

## **DETERMINING THE TOTAL NUMBER OF MICROORGANISMS IN RAW MILK**

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### **Abstract**

Obtaining a quality dairy product is conditioned primarily by the production of raw milk of satisfactory quality. The application of microbiological criteria to raw milk aims to produce dairy products of satisfactory quality. Hygienically correct milk means primarily milk with a small number of microorganisms, low content of somatic cells and residues. Therefore, to determine the quality of raw milk there are three main factors: the number of microorganisms, the number of somatic cells and the chemical composition of the milk. The raw milk obtained from dairy cows as basic product is examined within the framework of the quality control system for raw milk. Cow raw milk should satisfy the criteria, i.e. to contain  $\leq 100,000$  CFU/ml. The number of micro-organisms is calculated from the results obtained during the one-year period, with at least 4 samples per month. The number of somatic cells is calculated from the results obtained during the one-year period, with at least 3 samples per month.

The samples of raw cow milk originate from two dairies (dairy (1 and 2), which buy raw milk from the territory of the municipality of Tetovo and are taken samples from one purchase line. During the one-year period, 6379 (N = 6379) raw milk samples. For the microbiological examination of raw milk, the method for determining the total number of bacteria with the BactoScan instrument, while for the number of somatic cells (SCC) the Fossomatic 5000 apparatus, was used.

The geometric mean of the results of the testing of raw milk on the number of micro-organisms, over a year, amounted to  $\leq 100,000$  CFU/ml, with over 95% of the collected raw milk. This indicates that the geometric mean of the number of microorganisms in the raw milk for the collection line does not meet the criteria given in the quality rule for fresh raw milk.

**Keywords:** raw milk, total number of bacteria, somatic cage, microorganisms, dairy.

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### **1. INTRODUCTION**

Milk in its natural condition is very perishable food, because it is susceptible to rapid decay by the action of natural enzymes and contamination microorganisms. Good hygienic practice from farm to dairy products, effective cooling regimes, reduction of storage time and technologies for reducing spoilage and pathogenic microorganisms are basic measures that need to be achieved to maintain good quality raw milk (Sorhaug and Stepaniak, 1997). The provisions of the Law on Food Safety apply in all phases of production, processing, distribution of food and animal food (Law on Food Safety "Official Gazette of the Republic of Macedonia", No. 157, 2010). Under the Food Safety Law, Article 4, "Food" means any substance or product, in a processed, partially processed or unprocessed condition, intended to be or is expected to be consumed by the humans.

In order to produce milk of satisfactory quality in terms of microbiological status, microbiological criteria should be applied in raw milk. Raw milk, as a source raw material for the production of heat-treated milk, is tested within the framework of the quality control system for fresh raw milk. Raw milk is milk obtained from secretion of the mammary gland of one or more healthy animals from, which has not been heated to temperatures above 40°C or is not exposed to any treatment with the same effect. To process raw milk, it must:

- originate from a dairy animal that has at least 30 days to giving birth, or more than 8 days have passed since giving birth,
- depending on the species of the animal from which it is obtained, i.e. to be categorized as cow's milk, sheep's milk, goat's milk and buffalo milk,
- have a distinctive appearance, color, smell, taste and consistency
- in the case of the daily collection of raw milk, the milk should be cooled immediately to a temperature of not more than 8°C or not more than 6°C if the collection of raw milk is not done daily.

Pollution of milk with microorganisms occurs during secretion and after secretion of milk. Pollution of milk during secretion occurs in the milk canal. Milk originating from healthy cow udder contains less than 100,000 Cfu/mL and does not have a

significant effect on the total number of microorganisms in a larger amount of milk (Murphy S. C. and Boor K. J., 2006). However, milk from cows with latent infection or subclinical mastitis contains mastitis causer and leads to an increase in the total number of microorganisms in milk from the farm (Bramley A.J., Mc Kinnon C.H., 1990). Milk contamination after secretion originates from the surface of the udder and body of the animal, the accessories and the equipment for milking and storage of milk and from the air (Wallace L. R. 2009). According to its composition, milk represents an appropriate environment for the propagation of microorganisms (Vujicic IF, 1985).

