

## CHAPTER «VETERINARY SCIENCES»

### MICROBIOLOGICAL QUALITY OF SOUR MILK CHEESE MADE FROM RAW COW'S MILK

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DOI: <https://doi.org/10.30525/978-9934-26-355-2-32>

**Abstract.** The microbiological safety of food products is one of the priority tasks of every country, the solution of which is directly aimed at protecting the health of the population. All over the world, this problem has become widespread due to the increase in the number of diseases that occur as a result of the consumption of poor-quality food. It is believed that dairy products that are made at home are better in terms of biological completeness and nutrition. However, manufacturers of these products do not always comply with veterinary, sanitary and hygienic requirements for their production. In this regard, whole cow's milk, sour milk cheese and other products are classified as a high risk category, and their production must be under constant veterinary and sanitary control. Taking this into account, improving the methods of microbiological control of sour-milk cheese, which comes for sale on agro-food markets, is relevant and will provide an opportunity to increase its safety. *The aim* of the work was to improve the veterinary and sanitary control of sour-milk cheese, which is delivered to agro-food markets, according to microbiological indicators. *The research* was conducted using standard microbiological, physicochemical and organoleptic methods. According to the *results* of the study, it was established that the normal microflora of sour-milk cheese made from whole cow's milk is represented by lactic acid microorganisms and enterococci (*E. faecalis* species dominates among enterococci). It was also found that the quantitative content of bacteria of the genus *Enterococcus* in raw sour milk made from whole cow's milk, which is delivered to the agro-food markets, is a more stable indicator compared to the titer of coliform bacteria

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and objectively characterizes the sanitary conditions of its production, transportation and sale. In order to increase the safety and quality of sour-milk cheese, which is delivered to agro-food markets, it is necessary to examine it for the content of enterococci. The use of these indicators allows you to take appropriate corrective actions, which will increase the safety of sour-milk cheese.

### Introduction

The microbiological safety of food products is one of the priority tasks of every country, the solution of which is directly aimed at protecting the health of the population. All over the world, this problem has become widespread due to the increase in the number of diseases that occur due to the consumption of poor-quality food. Thus, according to data [1; 8; 16], emerging food pathogens (*L. monocytogenes*, *Salmonella*, *E. coli*, *S. aureus*, *Enterococcus*, *E. sakazakii*, etc.), which contaminate food products and environmental objects.

Currently, in connection with the difficult economic situation in Ukraine, the price policy contributes to the fact that a large part of the population prefers dairy products that are sent to agro-food markets for sale. It is also traditionally noted that dairy products made at home are better in terms of biological quality and vitality. Protests of manufacturers of these products do not always comply with veterinary, sanitary and hygienic requirements for their production [4; 27; 28]. In this regard, whole cow's milk, sour cream, sour milk and other products are classified as high risk, and their production must be under constant veterinary and sanitary control.

In some countries of the European Union, these products are allowed for free sale, but the control of their production is a scientifically based system that guarantees the quality and safety of these products at the state level [11; 42; 43].

At present, the state laboratories of veterinary and sanitary examination in agro-food markets do not examine the dairy products that arrive for sale according to microbiological indicators. Therefore, today it is important to develop scientifically based microbiological criteria for the safety of dairy products. To solve this problem, it is necessary to scientifically determine the main risks in the technology of their production and implementation, to develop a methodological basis for veterinary and sanitary control and

microbiological criteria for assessing the safety of these products using the most optimal indicator microorganisms.

Also, there are currently no monitoring studies on the species and quantitative composition of the microflora of whole cow's milk and fermented milk products that are delivered to agro-food markets. As a result, this microflora has not been studied enough, its main properties – production of pathogenicity factors, natural reservoir of existence, sources of entry into products and resistance to antibacterial drugs – have not been elucidated. The need for a comprehensive study of this problem is obvious, since there is a significant anthropogenic and environmental impact in the technological process of their production. In this regard, the problem of establishing the distribution of strains with variable properties and resistant to antibiotics among traditional contaminants of whole cow's milk and sour milk products sold in agro-food markets is relevant. This problem is associated with uncontrolled and systematic use of antibiotics in veterinary medicine [2; 14; 20]. The data obtained as a result of such complex studies will become the basis for effective veterinary and sanitary control of alimentary toxicoses and toxic infections caused by dairy products entering the agro-food markets.

Taking into account the above, the improvement of methods of microbiological control of cow's whole milk and fermented milk products, which are delivered to the agro-food markets, is urgent and will provide an opportunity to increase their safety.

### **1. Research prerequisites**

#### **1.1. Scientific approaches to microbiological quality control of raw cow's milk and dairy products**

The microbiological safety of food products is exclusively one of the priority hygiene tasks of each country, which are aimed at protecting the health of the population. All over the world, this problem has become widespread due to the increase in the number of diseases caused by the consumption of insufficient food [9; 21].

Milk and dairy products form the basis of the diet for most people. In addition to the benefits, milk and dairy products are also a good nutrient medium for the development of pathogenic microorganisms. If the sanitary conditions of obtaining milk, storing it and processing it into dairy products are violated, they can become the cause of various alimentary diseases [15;

26]. Therefore, today there is a need to analyze the risks of obtaining whole cow's milk and manufacturing dairy products. Microbiological control of products allows to give an objective assessment of the quality and safety of dairy products [1; 8; 12]. Increasing the requirements for quality and safety indicators of milk and dairy products is an effective and efficient means of improving the culture of dairy farming [8].

The quality and safety of food products is determined by a complex of organoleptic, physicochemical and microbiological indicators [3; 7; 25]. In order to assess the microbiological safety of any product, it is necessary to determine and establish microbiological standards and indicators for it [5].

Microbiological indicators are established for such groups and types of microorganisms that characterize the general sanitary and epidemiological condition of the product, the conditions of its production, storage and sale. As a mandatory evaluation criterion, the WHO defined the control of the number of mesophilic-aerobic and facultative-anaerobic microorganisms, coliform bacteria, as well as the absence of pathogenic microorganisms [5].

In each country, such criteria are established in the relevant legislative and regulatory documents. In the European Union, not only general normative and legal acts are effective, but also a large list of specific requirements and norms, the purpose of which is to ensure the safety of food products. Three government structures are involved in supervision: the Ministry of Agriculture, the Ministry of Social Affairs, and the Ministry of Economy and Communications [33–44].

The main principles of regulation of legal norms in the countries of the European Union in the field of food products are contained in the following documents:

- general document on food products – Regulation 178/2002 of the European Parliament and of the Council of January 28, 2002 on the establishment of general principles and requirements of food law, the establishment of the European Food Safety Authority and the establishment of procedures in matters related to food safety products;

- package of hygiene requirements (risk analysis and critical control points of HACCP) – Regulation of the European Parliament and Council 852/2004 of April 29, 2004 on food hygiene, Regulation of the European Parliament and Council 853/2004 of April 29, 2004 on establishment of hygiene requirements for food products of animal origin;

– official control mechanisms – Regulation 882/2004 of the European Parliament and of the Council of April 29, 2004 on official control measures used to ensure compliance with feed and food legislation, animal health and welfare rules; Regulation of the European Parliament and of the Council 854/2004 of April 29, 2004, which establishes special rules for the organization of official events regarding products of animal origin intended for human consumption.

All resolutions are effective in member countries, that is, without their discussion in the legislation of each individual country.

