

## INVESTIGATIONS REGARDING THE PRESENCE OF MICROORGANISMS IN THE MILK FOOD PRODUCTS COMMERCIALIZED IN REPUBLIC OF MOLDOVA

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### Abstract

The scientific research reflected in this study constituted the determination of microorganisms in some categories of milk used in human consumption: raw cow milk and raw goat milk, pasteurized milk sold in the store according to the indicators of the total number of germs, microscopy and bacteriology. The recorded results of the number of bacterioscopic microorganisms, microbial colonies after the classification results and milk categories were evaluated, which recorded various microbiological values of a normal bacterial microflora in the milk food product. The bacterioscopic and bacteriological values obtained allow consumers to be safe and use this food product in their consumption.

**Key words:** Milk, Bacteriology, Bacterioscopy, Microorganisms, Microflora.

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Milk is one of the oldest and main foods consumed by humans. It is presented as an emulsion of fat in water, in which other chemical substances are also dissolved and comes from the mammary gland of mammals (cow, sheep, buffalo, goat or mixture). Due to its varied chemical composition and rich in the main groups of necessary nutrients, milk is the most complete food, and the caloric value of milk and dairy products obtained from milk processing is very high due to the content of water, gases, dry matter [2, 5, 4, 7, 9].

The presence of microorganisms in milk is of particular importance for quality, sanitation and freshness. These microorganisms can increase or decrease the quality of products or make them inedible, either through their pathogenic action or through their degradation and production of toxic metabolites [11, 13].

It is considered that the microorganisms in milk are important from the following points of view: some produce flavor and desired physical changes and are used to obtain different dairy products; others can be pathogenic or potentially pathogenic and toxic to humans, which is why their presence in milk must be known and prevented; many can cause various defects in color, consistency, smell and taste in milk and dairy products or create difficulties in their manufacturing technology [10; 14, 15].

The issue of milk microorganisms refers to most of the groups of microorganisms described in

the existing determinants, which require specialists in the field of food microbiology to know the rationale for using useful ones and removing useless or harmful ones. Therefore, from this point of view, the useful lactic microflora is represented by the lactic bacteria, which belong to two different families: the family Lactobacillaceae and the family Streptococcaceae, comprising the most important microorganisms for the milk industry [1, 3, 6, 8, 12].

The purpose of the study is to determine the microorganisms in some categories of milk used in human consumption: raw cow milk and raw goat milk, pasteurized milk sold in the store according to the indicators of the total number of germs, microscopy and bacteriology.

### MATERIAL AND METHOD

The presence of microorganisms in raw and pasteurized cow and goat milk assortments regarding the quantitative study was investigated by using microbiological techniques for sampling representative milk samples from peasant raw cow milk and raw goat milk procured from the central market and pasteurized milk procured from a supermarket from Chisinau.

The microbiological tests were carried out according to the microbiological laboratory methodology in order to identify the saprophytic and pathogenic microflora. Milk samples were taken, which were subject to investigations in the Microbiology Laboratory of the Food Safety and

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Public Health Department, Faculty of Veterinary Medicine, Technical University of Moldova. The bacterioscopic and bacteriological study constituted of the determination of the amount of saprophytic microflora existing in the milk samples, the presence of coliform, salmonella, staphylococci bacteria. Developmental characters of microbial cultures on simple and special culture media were visualized and their characteristics were expressed. At the same time, smears from the native samples and cultures were performed, stained according to the Gram method, the counting of microbial colonies and microscopic visualization with immersion, objective 90, in order to determine the species of microscopic microorganisms and their definition.

## RESULTS AND DISCUSSIONS

The microbiological research of different types of milk allowed us to analyze the results of figure 1, where the microscopy of raw cow's milk samples is shown. The results of this study allow us to analyze the contamination of raw cow's milk. It is observed that sample 3 of raw cow's milk is more polluted with microorganisms constituting 6 cocci under microscopy, compared to sample number 2 where the number of microbial cells/cocci constituted 3 microbial cells and sample number 1 constituted 2 microbial cells. These samples of raw cow's milk constituted classes III and IV. These reports confirm the fact that the contamination of these samples of raw cow's milk presents insignificant values, which do not denote pathogenic species, but on the contrary reflect insignificant numbers of saprophytic cocci, which can normally be found in milk.

