

JUSTIFICATION OF THE SHELF LIFE OF DAIRY PRODUCTS BASED ON GOAT'S MILK

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ABSTRACT

The article presents the results of studies on the number of survival of lactic acid bacteria, which determine the shelf life of dairy products during storage. The studies were carried out at the Department of Food Biotechnology of the Almaty Technological University, as well as in the laboratory of biotechnology of specialized products and dietary supplements of the Kazakh Academy of Nutrition. In the preparation of fermented milk products, a starter consisting of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus fermentum 14* was used. *Lactobacillus fermentum 14*, as a probiotic, exhibits antagonistic properties, which helps to extend the shelf life and does not allow yeast and mold to grow in the final products. *Lactobacillus bulgaricus*, in turn, synthesizes a specific enzyme, peptidoglycan hydrolase, which is responsible for the hydrolysis of peptidoglycan which is an important component of the bacterial cell wall. When dairy products are enriched with biologically active substances of berry syrups *Lactobacillus bulgaricus* actively synthesizes extracellular polysaccharides, which improve the structure of the clot, increase stability and prevent syneresis. *Streptococcus thermophilus* is a mild curing thermophilic culture with a high degree of viscosity that promotes the formation of a good clot. Rosehip, hawthorn and mountain ash syrups, grape peel extract enriched dairy products with biologically active substances, which improved the growth and development of lactic acid microorganisms. The addition of phytofillers not only *enhance* the microscopic picture, but also improves the consistency and odors of the products.

Keywords: goat milk, dairy product, starter culture, lactic acid microorganisms, microbiological indicators, plant fillers, syrup.

INTRODUCTION

Milk and dairy products are staples of daily consumption. The importance of milk and dairy products in human nutrition is significant, because they are a products of high nutritional value and the basis for the development of intestinal microflora, rich in trace elements, vitamins and amino acids [1, 2].

In terms of global consumption and production, goat's milk is second only to cow's. At the same time, a goat needs 6 times less food than a cow for normal life, while it produces enough milk to meet the needs of one average family: 3.5 - 3.8 liters per day. Goats rarely get sick, are not susceptible to brucellosis and tuberculosis. Goat's milk has excellent taste qualities without extraneous odors. Goats have the ability to convert feed carotene into vitamin A, so goat's milk is paler in color than cow's. Compared to cow's milk, goat's milk has a higher content of fat, protein and calcium; vitamin A, which has a positive effect on the condition of the eyes and skin; vitamin PP, which affects the course of redox processes in the body. Goat milk contains 6 times more cobalt, which is part of cobalamin (vitamin B₁₂), which is responsible for hematopoiesis and maturation of erythrocytes (erythrocytes). Also goat's milk contains less lactose (milk sugar) than cow's milk, so it is suitable for people with a lack of lactase [3-6].

Dairy products have a beneficial effect on the formation of intestinal microflora, increase the secretion of gastric glands, normalize intestinal motility, its microflora, and inhibit the growth and development of putrefactive bacteria. In addition, they contribute to better absorption of calcium, magnesium, phosphorus and iron. The outstanding Russian physiologist I. Mechnikov was convinced that the aging process of the body

is caused by putrefactive bacteria of the intestinal microflora and that lactic acid bacteria, in particular, Bulgarian bacillus, can have a beneficial effect on life expectancy. Therefore the daily intake of dairy products optimally affects the formation of intestinal microflora and will avoid colitis, chronic constipation and dysbacteriosis [7, 8].

In recent years, there has been a trend to create a new generation of food products, the main characteristics of which are a balanced composition, a high content of protein, vitamins, as well as probiotic properties [9, 10]. However, with the modern rhythm of life, it is sometimes not easy to independently balance your diet according to all the rules, then modern technologies come to the rescue. The use of modern biotechnological techniques in combination with traditional food technologies makes it possible to create dairy products with desired properties [11]. This can be achieved by combining dairy and vegetable components [12-14].

