**HEALTH TESTING OF RAW MILK FROM LOCAL AGRICULTURAL FARMS OF SAUTHEAST SERBIA**

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***Abstract.***The aim of this scientific article is to control the quality of raw milk from local farms in four districts of southeastern Serbia. Selected test parameters (milk fat, milk density, pH value, acidity, alcohol test, number of somatic cells and number of microorganisms) were performed in accordance with the Rulebook on the quality of raw milk (Official Gazette of RS, 106/2017). Food spoilage microorganisms were also tested according to ISO standards. The presence of afla toxins was also examined by rapid tests. The results show a milk density of 1.028 g / cm 3 at a temperature of 20 ° C, a pH value of 6.58 on average, an acidity ranging from 6.6 to 6.9 ° SH, milk fat from 3.3 to 4, 4%. All samples showed a negative alcohol test with 72% ethyl alcohol. Microbiological methods performed to examine the frequency of *Salmonella* spp; *Listeria* monocytogenes; *Enterobacteriaceae* showed the absence of *Salmonella* spp. and *Listeria* monocytogenes and the presence of *Enterobacteriaceae* less than 10. The presence of aflatoxins was not confirmed in any of the samples.

***Key words:*** *milk, chemical composition, microbiological quality control, aflatoxins, somatic cells*

**Introduction**

Milk is considered part of a healthy and balanced diet because it contains a number of nutrients that play an important role in meeting the daily needs of the body. In a broader sense, it is a white liquid, with a specific smell and taste, intended for feeding pups [1]. Nutritionally, it is a very valuable food of complex composition when it consists of water, proteins, fats, carbohydrates, minerals, vitamins, etc. It differs from all other liquids of plant and animal origin, similar purpose, and has great biological and technological value. Milk of different types contains the same components, but the amount of certain ingredients as well as their mutual relationship may be different. The chemical composition of domestic beef milk in the Simmental type is 87.3% water, 12.7% dry matter; 3.8% milk fat; 3.55% protein, 3.0% casein; 4.7% milk sugar. It is important to note that milk should not be viewed as a set of different substances dispersed in water and that differences in composition can occur due to different influences, such as the change of seasons and lactation period [1].

The correctness of milk is key to the health of the consumer, which is why strict quality control is necessary. Milk, due to its composition, is a suitable environment for the development of both saprophytic and pathogenic microorganisms. According to the Rulebook on the quality of raw milk, there are classes of milk determined on the basis of the total number of microorganisms and on the basis of the number of somatic cells [2]. Somatic cells are normally present in milk, and an increase in their number indicates that the milk originates from diseased animals, animals in which milk retention has occurred or is related to the stage of lactation [3].

An increasing number of studies indicate elevated concentrations of aflatoxins in milk and dairy products [4]. Aflatoxins are mycotoxins produced by the molds Aspergillus flavus and *Aspergillus parasiticus* [5]. Aflatoxins from milk are the strongest known carcinogens in nature that are related to the development of cancer. The most important aflatoxins in milk are M groups, ie M-1 and M-2. AFM1 in raw milk and dairy products is stable and generally remains unchanged in the process of pasteurization and production of yogurt and cheese. Recent data indicate that AFM1 in milk is bound to casein [6]. According to [7], the only way to ensure the safety of milk and milk products for human consumption is to avoid contamination and prevent contamination of raw materials and animal feed with aflatoxin B-1.

The number of somatic cells was determined by a direct microscopic method. Microscopic slides were stained and somatic cells were counted directly under a microscope. The number of somatic cells was determined by the product of the mean value of the number of somatic cells in one field of view, the microscope factor and the number 100.

Proving the presence of bacteria L. *monocytogens, Salmonella* sp. and *Enterobacteriaceae* is carried out according to international standard methods, ISO 11290-1: 2017 [8]; ISO 6579-1: 2017 [9] and ISO 21528-2: 2017 [10].