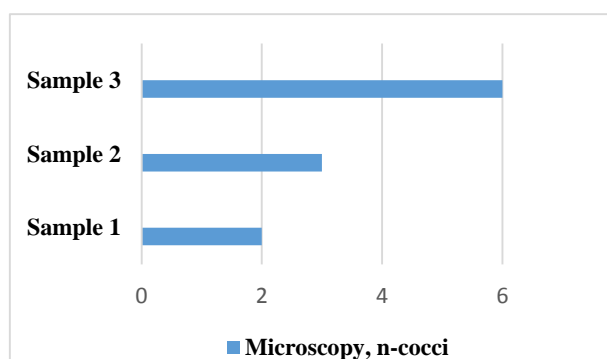


Figure.1. Microscopy of raw cow's milk samples

Source: elaborated by the authors

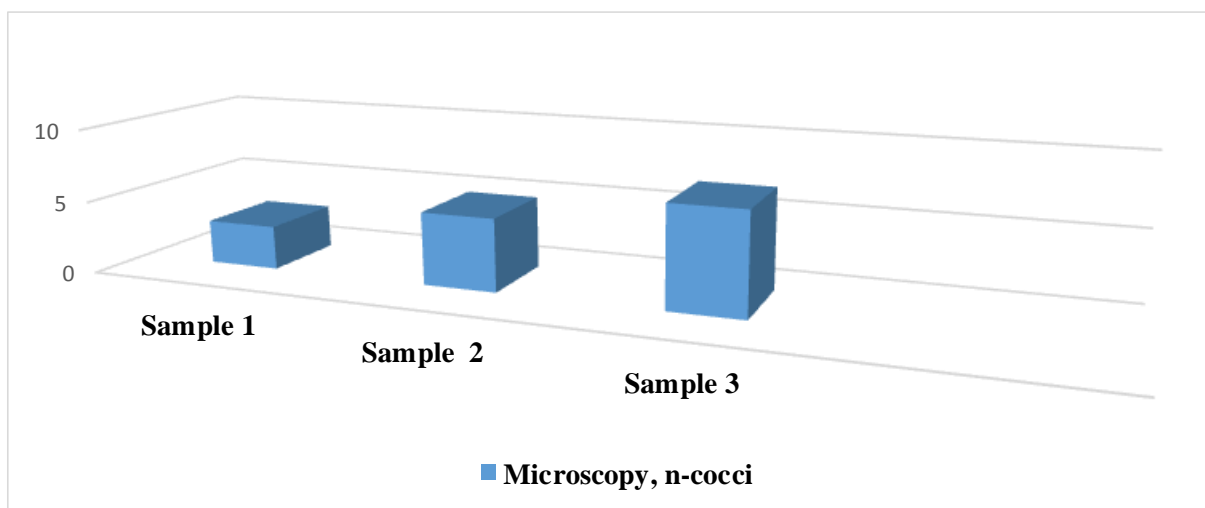
This detection and isolation is important and allows us to conclude that it is not dangerous for food use. These tests are usually practiced in microbiological laboratories because it is also important to test for the presence of Salmonella pathogenic species in any ready-to-eat food.

Important characteristics regarding the microscopy of the raw goat milk samples performed are reproduced in figure 2, where it is observed that the number of cocci is a higher number in sample number 3, constituting 7 cocci under microscopy, followed by a number of 5 cocci determined by microscopy in sample number 2 and 3 cocci determined by microscopy in sample number 1.

These raw goat milk samples constituted classes III and IV. Based on these data, according to the microbiological laboratory conduct, the scientific research were focused on the bacterioscopic investigations by performing bacterial smears from the samples of milk samples and simple staining with the methylene blue dye. From the point of view of these aspects, the number of microorganisms was determined on the colored preparations according to the simple method, which according to the morphological aspects were spherical representing bacterial cocci.