To increase the medicinal properties of fermented milk products, it is advisable to enrich them with berries and vegetable fillers. Hawthorn, wild rose, rowan are among the most popular berries, including a wide range of active ingredients such as essential oils, phenolic, proteolytic enzymes, vitamins and trace elements [15-17]. Rosehip berries have a bactericidal and antioxidant effect and the introduction of hawthorn syrup into the composition of products was due to the fact that it is an important botanical cardiotonic and is used in diseases of the heart and blood vessels [18]. The strong antioxidant and protective abilities of hawthorn against biological tissues and cell membrane lipids against toxicity are manifested in dose-dependent free radical scavenging activ-

ity. Rowan berries are a rich source of antioxidants such as rutin, quercetin-3-glucoside, and quercetin-3-D-galactoside. Scientific studies have proven anti-inflammatory and anti-diabetic effects, which are determined by a noticeable amount of ascorbic acid, organic acids, phenolic compounds, and carotenoids. These properties allow the use of berry syrups as additives to dairy products to enrich them with biologically active substances and enhance their taste [19].

As any fermented milk product, yogurt is certainly useful (especially with various additives), but live bacteria remain in it for no more than one or two weeks [20, 21]. Therefore, obtaining a fermented milk product with a plant fillers that retains beneficial bacteria at the end of the shelf life is an urgent task.

In this regard, the purpose of our work was to determine the quantitative composition of lactic acid microorganisms in dairy products at different periods of storage (at the beginning and end of the shelf life). Research on the development of the composition, formulations and technology of fermented milk (dairy) products with plant fillers was carried out at the Department of Food Biotechnology of the Almaty Technological University, as well as in the laboratory of biotechnology of specialized products and biologically active additives of the Kazakh Academy of Nutrition.

MATERIALS AND METHODS

Goat milk was used for the study from the Almaty region. For the study, goat milk from the Almaty region, that was pasteurized at $(84 \pm 2)^{\circ}\text{C}$ with an exposure of 15-20 seconds.

A combination of Streptococcus *Streptococcus salivarius subspecies termophilus* T45 (Danisco, Germany), *Lactobacillus delbruki subspecies bulgaricus* B6 (University of Food Technologies, Plovdiv, Bulgaria) strains and *Lactobacillus fermentum* 14 was used as starter culture. *Lactobacillus fermentum* 14 was isolated from goat milk and deposited in the Republican Collection of Microorganisms (Astana, the collection number of the strain is B-RCM 1020).

In order to completely replace sugar and increase the nutritional value of yoghurts, the following plant fillers were introduced into experimental samples of yogurts: syrups (PC «Firm» Kyzylmay», Kazakhstan), limited liability company «Zdorov'e Altaya» (Barnaul, Russia); grape peel extract (powder), (Healthlife Biotechnology Co., Ltd, China);

We have investigated 5 samples of the fermented product. Rosehip, hawthorn and mountain ash syrups in the amount of 5%, as well as grape peel extract were used as a plant fillers for fermented milk products.

We used selective medium MRS (Merck KGaA, Germany), the main feature of which is a low pH (<5.4) and a high concentration of acetate ions, which is an inhibitor of many microorganisms. The total bacterial contamination (reductase test, determination of the *Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms*, QMA-FAnM) was carried out according to GOST 9225-84 "Milk and dairy products. Methods of microbiological analysis". The number of lactic acid bacteria in samples of dairy products was determined according to GOST 10444.15-94. The method of limiting dilutions was used, followed by the calculation of the most probable number of lactic acid microor-

ganisms. Determination of belonging of isolated bacteria to the genus *Lactobacillus bulgaricus* was carried out according to GOST 10444.11-89 "Food products. Methods for the detection of lactic acid microorganisms" in relation to Gram stain, mobility. Bacteria of the genus *Lactobacillus* included microaerophilic, Gram-positive, rod-shaped, immobile, non-spore-forming microorganisms.

Seeding from each dilution was inoculated with at least two volumes of 0.1 cm^3 on the agar surface, incubated for 2-3 days at 37°C under anaerobic conditions using anaerobic balloons and gas generating units (Merck KGaA, Germany). All analyzes were carried out in duplicate. The content of lactic acid bacteria in products should be at least 10^7 CFU/cm^3 , with additives - at least 10^5 CFU/cm^3 , and yeast - not more than 50 CFU/cm^3 .

The grown colonies were counted in 1, 8, 15, 20, and 26 days of incubation in a thermostat. To calculate the number of microorganism cells in 1 ml of the initial suspension, the results of parallel inoculations from one dilution were summarized and the average number of colonies for this dilution was determined. The best dilution was considered to be the one that, after inoculation on an agar nutrient medium, formed 30 colonies or more. The data obtained was substituted into the formula:

$$M = \frac{a * 10^n}{V}$$

where M is the number of cells in 1 ml; a is the average number of colonies when seeded from a given dilution; V is the volume of the suspension in ml, taken for inoculation; 10 - dilution factor; n is the serial number of the dilution.