For revitalization of bacteria L. *monocytogenic* broth with a reduced concentration of inhibitors, Fraser's broth (Biocar, France) was used. Sowing of incubated cultures from broth is performed on ALOA (Biocar, France) and PALCAM agar (Biocar, France) by dilution procedure to obtain individual colonies. The colonies on PALCAM agar are tiny grayish with a concave center and blackwash. Colonies after 48 h of incubation are darker and have an olive green sheen, while on ALOA agar they are green-blue in color [8].

Detection of *Salmonella* spp. encompasses four consecutive phases: in the first phase enrichment is performed in non-selective liquid medium BPW (Himedia, India), followed by selective enrichment using selective RVS broth (Himedia, India). Isolation on selective solid substrates is performed using XLD agar (Torlak, Serbia). *Salmonella* colonies have a black precipitate in the center due to the formation of hydrogen sulfide, while the substrate indicator changes the color of the substrate to red. Biochemical confirmation is performed by sowing pure culture on appropriate substrates of biochemical sequence (TSI agar, urea agar, lysine decarboxylation substrate). Serological confirmation 14 is performed by agglutination on a microscope plate using appropriate antisera [9].

VRBG agar (Biocar, France) was used to determine Enterobacteriaceae [10].

Detection of afla M1 mycotoxin in milk was performed by Charm MRL Afkatoxin M1 quantitative test, the sample is added without prior preparation and the result is read by ROSA Pearl Reader, which uses light reflection to analyze the results.

**RESULTS AND DISCUSSION**

A total of 80 samples were sampled over a period of 28 days. From a total of 16 households from four different districts of southeastern Serbia, 5 samples of milk from the morning milking were sampled. Tables 1 to 5 present the variability of milk chemical parameters by sampling dates. Based on the presented results, it is clear that the share of milk fat ranged from 3.3% to 4.4%, milk density in the range from 1.027 to 1.031 g / cm3, pH value in the range from 6.52 to 6.61 , acidity expressed in ° SH in the range of 6.6 to 6.9.

Table 6 shows the mean value of all measured parameters where the mean value of milk fat content is 3.73%, the mean value of milk density is 1.028 g / cm3 at a temperature of 20 ° C, the mean value of pH 5.58 and the mean value of ° SH 6 , 74. All samples were negative in reaction with 72% alcohol.

**Table 1:** Value of tested chemical parameters and number of somatic cells on June 1, 2021

|  |  |  |  |
| --- | --- | --- | --- |
| County | Sample number | Chemical parameters | Somatic cell count |
| Milk density | pH value | Degree of acidity | Milk fat content |
| Toplički | 1 | 1.028 | 6.54 | 6.6 | 3.6 | 192850 |
| 2 | 1.028 | 6.58 | 6.7 | 3.9 | 82650 |
| 3 | 1.029 | 6.59 | 6.9 | 4.0 | 101935 |
| 4 | 1.027 | 6.61 | 6.9 | 3.8 | 102329 |
| Nišavski | 5 | 1.028 | 6.53 | 6.7 | 3.8 | 119383 |
| 6 | 1.028 | 6.52 | 6.8 | 3.8 | 110200 |
| 7 | 1.030 | 6.53 | 6.9 | 4.1 | 122680 |
| 8 | 1.031 | 6.58 | 6.9 | 4.4 | 238766 |
| Jablanički | 9 | 1.027 | 6.60 | 6.6 | 3.6 | 173565 |
| 10 | 1.027 | 6.61 | 6.6 | 3.3 | 181830 |
| 11 | 1.028 | 6.54 | 6.7 | 3.7 | 137750 |
| 12 | 1.028 | 6.60 | 6.9 | 3.6 | 118300 |
| Pirotski | 13 | 1.027 | 6.54 | 6.8 | 3.4 | 119380 |
| 14 | 1.028 | 6.55 | 6.7 | 3.8 | 100830 |
| 15 | 1.028 | 6.61 | 6.7 | 3.7 | 247950 |
| 16 | 1.028 | 6.57 | 6.7 | 3.7 | 165333 |
| Average value of parameters: | 1.028 | 6.57 | 6.76 | 3.76 | 144733.19 |
|
| Standard deviation parameters: | 0.001087811 | 0.032634338 | 0.115289491 | 0.26299556 | 50044.1789 |
|