If the number of microorganisms in raw milk is increased, it will greatly affect the technological characteristics of the milk for processing or the quality of dairy products. This effect is multiple and is reflected in the disruption of hygienic correctness, chemical composition and changes in organoleptic properties, which leads to the decomposition of milk proteins and fats, the occurrence of bitter taste, changes in consistency, increase in the degree of acidity, separation of whey, the variability in the characteristics and the quality of the product, the reduction of the expiration date of the product, etc., These changes occur during the manufacturing process or during the storage period of the product. The total number of microorganisms, among other things, is an indicator of the health status of the herds, maintenance of farm hygiene, a procedure at that time of milk and milk for cooling (Berry D. P., Brien B. O., Callaghan E. J. O., Sullivan K. O. and Meaney W. J., 2006). Bacteriological quality of milk is an indicator of hygiene in primary production. Non-compliant sanitary procedures in milk production contribute to high variability and high index of variation of CFU/ml in all seasons (Trajkovska B., et al. (2015). For this reason, important parameters for determining the quality of milk are the total number of microorganisms and the number of somatic cells. According to many authors, SCC <100,000 cells/ml is normal in healthy mammary glands (Sordillo, LM et al., 1997), while SCC > 200,000 cells/ml refers to a bacterial infection (Schepers, AJ, et al., 1997).

If all appropriate hygienic procedures are carried out in the manipulation and cooling of milk after milking, it effectively inhibits the growth of microorganisms, but they multiply during transport in a mode of inadequate cooling or during inappropriate conditions of purchase. If the milk is kept at a temperature of 1-2 ° C, an increase in the number of microorganisms may slow down, but not unlimited for a long time (Murphy SC and Boor KJ (2000). In order for milk to be suitable for further processing, the growth of microorganisms in raw milk should be prevented. The growth of microorganisms in raw milk is achieved by cooling the raw milk in two ways. If the milk is collected for less than two hours it must be cooled down to 8 ° C or less in the case of daily collection, or at a temperature of 6 ° C if collection is not happening on daily basis (Regulation (EC) No 853/200 4).

Raw cow milk, which will be thermally processed in further processing, in terms of the number of microorganisms must meet the criteria, this means that it has have  $\leq 100,000$  CFU / ml.

The number of microorganisms is calculated from the individual results obtained for a period of one month, with at least two samples per month, using the standard method for determining the number of microorganisms (ISO 4833: 2003). In many countries, milk buyers use exclusively flow cytometry to determine the total number of microorganisms in raw milk. Most dairy producers in the EU produce milk with a small number of microorganisms of 100,000 CFU/ml without major problems. The national average is very often less than 10 000 CFU/ml (Hillerton JE and Berry EA (2004)). For evaluation of the milk based on the number of microorganisms, there are valuation standards ranging from 20,000 CFU/ml in Austria and the United Kingdom, up to 300,000 CFU/ml in Japan, and the most frequently selected number is between 100,000 and 300,000 CFU/ml.

The number of somatic cells in milk (SCC) is a key measure of milk quality, reflecting the health status of the mammary gland and the risk of non-physiological changes in the composition of milk (Hamann, 2005). As a result of the increase of somatic cells and changes in the chemical structure, the milk has decreased technological quality, the yield of cheese has decreased, the shelf life of pasteurized milk is shortened and undesired scents with these products can occur. (Trajkovska B., et al. 2011). As a result of the increase in the number of somatic cells, milk has decreased technological quality, the yield and quality of cheese is reduced, the pasteurized shelf life of milk is shortened, and with these products there may be unwanted odors (Kochoski L., et al. 2011).

It is reduced and they consist mainly of epithelial cells of the udder and cells originating from the blood (leucocytes). Of the total number of 70% are epithelial cells of the udder (skin, alveoli, milk canals and milk cisterns), and the rest of 30% make the cells from the blood and other cells. The normal number of somatic cells in healthy udder is on average 20,000 in ml. Any increase in the number of somatic cells above 250,000 or 300,000 in ml of milk is considered as an indicator of mastitis. In the study of the pathology of the mammary gland and the diagnosis of infected udder is used to determine the number of somatic cells in milk. This parameter is considered the best indicator for the health of the mammary gland.

