 and L2 respectively. Then the number of *FD* viable cells in diapason 14–42 days of storage decreased to 6,9 and 6,7 lg CFU/g (**Fig. 5, a**).

The best viability on 42 day of storage was inherent to the sample L1 at using *FD* independently at fermentation temperature (30±1) °C. All other samples in this period were characterized with less number of *FD* viable cells.

The most stable at storage are *La-5* cells (**Fig. 6, a**). The phase of their active growth in sour-cream butter was 14 days. During this period in L2 sample the number of viable cells increased from 8,5 to 8,9 lg CFU/g. then the number of cells gradually decreased and on 42 day reached 6,9 lg CFU/g. Such number of viable cells doesn't refer the product to probiotic ones, so it is expedient to store it no longer than 35 days at temperature 0...-5 °C. On 35 day of storage the number of cells in L2 was 7,6 lg CFU/g.

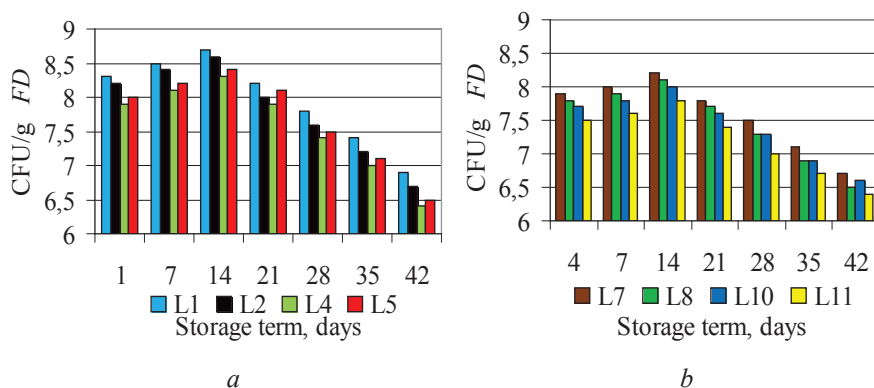


Fig. 5. Change of the number of *FD* viable cells at sour-cream butter storage at temperature 0...-5 °C in spring-summer period of time: *a* – in I and II group; *b* – in III and IV group

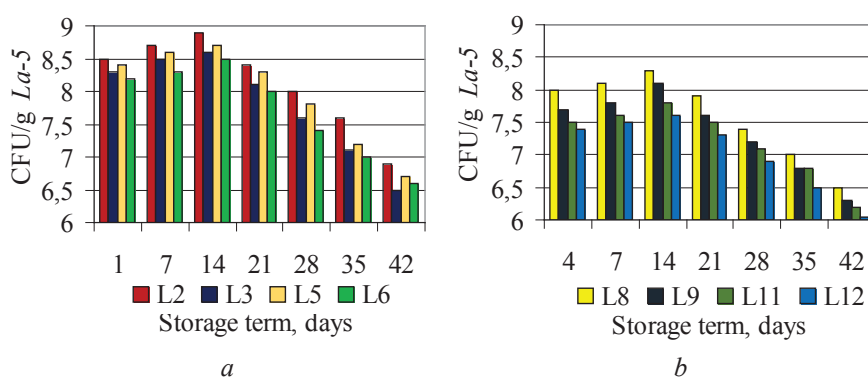


Fig. 6. Change of the number of *La-5* viable cells at sour-cream butter storage at temperature 0...-5 °C in spring-summer period of time: *a* – in I and II group; *b* – in III and IV group

As to the features of summer and winter periods, cream fermentation is more active in summer one that is indicated by more number of cells of both microbial cultures that is completely regular due to the higher content of growth factors in summer milk. In spring-summer period of time the number of *La-5* viable cells in L2 sample of ready product was by 10,4 % more comparing with autumn-winter one.

4. Conclusions

The most number of *Lactobacillus acidophilus La-5* viable cells was registered for the sample, fermented at temperature (30±1) °C at combination of *FD* and *La-5*. The storage term of cultured butter with probiotic properties is 35 days at temperature 0...-5 °C.

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