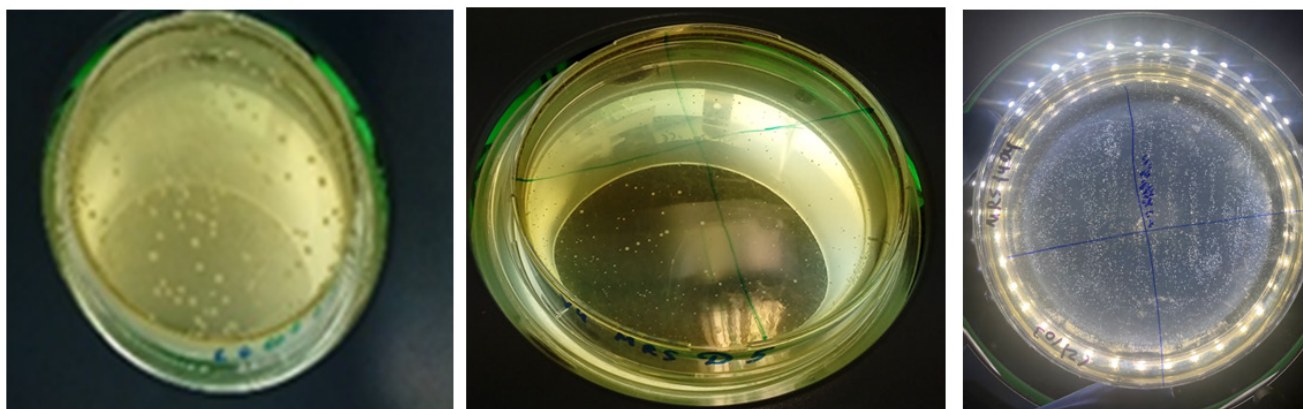
To determine the water-holding capacity, a centrifuge with a separation factor of 2000 was used. 10 cm^3 of a premixed clot was added to a centrifuge tube placed in a centrifuge. The test tubes were arranged one against the other in an even number. Then the test tubes with the test sample were centrifuged for 10 minutes. To measure the volume, the serum was temporarily poured into a graduated tube. At the end of the process, a graph of serum separation was built, and the amount of serum released from 10 cm^3 of the test sample ($\text{cm}^3/10\text{ cm}^3$) was taken as the final option. The results were recorded as a percentage.

All experiments were performed in 3 replicates. The reliability of the results was determined by Student's t-test, $p < 0.05$.

RESULTS

The following five samples of dairy products (yogurts) have been developed: control sample; sample 1 is dairy product with the addition of hawthorn syrup and grape peel extract; sample 2 is dairy product with the addition of rosehip syrup and grape peel extract; sample 3 is dairy product fermented milk product with the addition of rowan syrup and grape peel extract and sample 3 is dairy product with grape peel extract.

Five samples from each dilution were prepared and analyzed for each variant of nutrient medium.

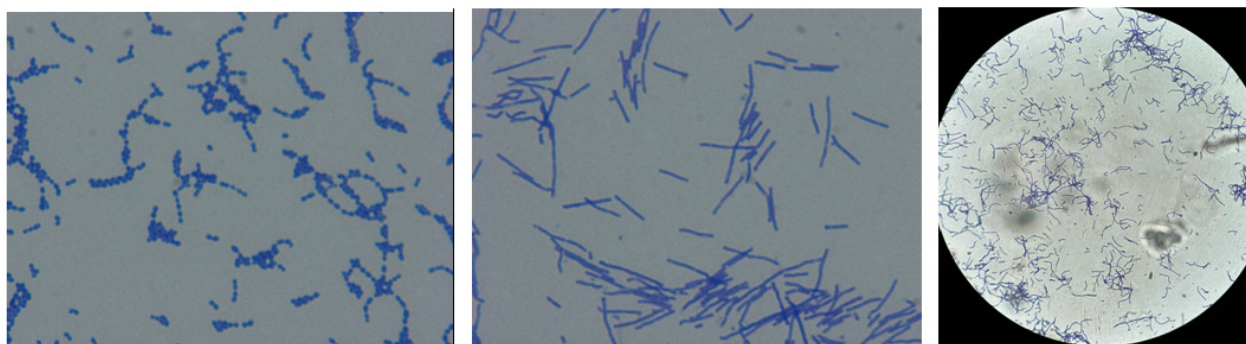


a

b

c

Picture 1 is demonstrated the result of the cultivation of probiotic microorganisms on the MRS 5.4 nutrient medium: Picture 1 - Cultivation of probiotic microorganisms, delution 1:10⁻⁷. on the MRS at pH 5.4 agar: a) *Lb. bulgaricus*, b) *Str. Thermophilus*, c) *Lb. fermentum 14*



