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TECHNOLOGICAL FEATURES OF GOAT'S AND COW'S HARD CHEESE PRODUCTION USING BIOLOGICAL PROCESSING OF MILK

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ABSTRACT

The article presents a study of biological processing of milk of cows, goats and the mixture of cow's and goat's milk (50:50) by applying the cultures of *L. acidophilus* during the production of cheeses with a low temperature of the second heating. This approach accelerates the technological process by 3-4 times and guarantees a long-term storage of products without deteriorating their quality.

The reduction of the time of rennet curd formation, whey removal, kneading, hot self-pressing, salting and ripening were specific features of the production process.

The study showed the dynamics of fermenting microbial flora's quantity as well as the content of benzoic and sorbic acids, which guarantee the long-term storage of cheeses.

We have studied the influence of our technology on physicochemical and rheological parameters of cheeses in relation to the type of milk.

1. Introduction

The demand for wider selection of cheeses is growing, thereby giving rise to measures aimed at changing and stabilizing biochemical and technological parameters of raw milk; triggering the development of special fermenting agents, various physicochemical and biological methods of raw material processing, and so on.

In order to improve the technological and microbiological properties of raw milk, additional measures are used: bacteriological purification by cream settling and bacterial separation, introduction of nitrates, lysozyme, inhibitory yeasts, nisin, exogenous enzymes and peroxide-catalase treatment.

The quality of milk is important because there is a risk of contamination by spontaneous microbial flora during storage of raw chilled milk (Richard & Auclair, 1989). The use of low-quality raw milk in cheese-making is possible only if, in addition to traditional pasteurization, supplemental methods of neutralization of foreign microflora are introduced, particularly the additional heat treatment of milk and its ripening. These methods help to effectively destroy foreign microflora of raw materials without adversely affecting the technological properties (Shulga, 2003).

Ripening of milk reduces the duration of technological stages, as it intensifies biochemical processes, which increase the content of total, soluble and non-protein nitrogen. One of the promising areas of milk ripening could be a method of introducing microorganisms, enzymes lactase (β-galactosidase) or protease. The combinations of drugs including *Lactococcus lactis* ssp. *lactis, L. lactis* ssp. *cremoris, L. lactis* subsp. *lactis*

biovar. *diacetylactis* are used during the ripening of milk (Richard & Auclair, 1989; Davidson et al., 2015). Samples with the strains of *Lactobacillus plantarum*, *L. lactis* ssp. *lactis*, which have an antagonistic effect on pathogens of butyric fermentation in cheese are also applied as preventive measures during the production of cheeses with a high temperature of the second heating (Kuznetsov & Shiler, 2003; Hassan et al., 2021).

To increase the term of storage flavoring and preserving agents of chemical origin are being used more and more in food industry, particularly in cheese production (Beltyukova & Liventsova, 2013).

At the same time healthy diet is a world tendency which encourages refusing chemical treatment of milk replacing it with biological treatment during cheese-making (Beltyukova & Liventsova, 2013; Davidson et al., 2015).

European Union regulates the usage of food additives with the Regulation No.1333/2008 from Dec 16, 2008, emphasizing that it is important to inform consumers about food additives in food.

In cheese-making it is preferably to exclude the chemical additives like nitrates (e.g. E251, E252). Potassium nitrate (E252) in cheeses could reduce to potassium nitrite (E249) with a participation of xanthinoxidase or nitrate reductase of milk (Munksgaard & Werner, 1987).

As a result the accumulated nitrites inhibit unwanted and dangerous microbiological processes, particularly a growth of *Clostridium* bacteria, and prevent a raw cheese detachment, but also may have a negative influence on the fermenting flora (Park et al., 2016; Hassan et al., 2021).

The preservatives in the form of potassium nitrate or sodium nitrate with a standard of up to 20 g/100 kg of milk are allowed in EU countries, except France, Greece, Italy and Switzerland (Park et al., 2016).

In addition to nitrates, lysozyme is widely used in cheese-making as it prevents the development of butyric acid bacteria by breaking the bonds in the molecules of cell walls. This is a fundamental difference between the mechanisms of action of lysozyme and nitrates (Davidson et al., 2015).

There are acceptable prices for the other permitted preservatives for surface treatment of cheeses: sorbic acid (E200) and its salts (maximum level in the product is 1 mg/kg); benzoic acid and its salts; dehydroacetic acid, at the maximum level of 5 mg/kg. They could be of chemical rather than natural origin. These additives are effective in inhibiting the growth of yeast, mold, and cause an inhibitory effect on a wide range of bacteria (Tfouni & Toledo, 2002; Mroueh et al., 2008).

Benzoic acid is widely used in the food industry due to its low cost, colorlessness, lack of taste and relatively low toxicity (Beltyukova & Liventsova, 2013; Del Olmo et al., 2017; Bartáková et al., 2021). However, it is not allowed as an additive to dairy products in most countries, including Ukraine (Order of the Ministry of Health No. 222 of 23.07.96 On Approval of "Sanitary Rules and Norms for the Use of Food Additives").

A number of scientists (Kurisaki et al., 1973; Sieber et al., 1995; Tfouni & Toledo, 2002; Urbienė & Leskauskaitė, 2006; Esfandiari et al., 2013; Horníčková et al., 2014; Gucer et al., 2016; Han et al. 2016; Yerlikaya et al., 2021) investigated the formation of benzoic acid in the production of dairy products and cheese.

It is known, that benzoic acid is formed by various biochemical pathways: a) the formation of benzoic acid from hippuric acid in cheese production; b) through the hydrolysis of phenylalanine as an alternative pathway in cheeses (Sieber et al., 1995; Urbienė & Leskauskaitė, 2006; Horníčková et al., 2014); c) autooxidation of benzaldehyde produced by some strains of lactic acid bacteria and yeast (Mroueh et al., 2008; Gucer et al., 2016). Therefore, benzoic acid can be considered as one of the natural components of milk and dairy products.

The content of benzoic acid in fermented dairy products and cheeses depends on the applied lactic acid cultures, the origin of raw milk, duration and temperature of the fermentation process. It is known that strains of

Lactococcus lactis ssp. *lactis, L. lactis* ssp. *cremoris, L. lactis* ssp. *lactis* biovar. *diacetylactis, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus, Streptococcus thermophilus* produce benzoic acid in milk (Garmiene et al., 2010; Han et al. 2016; Bartáková et al., 2021).

The concentration of benzoic acid in dairy products can range from 2-5 mg/kg to 50 mg/kg (Urbienė & Leskauskaitė, 2006; Esfandiari et al., 2013), and in hard cheeses - from 1.6 to 90 mg/kg (Kurisaki et al., 1973; Horníčková et al., 2014).

Because numerous studies have shown the presence (Iammarino et al., 2011) of benzoic acid at the level of 20.5÷28.7 mg/kg in hard, semi-hard, soft, semi-soft, pickled and grated cheeses in which there were no chemical preservatives, it was proposed to introduce the maximum allowable level of benzoic acid - 40.0 mg/kg.

According to the data of Gucer et al. (2016), in Turkish cheeses (White Pickled, Kashar, Tulum), yogurts, ayran, butter, the content of benzoic acid varied in the amount of 2.3-160; 6.4–83; 0.6–12.8 and 0.0–7.3 mg/kg, respectively. All dairy products were made from the milk of cows, goats, sheep or mixtures thereof.

Milk usually contains a small amount of benzoates, but there are exceptions. There is evidence that this is due to contamination by foreign microflora, the use of veterinary drugs and feeds (soybean broth and especially flavorings of fruit pulp containing benzoic acid) (Sieber et al., 1995; Urbienė & Leskauskaitė, 2006).