In Ukraine, microbiological safety indicators are regulated by the Law of Ukraine "On Basic Principles and Requirements for the Safety and Quality of Food Products" [36]. It brings Ukraine closer to Europe in terms of food safety standards.

The law contains many innovations that affect food producers at all stages from production to sale. This document provides for the introduction in Ukraine of the European model of the food safety and quality assurance system based on Hazard Analysis and Critical Control Point (HACCP) procedures [5; 17]. This law establishes a fundamentally new approach to food safety. The main responsibility for safety lies with manufacturers, and state control is directed not at the finished product, but at production and circulation.

The Law of Ukraine "On Milk and Dairy Products" defines the legal and organizational basis for ensuring the safety and quality of milk and dairy products for the life and health of the population. The norms of this law apply only to milk processing enterprises [37].

Modern international requirements for the assessment of quality and safety provide for the establishment of microbiological criteria related to a specific product. They must contain the name of the microorganisms and/or their toxins that can cause harm to the human body, the name of the method that should be used to determine the number of the specified microorganisms and the maximum permissible levels established for a specific product. In addition, the values " $M$ ", " $m$ ", " $c$ " should be set. The " $m$ " value is the minimum permissible level, and the " $M$ " value is the maximum permissible level of microorganisms that are determined in the sample. Exceeding the " $M$ " value means a dangerous level of microorganisms, and the product in which it is detected cannot be allowed for consumption. The

indicator "c" means the number of "positive" samples in which the level of contamination with these microorganisms between the value "m" and "M" was detected. The conclusion regarding compliance with microbiological criteria should be formed based on the results obtained for each of the point samples that form the total sample. For each specified sample "n", which is formed in the above-mentioned way from each homogeneous batch of products, it is necessary to conduct microbiological studies on each spot sample. The results should highlight data on the number of "positive" samples "c". The indicator "c" indicates the number of positive samples in which microorganisms or a certain number of them were detected.

Today, in connection with the deterioration of the economic situation in Ukraine, the number of sellers of whole cow's milk and dairy products produced on private farms, as well as its buyers, has increased in the agro-food markets. This is due to the lower price of these products, compared to dairy products sold through a chain of stores and supermarkets.

The rules of veterinary and sanitary examination of milk and dairy products and requirements for their implementation [7] require examination of whole cow's milk and fermented milk products (fermented milk cheese, sour cream) according to such indicators as: purity, acidity, density, bacterial insemination of milk (reductase sample), mass fraction of fat, protein, dry skimmed milk residue, moisture. The rules state that milk and dairy products produced in private peasant farms must meet the requirements according to DSTU [29–34]. However, as practice shows, these norms cannot be directly transferred without scientific justification to dairy products that are delivered to agro-food markets. This is due to the fact that the industrial technology of manufacturing such dairy products as sour cream and sour milk cheese involves heat treatment of milk and cream. Production of sour cream and sour milk cheese in private peasant farms is carried out from whole cow's milk, which is not subject to heat treatment.

Therefore, dairy products are sold on agro-food markets practically without the presence of microbiological criteria and legal documents that regulate their safety.

Considering the mentioned problems, it is urgent to provide consumers with safe food products, in particular, whole cow's milk and fermented milk products, which are sold in agro-food markets, according to indicators of microbiological safety.

## 1.2. Contamination of raw cow's milk and dairy products with pathogenic microflora

Currently, the epidemiological safety of food products of both animal and vegetable origin is determined primarily by microbiological indicators. This is not surprising, since among all agents that cause food poisoning in humans, 70% are pathogenic bacteria. In the technology of production of milk and dairy products, one of the most important dangers is the risk of food poisoning caused by opportunistic and pathogenic microorganisms [9; 13; 22].

Conditionally pathogenic microorganisms are microorganisms that are constantly in the environment and most of which are "residents" in the intestines of humans and animals, which under normal conditions do not cause diseases [8]. Microorganisms such as salmonella, streptococci, staphylococci, etc., which multiply and accumulate in food products, do not change their organoleptic properties [28]. According to scientists [1; 5; 14; 17], emergent food pathogens (*Listeria*, *Salmonella*, enterohemorrhagic *Escherichia coli*, *Staphylococcus aureus*, etc.) that contaminate food products and environmental objects are of particular importance.

Research by the authors [18; 21] shows that mycobacteria, *Brucella*, *Listeria*, *Staphylococcus aureus*, *Escherichia*, and *Salmonella* are most often isolated from milk and dairy products. The most frequently registered food poisoning in humans is associated with contamination of milk and dairy products with bacteria such as *Salmonella*, *Staphylococcus aureus*, and enterohemorrhagic *Escherichia coli* [28; 32; 34].

Salmonellosis is one of the most widespread bacterial foodborne pathogens worldwide [14]. This disease is most often registered in North America and Europe. Thus, in the USA, salmonellosis accounts for almost 9% of all foodborne infections [5]. In Ukraine, cases of salmonellosis are registered in almost all administrative territories [30]. *Salmonella* can cause gastrointestinal disease. The main sources of transmission are water, eggs and raw foods [6]. Thus, scientists [1–3] managed to detect salmonella in raw milk, which was sold on local markets. The obtained results showed that 21% of samples of milk and dairy products were positive for *Salmonella*, and their content in raw milk was up to 20%.

A number of authors [16; 44] claim that milk and milk products should be considered as the main source of *Staphylococcus aureus*. In Europe, 5% of outbreaks of staphylococcal toxicosis occurred as a result of

consumption of milk and dairy products. In Ukraine, food staphylococcal intoxication is one of the most common food poisonings of a microbial nature [10]. According to a number of authors [15], about 75% of cases of staphylococcal toxicosis occur in milk and milk products infected with *S. aureus* bacteria. However, not every type of *Staphylococcus aureus* can cause the disease. Most of them are resident microflora of the skin and mucous membranes of humans and animals, being part of many microbiocenoses [14; 20]. It is believed that the most pathogenic and enteroxygenic are coagulase-positive staphylococci, the main representative of which is *S. aureus* [19]. Studies conducted by scientists in our country and abroad [3] indicate that staphylococci are present in raw milk almost constantly and their number ranges from  $5 \times 10^2$  to  $2.8 \times 10^5$  CFU/cm<sup>3</sup>.

Staphylococci that produce the toxin can enter dairy products from the udders of sick animals, from people who carry the bacteria, or from contaminated equipment. The content of *S. aureus* bacteria in the milk of cows suffering from mastitis increases especially, reaching the amount of  $10^8$  CFU/cm<sup>3</sup> and more. Improper storage and insufficient cooling contribute to the reproduction and development of these microorganisms and, accordingly, the formation of toxins in products. The presence of the toxin does not affect the taste or appearance of the product [2; 19; 28].

6 serological types of staphylococcal enterotoxins are described: A, B, C, D, E, and F. Not all pathogenic strains of staphylococci are capable of producing enterotoxin. Individual pathogenic strains of staphylococci produce not one type of toxin, but two or more [2; 14]. Most cases of staphylococcal food poisoning are caused by enterotoxin type A and less often by type D [7; 14].