**Table 2*:*** Value of tested chemical parameters and number of somatic cells on June 7, 2021

|  |  |  |  |
| --- | --- | --- | --- |
| County  | Sample number | Chemical parameters | Somatic cell count |
| Milk density | pH value | Degree of acidity | Milk fat content |
| Toplički | 1 | 1.028 | 6.54 | 6.6 | 3.6 | 194957 |
| 2 | 1.028 | 6.59 | 6.6 | 3.8 | 129485 |
| 3 | 1.028 | 6.60 | 6.8 | 3.8 | 137750 |
| 4 | 1.027 | 6.57 | 6.8 | 3.4 | 156116 |
| Nišavski | 5 | 1.027 | 6.55 | 6.8 | 3.3 | 198650 |
| 6 | 1.027 | 6.55 | 6.8 | 3.3 | 156116 |
| 7 | 1.028 | 6.56 | 6.8 | 3.6 | 118300 |
| 8 | 1.028 | 6.59 | 6.9 | 3.8 | 219383 |
| Jablanički | 9 | 1.028 | 6.60 | 6.6 | 3.9 | 180830 |
| 10 | 1.028 | 6.60 | 6.6 | 3.9 | 191200 |
| 11 | 1.028 | 6.53 | 6.6 | 3.8 | 90915 |
| 12 | 1.029 | 6.60 | 6.8 | 4.0 | 303050 |
| Pirotski | 13 | 1.028 | 6.55 | 6.7 | 3.9 | 257133 |
| 14 | 1.028 | 6.57 | 6.7 | 3.9 | 128813 |
| 15 | 1.029 | 6.61 | 6.8 | 4.0 | 289456 |
| 16 | 1.029 | 6.59 | 6.7 | 3.9 | 200657 |
| Average value of parameters: | 1.028 | 6.58 | 6.73 | 3.74 | 184550.69 |
|
| Standard deviation parameters: | 0.000632456 | 0.025560386 | 0.1 | 0.23371992 | 60693.9472 |
|

**Table 3*:*** Value of tested chemical parameters and number of somatic cells on June 14, 2021

|  |  |  |  |
| --- | --- | --- | --- |
| County  | Sample number | Chemical parameters | Somatic cell count |
| Milk density | pH value | Degree of acidity | Milk fat content |
| Toplički | 1 | 1.027 | 6.56 | 6.7 |  3.4 | 259683 |
| 2 | 1.028 | 6.60 | 6.6 |  3.6 | 101833 |
| 3 | 1.029 | 6.58 | 6.7 |  3.9 | 158900 |
| 4 | 1.028 | 6.54 | 6.7 |  3.6 | 160855 |
| Nišavski | 5 | 1.028 | 6.57 | 6.7 |  3.6 | 158455 |
| 6 | 1.030 | 6.56 | 6.7 |  4.1 | 110853 |
| 7 | 1.029 | 6.58 | 6.8 |  4.0 | 194650 |
| 8 | 1.031 | 6.60 | 6.8 |  4.4 | 254660 |
| Jablanički | 9 | 1.028 | 6.59 | 6.6 |  3.7 | 303050 |
| 10 | 1.027 | 6.53 | 6.7 |  3.3 | 293866 |
| 11 | 1.027 | 6.55 | 6.7 |  3.3 | 238766 |
| 12 | 1.028 | 6.59 | 6.8 |  3.9 | 179565 |
| Pirotski | 13 | 1.028 | 6.57 | 6.7 |  3.8 | 101935 |
| 14 | 1.029 | 6.56 | 6.8 |  3.9 | 183666 |
| 15 | 1.029 | 6.60 | 6.7 |  3.9 | 100655 |
| 16 | 1.030 | 6.58 | 6.7 |  4.1 | 239430 |
| Average value of parameters: | 1.029 | 6.57 | 6.71 | 3.78 | 190051.38 |
|
| Standard deviation parameters: | 0.001154701 | 0.021447611 | 0.061913919 | 0.30598203 | 68402.9678 |
|