a

b

c

Picture 2 - Microscopy of lactic acid bacteria: a) *Str. Thermophiles*, b) *Lb. bulgaricus*, c) *Lb. fermentum 14*, at 1000x magnification

Table 1 – Water holding capacity of yogurt samples

The storage time of yogurt, days	The percentage of whey released during centrifugation				
	Control sample	Sample 1	Sample 2	Sample 3	Sample 4
1	no	no	no	no	no
8	0.8	0.6	0.7	0.5	0.4
15	1.6	1.4	1.5	1.3	1.2
20	3.1	3.3	3.2	3.3	3.4
26	5.0	4.8	4.9	4.8	4.7

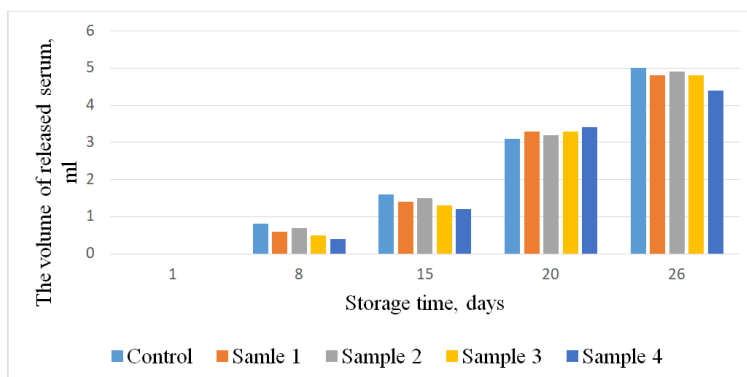


Figure 1 - Water-holding capacity of test samples in comparison with control afetr centrifuging at a rotation speed of 3000 min⁻¹

Lb. bulgaricus, *Str. Thermophilus* and *Lb. fermentum* 14. Picture 2 is demonstrated the microscopy of samples diluted $1:10^{-7}$ on MRS 5.4 medium.

Medium-sized gray-white colonies with a smooth edge, convex shiny with a spherical surface are observed on a nutrient medium. The microscopic picture is as follows: large lactic acid bacteria *Lactobacillus bulgaricus*, an accumulation of small rods *Lactobacillus fermentum* 14 and *Streptococcus thermophilus* are clearly visible.

Current trends towards increasing the shelf life of products put forward the requirements for maintaining a good consistency during long-term storage. In this regard, studies of the water-holding capacity of clots were carried out, which were determined by the volume of whey released during centrifugation of dairy products for 10 minutes at a speed of 3000 min^{-1} at a temperature of $20 \text{ }^{\circ}\text{C}$. The water-holding capacity

of the test samples in comparison with the control is presented in Table 1 and Figure 1.

As can be seen from the data presented in Figure 1, during the storage period, no significant difference was observed in the control and in the products during centrifugation for 10 minutes and measurement of the serum separation rate. The volume of released whey in products after 26 days of storage is $4.4\text{-}4.7 \text{ cm}^3/10 \text{ cm}^3$.

According to the data obtained, the clot based on berry syrups and grape skin extract has good water-retaining properties. This is apparently due to the increase in the content of whey proteins in the milk base, pectin substances in syrups from mountain ash and hawthorn, as well as grape skin extract, which, due to their high hydrophilic properties, increase the water-retaining capacity of casein and slow down the separation of whey. The change in titratable and active acidity of