According to the authors [17], from the total number of enterotoxigenic strains of staphylococcus isolated from milk, 21.2% of microorganisms had the ability to produce type A enterotoxins in amounts from 2 to 250 ng/cm<sup>3</sup>. Producers of type B staphylococcal enterotoxin were practically absent (less than 1.5%), which may be explained by the lack of favorable conditions for the survival and reproduction of enterotoxigenic staphylococci that synthesize type B SET in these products. Also, a strain of *S. aureus* was isolated, in the culture filtrate of which enterotoxins of types A and B were detected at the same time (170 and 200 ng/cm<sup>3</sup>, respectively), while staphylococci producing SET of type C were not detected.



Enteropathogenic strains of *S. aureus* bacteria isolated from humans differ in some properties from animal strains [2]. There are four main biotypes of *Staphylococcus aureus*, mostly related to the types of carriers, which, in turn, differ among themselves. Unfortunately, the authors of many works [15; 23], which are devoted to the detection of *Staphylococcus aureus* in milk and dairy products, are limited to stating the very fact of isolation of these microorganisms without establishing the biotype of *Staphylococcus aureus*, which directly indicates the source of contamination.

Thus, knowledge and control of potential sources of contamination of unprocessed food products with *Staphylococcus aureus* is the key to effective prevention of food toxicosis of staphylococcal etiology.

One of the sanitary-indicative microorganisms that can lead to various types of toxicosis and intestinal poisoning are bacteria of the group of *Escherichia coli*. The most pathogenic representative of Coliform bacteria is *E. coli* bacteria [6; 24]. A number of scientists [17] isolated enterohemorrhagic *Escherichia coli* in 26 to 30% of cases from samples of milk and dairy products that were sold in the markets, and from 16 to 20% of milk produced on the farm. At the same time, other scientists isolated *E. coli* from raw milk in markets in 63% of cases, and in 41.2% on farms [6; 24].

The authors explain the high level of *Escherichia coli* in milk and milk products that were sold in the markets, compared to milk received at milk processing enterprises, by the fact that they are more in contact with the environment and do not always comply with the sanitary and hygienic conditions of receipt, storage and sale [13].

An important and significant group of microorganisms in whole cow's milk and dairy products are bacteria of the genus *Enterococcus*. These microorganisms are always present in milk and make up the so-called primary microflora [24]. Enterococci are used as probiotics and are part of biologically active substances [14]. Recently, the importance of enterococci in the process of formation of antibiotic resistance of these bacteria, which can cause diseases in humans, has increased [21]. According to researchers [20], enterococci were detected in 96% of raw milk samples, and their number was 2.48 log CFU/cm<sup>3</sup>.

Therefore, studies indicate that milk and dairy products are significantly contaminated with opportunistic and pathogenic microflora, which can pose a threat to the health of consumers. This especially applies to milk

and dairy products that are manufactured in violation of veterinary, sanitary and hygienic requirements. Such dairy products include dairy products delivered to agro-food markets, the manufacturing process of which cannot be controlled, and the produced products are practically not tested for safety indicators. In addition, Ukraine lacks the latest data on the quantitative and qualitative composition of the microflora of dairy products sold on agro-food markets. The study of the microflora of these dairy products (sour cream, sour milk cheese) will allow to propose sanitary-indicative microorganisms and their quantitative values for the purpose of hygienic assessment of their quality and safety during sale on agro-food markets.

## 2. Methodology

The main direction of this study was to investigate whole cow's milk and sour milk products (sour cream, sour milk cheese), which are delivered to agro-food markets, according to organoleptic, physico-chemical and microbiological indicators, to improve the control of these products according to microbiological indicators.

### 2.1. Microbiological methods

Sampling of samples of whole cow's milk, fermented milk products, washes from the hands of sellers, trade equipment and their delivery to the laboratory was carried out in accordance with regulatory documents. Preparation of samples and washes, their dilution for research was carried out in accordance with DSTU 4834:2007, DSTU IDF 122C:2003 [31; 32]. Microbiological studies of products were carried out no later than 4 years after sampling.

Samples of sour cream and sour milk cheese were mixed and neutralized before the research. For this sterile flask, 10 cm<sup>3</sup> of the product was taken and 1 cm<sup>3</sup> of a sterile sodium bicarbonate solution with a mass fraction of 100 g/dm<sup>3</sup> was added, the contents of the flask were left to mix.

From samples of milk, dairy products, washes from sellers and trade equipment, a number of tenfold dilutions were carried out in accordance with DSTU IDF [32]. A sterile sodium chloride solution was used for dilution. The number of mesophilic aerobic and facultatively anaerobic microorganisms was determined according to DSTU 7357:2013 and DSTU IDF 100B:2003 [31; 32].

Lactic acid microorganisms were isolated on MRS medium. The study of the composition of lactic acid microflora was carried out as follows: isolation of a pure culture, determination of its belonging to the coccal forms of microorganisms, gram-positive and gram-negative rods.

The content of enterococci was determined on enterococcal medium. Bacterial, gram-positive, and catalase-negative forms that met the requirements of Sherman's tests were assigned to the *Enterococcus* genus: they grew in nutrient broth at a temperature of +45 °C; in a medium containing 6.5% sodium chloride; at pH 9.6 units; with a content of 40% of bile and kept at a temperature of 60 °C for 30 min. An additional type of identification was carried out using the test system EN-COKKUS test ("ERBA-Lachema Diagnostika", Czech Republic).

Isolation of staphylococci from whole cow's milk, sour cream and sour milk cheese was carried out on agar with 5% bovine blood and 5% sodium chloride. The genus *Staphylococcus* included cocci forms of bacteria that stained positively by Gram, produced catalase and fermented glucose in Hugh-Leifson medium. The ability to coagulate plasma was determined by the classic method using rabbit plasma. Identification of staphylococci was carried out on the basis of their biochemical activity using commercial test systems: "STAPHY-test 16", (LACHEMA, Czech Republic). Biotyping of *Staphylococcus aureus* was determined according to Meyer. The Ilek-Levy method was used to determine the type of staphylococcal hemolysins. Phosphatase was determined according to the method described by A. K. Akatov and T. M. Samsonova. Determination of deoxyribonuclease (DNAase activity) of staphylococci was carried out according to methodical recommendations.

To determine staphylococcal enterotoxins, the RIDASCREEN®SET A, B, C, D, E test system (R-Biopharm AG, Darmstadt, Germany) was used according to methodological recommendations.

The titer of BGKP and the content of *E. coli* bacteria were determined according to DSTU 7357:2013 [29]. Determination of the content of fungi and yeast was carried out according to DSTU ISO 7954:2006. The determination of the bacterial genus *Salmonella* was carried out according to DSTU IDF 93A:2003 [29], and the bacterial species *Listeria monocytogenes* according to DSTU ISO 11290-1:2003 and DSTU ISO 11290-2:2003.

Spore-forming bacteria were determined by sowing a sample of milk and dairy products and diluting them on MPA followed by incubation at 30°C for 72 hours. The samples were previously kept in a water bath at a temperature of 85 °C for 10 minutes.

## **2.2. Organoleptic methods**

Samples of milk for taste were carried out after pasteurization. The color was determined in a cylinder made of transparent glass in the rays of reflected daylight, the consistency and appearance – by the trace left on the wall of the cylinder. Organoleptic indicators of sour cream and sour milk cheese were studied according to DSTU 4554:2006 and DSTU 4418:2005.