## **2. TEST OBJECTIVES**

The purpose of this research is to evaluate the quality of raw milk and the conditions of raw milk on the basis of the obtained results from the main chemical components and the total number of microorganisms and the number of somatic cells of raw cow milk for a period of one year in two dairies from Tetovo region was assessed the quality of raw milk and hygienic conditions in the production process.

### 3. MATERIAL AND METHODS

#### 3. 1. Material

The research of the raw milk tests was conducted during the period from September 2017 to September 2018. The data from raw cow milk come from 2 dairy farms who collect milk from the territory of the municipality of Tetovo. One is located on the east side and the other on the western side of the city of Tetovo. To determine the number of microorganisms, 6379 samples were used, and 6229 of raw milk obtained from individual producers were used to determine the number of somatic cells. In dairy farm 1, samples of raw milk were taken from one collection line.

Sampling is carried out at least two samples per month, from dozens of collection points, three times a month. The samples were collected in sterile plastic bottles in which Azidiol preservative was added and transported to the laboratory for the quantity of raw milk at the Faculty of Veterinary Medicine in Skopje, kept at a temperature of + 4°C. It is common practice for samples to be tested no later than 72 hours after taking the subcontractors.

#### 3. 2. Test methods

Laboratory tests of the samples were carried out at the Faculty of Veterinary Medicine in Skopje. The laboratory is accredited according to ISO 17025: 2005 standard. The BactoScan instrument was used to count the total number of microorganisms, by using the procedure according to the ISO 21187: 2004 standard. For the counting of somatic cells, the Fossomatic 5000 was used, while the procedure for counting somatic cells was performed in accordance with the standard ISO 13366-2: 2006.

Within the microbiological criteria for raw milk, in the rulebook on raw milk and the rulebook on quality requirements for raw milk foresees mandatory control of raw milk that will be further processed thermally. With this procedure, raw milk should meet the requirements for the number of microorganisms and the number of somatic cells. The examinations and the examination for fulfilling the requirements for raw milk were carried out in an authorized accredited laboratory at the Faculty of Veterinary Medicine in Skopje in accordance with the Law on Veterinary Health.

According to the Rulebook on requirements for safety and hygiene "Official Gazette of the Republic of Macedonia" No. 197/2016, for the number of microorganisms and the number of somatic cells, in raw cow milk the number of columns at 30°C (per ml) should be  $\leq 100,000$ , while the number of somatic cells is  $\leq 400,000$ . Examination of samples of raw milk was monitored from September 2017 to September 2018. In September, 539 samples were taken from both dairies, in October 519, in November 524, in December 558, in January 511, in February 493, in March 504, in April 546, in May 547, in June 531, in July 541 and in August 566 copies. In the period from September 2017 to September 2018, a total of 6379 samples of raw milk were taken, and the test was carried out to determine the total number of microorganisms in raw milk.