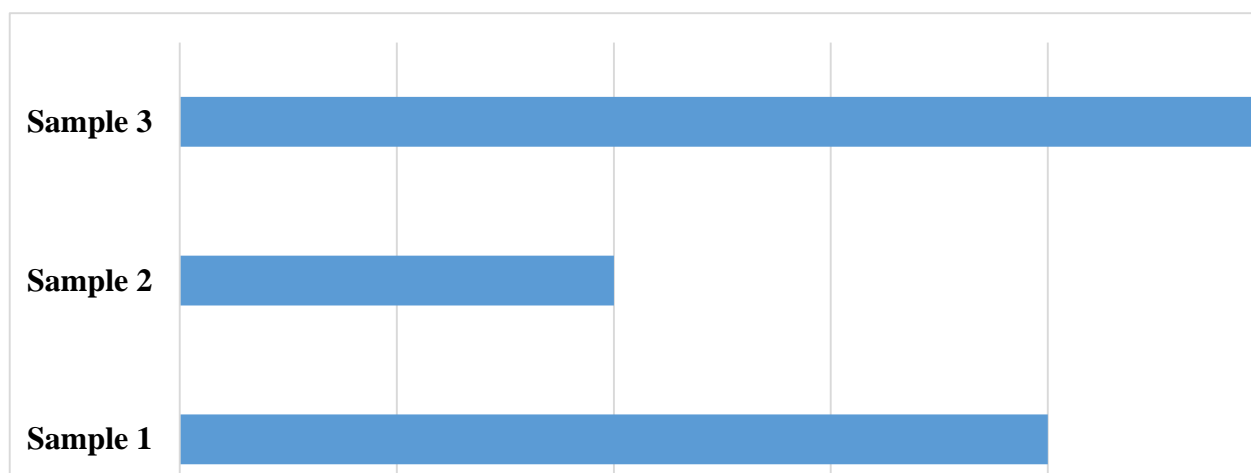
The analysis of the indices presented in figure 3 shows that, as a result of the microbiological research carried out in the samples of pasteurized milk sold in the store, the microflora consists of 3 cocci bacteria under microscopy in the milk sample number 3, compared to the pasteurized milk samples number 1 and 2 where the cocci bacterial microflora constituted in the sample 1 - 2 cocci and the sample 2 - 1 cocci.

The classes of the milk samples according to the microscopic results of the number of detected cocci were classified in the class I category of all the investigated milk samples. As a result of the microscopy research of smears from pasteurized milk, the simple/methylene blue coloration, the morphological aspects of the cocci germs, which were presented on the microscopic field, Gram positive constituting a normal microflora of the investigated pasteurized milk, are revealed.



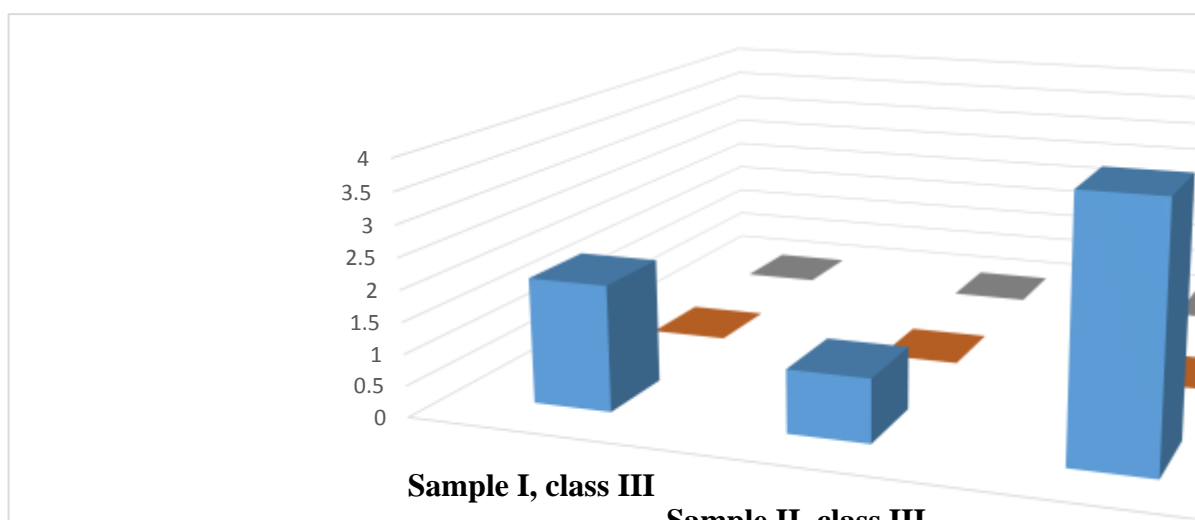
**Figure.2. Microscopy of raw goat milk samples**

Source: elaborated by the authors



**Figure.3. Microscopy of pasteurized milk samples**

Source: elaborated by the authors



**Figure.4. The number of microbial colonies in the classes of raw cow's milk samples**

Source: elaborated by the authors

The bacterioscopic investigations of milk samples of various commercial categories according to the studies carried out demonstrate the fact that all the categories of milk investigated from the bacterioscopic point of view are favorable and beneficial to be used in food.