Table 2 - Change in titratable and active acidity of the product during storage

Storage duration, days	Control		Sample 1		Sample 2		Sample 3		Sample 4	
	$^{\circ}\text{T}$	pH	$^{\circ}\text{T}$	pH	$^{\circ}\text{T}$	pH	$^{\circ}\text{T}$	pH	$^{\circ}\text{T}$	pH
1	75±0.5	4,83±0.05	76±1.5	4,82±0.05	81±1.3	4,80±0.05	80±1.7	4,79±0.05	78±1.2	4,81±0.05
8	90±0.7	4,76±0.05	96±2	4,72±0.07	98±2	4,71±0.06	102±0.9	4,68±0.05	94±0.7	4,73±0.07
15	103±0.5	4,70±0.02	111±0.7	4,66±0.05	113±1.5	4,65±0.03	119±2	4,62±0.07	107±1.5	4,67±0.04
20	114±1.2	4,68±0.05	123±0.9	4,63±0.05	125±0.5	4,62±0.04	128±0.7	4,59±0.05	118±1.6	4,65±0.03
26	128±2.0	4,63±0.03	134±2.0	4,56±0.09	136±1.2	4,54±0.07	140±1.5	4,50±0.05	130±2.0	4,60±0.02

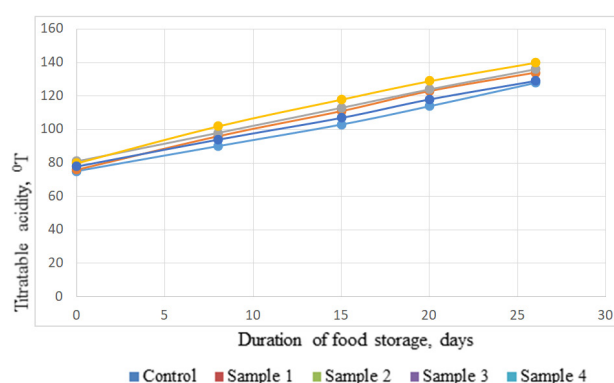


Figure 2 - Dynamics of changes in the titratable acidity of the samples.

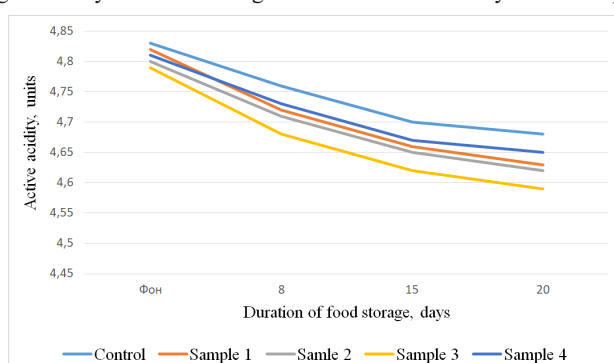


Figure 3 - Dynamics of changes in the active acidity of the samples

products during storage is presented in Table 2.

The dynamics of changes in the titratable and active acidity of the control and experimental samples are shown in Figures 1 and 2.

As can be seen from the data presented in Figures 2 and 3, there were no significant differences in the dynamics of acid accumulation of titratable and active acidity between the control and experimental samples with vegetable fillers introduced after pasteurization. The dynamics of acid accumulation in the experimental and control samples was similar. During storage, the titratable acidity reached 128-140 °T, the active acidity was pH 4.50-4.63.

An important step in justifying the shelf life of a product is a microbiological study. Given the fact that an acidic environment is favorable for the development of yeast and mold fungi, it is advisable to study the dynamics of their content during the storage of fermented milk products. The results showed a change in the number of probiotic microorganisms that make up the sourdough of the studied products during the shelf life.

The results of the microbiological study of dairy products during the shelf life are shown in Tables 3 and 4.

Dairy products during storage must be subjected to a study on microbiological quality indicators. The cause of microbiological spoilage of dairy products is usually the reproduction of yeast and mold.

At the end of the shelf life the number of lactic acid bacteria in all samples decreased by three orders of magnitude as follows: in the control sample - from 4.9×10^9 CFU/cm³ decreased to 1.0×10^7 CFU/cm³, sample 1 - from 6.3×10^9 CFU/cm³ to 1.5×10^7 CFU/cm³, sample 2 - from 6.4×10^9 CFU/cm³ to 1.6×10^7 CFU/cm³, sample 3 - from 6.5×10^9 CFU/cm³ to 1.4×10^7 CFU/cm³ and sample 4 from 5.3×10^9 CFU/cm³ to 1.3×10^7 CFU/cm³.