The properties of sorbic acid are very similar to the properties of benzoic acid, but its content in dairy products is much lower. Sorbic acid (E200) and its salts - sorbates (E201-209) are also considered GRAS additives. In Ukraine, it is used in the production of condensed milk in the amount of 2000 mg/kg, and also during the manufacture of maturing cheeses and whizzed cheeses in the amount of 1000 mg/kg (Ministry of Health of Ukraine, 1996, No. 222). It has low toxicity because, it is rapidly metabolized by pathways similar to free fatty acids. There are data on the intolerance of sorbic acid in humans (Mroueh, et al., 2008; Park et al., 2016).

Dehydroacetic acid (E265) and its sodium salt (E266) are used for surface treatment of cheeses and cheese rind. These additives are effective against the development of mold, fungi and yeast. They can be added to animal feed (hay). E265 is active at pH values higher than benzoic acid. Although it is excreted in the urine, this process is very slow, therefore because of a constant consumption it is accumulated in the body. The daily consumption level should not exceed 3 mg/kg of body weight. In the EU (Official Journal of the European Union 354, 2008), the use of E265 and E266 is prohibited, in USA they are used for canning fruit (21CFR172.130, 2020), but in Ukraine they are allowed in food production (Ministry of Health of Ukraine, 1996, No. 222).

To avoid the use of chemicals in cheese manufacture, Ukraine has developed a method of biological treatment of milk with L. acidophilus of non-mucous race during the production of soft, semi-hard and hard cheeses (Fedin et al., 1985; Kolesnikova et al., 1991, Kolesnikova & Gening, 1994; Kolesnikova, 2000). At the same time, there is a trend of increasing the range of cheeses by using different raw materials: milk of cows, goats, buffaloes, sheep, deers, yaks and other animals.

The popularity of dairy products from goat's milk is due to their hypoallergenic properties, as well as due to the specific fatty acid and protein composition, which is good for people having gastric disorders, anemia, epilepsy, asthma, atherosclerosis and more.

Relevance: The development of technology for the production of cheeses capable of long-term storage, in which the content of chemical preservatives, dyes and other artificial substances is minimized or completely absent, is an urgent issue, especially taking into account the growing demand for this category of food.

Moreover, the population of goats and sheep in Ukraine has been increasing in recent years, which in turn highlights the need to create mini-enterprises and introduce technologies for the

production of various types of cheese from goat's and sheep's milk.

The purpose of our study were: to study the effect of biological treatment of milk using cultures of *L. acidophilus,* to show how it changes physicochemical and microbiological parameters of hard cheeses made from cow's milk, goat's milk and their mixture (50:50) during ripening and storage.

2. Materials and methods

The research was conducted in the Laboratory of the Institute of Post-Diploma Training of NUFT and the Department of Analytical Research and Food Quality of the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine.

2.1. Materials

2.1.1. Samples

The objects of research were cheeses made from milk of cows and goats, obtained from the farm "Ostrivske" located in Bila Tserkva district of Kyiv region. The research was carried out during the indoor period (June – September) in 2017-2018.

The samples of milk were labeled as cow's milk - K-1, a mixture of milk from cows and goats - K-2, goat's milk - K-3.

During the production of cheeses, two starters' preparations produced by DDP IPR NAAS, Ukraine, were used: 1) dry lyophilized starter culture of *Lactobacillus acidophilus*; 2) the preparation "Iprovit-Active". The culture of *L. acidophilus* contained not less than $1.0 \cdot 10^9$ CFU/g (Colony-Forming Units per gram). The Iprovit-Active contained the cultures of *Lactococcus lactis ssp. lactic, Lactococcus lactis ssp. cremoris, L. lactis ssp. lactis biovar. diacetylactis, Lactobacillus casei* ($2.0 \cdot 10^{10}$ CFU/g).

Also, enzyme rennet (Semenko LLC, Ukraine) and calcium chloride were used in cheese production.

2.2. Methods

2.2.1. Preparation of starter cultures L. acidophilus and Iprovit-Active

According to the proposed technology, the experimental cheeses were produced using starter's preparations, which were applied in the activated state.

To activate 3 g of dry culture, *L. acidophilus* was added to 100 cm^3 of sterilized skim milk heated to a temperature of (38±1) °C.

The mixture was thermostated at (38±1) °C for (6.0±0.5) hours until the curd formation. The number of viable *L. acidophilus* cells in the starter culture was $5.0 \cdot 10^8$ CFU/cm^3.

Separately, up to 100 cm^3 of sterilized milk, and 5 g of dry preparation Iprovit-Active was added and kept at a temperature of (35 ± 1) °C for 9 hours until the curd formation. The number of viable Iprovit-Active cells in the activated state was equal to $(4.5\text{-}6.0) \cdot 10^9$ CFU/cm^3.

The cultures were then cooled to room temperature and stored at (5±1) ℃ before use for making hard cheese as described below.

2.2.2. Cheese making

Experimental cheeses (n = 5) were produced by the method of Kolesnikova, (2000). Cheeses were made from different types of milk. The samples were labeled as cheese No.1 (control) - from cow's milk; cheese No.2 - from a mixture of milk of cows and goats (1:1); cheese No.3 - from goat's milk.

The method involved pasteurization of milk (65°C for 30 minutes), cooling to coagulation temperature (32-34°C), introduction of a culture of *L. acidophilus* (1.5-2.5)%, calcium chloride, the preparation Iprovit-Active (1.5-2.5)% and rennet. After the formation of the rennet curd, the following steps were performed: cutting, setting the curd grain, draining the whey, the second heating at a temperature of 38-42°C, self-pressing at two temperatures: at room temperature and then heating the cheese head surface with hot water (65-75)° C, salting and ripening at (12±1) °C for 15 days (Figure 1). (Kolesnikova, 2000). The obtained finished cheese heads were coated with latex and stored for 2 months at a temperature of (5±1)°C.

2.2.3 Microbiological tests

Sampling and preparation of samples for microbiological studies were performed in accordance with the Ukrainian standard DSTU 7357:2013. The number of bacteria was determined by seeding dilutions in agar culture medium: lactic acid bacteria according to the standards GOST 10444.11-89 and DSTU 7999:2015; bacteria of the genus L. acidophilus on - MRS medium with 2% glucose or Rogoza (Rogosa et al., 1951), bacteria of the Escherichia coli group (coliforms) according to DSTU IDF 73A:2003; yeast and mold - DSTU 8447:2015; spore aerobic rod bacteria - DSTU 5093:2008.

Figure 1. The diagram of cheese production based on various raw milk in accordance with the experimental technology.

2.2.4. Physico-chemical analysis

The titratable acidity was determined according to the standard GOST 3624-92; active acidity - according to DSTU 8550:2015; mass fraction of fat according to DSTU ISO 1735:2005; mass fraction of dry matter - according to DSTU ISO 5534:2005; mass fraction of total protein and nitrogen-containing compounds by Kjeldahl method on the digester and distiller Fisher Bioblock Scientific according to DSTU ISO 8968-2:2005, DSTU ISO 8968-4:2005; DSTU ISO 17997-1/IDF 29-1:2009; DSTU 5038:2008.

The rheological index of shear force was performed on a SANS test machine series CMT2503 (Shenzhen SANS Testing Co. Ltd.).

2.2.5. Analysis of benzoic and sorbic acids

Sorbic and benzoic acids were detected using high-performance liquid chromatography - HPLC LC-20 (SHIMADZU Corp, Japan) with reversed phase and diode array detector.

The following work was performed to remove fat and proteins from the samples. A portion of the sample weighing 3 g was dispersed in 10 cm^3 of distilled water and then quantitatively transferred to a 100 cm^3 capacity measuring flask with two portions of 5 cm^3 of water. After that 25 cm^3 of 0.4% sodium hydroxide solution was added to the resulting solution. Then the resulting mixture was placed in an ultrasonic bath heated during 15 minutes at the temperatures of 70° C. Thereafter it was cooled to room temperature, and 0.5 normal sulfuric acid solution was added to reach pH (8.0±0.1). Then, to precipitate proteins and fats, 2 cm^3 of 10.6% potassium hexacyanoferrate (II) solution and 2 cm^3 of 21.6% zinc acetate solution were added to the reaction mixture. The flask was then shaken for 10 minutes and left alone for 15 minutes. After shaking, the flask was adjusted to the mark with methanol, stirred and allowed to stand for another 15 minutes. The supernatant was filtered through a membrane filter and used for the study.

