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.

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