## **2.3. Physico-chemical methods**

Determination of the mass fraction of protein, fat, density and water content in milk was carried out on the "Laktan" device. The acidity of milk and dairy products was determined by the titrometric method. Inhibitors in milk were determined using the BRT test and the ROSA Milk test according to DSTU ISO 13969:2005. Determination of the number of somatic cells was performed by direct counting using the Prescott-Breed method in accordance with DSTU ISO 13366-1/IDF 148-1:2014.

## **2.4. Statistical methods**

The obtained research results were processed statistically using Microsoft Excel and Statistika 99 Edition programs on a personal computer. The difference was considered probable at  $p \leq 0.05$ ,  $p \leq 0.01$ , and  $p \leq 0.001$ .

# **3. Description of research results**

## **3.1. Improvement of veterinary and sanitary examination of sour milk cheese made from raw cow's milk**

As a result of the deterioration of the economic situation in Ukraine, the number of both sellers of milk and dairy products produced in private farms and buyers has increased in the agro-food markets. This is due to the lower price of these products, compared to dairy products that are sold through a retail chain of stores. It is also traditionally believed that dairy products made from thermally untreated milk are better in terms of biological quality and nutrition.

According to the Rules of veterinary and sanitary examination of milk and dairy products and requirements for their implementation [36], dairy products obtained from animals kept in personal peasant farms of the population are allowed for sale on the agro-food market, provided that their quality and safety are confirmed by an accredited laboratory. Also, the rules state that milk and dairy products must meet the requirements of normative legal acts – DSTU.

Analysis of the activity of state laboratories of veterinary and sanitary examination in agro-food markets showed that they examine sour-milk cheese organoleptically and for acidity, and in some cases for the content of the mass fraction of fat, moisture and soda impurities.

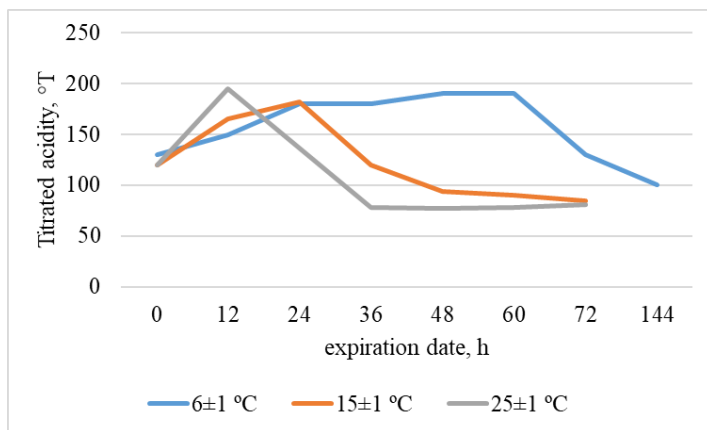
The norms of DSTU cannot directly apply to dairy products made from whole cow's milk, since the industrial technology for making sour milk cheese involves the fermentation of pasteurized milk. In raw fermented milk of industrial production, the content of Coliform bacteria is allowed in 0.001 g of the product [44]. During the production of sour milk cheese in the conditions of private peasant farms, sour milk is subjected to heat treatment at a temperature of  $80 \pm 5$  °C, at which Coliform bacteria die. At the same time, enterococci were found in this cheese as heat-resistant microflora.

Therefore, taking into account the above, in order to increase the safety level of sour-milk cheese, which comes for sale on agro-food markets, it is relevant to study the identification and selection of additional sanitary-indicative microorganisms that would characterize the general sanitary-hygienic state of production, storage and sale conditions.

### **3.1.1. Microbiological and biochemical processes in sour-milk cheese made from raw cow's milk in laboratory conditions**

In order to give a full veterinary and sanitary assessment of sour milk cheese, which comes for sale on agro-food markets, at the first stage of research, indicators of the quality and safety of sour milk cheese, produced in laboratory conditions in compliance with all sanitary and hygienic requirements, were determined. The data obtained in this way will serve as a reference point for the assessment of sour milk cheese made from whole cow's milk, which is delivered to agro-food markets.

Figure 1 shows the results of research on changes in titrated acidity in sour-milk cheese made in laboratory conditions.



**Figure 1. Titrated acidity of sour-milk cheese during storage at different temperatures**

As can be seen from Figure 1, the initial acidity in freshly made sour-milk cheese was  $130 \pm 10$  °T. During exposure for 48 hours at a temperature of  $6 \pm 1$  °C, the acidity of the cheese increased by 1.4-1.6 times ( $p \leq 0.05$ ). From the second day, titrated acidity began to decrease, and on the 7th day of exposure, its content was  $100 \pm 10$  °T, which is 1.9 times less ( $p \leq 0.01$ ), compared to after 48 hours.

Changes in the titrated acidity of cheese samples stored at temperatures of  $15 \pm 2$  and  $25 \pm 3$  °C were also studied, that is, at temperatures that reflect the real conditions under which fermented milk cheese is manufactured and sold. A similar trend of titrated acidity changes was observed at temperatures of  $15 \pm 2$  and  $25 \pm 3$  °C, as in the previous experiment at temperatures of  $6 \pm 1$  °C. However, the time for a rapid increase in acidity to 165-195 °T was reduced to 12-18 hours depending on the storage temperature and was stably maintained at the highest level for up to 6 hours. Then there was a rapid decrease in the acidity of the cheese, and after 36 hours of storage at a temperature of 25 °C it was  $78 \pm 5$  °T and at a temperature of 15 °C –  $120 \pm 6$  °T.

Therefore, the obtained results of a laboratory study of changes in the titrated acidity of sour-milk cheese at different temperatures indicate that

the dynamics of the change in acidity is wave-like and is characterized by a maximum rise to 200 °T during the day, depending on the storage temperature, and is maintained at this level for up to 48 hours when stored at a temperature of  $6\pm 1$  °C, up to 8 p.m. at a temperature of  $15\pm 2$  °C and up to 6 p.m. at a temperature of  $25\pm 3$  °C, and then for 12–24 h it decreases to 80–100 °T.

When studying the quantitative and morphological composition of the microflora of sour-milk cheese, it was established that the microflora of fresh sour-milk cheese is represented by lactic acid bacteria and enterococci. However, the main part consists of lactic acid bacteria – 99.2%, which are attached by cocci forms. After a day of storage, the content of lactic acid bacteria increased by 2.5 times ( $p \leq 0.01$ ), enterococci – by 1.9 times ( $p \leq 0.01$ ), and the morphological composition remained unchanged.

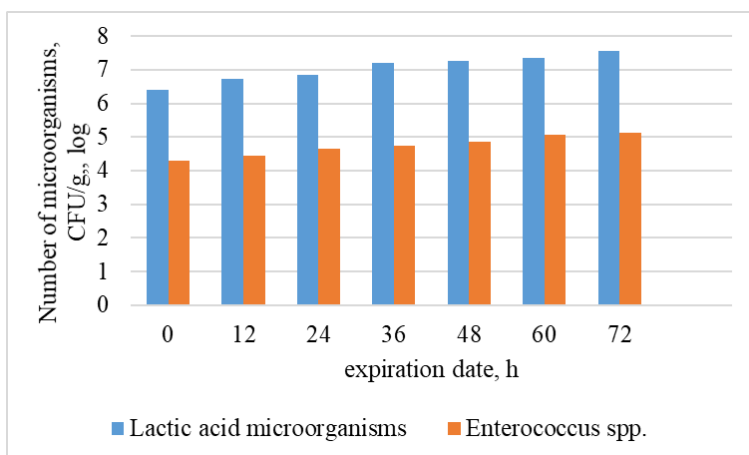
After two days of cheese storage at a temperature of  $6\pm 1$  °C, a quantitative increase in the total number of lactic acid microflora by 1.9 times ( $p \leq 0.01$ ), enterococci by 1.2 times ( $p \leq 0.05$ ) and changes in the morphological composition was noted lactic acid bacteria. The content of cocci forms of bacteria decreased by 1.6 times ( $p \leq 0.01$ ) and they accounted for 55%, and rod-shaped forms of microorganisms increased by 4.5 times ( $p \leq 0.001$ ) and amounted to 45%.