### 4. RESULTS AND DISCUSSION

#### 4. 1. Number of microorganisms

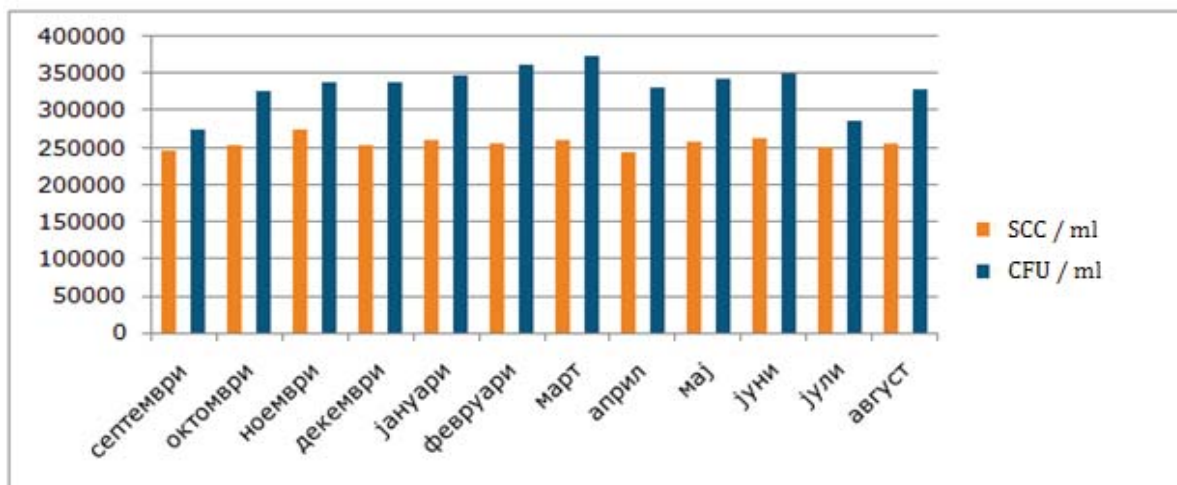
Regarding the number of microorganisms in raw milk, in both dairies for twelve months, starting from September 2017 to September 2018, 6379 samples were tested, from which the following results were obtained. Of all the examined samples (N = 6379), we noticed that the lowest number of microorganisms in raw milk was found in May 2018, amounting to 101500 CFU/ml, and the highest number of microorganisms was found in March 2018, amounting to 789500 CFU/ml. While the results of the average number of microorganisms are shown in Table 1. In all the samples tested, the results do not vary a lot. The average number is the lowest in September 2017, amounting to 275476 CFU/ml, and the highest in March 2018, being 375019 CFU/ml.

**Table 1** - Average values of the number of microorganisms and the number of somatic cells in raw milk

Month	CFU /mL	SCC /mL
<b>2017</b>		
September	275476	246540
October	325560	252230
November	338748	274081
December	339247	254428
<b>2018</b>		
January	347776	259899
February	361585	255259
March	375019	261081
April	332148	243956

<b>May</b>	342264	258219
<b>June</b>	349832	263249
<b>July</b>	285323	250079
<b>August</b>	328610	255650

**Chart 1** - Average values of the number of microorganisms and the number of somatic cells in raw milk



Regarding the number of somatic cells in raw milk, during the twelve-month period, starting from September 2017 to September 2018, 6229 samples were tested, and the following results were obtained. The lowest number of somatic cells in raw milk, out of all test samples, was founded in May 2018 and it amounted to 11500 SCC/ml, and the highest number of somatic cells was found in May 2018, at 73100 SCC/ml. In Table 1, the average number of somatic cells is reported by months. Of all tested samples, the number of somatic cells is not very variable. The average number is lowest in April 2018, amounting to 243956 SCC/ml, and the highest in November 2017, being 274081 SCC/ml.

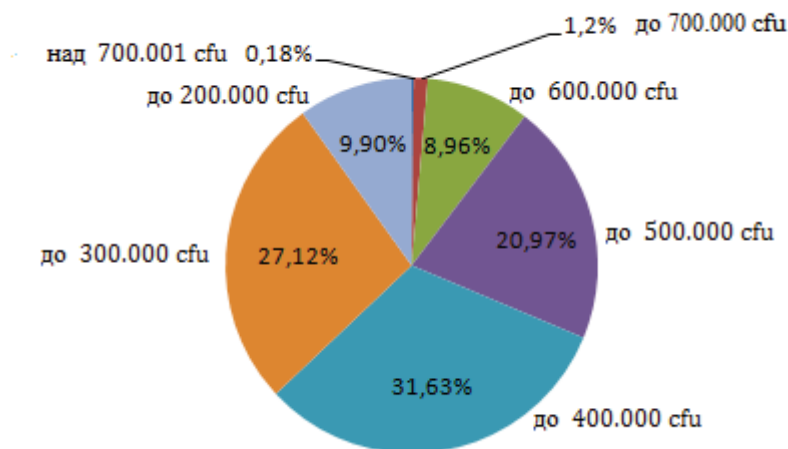
From Table 2 it can be concluded that from the total number of tested samples of 6379, that in relation to the number of microorganisms ( $\leq 100,000$  CFU/ml) no sample meets the criteria of the Rulebook on safety and hygiene requirements "Official Gazette of the Republic of Macedonia" RM "26/2012 and the criteria of the European Union (Council Directive 92/46 EEC).