DISCUSSION

The results show that the largest number of lactic acid bacteria by the beginning of storage has grown in sample 3 (with rowan syrup and grape skin extract), which is 6.5×10^{10} CFU/cm³. At the beginning and at the end of the shelf life, yeast and mold were not found in the samples. The number of lactic acid microorganisms in all samples during storage remained within the permissible norm: in samples with syrups and grape skin extract, the content of lactic acid microorganisms is 13-50% higher compared to the control and is 0.4-1.3 CFU/cm³.

This may be due to the continued development of lactic acid bulgaric and *Lb. fermentum*, which are part of the leaven. The safety indicators of dairy products comply with the requirements of GOST: pathogenic bacteria, mold and yeast were not detected.

In the manufacture of syrup, berries are not subjected to long processing, so they retain their useful properties and vitamins, which makes it possible to continuously provide food production with valuable sources of biologically active substances. Only natural milk was used in the formulation of the products, without the addition of powdered milk, which favorably affects the microflora of the starter culture.

It has been established that the introduction of plant-based fillers into the mixture contributes to the intensification of the fermentation process, increases the moisture-retaining ability and improves the structural and mechanical properties of the clots. *Lactobacillus bulgaricus* and *Lactobacillus fermentum* synthesize a specific enzyme peptidoglycan hydrolase, responsible for the hydrolysis of an important component of the bacterial cell wall (peptidoglycan) and for growth on nutrient media they need vitamins throughout the development period. Apparently, when enriching dairy products with biologically active substances of berry syrups, lactic acid bacteria actively synthesize extracellular polysaccharides, which improve the structure, increase stability and prevent synere-

Table 3 - Microbiological studies of dairy products by the beginning of the shelf life

Sample No.	Number of microorganisms, CFU/cm ³	
	lactic acid bacteria	Yeast
Control	$4,9 \times 10^9$, n=9	-
1	$6,3 \times 10^9$, n=9	-
2	$6,4 \times 10^9$, n=9	-
3	$6,5 \times 10^9$, n=9	-
4	$5,3 \times 10^9$, n=9	-

Table 4 - Microbiological studies of dairy products at the end of the shelf life

Sample No.	Number of microorganisms, CFU/cm ³	
	lactic acid bacteria	Yeast
Контроль	$1,0 \times 10^7$, n=7	-
2	$1,5 \times 10^7$, n=7	-
3	$1,6 \times 10^7$, n=7	-
4	$1,4 \times 10^7$, n=7	-
5	$1,3 \times 10^7$, n=7	-

sis of the final products.

It is known that the proto-cooperation of yogurt bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* consists in the production of amino acids and small peptides by lactic acid bacteria and the production of formic acid by *S. thermophilus*. These compounds are the limiting substrates in milk [22, 23]. However, the proto-cooperation of yogurt bacteria cannot be fully explained by the production of these aforementioned compounds. It was found that *S. thermophilus* in milk produces an excess of carbon dioxide necessary for optimal growth of *L. bulgaricus*. It has been established that carbon dioxide is not removed from the milk mixture during heat treatment (or vacuuming) [24].

CONCLUSIONS

Thus, the study showed that the number of viable lactic acid bacteria cells in freshly processed products exceeds the level established by TR CU 033/2013 «On the safety of milk and dairy products» by several orders of magnitude. A high survival rate of lactic acid bacteria was also established after the expiration date of 20 days. Taking into account the fact that the acidic environment is favorable for the development of yeast and mold fungi, it was advisable to study the dynamics of their content during the storage of fermented milk products. The main criterion for a positive sanitary and epidemiological assessment of the shelf life of the developed dairy products is the absence of negative dynamics of the studied indicators in the samples. The addition of vegetable fillers does not worsen the microscopic picture of products and allows them to be enriched with biologically active substances of hawthorn, rosehip, rowan and grape peel extract.

It is determined that the moisture-retaining ability, as well as the viscosity of the samples of fermented milk products, is directly dependent on the composition of the milk base and with an increase in the number of plant fillers in the samples, the moisture-retaining ability increases. This is due to the properties of syrups and dry grape peel extract, which give the final products not only a pleasant taste, but also a moisture-retaining ability, preserving their consumer properties during the shelf life. Thus, the addition of vegetable filler increases the shelf life of fermented milk products, and the number of beneficial bacteria at the end of the shelf life meets the requirements of GOST for milk and dairy products [25].