On the sixth-seventh day of storage, the quantitative content of lactic acid microorganisms increased 14 times ( $p \leq 0.001$ ), in comparison with the initial amount, enterococci increased by 4.65 times ( $p \leq 0.001$ ), and the morphological composition of the microflora was transformed from coccal to rod-shaped, which was 90%.

The results regarding the reproduction of lactic acid microorganisms and bacteria of the genus *Enterococcus* in sour milk during 72 hours of storage are shown in Figure 2.

As can be seen from Figure 2, intensive reproduction of lactic acid microflora occurred already during 12 hours of storage of sour-milk cheese. Its content increased at a temperature of  $15\pm 2$  °C by 2.2 times ( $p \leq 0.01$ ), and at a temperature of  $25\pm 2$  °C by 5.0 times ( $p \leq 0.001$ ). The number of enterococci during this period at these temperatures increased by 1.35 times ( $p \leq 0.05$ ) and 3.2 times ( $p \leq 0.001$ ), respectively.

During this period, the microflora of sour-milk cheese is in the logarithmic phase of development, as a result of which there is a rapid increase in titrated



**Figure 2. The composition of the microflora of sour-milk cheese when stored at a temperature of  $15\pm 2$  °C for 72 hours**

acidity. The duration of the logarithmic phase for lactic acid microflora and enterococci in sour milk at a temperature of  $15\pm 2$  °C was up to 36 hours on average, as a result, the number of lactic acid bacteria increased 6.3 times ( $p\leq 0.001$ ) to 7.19 log, CFU/g, and enterococci – 2.8 times ( $p\leq 0.01$ ) to 4.74 log, CFU/g.

At a storage temperature of  $25\pm 2$  °C, the maximum intensity of development of lactic acid bacteria and enterococci occurred within 24 hours. During this time period, lactic acid microflora increased 8.3 times ( $p\leq 0.001$ ) to 7.31 log, CFU/g, and Enterococcus bacteria – 5.1 times ( $p\leq 0.001$ ) to 5.0 log, CFU/g of cheese

After 36 hours of storage of sour milk cheese at a temperature of  $15\pm 2$  °C and after 24 hours at a temperature of  $25\pm 2$  °C, a decrease in the intensity of development of lactic acid bacteria by 2.3-2.4 times ( $p\leq 0.01$ ) and a decrease in the reproduction of enterococci by 1.6 – 2.4 times ( $p\leq 0.01$ ), which indicates the entry of microflora into the stationary phase of development.

Also, the data of Figure 2 indicate that the rate of development of lactic acid microflora and enterococci at a temperature of  $25\pm 2$  °C is, on average, 2-3 times faster, compared to a temperature of  $15\pm 2$  °C. This indicates that as the ambient temperature increases, biochemical processes will take place



more intensively in sour-milk cheese, as a result of which the organoleptic properties and indicators of quality and safety will deteriorate.

Therefore, the conducted laboratory studies revealed that the increase in titrated acidity occurs in freshly made sour-milk cheese at the expense of coccal lactic acid microflora, the number of which increases maximally on the second day of storage at a temperature of  $6\pm 1$  °C. Further storage at this temperature leads to a change in the composition of lactic acid microflora, namely: cocci lactic acid bacteria gradually die, and rod-shaped forms of lactic acid bacteria multiply. These bacteria metabolize accumulated lactic acid and, as a result, its content rapidly decreases (by 1.9 times ( $p\leq 0.01$ )). After 48 hours of storage of sour-milk cheese at a temperature of  $6\pm 1$  °C, the content of sticks gradually increases even on the seventh day their content is 90%, and the titrated acidity remains at the same level. A practically similar trend in the dynamics of the microbiological and biochemical process is also noted at higher cheese storage temperatures of  $15\pm 2$  and  $25\pm 2$  °C, however, the time for the increase of acidity is significantly reduced, and the intensity reproduction of microflora increases tenfold.

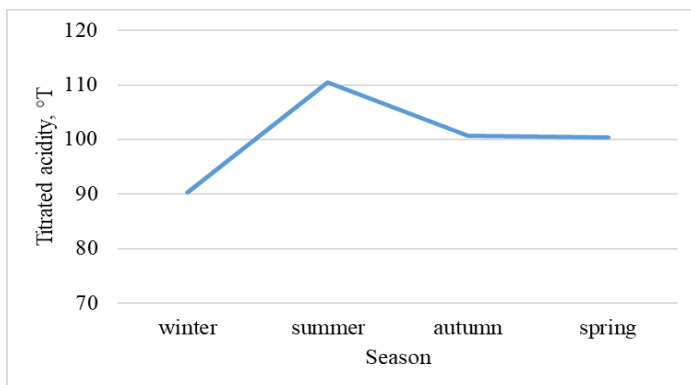
In general, the obtained data of experimental studies indicate that the determination of the value of titrated acidity in raw sour milk, which is delivered to the agro-food markets, will not characterize its freshness, shelf life, and production time.

### **3.1.2. Microbiological and biochemical processes in raw sour milk, which is delivered to agro-food markets**

At the second stage of the work, our research was aimed at determining the titrated acidity, quantitative, generic and species composition of the microflora of sour-milk cheese made from raw cow's milk, which is delivered to agro-food markets.

The results of studies on the determination of the titrated acidity of sour-milk cheese, which is sold in agro-food markets, during the year are shown in Figure 3.

As can be seen from Figure 3, the value of the titrated acidity in sour milk during the year practically did not change and was, on average,  $100.5\pm 9.2$  °T. If we compare these results with the data shown in Figure 3, it can be noted that sour-milk cheese is sold on agro-food markets freshly made or after 24 h of storage, due to the fact that the titrated acidity of



**Figure 3. Titrated acidity of sour-milk cheese, which is delivered to agro-food markets during the year**

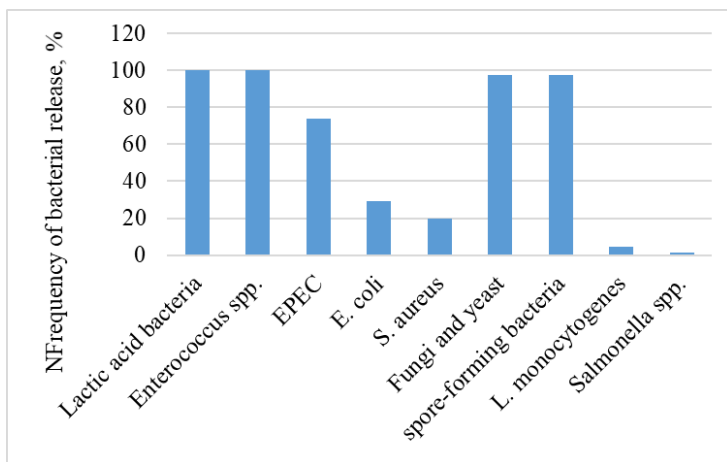
freshly made cheese was, on average,  $130 \pm 15$  °T. We did not detect a single sample of cheese taken from agro-food markets with a content of titrated acidity in the range of 180-200 °T.