Sample preparation was performed according to the standard ISO 9231:2008 (IDF139:2008). A Shim-Pack Velox C18 chromatographic column (5 μm, 4.6 × 150 mm) (SHIMADZU Corp, Japan) was used to separate the acids. The mobile phase consisted of 10 volume parts of methanol (A) and 90 parts of phosphate buffer pH 6.7 (B). Isocratic elution was performed at room temperature for 10 minutes at a flow rate of 0.8 cm^3/min Target components were detected at a wavelength of 227 nm and 250 nm. Sorbic and benzoic acids (Sigma-Aldrich, USA) were used as standards, eluting after 1, 2, 3 and 7 minutes, respectively.

Stock solutions of benzoic acids were prepared in distilled water (1000 mg/dm^3). Working standard solutions in the concentration range from 15 to 500 mg/dm^3 were obtained by diluting the starting material. The linearity of the procedure was determined by introducing a standard solution with a concentration from 3 to 500 mg/dm^3. The method of additives was used to determine the accuracy.

Similar procedures were performed for working standard solutions of sorbic acid with a concentration of from 0.02 mg/dm^3 to 2 mg/dm^3.

2.2.6. Statistical analysis

Analyzing the results of the main indicators of the three variants of experimental cheeses (n=5) was performed using Microsoft Excel 2010. Statistical analysis of the data was carried out by the methods of variation statistics; we used Statistica 6.0 software packages (StatSoft, Inc., 2001; www.statsoft.com).

3. Results and discussions

Cheeses made by traditional technologies have been in steady demand for decades, and in some cases even a century. Today there is a tendency to supplement the traditional range of cheeses with new varieties that are created taking into account the basic laws of technological processes of cheese making, which are fully consistent with modern conditions. In recent years, products from small cheese factories from farms and cooperatives have become popular.

The experimental technology of cheese-making presented in this article can be used at small cheese-making businesses as it provides hygienic reliability of finished products and the ability of cheeses to be stored.

3.1. Technological parameters of biological treatment of raw milk

The rennet coagulation ability of milk depends not only on the differences between the raw materials, but also on the processing of milk on the eve of the cheese-making process. The decisive effect of the acidity (pH) of milk on the rate of coagulation is still underestimated in the preparation of cheese at home and on mini-farms.

The whole milk obtained from the farm was pasteurized (Figure 1). As is known, the mode of pasteurization is chosen depending on the bacterial contamination of raw milk and the desired properties of the cheese curd.

The optimal mode of pasteurization of milk is heating to a temperature of 70-72 °C for 20-25 seconds. For the production of some types of cheese it is possible to use a higher temperature, it depends on the technology of production of a particular type of cheese. However, this type of pasteurization involves the appropriate equipment, that is advisable to apply for large volumes of products.

According to the experimental technology we applied a long-term pasteurization temperature (63-65 °C for 30 minutes) with constant stirring, which can be used on small farms. Then the milk mixture was quickly cooled to a temperature of (33-35) °C. The importance of following this regimen is to preserve the structure of milk protein and organoleptic properties of the finished product.

After adding a solution of calcium chloride at the rate of 20 g per 100 kg of milk to the milk mixture, activated acidophilic culture was added (Figure 1). Thus, the process of biological treatment of the milk mixture with constant stirring took place.

To determine the method and dose of *L. acidophilus* (1%, 2%, 3%) for biological treatment of milk with this culture, a number of previous model experiments were performed with different types of raw milk.

It was shown that when using 1% of the sample of *L. acidophilus* and holding for 30 minutes, there was a too slow decrease in acidity ($\Delta pN = 0.18 \div 0.22$), which resulted in longer subsequent period of rennet coagulation of milk mixture – up to 30-40 min.

When 2% acidophilic culture was added in milk with a processing time of up to 30 minutes, we observed an intensive increase in active acidity ($\Delta pH = 0{,}30 \div 0{,}42$), which allowed to obtain a rennet curd during 20 min. The produced cheese had a pleasant milk flavor.

When 3% acidophilic culture was added to milk with a processing time of up to 30 minutes, we observed an intense increase in active acidity ($\Delta pN = 0.58 \div 0.72$), which accelerated the rennet curd to 15 minutes. The result was a sour milk cheese with a sour flavour.

Therefore, the process of biological processing of milk should be carried out with 1.5-2% acidophilic culture for (30±2) minutes in the manufacture of this type of cheeses. The use of a culture of *L. acidophilus*, which does not form carbon dioxide during fermentation, and promotes the hydrolysis of a significant amount of lactose (70%) immediately after the grain is removed together with the whey, prevents swelling of the future product.

It is known that when using dry lyophilized cultures of direct application in cheese-making, it is necessary to increase the period of time from the moment of adding the starter to the moment of application of the coagulating enzyme (Kuznetsov & Shiler, 2003; Dağdemir et al., 2003; Shingareva et al., 2007).

According to the proposed technology, the experimental cheeses were produced using two fermenting cultures *L. acidophilus* and Iprovit-Active, which were applied in the activated state. The activation of fermenting cultures was carried out to avoid the prolonged lag phase of the cultures in milk raw materials.

The number of introduced cells of the fermenting microflora correlated with the data of active and titrated acidity. Figure 2 shows that the duration of the latent phase of cultures of Iprovit-Active and *L. acidophilus* was about 2 hours, after which the phase of active growth began. The obtained data coincide with Shingareva et al., 2007.

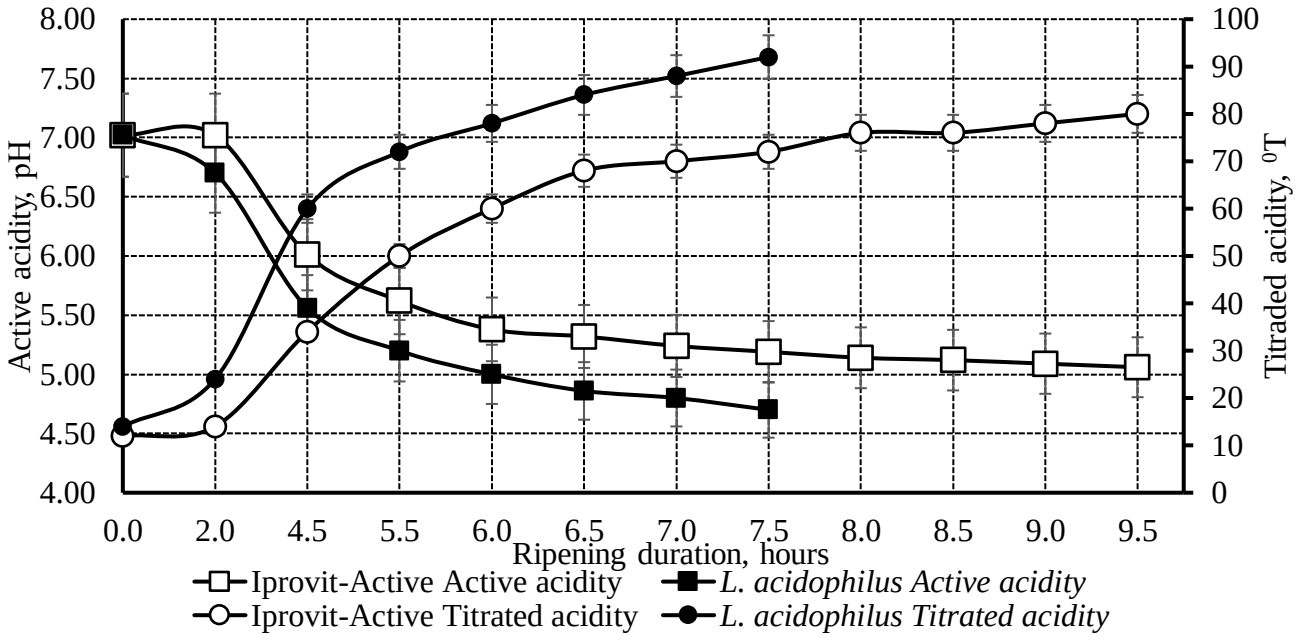

Figure 2. Active and titrated acidity during the activation of cultures.