Of significant importance are the samples of pasteurized milk sold in the store, which in our microbiological research study confirmed that the pasteurized milk food product sold in the store after the conduct of the microbiological examination confirms that this category of milk is polluted with a lower number of saprophytic microorganisms represented by Gram positive cocci.

The data in figure 4 shows some quantitative accounts of the number of colonies highlighted in the bacterial microflora of raw cow's milk recorded by the number of microbial colonies visualized in three samples of raw cow's milk sold on the market. Thus, the indications in figure 4 indicate the detection of 4 bacterial colonies in the microflora of raw cow's milk sample number 3, compared to sample number 1, where the number of colonies constituted 2 colonies and sample number 2, where the number of microbial colonies constituted 1 colony. According to the number of microbial colonies, the samples of raw cow's milk constituted classes III and IV.

Figure 5 shows the quantitative study of the number of microbial colonies in the classes of raw goat milk samples and reveals that the classes of these samples of raw goat milk are of category III and IV.

Passages were performed on simple agar culture media, broth and special media for the purpose of identifying pathogenic species of Endo, Saburov microorganisms. Analyzing the obtained results reported in figure 5, it is observed that a higher number of colonies were recorded in the raw goat milk sample nr 3, which constituted 5 microbial colonies, compared to samples one and two where the number of colonies in sample nr. 2 constituted 3 microbial colonies and in sample nr 1 constituted 1 microbial colony. Pathogenic colonies did not develop on Endo and Saburov media.

Important characteristics are highlighted on the culture media in the investigated pasteurized milk samples that can be seen in figure 6.

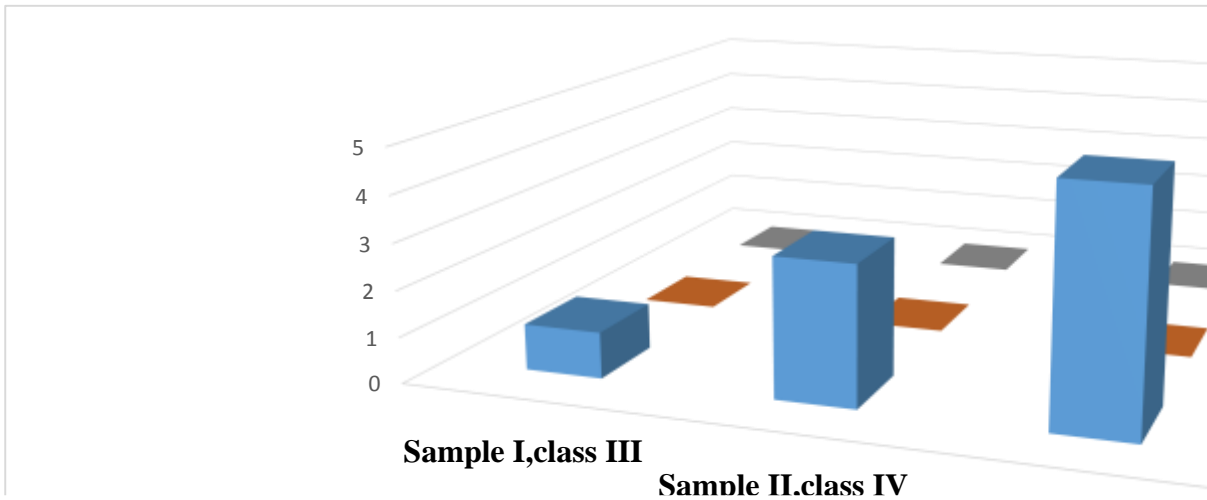
The data of figure 6 determine quantitative indices of the number of colonies in these three samples of pasteurized milk sold in the store, which constitute in all three samples the first class of milk. As a result of the investigations, the highest number of colonies was determined in milk sample number 1, which constituted 2 microbial colonies, followed by samples 2 and 3 where the number of microbial colonies in both samples constituted 1 microbial colony each. No microbial colonies were detected on Endo and Saburov media.

Among the EU member states, milk production per cow is the highest in Denmark (10,097 kilograms) and Estonia (10,020 kilograms) and the lowest in Bulgaria (3,628 kilograms) and Romania (3,362 kilograms) (figure 7; figure 8).

In 2021, just over a fifth (20.9%) of EU raw cow's milk was produced on German farms and a similar proportion (21.8%) was processed at German dairies. Together, Germany, France, the Netherlands, Poland and Italy are responsible for two-thirds (64.2%) of the EU's cow's milk production in 2021 and two-thirds (65.2%) of the cow's milk collected by dairy factories.

