Original scientific paper
UDC 637.354.055(497.775)

MICROBIOLOGICAL PROPERTIES OF ARTISANAL CHEESE (BIENO SIRENJE)

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Abstract

Samples from six cheeses made from raw ewe's milk, in two farmhouses, located in the village Peshtani, region of Mariovo during the complete production process were collected. Microbiological analyses were conducted including mesophylic counts, LAB, coliform bacteria, *Salmonella sp*., yeasts and moulds. The quality of the milk used for cheese production in the both farmhouses was comparable.

The prevalence of microbiological groups in milk, vary from 6.72-7.10 log cfu/mL for mesophylic counts; 6.52-7.13 log cfu/mL for lactococci; 6.29-6.52 log cfu/mL for lactobacilli; 5.37-5.80 log cfu/mL for coliform bacteria and 4.09-4.38 log cfu/mL for yeasts. The presence of *Salmonella* sp. was not detected in neither of collected samples. The increasing of the number of all analyzed microorganisms during cheeses maturing was recorded in all cheese samples. During maturation, the dominant group of microorganisms is LAB. The trend of prevalence decreasing of analyzed microorganisms was noticed during the brining (increased concentration of salt and lowering pH value). Moulds have discontinued occurrence in the cheese, being more frequently recorded during brining.

To gain an artisanal cheese with good quality, it is necessary to monitor the process of production, sanitation of equipment and tools as well as introduction of good manufacturing practice.

***Key words**: Artisanal cheese, mesophilic count, LAB, coliform bacteria, yeasts and moulds*.

1. Introduction

Manufacturing of dairy products on traditional way especially cheese is a feature for South Europe and Mediterranean countries (Cogan *et al*. [1]). The cheese production in Republic of Macedonia is carried out on traditional manner as well. In general, in Macedonia three types of cheese are produced: white brined cheese, kashkaval and beaten cheese. Beaten cheese has been produced almost on the completely state territory mainly from raw cows, ewes and goats milk. The process of cheese making is variable in different regions of production that results in appearance of different cheese varieties. Characteristics of milk used for cheese production are also variable and depend on the geographical region, season of milking and the nutrition method. Such differences have strong influence on characteristics and appearance of cheese varieties.

Beaten cheese has yellowish color, hard consistence, pleasant aroma and particularly salty taste (5-10%). Different size halls with random arrangement are visible on the cross-section. These features of beaten cheese are constant and not alter from the production region. According to Kapac-Parkačeva *et al*. [2], no standardized technology utilization and poor sanitary conditions during the manufacturing and ripening are responsible for the variation of the chemical composition and reological characteristics beaten cheese.

There are few published data concerning microbiological characteristics of beaten cheese. The available data about microbiology of beaten cheese originate from the investigations conducted on few varieties of beaten cheese made from row cow milk in several dairies in Kumanovo, Trubarevo and Tetovo.

So far, there are no published data about microbiological characteristics of the beaten cheese made in a traditional manner in farmhouse conditions. The aim of this study was estimate the counts of microbial groups of technological interests during the whole production process, to monitor the changes of the counts during the storage and ripening in farmhouse conditions as well as to determine the influence of sanitary conditions on cheese microbiological quality.

2. Materials and Methods

Cheese manufacture and sampling

Microbiological analyses were conducted on beaten cheese handmade in two farmhouses in Peshtani village, Mariovo region, in the traditional manner without utilization of modern dairy equipment. Samples of milk, curd, steaming curd, cheese during dry ripening, salting and brine ripening were analyzed during the summer period (July and August) in 2010. The samples were taken during three different cheese production procedures in each farmhouse. The cheese production technology was similar in the two farmhouses. Microbiological analyses were carried out at least in duplicate for each batch and the values were averaged.