3.2. Physico-chemical and biochemical parameters of hard cheeses during ripening and storage

After biological treatment of raw milk (the stage of rennet coagulation of the milk mixture) we applied the activated culture "Iprovit-Active" in the amount of (2.0 ± 0.5)% of the total amount of milk mixture and an aqueous solution of rennet enzyme in the amount of 1.9-2.0 g/100 kg (see Fig. 1).

The dependence of clotting time for milk coagulation and the formation of rennet curd on the applied raw material was established (Table 1).

Table 1. Technological data of curd grain and cheese mass characteristics

The name of data	Raw material		
	Cow's milk (K-1)	Mix of cow's milk and goat's milk (K-2)	Goat's milk (K-3)
Time of rennet curd formation, min.*)	23.25±1.95	20.11±1.50	18.82±1.63
Duration of curd grain stirring before the second heating, min.	7.5±2.5	7.5±2.5	7.5±2.5
Temperature of the second heating, °C	40±1	40±1	40±1
Active acidity of the cheese mass, units. pH**):			
- before self-pressing	6.24±0.03	6.20±0.02	6.17±0.02
- after self-pressing	5.85±0.02	5.80±0.02	5.73±0.03
Mass fraction of moisture in cheese mass, %**):			
- before self-pressing	57.1±0.5	58.3±0.4	60.1±0.6
- after self-pressing	47.8±0.2	48.5±0.20	49.0±0.3

*) Mean ± standard deviation (S_r) of five repetitions.

**) Mean ± standard deviation (S_R) of five repetitions.

It is known, that the ability and duration of milk to rennet coagulation largely depends on the composition of the casein complex, because it is known that there is a close relationship between the duration of coagulation and the content of β-casein, while the density of the gel

is more related to α_s-caseins. It has been shown, that the content of β-casein fraction in goat's milk can be 30% higher than the similar fraction in cow's milk, which is reflected in the duration of rennet coagulation, lower density and higher moisture retention capacity (Brule & Lenoir 1989; Beux et al., 2017).

However, under the research conditions, in samples based on goat's milk there was a tendency to reduce the duration of curd formation by 3-5 minutes (by 7-24 % comparing to the mixture of cow's milk and goat's or milk the cow's milk) ($P \leq 95.0\%$). This can be explained by pre-treatment of raw milk with *L. acidophilus*, as this culture developed more intensively in goat's milk, which allowed to increase the low titrated acidity of goat's milk 15-16°T to the characteristic of cow's milk 19-21°T.

The increase in the rate of curd formation at the time of decreasing pH of raw milk was observed to a certain extent (6.17-6.24) pH units (Table 1), because 6.2 pH units is optimal for the action of rennet enzyme. And a further decrease in acidity resulted in the acceleration of milk coagulation not due to the activation of rennet enzyme by hydrogen ions, but due to casein coagulation with acid (Beux et al., 2017).

So, the duration of coagulation of all milk mixtures with the use of both fermentation cultures ranged from 18.82±1.63 to 23.25±1.95 minutes, which differs from the technological regulations of cheeses produced by traditional technology for 15-30 minutes. The previous laboratory production of cheeses have shown, that a longer formation of rennet curd is impractical, because the lengthening of the process to 40 minutes was characterized by the formation of uneven grains and the curd began to crumble into fragments, which led to turbidity of the whey and an increase in protein in it, which reduced the yield of cheese.

As a result of organoleptic evaluation of the experimental samples after rennet coagulation of the milk base, the formation of curds with qualitative structural and mechanical parameters was recorded. In particular, the curds in all samples were dense, homogeneous, tender, they gave a split with sharp edges without the formation of protein flakes with a shiny surface, without the formation of cheese dust. And the taste of curds was pure fermented-milk. Goat's milk samples lacked the specific taste and odor of goat fat. The resulting curds were ready for further processing.

Particular attention was paid to the stage of curd grain formation. From the formed curds, which were cut by special cutting devices into uniform small cubes 2-4 mm in size, a curd grain was formed within 6±1 min. (Table 2).

Table 2. Technological data of separate operations during cheese manufacture

Technological operations*)	**Traditional technology**	**Experimental technology**
Rennet curd formation, min.	35±5	25±5
Grain formation, min.	17.5±2.5	6±1
Draining whey after the grain formation, min.	10±1	10±1
Grain stirring before second heating, min.	25±5	7.5±2.5
Draining whey removal before second heating, min.	10±1	Nil
Second heating at temperature 40±1°C, min.	25±5	10±1
Mixing after second heating, min.	30±10	10±1
Curd grain formation by stacking, min.	20±1	20±1
Self-pressing, min.	90±30	120±10
Pressing, min.	210±30	Nil
Duration of production until we get the pressed cheer of cheese, totally hours	7.78±1.47	3.48±0.25
Salting duration, hours	10.0±2.0	10.0±2.0
Ripening duration, days	55±5	17,5±2.5

*) Mean ± standard deviation (S_r).

The cutting was performed at a time when the curd was still highly mineralized and not very compacted to avoid the appearance of individual particles of the curd (cheese dust). Further syneresis proceeded for another 10±1 min. at rest, resulting in the formation of a rind on the curd grains (Figure 3).

Figure 3. Formed curd grain (size 2-4 mm) after rennet clotting of milk base.

After grain precipitation, the whey was removed in one step in the amount of (65±5) %. The whey released during the cutting of the curd was transparent, having a light green color and a sufficient level of acidity (increase in titrated acidity was 2 °T), turbidity of the whey was not visually observed.

According to traditional technology, the process of removing whey takes place in two stages: the first portion (30% by weight of the mixture) - after setting the grain, and the second (25-30) % - after mixing to the second heating (see Table 2). The reason to apply this approach is because of the slow rate of acidity due to the low dose of fermentation of mesophilic microorganisms (Kuznetsov & Shiler, 2003).

According to the experimental technology, the acidity quickly acquires maximum values, so the removal of whey is carried out in one step (Kolesnikova, 2000).

According to the experimental technology under the conditions of biological treatment of *L. acidophilus*, the curd grain with the rest of the whey was intensively mixed until the lumps disappeared, and then the second heating of the curd grain was carried out for (10±1) minutes applying hot water (70±5) °C in the amount of 15% of total weight of the milk mixture with increasing temperature to (40±1)°C in whey. Then partial salting in the grain was performed - a solution of sodium chloride (21±1) % (0.2% of the amount of the processing mixture) was added to the milk mixture and the curd grain was kneaded for 10 min and sent to perforated molds. Partial salting in grain shortens the salting process in brine.

The higher temperature regime provided uniform drying of curd grain in all samples. After self-pressing we obtained the required moisture content and the level of active acidity: 46.5÷49.2 % and 5.65÷5.78 pH units, respectively.

It was found that the level of active acidity could depend on the water content in the cheese mass: the cheese mass with a higher mass fraction of moisture had lower pH values (see Table 1). In particular, the acidity of the cheese mass in the sample made from goat's milk (K-3) was by (1.2-2.0) % lower, and the mass fraction of moisture was by (1.0-2.5) % higher compared to the samples from cow's milk (K-1) and the mixture (K-2).

According to this technology, there was one process of hot self-pressing, which accelerated the processes of self-pressing and pressing by 2.2-2.7 times comparing to the traditional technology (see Table 2). This process took place by heating the surface of the wheel of cheese with hot water (70±5) °C accompanied with rolling over the cheese perforated molds every 20 minutes for (120±10) minutes After that we cooled the wheel of cheese with cold water to room temperature and sent for salting.