**Table 2** - The obtained results for the number of microorganisms and number of samples by category and according to the Rulebook

Number of microorganism CFU/ml	Sample number	%
over 700.001	12	0.18
up to 700.000	77	1.20
up to 600.000	572	8.96
up to 500.000	1338	20.97
up to 400.000	2018	31.63
up to 300.000	1730	27.12
up to 200.000	632	9.90

Graph 2 - Percentage share of samples according to the number of microorganisms given in the Rulebook expressed in percentage 103

Up to 200,000 CFU/ml has 632 samples or 9.90% that meets the criteria according to the criteria required in 2011. Up to 400,000 CFU/ml has 3748 samples or 58.75%, which is the highest number that meets the requirements under the 2010 rulebook.



According to our findings, the reasons for not meeting the criteria required in 2012 are insufficient hygiene of the udder, poor hygiene of milking appliances, and most importantly, this is the lack of lactofreezers for storing milk in a large number of individual milk producers collecting raw milk before transport.

Deficiency of the lactofreezer, i.e. a milk cooling device for storing milk before transport, especially during the summer months, leads to further contamination of raw milk, and an increase in the number of microorganisms that can spoil the milk and make it inappropriate for further processing. Our recommendation for individual milk producers is to improve the hygiene of the udder, milking and lactofreezing equipment in order to avoid additional contamination of raw milk. Operators collecting or processing milk shall control milk-producing breeders to create conditions for hygienic collection and storage of raw milk before it is transported for processing in a dairy.

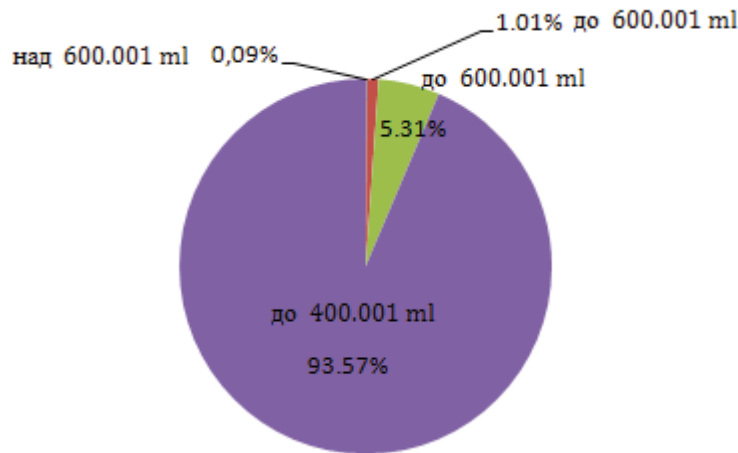
#### 4. 2. Number of somatic cells

From the obtained results of the number of somatic cells, it can be concluded that the bulk of tested samples meets the criteria of the Rulebook on safety and hygiene requirements "Official Gazette of the Republic of Macedonia" 26/2012 and the criteria of the European Union (Council Directive 92/46 EEC) in terms of the number of somatic cells in milk. From Table 3 it can be concluded that a large number of samples 5829, or 93.57%, meet the criteria for the maximum number of somatic cells, while on the opposite side only 400 samples or 6.41% are more than 400.000 somatic cells per ml. From this it can be concluded that in terms of the number of somatic cells, the tested raw milk, in hygienic aspect meets all the criteria for further processing.

**Table 3** - Results obtained for the number of somatic cells and the number of samples by category and according to the Rulebook

Number of somatic cells per ml	Sample number	%
over 600.001	6	0.09
500.000 to 600.000	63	1.01
400.000 to 500.000	331	5.31
up to 400.000	5829	93.57
<b>Total</b>	<b>6229</b>	<b>99.98</b>

**Chart 2** - Percentage sample participation according to the number of somatic cells given in the Rulebook, expressed in percentage



Analyzing both parameters, i.e. the total number of microorganisms and the number of somatic cells in raw milk from both tested dairies, we came to the conclusion that from the aspect of the number of somatic cells, a large number of samples of raw cow milk meets the requirements of the Rulebook on safety and hygiene requirements, which means that they have  $\leq 400,000$  somatic cells per ml.