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ӘОЖ 579.678

ЕШКІ СҮТІНЕН АЛЫНҒАН СҮТ ӨНІМДЕРІНІҢ ЖАРАМДЫЛЫҚ МЕРЗІМІНІҢ НЕГІЗДЕМЕСІ

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ТҮЙІН

Мақалада сақтау процесінде ашытылған сүт өнімдерінің жарамдылық мерзімін анықтайтын лактобактериялардың өмір сүруін анықтау бойынша зерттеу нәтижелері келтірілген. Зерттеулер Алматы технологиялық университетінің «Тағамдық биотехнология» кафедрасында, сондай-ақ Қазақ тағамтану академиясының мамандандырылған өнімдер мен биологиялық белсенді қоспалар биотехнологиясы зертханасында жүргізілді. Ашытылған сүт өнімдерін дайындауда *Streptococcus thermophilus*, *Lactobacillus bulgaricus* және *Lactobacillus fermentum 14*-тен тұратын ашытқы қолданылды. *Lactobacillus fermentum 14* пробиотик ретінде антагонистік қасиеттерді көрсетеді, бұл сақтау мерзімін ұзартуға көмектеседі және соңғы өнімдерде ашытқы мен көгерудің өсуіне жол бермейді. *Lactobacillus bulgaricus* өз кезегінде бактериялардың жасуша қабырғасының маңызды компоненті – пептидогликанның гидролизіне жауап беретін арнайы пептидогликан гидролаза ферментін синтездейді. Ашытылған сүт өнімдерін жидек сироптарының биологиялық белсенді заттарымен байыту кезінде Болгар таяқшасы тромб құрылымын жақсартатын, тұрақтылықты арттыратын және синерезистің алдын алатын жасушадан тыс полисахаридтерді белсенді түрде синтездейді. *Streptococcus thermophilus* - тұтқырлығы жоғары жұмсақ ашытылатын термофильді дақыл, Жақсы сүт ұйығышының пайда болуына ықпал етеді. Итмұрын, долана және тау күлінің сироптары, жүзім қабығының сығындысы ашытылған сүт өнімдерін биологиялық белсенді заттармен байытып, сүт қышқылды микроорганизмдерінің өсуі мен дамуын және өнімдерінің консистенциясын жақсартады.

Негізгі сөздер: ешкі сүті, сүтқышқылды өнім, ашытқы, сүт қышқылды микроорганизмдер, микробиологиялық көрсеткіштер, фито толтырғыштар, сироптар.

УДК 579.678

ОБОСНОВАНИЕ СРОКА ГОДНОСТИ КИСЛОМОЛОЧНЫХ ПРОДУКТОВ НА ОСНОВЕ КОЗЬЕГО МОЛОКА

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АННОТАЦИЯ

В статье представлены результаты исследований по определению выживаемости лактобактерий, обуславливающих сроки годности кисломолочных продуктов в процессе хранения. Исследования проведены на кафедре «Пищевой биотехнологии» Алматинского Технологического Университета, а также в лаборатории биотехнологии специализированных продуктов и биологически активных добавок Казахской академии питания. При приготовлении кисломолочных продуктов использовали закваску, состоящую из *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus fermentum 14*. *Lactobacillus fermentum 14* как пробиотик, проявляет антагонистические свойства, что способствует продлению сроков хранения и не позволяет расти дрожжам и плесени в конечных продуктах. *Lactobacillus bulgaricus*, в свою очередь, синтезирует специфический фермент пептидогликангидролазу, ответственного за гидролиз важного компонента клеточной стенки бактерий – пептидогликана. При обогащении кисломолочных продуктов биологически активными веществами ягодных сиропов болгарская палочка активно синтезирует внеклеточные полисахариды, которые улучшают структуру сгустка, повышают стабильность и предотвращают синерезис. *Streptococcus thermophilus* - мягкосквашивающая термофильная культура, обладающая высокой степенью вязкости, способствует образованию хорошего сгустка. Сиропы ягод шиповника, боярышника и рябины, экстракт виноградной кожуры обогатили кисломолочные продукты биологически активными веществами, что улучшило рост и развитие молочнокислых микроорганизмов. Добавление фитонаполнителей не только не ухудшает микроскопическую картину продукта, но и улучшает консистенцию продукта.

Ключевые слова: козье молоко, кисломолочный продукт, закваска, молочнокислые микроорганизмы, микробиологические показатели, фитонаполнители, сиропы.