Earlier in model laboratory experiments it was found that the duration of self-compression less than 100 minutes was insufficient, because the mass fraction of moisture in fresh and mature cheese can increase to 60% and over 45%, respectively, which is not typical for hard cheeses. And self-pressing over 130 minutes is impractical because (120±10) minutes is sufficient to close the surface of the wheel of cheese and achieve a mass fraction of moisture in the cheese after self-pressing and mature -

(48±2) % and (45±2) %, respectively, which corresponds to the technology of pressing hard cheeses.

The process of cheese ripening was carried out at a temperature of (12±1) °C and relative humidity of (80÷85) % for 15 days and storage at a temperature of (5±1) °C for 60 days.

It should be noted that there were no differences in the technological mode between different variants of experimental cheeses produced from different raw materials at the stages of self-pressing, salting and ripening.

Thus, biological processing of milk using *L. acidophilus* allowed reducing almost twice the duration of curd grain production, its formation in cheese wheels. Because *L. acidophilus* activated the fermenting lactic acid bacteria of the Iprovit-Active, the process of syneresis was active with the production of small grains from a delicate rennet curd. The grain settled quickly, which made it possible to remove (65±5) % of whey after the grain formation.

Starter culture of *L. acidophilus*, due to its probiotic properties is suitable for use in cheese production. Cultures of *L. acidophilus* produce natural antibiotics - acidophilus, lactocidin, acidolin and lactobacillin which are capable of inhibiting streptococci of serological groups A, B, C and H, and also staphylococci, *Proteus*, *Shigella*, *Salmonella*, *Mycobacteria* and moulds. The titer of antagonist activity toward *E. coli* i *Cl. butyricum* is greater than 1 (Ahmed et al., 2010, Chichik & Irkitova, 2013).

This approach is well established in the production of soft, semi-hard and hard cheeses in order to prevent their early and late swelling. In addition, in the manufacture of hard cheeses, this technology is effective, and particularly is cost-effective, as it accelerates by 2-3 times the entire process – from the curd grain formation to cheese ripening while maintaining the quality of cheese during long-term storage (Fedin et al, 1985; Kolesnikova et al, 1991, Kolesnikova & Gening, 1994; Kolesnikova, 2000).

During the ripening and storage of experimental cheeses, a change in their physicochemical parameters was recorded (Table 3).

Table 3. Physico-chemical and rheological parameters of experimental cheeses *)

Stages of the technological process	Cheese	Active acidity, pH units	Mass fraction of moisture, %	Mass fraction of fat in dry matter,%	Total nitrogen content,% of dry matter content	Cutting force, кН/м^2
Finished cheese (VII – cheese ripening 15 days)	No.1	5.50±0.03	45.50±0.17	48.57±0.15	7.21±0.20	27.34±1.05
	No.2	5.44±0.04	45.80±0.14	48.84±0.21	7.36±0.18	25.25±0.95
	No.3	5.39±0.03	46.20±0.15	49.00±0.20	7.43±0.16	23.41±1.20
30 days storage	No.1	5.37±0.03	42.80±0.20	48.90±0.18	7.17±0.18	36.50±0.85
	No.2	5.34±0.04	43.20±0.11	48.93±0.21	7.25±0.19	33.54±0.91
	No.3	5.22±0.04	43.90±0.13	49.01±0.17	7.46±0.20	31.42±0.96
60 days storage	No.1	5.30±0.02	42.10±0.18	49.14±0.20	7.14±0.18	49.15±0.70
	No.2	5.26±0.03	42.50±0.17	49.06±0.19	7.21±0.17	42.80±0.85
	No.3	5.16±0.02	43.00±0.17	49.20±0.18	7.44±0.20	40.63±0.83

Note*): the mass of the cheese wheels was 140±5 g.

**) Values are displayed as the mean ± standard deviation (S_R) of the five replications ($P < 0.05$).

Hard cheeses were characterized by a mass fraction of fat in the dry matter of not less than 40.0%, a mass fraction of moisture of not more than 47.0% according to the standard DSTU 6003:2008.

On the 15th day, organoleptic analysis of three types of cheese was performed. They had a pure fermented-milk taste and aroma with the features intrinsic to each type of cheese; dense, plastic consistency; appearance - the rind is thin,

dense, clean, had an imprint of perforation. In section the cheeses were characterized by a uniform pattern, consisting of holes (eyes) of regular shape with a size of 2 to 4 mm, the dough color was uniform throughout the mass: Cheese No.1 - bright yellow, Cheese No.2 - slightly yellow; Cheese No.3 - white.

According to the results of rheological studies obtained on a universal mechanical test machine "SANS" of SMT series, the density of the studied products significantly depended on the type of raw materials from which the cheese was produced (Table 3).

The process of cheese ripening takes place under the influence of living microorganisms, which gradually die, but the ripening process itself continues. It is caused by bacterial endoenzymes secreted from bacterial cells. This process can occur more intensively, in the case of increased moisture in the cheese mass (Blaya et al., 2018).

In the studied cheeses, the mass fraction of moisture decreased during ripening, and it resulted in the concentration of components.

The change in acid formation was observed after pressing in all cheeses in the range from 5.73 ÷ 5.85 pH units up to 5.50 ÷ 5.39 pH units after 15 days of ripening. The level of active acidity continued to decrease by 9.4-10.0 % in all cheeses until the 60th day compared with the cheeses after self-pressing.

The cheese No.3 made from goat's milk had a higher moisture content compared to other samples, so it resulted in a more intense change in the active acidity of the cheese mass compared to the rest of the samples (Table 3).

Finished cheeses lost moisture in the range of 6.9-7.5 % after two months of storage.

The cheeses had a good storage capacity, which was in line with the requirements for hard cheeses. The finished test products had a clean flat surface, pronounced, slightly acidic, sharp clean taste and smell, delicate, plastic, homogeneous consistency that corresponded to the highest quality products.

The main biochemical processes, due to which the specific characteristics of cheese are formed, are the reactions of splitting of protein and fat components of curd mass. As a result of the action of proteolytic enzyme systems of the microflora, caseins are broken down to form large peptide fragments, which in turn are hydrolyzed to low molecular weight peptides and free amino acids.

It is known that the process of formation of soluble nitrogen fractions and a pool of free amino acids depends on the proteolytic activity of bacteria that are part of the fermentation culture (McSweeney, 2004; Gudkov, 2004; Blaya et al., 2018).

One of the most important indicators in the study of cheeses is the amount of soluble nitrogen-containing compounds, as their level largely characterizes the degree of ripening and organoleptic properties of the finished product (Figure 4).

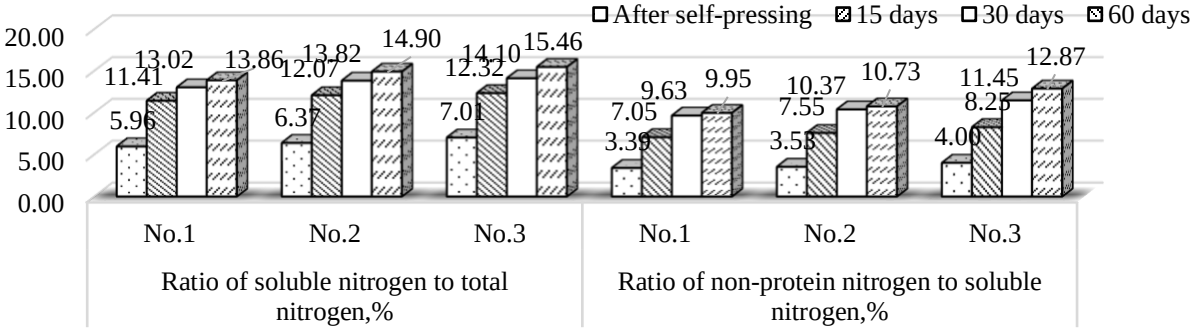

Figure 4. Dynamics of changes in the content of nitrogen-containing compounds during cheese ripening and storage *)

No.1 - cheese made from cow's milk; No.2 - cheese from a mixture of cow's milk and goat's milk (1: 1); No.3 - goat's milk cheese; 15 days - duration of cheese ripening; 30 days, 60 days - the duration of storage of cheese. Note*): the mass of the cheese wheels was 140±5 g.