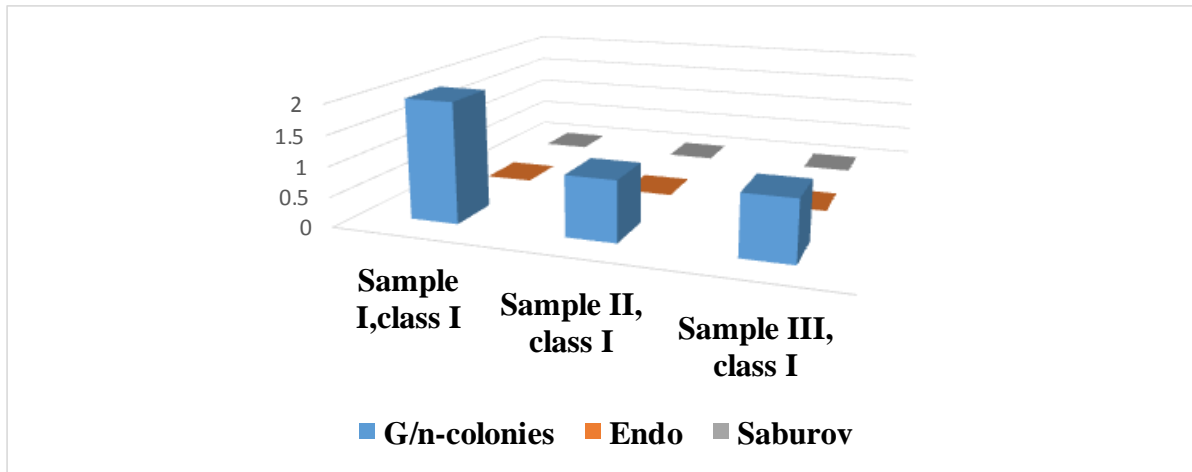
Eurostat data also show that there are several EU member states where animals other than cows have an important contribution to the total milk production. For example, in 2021 Spain produced one million tons of goat milk, Greece 900,000 tons and France 800,000 tons. Italy produced 700,000 tonnes of milk from animals other than cows and this included almost all of the EU's buffalo milk production.

In 2021, 149.5 million tonnes of milk were processed at EU dairies, of which 71% to produce cheese and butter. In total, the EU produced 2.3 million tonnes of butter, which required 44.4 million tonnes of milk, 10.4 million tonnes of cheese, out of 61.4 million tonnes of whole milk and 16.4 million tons of skimmed milk, 23.2 million tons of drinking milk and 10 million tons of skimmed milk.



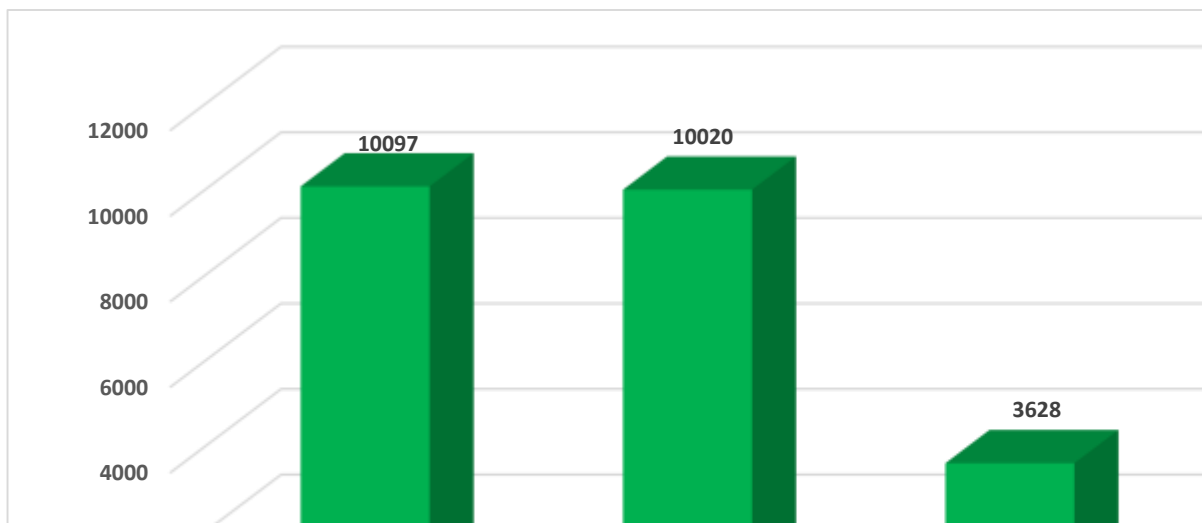
**Figure.5. The number of microbial colonies in the classes of raw goat milk samples**