These data confirm the results of laboratory studies and indicate that the amount of titrated acidity in raw fermented milk, which arrives for sale on agro-food markets, is not an indicator of its freshness.

The results of studies of the frequency of isolation of various species and genera of microorganisms from sour milk cheese are shown in Figure 4.

As can be seen from Figure 4, the permanent microflora of sour milk cheese, which was isolated in 100%, can include lactic acid bacteria and enterococci, fungi and spore-forming microorganisms (isolated in 97.7% of cases). 73.8% of the examined cheese samples were contaminated with BGCP and 29.4% with *E. coli* bacteria. *Staphylococcus aureus* was isolated from approximately 20% of sour-milk cheese samples, and pathogenic microorganisms *Listeria monocytogenes* and *Salmonella* spp. were isolated from 4.8 and 1.6% of samples, respectively.

The most numerous and always present lactic acid group of the microflora of sour milk cheese was isolated in the amount of  $10^6$  CFU/g in  $86.5 \pm 6.3\%$  of cases. Enterococcus bacteria, which are also the normal microflora of sour milk cheese made from whole cow's milk, are present in the amount of 105-104 CFU/g.



**Figure 4. The frequency of isolation of microorganisms from sour-milk cheese, which is delivered to agro-food markets during the year**

Sanitary indicator microorganisms of Coliform bacteria were isolated from samples of sour milk cheese in different quantities. According to DSTU 4554:2006, Coliform bacteria should not be released from 0.001 g in sour-milk cheese during storage for 72 hours. Our research revealed that  $89.3 \pm 5.1$  samples of sour-milk cheese were sold with an above-standard content with a titer from 0.001 to 0.00001, and the largest number of them was  $10^6$  CFU/g in  $3.2 \pm 0.61\%$  of samples. *Escherichia coli*, which indicates fecal contamination of the product and is an indicator of the sanitary-epidemiological state, was isolated in the amount from  $10^1$  to  $10^5$  CFU/g.

*Staphylococcus aureus* should not be isolated from 0.01 g of sour milk cheese. Main amount of  $88.0 \pm 3.78\%$  of sour-milk cheese samples contained *Staphylococcus aureus* in  $10^2$ - $10^3$  CFU/g, and its maximum amount was  $10^4$  CFU/g in  $8.0 \pm 0.74\%$  of samples. Only  $8.0 \pm 0.56\%$  of sour milk cheese, which were contaminated with *Staphylococcus aureus*, met the requirements of DSTU 4554:2006 regarding its quantity.

Fungi uniformly contaminated sour milk cheese from  $10^1$  to  $10^6$  CFU/g. Their allocation in the amount of more than  $10^2$  indicates non-compliance with sanitary and hygienic conditions of production or the sale of a

product that has been stored for a long time. Pathogenic microorganisms *Listeria monocytogenes* and *Salmonella* spp. were released in the amount of  $10^2$  CFU/g, which cannot fail to attract attention, as they are causative agents of foodborne infections.

The organoleptic indicators of sour milk cheese depend on the season. Most of the good-quality sour milk cheese with satisfactory and acceptable organoleptic indicators was sold in the winter-spring period – 96.5-97.5%. In the summer and autumn periods, the amount of cheese with such organoleptic properties was 93.4-92.9%. Also, in the summer-autumn period, the number of samples with questionable organoleptic properties increased by 1.5-2.7 times ( $p \leq 0.01$ ). The increase in samples of sour milk cheese with questionable organoleptic properties in the summer-autumn period can be explained by the increased temperature of the environment, which contributes to the development of microflora and spoilage of the product.

Sour milk cheese, which was sold in agro-food markets with a mass fraction of fat of 18%, contained moisture at least 65%, with a mass fraction of fat of 9% – 73% and low-fat – 80%, which meets the requirements of regulatory documents.

Therefore, the conducted microbiological studies on the quantitative and qualitative contamination of sour-milk cheese indicate that sour-milk cheese made from whole cow's milk, the normal microflora of which consists of lactic acid microorganisms and enterococci, is sold on agro-food markets. Other microorganisms were isolated in different quantities and, obviously, in order to determine the safety of sour-milk cheese, it is necessary to take a comprehensive approach to the interpretation of sanitary-indicative microorganisms. The absence of 25% of samples or the selection of a small amount of Coliform bacteria does not guarantee the complete safety of the cheese. Currently, the state cannot refuse products produced in private peasant farms, which are sent to agro-food markets for sale. However, in order to increase the safety of dairy products, it is necessary to develop a new model of their research using modern microbiological criteria and the identification of additional sanitary-indicative microorganisms.

### **3.1.3. Contamination of sour-milk cheese with *Enterococcus* bacteria**

In order to substantiate the microbiological criteria of the safety of sour-milk cheese using *Enterococcus* bacteria, it was necessary to conduct a

study on their quantitative content in the product. Therefore, the next stage of the work was to conduct a comparative study to determine the content of enterococci and Coliform bacteria as sanitary indicator microorganisms in sour milk cheese, which is delivered to agro-food markets.

The results of studies of the content of Coliform bacteria in sour milk, depending on the number of lactic acid microorganisms, are given in Table 1.

Table 1

**The content of lactic acid microorganisms and the titer of Coliform bacteria in sour-milk cheese, %,  $M \pm m$ , n = 61**

Content of lactic acid microorganisms, CFU/g	Samples were studied, n	Title of Coliform bacteria					
		$\geq 1$	0,1	0,01	0,001	0,0001	0,00001
Up to 1 mln.	11	27,3 $\pm$ 2,45	18,2 $\pm$ 1,62	9,1 $\pm$ 0,7	27,3 $\pm$ 4,5	18,2 $\pm$ 1,7	0
From 1 mln. to 10 mln.	33	12,1 $\pm$ 1,08	12,1 $\pm$ 1,0	9,1 $\pm$ 0,8	36,4 $\pm$ 3,2	18,2 $\pm$ 1,8	12,1 $\pm$ 1,0
From 11 mln. to 30 mln.	17	0	11,8 $\pm$ 1,0	11,8 $\pm$ 1,0	23,5 $\pm$ 2,2	29,4 $\pm$ 3,4	23,5 $\pm$ 2,2

As can be seen from the table 1 the content of Coliform bacteria in sour-milk cheese did not depend on the quantitative content of lactic acid microorganisms. Coliform bacteria were almost uniformly isolated from sour milk cheese, both with the number of lactic acid microorganisms up to 1 million CFU/g, and from 11 to 30 million CFU/g. If we take into account the fact that the lactic acid microflora in freshly made fermented milk cheese is mainly 1-10 million CFU/g, then its increase indicates reproduction due to violation of storage conditions and term. At the same time, Coliform bacteria in sour-milk cheese is an indicator of compliance with sanitation and hygiene during production, storage and sale. In our opinion, according to the technology of production of sour milk cheese from thermally untreated milk and the conditions of its sale on the market, they can fall into any segment from production to sale. The detection of a significant number of them  $\geq 0.001$  in cheese does not necessarily indicate a multiplication process, because in this product they multiply slowly due to high acidity.