Analysis of the dynamics of accumulation of nitrogen-containing compounds in cheeses made from milk of cows, goats and their mixture showed differences in the intensity of proteolytic processes already in fresh cheese and increased during storage. Soluble nitrogen-containing compounds accumulated most actively at the beginning of cheese ripening.

The ripening index of cheeses, which some authors (Zhukova et al., 2006; Andronoiu et al., 2015) prefer to calculate as the ratio of soluble nitrogen to total nitrogen, showed that for experimental cheeses No.1, No.2, No.3 on the 15th day of ripening it was 11.41%, 12.07%, 12.32%, respectively (Figure 4).

The highest percentage of soluble nitrogen after pressing was characteristic of cheese No.3 from goat's milk, and the lowest - for cheese No.1 from cow's milk. A similar pattern was observed in the composition of soluble non-protein nitrogen (Figure 4). The amount of non-protein nitrogen in the finished cheeses No.1-No.3 increased after pressing by 3.7-4.0 times, respectively.

The ratio of non-protein to soluble nitrogen fractions is considered to be interesting as well. This ratio for cheeses No. 1-3 was 15 days - 7.05; 7.55; 8.25, respectively.

This Figure indicates a high degree of cheese ripening. The highest concentration of this nitrogen fraction was in goat's milk cheese, and the lowest - from cow's milk.

Cheeses made from a mixture of raw milk had average values between cheese from the milk of cows and goats. The obtained indicators confirm that all experimental cheeses are quite mature on the 15th day, with a pure fermented-milk cheese taste.

During 2 months of storage, it was found that the accumulation of nitrogen-containing compounds varied depending on the raw material. The amount of soluble nitrogen increased by 1.27-1.33 times, non-protein nitrogen rose by 1.78-2.04 times. It should be noted that the ratio of non-protein to soluble nitrogen indicates a more significant accumulation of non-protein nitrogen in cheeses from goat's milk compared to cow's milk for the same period of time. In this case, the active accumulation occurred after self-compression until the 15th day, as well as from the 30th to the 60th day, which is associated with the activity of bacterial enzyme systems.

3.3. Microbial assessment

The content of fermenting and spontaneous microflora during the production and storage of cheeses was studied.

It was found that after the introduction of activated cultures of *L. acidophilus* and cultures of the culture Iprovit-Active, the number of lactic acid microflora was about 10^6 CFU/g in variants of raw milk obtained from milk of cows, goats and their mixture (50:50) (Figure 5).

The development of lactic acid bacteria was positively influenced by maintaining the temperature (33±1) °C during the coagulation of the milk mixture and before the second heating (Figure 1).

Analysis of the dynamics of fermentation microflora during cheese making showed that from the stage I to the stage III (introduction of fermentation starters into the milk mixture and self-pressing of cheese mass) more intensive development was observed in *L. acidophilus* cultures. While after the stage IV (cheese mass after salting), the fastest growth was noticed among Iprovit-Active lactic acid bacteria. The increase in the number of cultures of *L. acidophilus* between the stages I and II occurred 20.4-48.9 times, and the culture Iprovit-Active only 2.6-4.2 times. Thus, the intensive development of *L. acidophilus* cultures can be explained by the introduced stage of the second heating of curd grain to 40°C (see Figure 1).

At the stage III (Figure 5) in the cheese mass we observed an increase in the number of *L. acidophilus* cultures by 42.7-102.3 times and the microflora of the culture Iprovit-Active by 7.4-12.0 times. The results of microscopy of the samples showed that at this stage the number of lactobacillus *(L. acidophilus* and *L. casei*) in different fields of view was 15-20% more than the coccus microflora of the culture Iprovit-Active (*Lactococcus lactis ssp. lactic,*

Lactococcus lactis ssp. cremoris, L. lactis ssp. lactis biovar. diacetylactis).

Depending on the type of raw material used, the results of the dynamics of lactic acid microflora development showed significant variation.

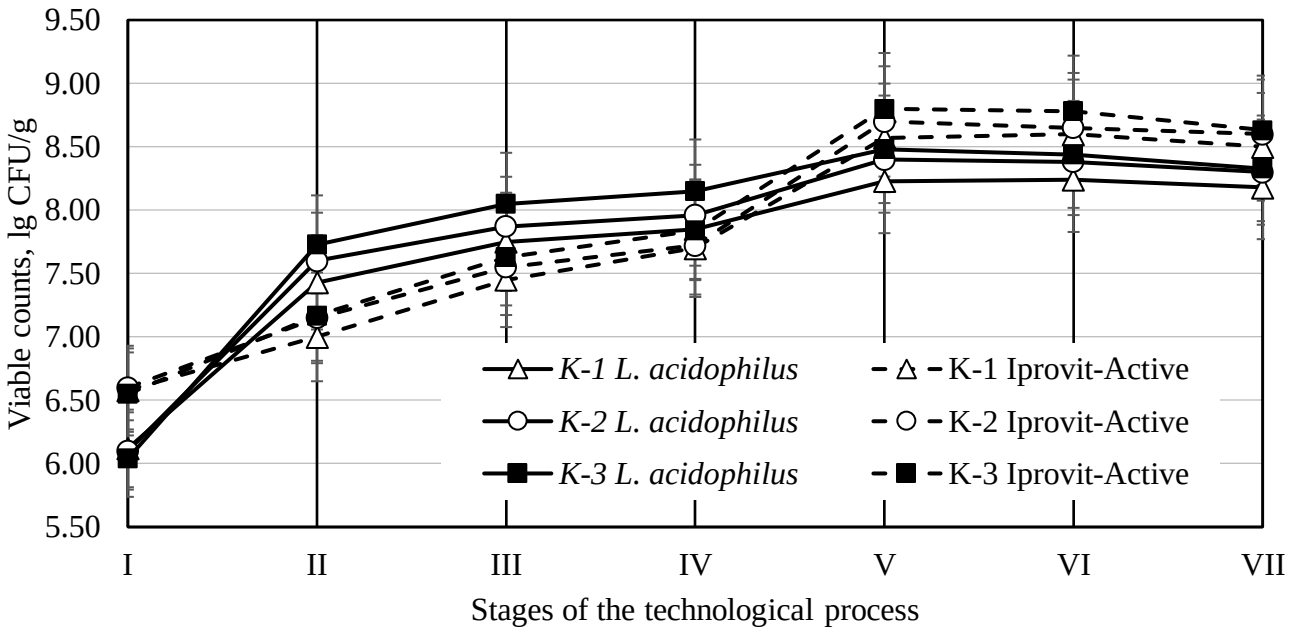

Figure 5. The change of lactic acid microflora during cheese production with the use of fermenting cultures

I - introduction cultures into the milk mixture; II - cheese mass after molding; III - cheese mass after self-pressing; IV - cheese mass after salting; V - 3 days' cheese ripening; VI - 7 days' cheese ripening, VII - 15 days' cheese ripening. Cow's milk - K-1, a mixture of milk from cows and goats - K-2, goat's milk - K-3.

The development of *L. acidophilus* cultures in goat's milk cheese was 139% and 101% more intense comparing to cow's milk and the mixture of two types of milk, respectively. The amount of lactic acid microflora of Iprovit-Active increased less actively than the culture of *L. acidophilus*. The maximum increase in Iprovit-Active lactic acid microflora was also revealed in goat's milk cheese, which was higher by 42-62% compared to other variants.