Source: elaborated by the authors



**Figure.6. The number of microbial colonies in the classes of pasteurized milk samples**

Source: elaborated by the authors



**Figure.7. The highest milk production per cow in EU countries**

Source: elaborated by the authors

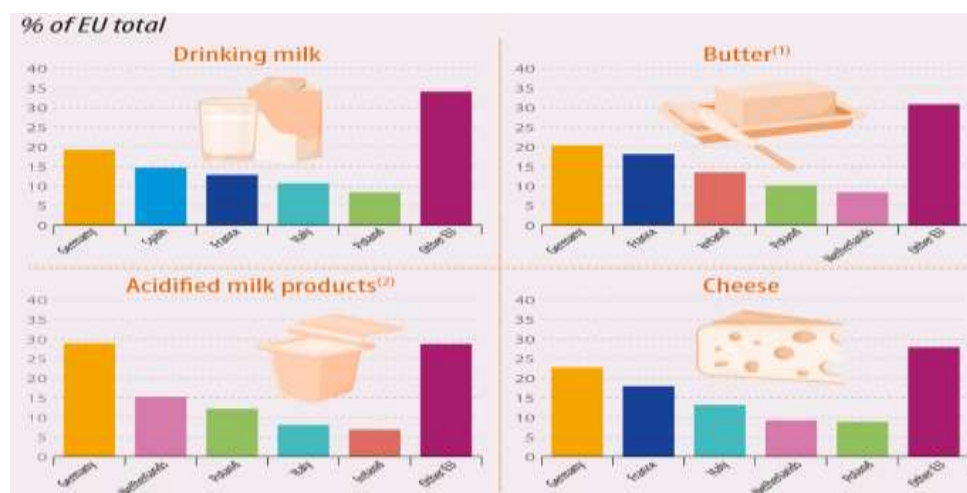


Figure 8. Top dairy producers in the EU, 2021 (by product)

Source: [16]

As for the Republic of Moldova, milk production contracted by 12%, to 217.3 thousand tons compared to the same period of the previous year. However, to ensure its own consumption, the Republic of Moldova needs 520 thousand tons.

Currently, 41 milk processing enterprises operate in the Republic of Moldova, of which only 2 factories import milk. These processing units produce about 90% of all milk products, the rest, 10%, belonging to small producers. According to National Bureau of Statistics, in the first half of 2022, processing enterprises produced 86,638.2 tons of dairy products, approximately 7% less than in the same period of 2021.

In 2022 compared to 2021 was registered a decrease of the milk production by 10,3% and of the production of the animals (live weight) by 2.4%

According to our investigations, the samples of pasteurized milk sold in the store constituted class I according to food standards.

Therefore, the results of investigations of the milk microflora of several categories of raw cow/goat milk and pasteurized cow's milk sold in the store confirmed to us during our investigations, that the presence of microorganisms in milk is of particular importance for the quality, sanitation and state of health freshness.

These microorganisms represented by non-pathogenic saprophytic cocci can grow but without reducing the quality of milk varieties and offer them the possibility of being edible, either through their saprophytic

action or through the non-pathogenic one which has a beneficial influence and does not have the property of producing toxic metabolites

## CONCLUSIONS

1. The bacterioscopic and bacteriological values obtained allow consumers to be safe and use this food product in their consumption.
2. The bacterioscopic conduct of the bacterial microflora in the investigated milk assortments determined a higher microflora of microbial cocci in the raw goat milk assortment - 7 microbial cells, compared to the raw cow milk assortments - 6 microbial cocci cells and pasteurized milk - 3 cells microbial cocci.
3. Microbial cultures according to the indices of cultural characters in the investigated milk varieties registered important microbiological indices according to the evaluation aspects which constituted 5 microbial colonies in the samples of raw goat milk, compared to samples of raw cow milk-4 microbial colonies and pasteurized milk -2 microbial colonies, constituting saprophytic cocci under microscopy.
4. Milk assortments are recommended to be stored in appropriate conditions to maintain microbiological parameters and organoleptic characteristics.

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