The results of research on the content of enterococci in cheese, depending on the number of lactic acid microorganisms, are shown in Table 2.

Table 2

**Content of enterococci and lactic acid microorganisms in sour-milk cheese, %,  $M \pm m$ ,  $n = 61$**

Content of lactic acid microorganisms, CFU/g	Samples were studied, n	Content of enterococci, thousand CFU/g			
		up to 10	from 10,001 up to 50	from 50,001 up to 100	More 100,001
Up to 1 mln.	11	18,2±1,6	63,6±5,72	18,2±14,56	0
From 1 mln. to 10 mln.	33	9,1±0,8*	36,4±2,9*	48,5±3,84*	6,0±0,48
From 11 mln. to 30 mln.	17	0	17,7±1,4♦	52,9±5,3**	23,4±1,45♦♦

Notes: \* –  $p \leq 0.01$ ; \*\* –  $p \leq 0.001$  – compared to samples up to 1 million CFU/g of lactic acid microorganisms; ♦ –  $p \leq 0.01$ ; ♦♦ –  $p \leq 0.001$  – compared to samples with up to 10 million CFU/g of lactic acid microorganisms.

When the number of lactic acid microorganisms in cheese is from 1 to 10 million CFU/g, the growth of enterococci was also noted. Thus, 9.1% of cheese samples contained enterococci up to 10,000 CFU/g, which is 2.0 times less ( $p \leq 0.01$ ) compared to samples with a content of lactic acid microorganisms up to 1 million CFU/g. With this content of lactic acid microorganisms, enterococci in almost 50% of the samples were from 50 to 100 thousand CFU/g, and only 6% had a content of more than 100 thousand CFU/g. The growth of lactic acid microorganisms in cheese up to 10 million led to a decrease in the number of cheese samples with enterococci content from 10 to 50 thousand CFU/g by 1.7 times ( $p \leq 0.01$ ) and an increase in samples with enterococci content from 50 to 100 thousand CFU/g by 2.7 times ( $p \leq 0.01$ ), compared to samples with the content of lactic acid microorganisms up to 1 million CFU/g.

In the samples of sour-milk cheese with the number of lactic acid microorganisms from 11 to 30 million CFU/g, we note a 3.9-fold increase ( $p \leq 0.001$ ) in the samples of sour-milk cheese with the content of enterococci more than 100 thousand CFU/g, the absence of samples with the content of enterococci up to 10 thousand and a 2-fold decrease ( $p \leq 0.01$ ) of samples with

enterococci content from 10 to 50 thousand CFU/g, compared to samples with the number of lactic acid microorganisms up to 10 million CFU/g.

The results of studies on the compliance of the Coliform bacteria titer and the content of enterococci with the organoleptic indicators of sour milk cheese, which is delivered to the agro-food markets, are shown in Table 3. The table includes data in which the Coliform bacteria titer was not lower than 0.001.

Table 3

**Comparative characteristics of enterococci content, *Coliform bacteria* titer and organoleptic indicators of sour-milk cheese, n=27**

Quality and safety indicators:			Quality and safety assessment
Organoleptic	Title of Coliform bacteria	Content of enterococci, CFU/g	
The consistency is soft or crumbly. The taste and smell are sour-milk, clean and gentle, without excessive acidity, extraneous tastes and smells. The color is white with a yellowish tint, uniform throughout the mass	1–0,01	up to 10 000	Satisfactorily
The consistency is soft or crumbly. The taste and smell are sour-milk, clean and gentle, without excessive acidity, extraneous tastes and smells. The color is white with a yellowish tint, uniform throughout the mass	1–0,01	from 10,001 up to 50,000	Satisfactorily
The consistency is soft or crumbly. The taste and smell are sour-milk, clean and gentle, without excessive acidity, extraneous tastes and smells. The color is white with a yellowish tint, uniform throughout the mass	1–0,01	from 50 001 up to 100 000	Reasonably
The consistency is soft or crumbly. Slightly sour taste with a bitter aftertaste. The color is creamy throughout the mass	1–0,01	more 100,001	Doubtful

As can be seen from the table 3, the Coliform bacteria titer is not a stable indicator that demonstrates the quality of sour-milk cheese. The content of Coliform bacteria was almost identically detected both in cheese samples with the number of enterococci up to 10,000 CFU/g and more than 100,000 CFU/g. At the same time, when the content of enterococci in cheese is more than 100,000 CFU/g, we detected organoleptic changes, which were manifested by a sour taste and a bitter aftertaste. This indicates that the amount of *Enterococcus* bacteria more objectively characterizes the quality of sour milk cheese.

Therefore, the conducted studies indicate that sour-milk cheese is suitable for sale on agro-food markets practically without the presence of microbiological criteria that regulate its safety.

Microbiological indicators, namely the titer of Coliform bacteria and others, specified in DSTU 4554:2006 "Sour milk cheese. Technical conditions" [44] cannot be directly transferred and fully characterize the sanitary conditions of production and sale of sour-milk cheese made from whole cow's milk. To provide this additional sanitary indicator microorganisms. In this case, bacteria of the genus enterococci could be suitable, which would complement the general sanitary picture of sour-milk cheese, as well as testify to its storage conditions and terms of sale.

#### **3.1.4. Development of a method of veterinary and sanitary examination of sour-milk cheese for the content of *Enterococcus* bacteria**

When determining the microbiological criteria for bacteria of the genus *Enterococcus* regarding the safety indicators of sour-milk cheese, which is delivered to the agro-food markets of Ukraine, we proceeded from the following considerations:

- bacteria of the genus *Enterococcus*, as heat-resistant microorganisms, belong to the normal microflora of sour milk cheese made from whole cow's milk, therefore a certain safe amount of them will always be present in the cheese and should not pose a threat to consumers;

- among the large number of species of bacteria of the genus *Enterococcus* in sour-milk cheese, two species *E. faecalis* and *E. fecium* dominate, which make up an average of 85% and are of fecal origin. These species are opportunistic bacteria and can cause food poisoning and



various inflammatory processes in people with reduced body resistance. The microbiological norm of the content of enterococci in sour-milk cheese must be determined taking into account the technological processes of production, and a large number of *Enterococcus* bacteria will be considered dangerous for consumers;

– the determined microbiological standard for the content of bacteria of the genus *Enterococcus* in sour-milk cheese made from whole cow's milk should characterize its quality and safety during implementation, as well as be an additional indicator to the already existing methods of assessing the quality and safety of sour-milk cheese.

Taking into account the results of our research, we found that enterococci were  $20.0 \pm 1.7$  thousand CFU/g in freshly prepared sour-milk cheese under laboratory conditions. Therefore, the number of enterococci within 25 thousand CFU/g in sour-milk cheese can be considered their constant content, which will always be present in sour-milk cheese. During the observance of the "cold chain" and the storage of sour-milk cheese at the standard cooling temperature of  $6 \pm 1$  °C for food products, the number of enterococci increased 1.9 times after a day to  $38.0 \pm 3.1$  thousand CFU/g, after 48 hours – up to  $47.0 \pm 4.4$  thousand CFU/h, and after 6-7 days on average up to 100 thousand CFU/h.