Keeping cheeses in salt brine (20% NaCl) for 10.0±2.0 h, at temperature (8±2) °C caused inhibition of growth of lactic acid microflora (Figure 5). In all the studied samples from the III to the IV stage of the technological process, no increase in the number of microflora was observed. It is significant that the survival rate of the microflora was the lowest (about 72%) during salting on the surface of the cheese mass in direct contact with the brine. However, already at a distance of (2±1) cm from the surface layer of cheese mass, this indicator increased significantly (up to 95%), and in the central part of the cheese mass the number of lactic acid microorganisms was up 12-15%.

At the stage IV after salting in all samples, the microflora of the culture Iprovit-Active overtook the development of *L. acidophilus* culture (7.99 lg CFU/g) and their average content in the cheese mass was about 7.75 lg CFU/g.

The maximum amount of microflora in all cheeses was observed on the 3rd day of ripening. During this period, the number of cultures of *L. acidophilus* reached the level $(1.7\text{-}3.0)\cdot10^8$ CFU/g and the microflora of the culture Iprovit-Active - $(3.7\text{-}6.2)\cdot10^8$ CFU/g.

At the V-VII stages of cheese ripening, from the 3rd to the 15th day (see Figure 5), a slowdown in the development of lactic acid microflora and a slight decrease in the number

of Iprovit-Active cultures and *L. acidophilus* by 1,2-1, 5 times and 1.1-1.4 times, respectively.

Thus, on the 15th day of ripening, the fermentation microflora increased in all types of cheeses, in particular Iprovit-Active cultures by 83.2-117.5 times, and *L. acidophilus* by 114.8-194.9 times in comparison with the initial content.

On the 15th day of ripening all cheeses made from milk of different origin had microbiological parameters that met the requirements of current regulations.

Therefore, the intensity of development of lactic acid microflora depended on the raw materials and fermenting cultures and could be different by much. It should be taken into account when developing technological modes of cheese production.

During 2 months of storage at a temperature of (5±1) °C (Figure 6) the gradual extinction of the fermenting microflora in all experimental cheeses was recorded.

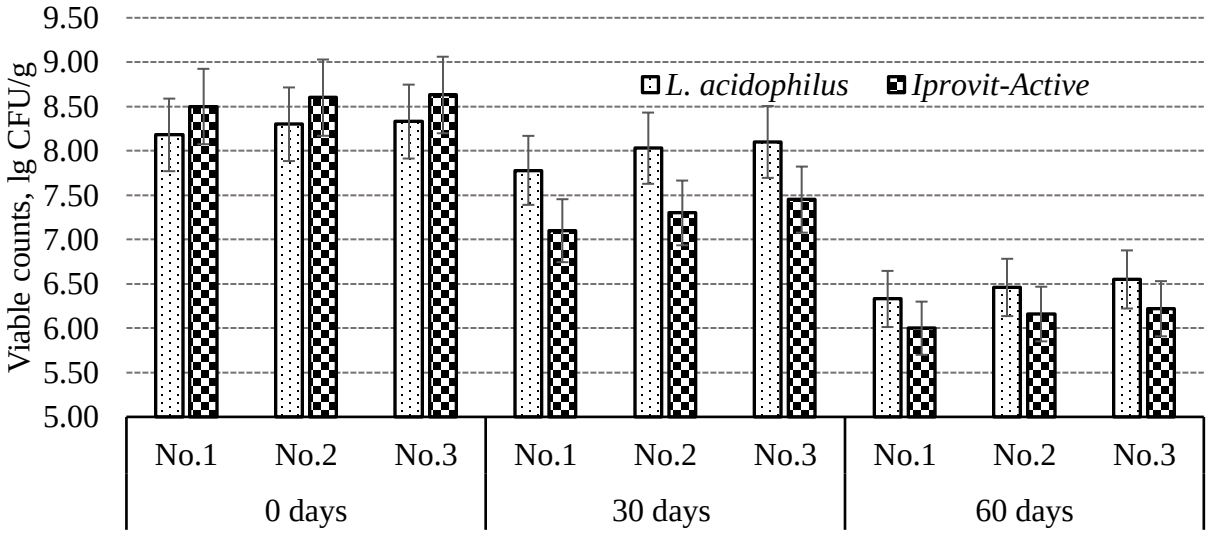

Figure 6. Dynamics of lactic acid microflora content during cheese storage.
No.1 - cheese made from cow's milk; No.2 - cheese from a mixture of cow's milk and goat's milk (1: 1); No.3 - goat's milk cheese.

The number of lactobacilli of the culture Iprovit-Active decreased more intensely by 4.0-4.4 times than the culture of *L. acidophilus* (Figure 6).

On the 60th day of storage in goat's milk cheese there was a minimal decrease in the number of both Iprovit-Active microflora and *L. acidophilus* culture. Thus, the slowdown was 18.7% and 5.8 % compared to cows' milk cheeses, and 14.9 % and 12.6 % compared to cheeses made from the mixture, respectively.

As a result, of this process, higher levels of benzoic and sorbic acids were observed in goat's milk cheeses.

In addition to studying the dynamics of fermenting microflora accumulation in cheeses, the analysis of the accompanying microflora was carried out, namely: coliform bacteria, spore-forming microorganisms, yeast and moulds after the self-pressing of cheese mass (the stage III), on the 15th day of ripening (the stage VII), as well as for 2 months of storage at a temperature of (5 ± 1) ° C (Figure 7).

During the production of cheeses from the III to the VII stage of the technological process, the content of foreign microflora decreased. This was especially true of spore-forming bacteria, the number of which in cheeses decreased by 3.1-3.3 times, which is explained by the use of cultures of *L. acidophilus* for biological processing of raw materials.

During storage at a temperature of (5±1) °C, a very slow development of foreign microflora was observed (Figure 7). The increase in spore-forming microorganisms occurred in 2.67-3.00 times, and yeast and mold - in 2.09-2.41 times compared with the content at the beginning of storage. This range of increase did not affect the organoleptic properties of cheeses and met the requirements of current regulations for these products according to DSTU 6003:2008.

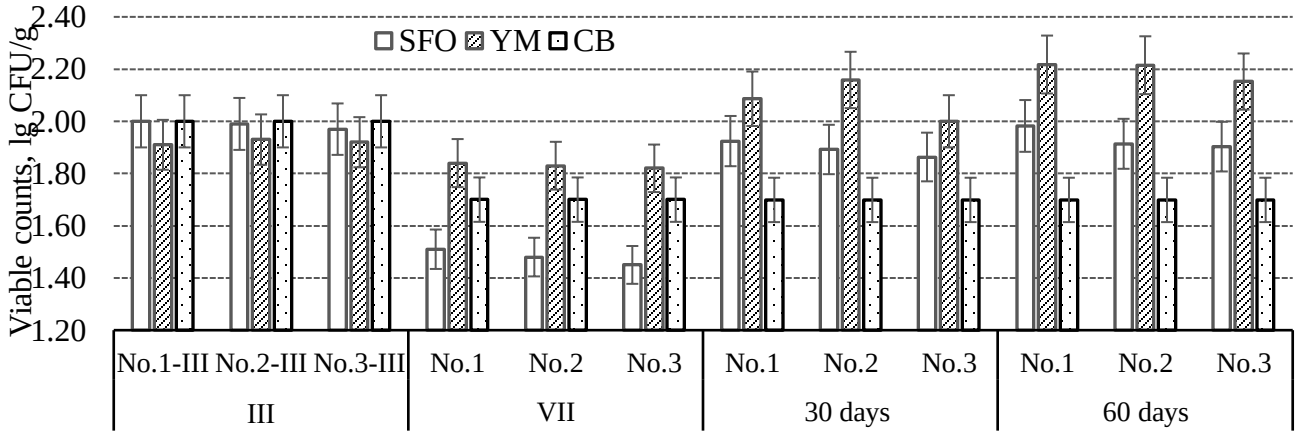

Figure 7. Changes of foreign microflora during ripening and storage of cheeses made from milk of different origin*)

III – cheese mass after self-pressing; VII –the 15th day of cheese ripening; No.1-III - cheese mass from cow's milk; No.2-III - cheese mass from a mixture of cow's milk and goat's milk; No.3-III - cheese mass from goat's milk; No.1 – cheese made from cow's milk; No.2 - cheese from a mixture of cow's milk and goat's milk (1: 1); No.3 - cheese from goat's milk, SFO – spore-forming organism; YM – yeast and moulds; CB – Coliforming bacteria.