**Table 4:** Value of tested chemical parameters and number of somatic cells on June 21, 2021

|  |  |  |  |
| --- | --- | --- | --- |
| County  | Sample number | Chemical parameters | Somatic cell count |
| Milk density | pH value | Degree of acidity | Milk fat content |
| Toplički | 1 | 1.029 | 6.55 | 6.7 | 4.0 | 348967 |
| 2 | 1.030 | 6.61 | 6.7 | 4.2 | 123260 |
| 3 | 1.027 | 6.58 | 6.7 | 3.4 | 173560 |
| 4 | 1.027 | 6.59 | 6.7 | 3.4 | 171720 |
| Nišavski | 5 | 1.029 | 6.56 | 6.8 | 3.8 | 147228 |
| 6 | 1.027 | 6.57 | 6.9 | 3.4 | 136650 |
| 7 | 1.028 | 6.56 | 6.8 | 3.8 | 156000 |
| 8 | 1.029 | 6.60 | 6.8 | 4.0 | 307733 |
| Jablanički | 9 | 1.027 | 6.61 | 6.7 | 3.5 | 247950 |
| 10 | 1.027 | 6.61 | 6.7 | 3.4 | 266250 |
| 11 | 1.028 | 6.55 | 6.8 | 3.8 | 292933 |
| 12 | 1.029 | 6.61 | 6.9 | 4.0 | 119300 |
| Pirotski | 13 | 1.029 | 6.57 | 6.9 | 3.9 | 144080 |
| 14 | 1.03 | 6.58 | 6.9 | 4.1 | 143500 |
| 15 | 1.029 | 6.60 | 6.7 | 3.9 | 198733 |
| 16 | 1.027 | 6.58 | 6.6 | 3.4 | 378714 |
| Average value of parameters: | 1.028 | 6.58 | 6.77 | 3.75 | 209786.13 |
|
| Standard deviation parameters: | 0.001125463 | 0.021823153 | 0.094648472 | 0.28751812 | 85042.7867 |
|

**Table 5:** Value of tested chemical parameters and number of somatic cells on June 28, 2021

|  |  |  |  |
| --- | --- | --- | --- |
| County  | Sample number | Chemical parameters | Somatic cell count |
| Milk density | pH value | Degree of acidity | Milk fat content |
| Toplički | 1 | 1.027 | 6.58 | 6.7 |  3.4 | 255100 |
| 2 | 1.028 | 6.60 | 6.6 |  3.5 | 101935 |
| 3 | 1.028 | 6.58 | 6.6 |  3.5 | 260183 |
| 4 | 1.028 | 6.58 | 6.7 |  3.5 | 174385 |
| Nišavski | 5 | 1.028 | 6.57 | 6.7 |  3.6 | 70843 |
| 6 | 1.027 | 6.57 | 6.8 |  3.4 | 90910 |
| 7 | 1.029 | 6.58 | 6.8 |  3.9 | 93273 |
| 8 | 1.029 | 6.56 | 6.8 |  3.9 | 238766 |
| Jablanički | 9 | 1.027 | 6.58 | 6.7 |  3.4 | 241817 |
| 10 | 1.028 | 6.57 | 6.8 |  3.5 | 173283 |
| 11 | 1.028 | 6.57 | 6.9 |  3.6 | 354963 |
| 12 | 1.029 | 6.58 | 6.9 |  4.0 | 119383 |
| Pirotski | 13 | 1.027 | 6.59 | 6.8 |  3.4 | 155100 |
| 14 | 1.030 | 6.58 | 6.8 |  4.2 | 109640 |
| 15 | 1.030 | 6.58 | 6.7 |  4.2 | 180420 |
| 16 | 1.027 | 6.58 | 6.7 |  3.3 | 315000 |
| Average value of parameters: | 1.028 | 6.58 | 6.75 | 3.64 | 183437.56 |
|
| Standard deviation parameters: | 0.001024695 | 0.009105859 | 0.089442719 | 0.2965777 | 85938.68676 |
|