According to DSTU 4554:2006, sour milk cheese is divided into those that are stored and sold for no more than 72 hours, and those that are sold for more than 72 hours, according to the shelf life at a temperature of 6 °C. Considering the first option, according to our research, the number of enterococci when stored at  $6 \pm 1$  °C after 48 hours was within 50 thousand CFU/g. This number of enterococci can be considered a sanitary-hygienic standard for sour-milk cheese, which was produced, stored and sold in ideal conditions in compliance with all hygiene rules.

However, in real production conditions, it is not always possible to comply with the conditions of the "cold chain" from the moment of production of sour milk cheese, and before and during its sale on agro-food markets. The cheese is stored and sold without refrigeration (depending on the ambient temperature). Taking into account this fact, the results of our research revealed that at a storage temperature of 15 °C, the number of enterococci after 24 hours was  $45.0 \pm 4.7$  thousand CFU/g, and after 48 hours –  $73.6 \pm 7.1$  thousand CFU/g. At a temperature of 25 °C, the number of enterococci after 12 hours was

63.8±5.9 thousand CFU/g, and after 24 hours – 102.0±9.5 thousand CFU/g. Based on the research data, we believe that when storing sour milk cheese at a temperature of 15 °C, it can be sold for 24 hours, and at a temperature of 25 °C for no more than 12 hours. Under these conditions, the content of enterococci will be within 50 thousand CFU/g, and the organoleptic properties of sour milk cheese will correspond to freshly made cheese. A further increase in the number of enterococci to 75-100 thousand CFU/g will characterize the deterioration of its quality and decrease in safety.

The results of our research also revealed that the indicator of the content of bacteria of the genus *Enterococcus* in sour-milk cheese made from whole cow's milk, which comes for sale on agro-food markets, is a more stable indicator, compared to the titer of Coliform bacteria, and more objectively characterizes its sanitary conditions production, transportation and sale.

Therefore, taking into account our principles and the European approach to establishing microbiological safety indicators for food hygiene, we have developed microbiological safety criteria for sour milk cheese made from whole cow's milk, which is delivered to agro-food markets.

Sour milk cheese research for producers who want to sell it for the first time on the agro-food market is carried out every 10 days during the month.

If the content of enterococci  $\geq M$  was detected during the first microbiological examination, such cheese is considered unacceptable and is not allowed to be sold. When, during the first microbiological examination of fermented milk cheese, the content of enterococci was found in the range between  $m$  and  $M$ , it is considered acceptable and is examined for a month every 10 days. If during this period the values were between  $m$  and  $M$ , or less than  $m$ , then it is examined twice a month. Provided that during the first microbiological examination of sour-milk cheese, the content of enterococci was found to be less than  $m$ , then it is considered satisfactory and is examined every 10 days for a month. And if during this period the values were less than  $m$ , then it is examined once a month.

Thus, the model developed by us for determining the microbiological safety of sour-milk cheese based on the content of bacteria of the genus *Enterococcus* well characterizes the entire complex of sanitary measures during the production and sale of sour-milk cheese and allows us to take appropriate corrective actions in order to correct the situation and prevent its occurrence. This hygienic standard for the content of enterococci in

Table 4

Safety criteria of sour-milk cheese made from raw cow's milk, which is delivered to agro-food markets

Category of food products	Microorganisms	Sampling plan		Permissible limits		The stage where the indicator is applied	Actions in case of unsatisfactory results
		n	c	m	M		
Sour milk cheese made from raw cow's milk	<i>Enterococcus</i>	3	2	50 000 CFU/g	100 000 CFU/g	during production and sale	prohibition of implementation; recommendations for improving production hygiene

Notes:  $n$  – is the number of samples taken from one manufacturer;  $c$  – is the number of samples whose parametric values are between  $m$  and  $M$ ;  $m$  – is the normative value of the content of enterococci in 1 g of sour milk cheese;  $M$  – is the maximum content of enterococci in 1 g of sour milk cheese.

sour-milk cheese complements the existing safety assessment methods and will increase the microbiological quality of sour-milk cheese made from whole cow's milk, which is delivered to agro-food markets.

#### 4. Conclusions

It was established that the titrated acidity in sour-milk cheese changes in a wave-like manner during the storage time of 6-7 days. During the first day, the acidity of the cheese increases to  $180 \pm 20$  °C and is maintained at this level for up to 48 hours at a storage temperature of  $6 \pm 1$  °C, up to 20 hours at  $15 \pm 2$  °C and up to 18 hours at  $25 \pm 3$  °C, and then decreases to 80-100 °T within 12-24 hours. The acidity of the cheese, which is sold on the agro-food markets, was  $100.5 \pm 9$  °T during the year. The amount of titrated acidity of cheese does not characterize its quality and freshness.

The microbiological composition of sour-milk cheese, which is produced in laboratory conditions, is represented by lactic acid bacteria and enterococci, the number of which was 6.39 log CFU/g and 4.3 log CFU/g, respectively. Storing cheese at a temperature of

15±2 °C for 36 hours leads to an increase in the number of lactic acid bacteria by 6.3 times ( $p \leq 0.01$ ), and enterococci by 2.8 times ( $p \leq 0.05$ ). The maximum intensity of development and increase in the number of lactic acid bacteria by 8.3 times ( $p \leq 0.01$ ) and enterococci by 5.1 times ( $p \leq 0.05$ ) occurs at a cheese storage temperature of 25±2 °C for 24 hours.

It was established that lactic acid bacteria and enterococci were isolated in 100% of the samples from sour-milk cheese, which is sold in agro-food markets, fungi and spore-forming bacteria were isolated in 97.7±1.2% of the samples, Coliform bacteria in 73.8±3.5 %, *E. coli* – 29.4±1.4%, KPS on average – in 20%, pathogenic microorganisms *L. monocytogenes* and *Salmonella* spp. – in 4.8±0.42 and 1.6±0.52% of samples, respectively.

*E. faecalis* species dominates among enterococci in raw fermented milk, which is delivered to agro-food markets, which is 73.4±6.71%, which is 1.37 times ( $p \leq 0.05$ ) more than in milk whole cow's milk. The remaining enterococci were represented by the species *E. faecium* – 12.1±1.08%, *E. durans* – 5.3±0.47 and unidentified – 9.2±0.82%. The sensitivity of *E. faecalis* isolated from sour milk cheese to antibacterial drugs was 1.3-37.0 ( $p \leq 0.05$ ) times lower than that of *E. faecalis* isolated from whole cow's milk.

Microbiological criteria for the safety of sour-milk cheese delivered to agro-food markets have been developed, based on the content of Enterococcus bacteria:  $n = 3$ ;  $c = 2$ ;  $m = 50,000$ ;  $M = 100,000$ , which characterize compliance with sanitary and hygienic requirements during the production and sale of cheese and allow appropriate corrective actions to be taken.

In order to improve the safety and quality of sour milk cheese made from raw cow's milk, which is delivered to agro-food markets, microbiological criteria for the content of Enterococcus bacteria are proposed:  $n = 3$ ;  $c = 2$ ;  $m = 50,000$ ;  $M = 100,000$ , which characterize compliance with sanitary and hygienic requirements during the production and sale of cheese and allow appropriate corrective actions to be taken.

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