*) Values are displayed as the mean ± standard deviation (S_R) of five replications. The average value in one row, followed by different indices, differed significantly ($P < 0.05$).

Thus, experimental cheeses can be stored for 60 days at a temperature of (5±1) °C while maintaining their quality, which exceeds the period specified in DSTU 6003:2008 for cheeses of this group for 15 days.

3.4. Investigation of benzoic and sorbic acids content during ripening and storage of cheeses

It has been found that some organic acids, in particular benzoic and sorbic, which are natural preservatives, may be present in milk and dairy products.

Benzoic acid is formed as a result of the activity of lactic acid microflora, and its content depends on the duration of fermentation, types of starter cultures, temperature and raw milk.

It was shown that during the fermentation of milk by starter cultures of S. *thermophilus, L. lactis* ssp. *lactis, L. lactis* ssp. *cremoris, L. lactis* ssp. *diacetylactis, Leuconostoc mesenteroides* ssp. *cremoris, Lactobacillus paracasei* the content of benzoic acid was 0.0÷12.5 mg/kg. At the same time the highest level of benzoic acid was observed in skim milk fermented with R707 (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) (Han et al., 2016).

According to Urbienė & Leskauskaitė (2006), the content of benzoic acid in milk can range from 2 to 5 mg/kg, and after fermentation of milk by starter cultures (La-5, ABT-2 and YC-180) - can be 14-23 mg/kg, and the content of sorbic acid - 0.06-0.09 mg/kg. The maximum formation of organic acids was detected at 3–6 h of fermentation of raw milk, ie during the log-phase, and depended on the type of lactic acid bacteria. The highest concentration of organic acids was found in milk for 7 hours of

cultivation by starter culture of *Lactobacillus acidophilus* La-5.

Bartáková et al., (2021) determined the range of benzoic acid in yogurts fermented with cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, in the amount (13.38±3.56); (21.31±5.66); (43.26±5.11) mg/kg depending on the raw material used - cow's, goat's or sheep's milk.

A study of cheeses (Cream, Curd, Cottage, Hard) found that the level of benzoic acid can reach 5.1-90 mg/kg (Gucer et al., 2016; Horníčková et al., 2014), and sorbic acid in Kashar and Fresh cheeses - 21.3÷511.3 mg/kg (Özdemir et al., 2020).

There are also data on a positive correlation of benzoic acid content with the number of lactic acid bacteria (LAB) in Feta cheese at (r = 0.827) (Heshmati et al., 2017), while there is no correlation for cream cheese. The concentration of benzoic acid was 41.80 mg/kg and 8.52 mg/kg in feta cheese and cream cheese, respectively.

Because the levels of benzoic and sorbic acids are controlled for food safety, in particular their chemical or natural origin, the study of the content of these compounds in fermented dairy products is of particular importance (Gucer et al., 2016; Özdemir et al., 2020; Bartáková et al., 2021; Yerlikaya et al., 2021).

Determination of the content of benzoic and sorbic acids in the process of making hard cheeses showed some differences depending on the applied raw milk. In the milk mixture No.3-I at the first stage of the technological process the content of benzoic acid was almost 4 times higher than in the milk mixture No.1-I. The content of sorbic acid was detected in trace amounts.

During the third stage, in the curd mass after self-pressing the increase in benzoic acid content occurred 3.7 times in cheeses No.1-III and 2.6 times in cheeses No.3-III compared to the first stage of the technological process; during the seventh stage, the cheese on the 15th day of ripening, the content of benzoic acid increased by 4.0 and 2.4 times, respectively, compared with the third stage (Table 4).

Table 4. Benzoic and sorbic acids content during cheese production (mg/kg)

The stage of the technological process	**Samples**	**Acid content, mg/kg**	
		benzoic	**sorbic**
I – adding fermenting culture in milk mixture	No.1-I	1.02±0.12	-
	No.2-I	3.13±0.48	-
	No.3-I	4.05±0.34	-
III – curd mass after self-pressing	No.1-III	3.79±0.62	0.060±0.004
	No.2-III	8.65±0.79	0.071±0.003
	No.3-III	10.55±1.24	0.092±0.002
VII – cheese, 15 days' ripening	No.1	14.80±1.76	0.068±0.003
	No.2	20.64±1.95	0.097±0.003
	No.3	25.44±1.83	0.123±0.003

Note. "-"- not found. Values are displayed as the mean ± standard deviation (S_R) of the five replications ($P<0.05$).

During storage, the accumulation of benzoic acid slowed down in all sample variants.

Compared with the finished cheese (at the seventh stage of manufacture), the content of this compound for 30 days of storage increased by 1.1-1.23 times and almost did not change during the next 30 days (Table 5).

The content of sorbic acid was much lower than benzoic acid in all samples of experimental cheeses at all stages of manufacture and storage. At the same time during storage of the first 30 days its content increased in comparison with finished cheeses. At the end of the next 30 days, a slight variation in the data was recorded, which

can be explained by the slowdown in the development of the fermenting microflora.

Table 5. The content of benzoic and sorbic acids in cheese during 2-months storage (mg/kg)

Storage period, days	Cheese samples	Acid content, mg/kg	
		benzoic	sorbic
30	No.1	18.37±0.45	0.067±0.004
	No.2	23.21±0.78	0.095±0.004
	No.3	28.20±0.52	0.120±0.004
60	No.1	18.41±0.53	0.062±0.003
	No.2	25.08±0.77	0.092±0.003
	No.3	29.77±0.60	0.118±0.002

Note. Values are displayed as the mean ± standard deviation (S_R) of the five replications ($P < 0.05$).

Thus, it can be assumed that organic acids are the main culture to prevent spoilage of cheeses during long periods of storage.

4. Conclusions

We have worked out the method of hard cheese production at a low temperature of the second heating using biological processing of raw milk, a shelf life was 60 days.

A feature of this approach is the step of introducing *L. acidophilus* cultures and keeping them in pasteurized milk for 30 minutes before adding the fermentation culture.

Comparing to the traditional technology, this approach has reduced the duration of rennet curd formation by about 10-15 minutes, grain formation by 10 minutes, and reduced protein and fat losses.

Also, the proposed approach involves the use of hot self-pressing, which reduces the stages of self-pressing and pressing by traditional technology by 180±50 minutes, i.e. by 2-3 times. The surface of the cheese wheels was treated with hot water (70±5) °C every 20 minutes and then there was a compaction and formation of a rind on the product's surface.

Our method of cheese making is valuable also because of the stages of grain mixing and whey draining. According to the proposed technology, the removal of whey occurs in one step in the amount of (65 ± 5) %, in contrast to the traditional technology which involves two stages.

Treatment of raw milk with cultures of *L. acidophilus* allowed to increase the shelf life of the finished product to 60 days without deterioration of organoleptic properties by preventing the development of spontaneous microflora.

It should be noted that the addition of two starter preparations, which included *L. acidophilus, L. lactis* ssp. *lactis, L. lactis* ssp. *cremoris, L. lactis* ssp. *lactis biovar. diacetylactis, Lactobacillus casei,* could also promote the accumulation of benzoic and sorbic acids in the experimental cheeses. At 60 days of storage, the content of benzoic acid was 18-30 mg/kg of cheese, which avoids additional measures for the treatment of finished cheeses with artificial preservatives.

It has been shown that this technology can be applied to cheeses made from various dairy raw materials - cow's, goat's or sheep's milk. However, the stage of biological treatment of raw milk with *L. acidophilus* culture should remain a mandatory stage. It was found that under these production conditions, the maximum content of benzoic acid was observed in cheeses made from goat's milk or a mixture. The peculiarity of this technology is the possibility of its implementation on mini-farms for small batches of products.

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