**Table 6:** Total mean examined chemical parameters and somatic cell count medium

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sampling date | Milk density | pH value | Degree of acidity | Milk fat content | Somatic cell count |
| 01. June | 1.028 | 6.57 | 6.76 | 3.76 | 144733 |
| 07. June | 1.028 | 6.58 | 6.73 | 3.74 | 184550 |
| 14. June | 1.029 | 6.57 | 6.71 | 3.78 | 190051 |
| 21. June | 1.028 | 6.58 | 6.77 | 3.75 | 209786 |
| 28. June | 1.028 | 6.58 | 6.75 | 3.64 | 183437 |
| Average value of parameters: | 1.028 | 6.58 | 6.74 | 3.734 | 182511 |
| Standard deviation parameters: | 0.000447214 | 0.005477 | 0.024083 | 0.055 | 23627 |

Tables 1 to 5 also provide an overview of the number of somatic cells in 1 ml of milk by sampling dates, while Table 6 gives the mean value of the number of somatic cells. According to our regulations, milk that contains more than 500,000 per ml of somatic cells is considered defective. The average number of somatic cells is 182511 / ml. The results indicate that musk deer do not suffer from clinical and subclinical mastitis, which significantly lead to a decrease in cow's milk yield. The obtained values ​​of all presented parameters are in accordance with the Rulebook on the quality of raw milk [2].

Table 7 shows the results of microbiological quality control performed in accordance with ISO standards. Microbiological methods performed to examine the frequency of *Salmonella* spp. (SRPS EN ISO 6579-1: 2017), *Listeria monocytogenes* (SRPS EN ISO 11290-1: 2017), *Enterobacteriaceae* (SRPS ISO 21528-2: 2017), showed the absence of *Salmonella* spp. and Listeria monocytogenes and the presence of *Enterobacteriaceae* less than 10.

**Table 7:** *Quality control results*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Microorganisms | Sampling plan | The limit values (cfu/ml) | Method designation | Determined value |
| n | c | m | M |
| *Salmonella spp.* | 16 | 0 | must not be in 25g | SRPS EN ISO 6579-1:2017 | no presence was established |
| *Enterobacteriaceae* | 16 | 0 | 10 | SRPS EN ISO 21528-2:2017 | < 10 cfu/g |
| *L. monocytogenes* | 16 | 0 | must not be in 25g | SRPS EN ISO 11290-1:2017 | no presence was established |

The synthesis of aflatoxin M1 occurs in mammals after the intake of aflatoxin B1 through contaminated food. A study [11] on aflatoxins in raw milk on the territory of Serbia from 2013, indicates that as many as 65% of the examined milk samples contain more than 0.05 µg / kg, and 13% more than 0.5µg / kg of aflatoxin M1. In contrast to this study, in all 80 examined samples, a rapid quantitative test for aflatoxins showed a negative result, which indicates that nutrition is not contaminated with group B aflatoxins.

**CONCLUSION**

Every day we encounter doubts about the health safety of the milk of small agricultural producers. The results of this work show that the selected chemical parameters are in accordance with the Rulebook on the quality of raw milk. The average density of milk is 1.028 g / cm3, pH value 6.58, degree of acidity 6.74, milk fat content 3.73%.

The average number of somatic cells is 182511 / ml, which according to the mentioned Ordinance classifies most of the samples as class I. Microbiological quality control performed according to ISO standards does not indicate the presence of *Salmonella* spp. and L. *monocytogens, Enterobacteriaceae* are present less than 10 cfu / ml. The presence of aflatoxin M1 was not confirmed in any sample by rapid quantitative test. Based on the performed chemical and microbiological analyzes, it can be concluded that the milk of small agricultural producers is completely safe for use.

**LITERATURE**

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