Within the microbiological criteria for raw milk, the rules (Rulebook on requirements for quality of raw milk "Official Gazette of RM", No. 96, 2011) foresee mandatory control of raw milk which will be further processed thermally. With this criterion, the number of microorganisms is determined in raw milk. The results of the examination of separate samples of raw milk in relation to the limit value of the number of microorganisms determined by the said rules for each month are shown in Table 4.

**Table 4** - Results of the testing of individual samples of raw milk in relation to the limit value of the number of microorganisms

Month	Samples with $\leq 100,000$ CFU/ml		Samples with $> 100,000$ CFU/ml	
	n	%	n	%
January	5	10,20	44	89,80
February	2	7,69	24	92,30
March	2	7,69	24	92,30
April	7	10,30	61	89,70
May	6	9,38	58	90,62
June	3	11,54	23	88,46
July	3	6,98	40	93,02
August	3	4,69	61	95,31

The results of the testing of the individual samples of raw milk per month are as follows: in January, 10.20% samples of raw milk had a number of microorganisms in milk  $\leq 100,000$  CFU / ml, in February 7.69%, in March 7.69% in April 10.29%, May 9.35%, and in June 11.53%, in July 6.97%, and in August 4.69% samples. During the eight-month period of testing samples of raw milk, each month over 88% of the samples had a number of microorganisms  $> 100,000$  CFU / ml. A total of 31 samples, or 8.56% of the samples, had a number of microorganisms  $\leq 100,000$  CFU / ml. The results obtained do not comply with the results of tests for the number of microorganisms in raw milk of the EU milk producers. As a recommendation to milk producers and dairies, they should improve the hygiene of milking, hygiene of the containers for keeping raw milk before transport.

The results of the chemical tests are shown in Table 5, and the chemical composition of the raw milk examined is in the quantities of the normal values.

**Table 5.** Average chemical composition of cow milk in the examined dairies

Essential ingredients	Quantity in milk	
	%	
Water	88,76	85,3-88,7

<b>Dry matter</b>	7,96	7,9-10,0
<b>Milk fat</b>	3,96	2,5-5,5
<b>Proteins</b>	2.96	2,3-4,4
<b>Lactose</b>	4,32	3,8-5,3
<b>Specific weight</b>	27,38	25,15-29,55

Although milk fat is the most variable component in milk, the tests carried out in both dairies have an average of 3.96%, and have a low index of variation. The average protein value in raw milk was 2.96%. The non-greasy dry matter of all tests according to the amount in raw milk was in variable form. The average quantity is within the legal minimum, which amounts to 7.96%. Some samples amounted to a low percentage of dry matter up to 6.99%. The amount of protein in the milk samples was 2.96%, which in all tests was within the limits of the normal values. Milk sugar or lactose in samples of raw milk amounted to 4.32%, which is within the limits of the normal value for cow milk. The specific weight of the milk measured with lactodensimeter at a temperature of 15°C was 27.38, with variations of 25.15-29, 55, indicating that it varies during lactation and is affected by the relationship between the fat component and the fat-free component.

## Conclusion

Analyzing the two parameters, i.e. the total number of microorganisms and the number of somatic cells in raw milk from both tested dairies we concluded that from the aspect of the number of total microorganisms, all tested samples do not meet the criteria of the Rulebook on milk safety and hygiene requirements, which means that they have  $\leq 100,000$  CFU/ml. On the other hand, with regard to the number of somatic cells in a large number of samples of raw cow milk, they meet the criteria of the Rulebook on safety and hygiene requirements, which means that they have  $\leq 400,000$  somatic cells per ml. This shows that the milk is produced from healthy udder without signs of mastitis or some other infections of the udder, i.e. hygienic milking, but after milking and collection, the number of microorganisms increases enormously. In our opinion, the increase of the number of microorganisms is due to poor hygiene in farming facilities, non-hygienic milking, poor personal hygiene of the milker, and lack of adequate equipment for longer milk storage, i.e. due to a lack of lactofreezers that store the milk at an optimum temperature of 4-6°C until the moment it is to be transported to the dairy.

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