Raw ewe's milk from evening and morning milking was used to manufacture the cheese. The renneting is done with liquid commercial rennet (Bitola yeast factory 1:5000) for 45-50 minutes without heating of milk. After the completion of curdling the curd is thoroughly stirred with wooden tool (3 series of 40-50 times each) and than left to settle down for 15 minutes. Then curd is steamed with hot water (80-90 ^{0}C). After steaming, the water is removed from the vat and the curd was pressed by hand to remove the whey and than transferred in cheesecloth. The cheesecloth was fastened and hung to drain for 24 h at the room temperature (~25 ^{0}C). Next day the curd was left for dry ripening 1-5 days (depending of ambient temperature) covered with cheesecloth in the same environment. Then the cheese was cut on rectangular slices 5-7 cm thick and salted on the surface with coarse-grained salt. Salted slices were transferred into tins and left in the same room for 1-2 days depending on the ambient temperature. Finally, the cheese slices are covered with brine (22% salinity) and left to brine ripening for 45 days in the same room.

Samples were taken under sterile conditions and put in plastic sterile dishes, kept in transport refrigerator on 4 ^{0}C. Microbiological analyses were made in Laboratory for Microbiology at the Faculty for Agricultural Sciences and Food in Skopje.

Microbiological analysis

Aliquot of 10 mL of milk or 10 g of cheese was homogenized with 90 mL of sterile 2% Na-citrate solution. Decimal dilution was made with sterile physiological solution.

Microbiological analyses were completed on following way: Total viable counts (TVC) on Plate count agar (BioMérieux) after incubation on 30 ^{0}C for 48 h; lactic acid bacteria (LAB) on M-17 agar (Biolife Italiana) on 30 ^{0}C for 48 h for cocci and MRS agar (Biolife Italiana) incubated under anaerobic conditions (GENbag anaer, BioMérieux) on 30 ^{0}C for 72 h; coliform bacteria on ENDO (Biolife Italiana) agar on 37 ^{0}C for 24 h; *Salmonella* on XLD Medium (Oxoid) on 37 ^{0}C for 24 h; yeast and moulds on YGC agar (Merck) on 25 ^{0}C for 5 days.

pH of samples were determinate with pH meter (HANNA instruments) and the ambient temperature and the temperature of samples were determinate with ordinary thermometer.

3. Results and Discussion

The average values of obtain results through the preformed microbiology assays in two farm houses are shown in Table 1 and 2.

Table 1. Microbiological contents (log cfu/mL or log cfu/g) of the milk and cheese samples from first farmhouse

Production phase	Total viable counts	Lactococci counts	Lactobacilli counts	Coliforms counts	Yeasts counts
Milk	7.10	7.13	6.29	5.37	4.09
Curd	7.22	7.49	6.54	5.43	4.27
Curd after beating	7.22	7.88	6.72	4.88	4.17
Curd after steaming	7.42	7.66	7.28	5.68	3.94
After draining	7.97	8.48	8.45	6.04	4.15
1 day dray ripening	8.44	8.77	9.28	6.48	4.49
2 day dray ripening	8.72	8.59	8.59	5.74	4.44
1 day salting	8.48	8.43	8.40	6.77	5.83
2 day salting	8.56	8.45	8.34	6.42	5.29
1 day brining	7.76	7.93	7.94	5.90	5.39
10 day brining	7.65	8.00	8.04	5.45	5.18
30 day brining	7.17	6.99	7.03	5.44	4.57
45 day brining	7.09	6.74	6.74	4.53	4.35

Table 2. Microbiological contents (log chum or log cuff/g) of the milk and cheese samples from second farmhouse

Production phase	Total viable counts	Lactococci counts	Lactobacilli counts	Coliforms counts	Yeasts counts
Milk	6.72	6.52	6.42	5.80	4.38
Curd	6.91	6.70	6.58	6.43	4.39
Curd after beating	7.01	7.05	7.39	6.05	4.26
Curd after steaming	7.21	7.12	6.90	5.68	4.18
After draining	8.73	8.61	8.63	7.45	4.64
1 dry ripening	8.86	8.84	8.78	7.30	4.94
1 salting	8.79	8.69	8.54	7.49	4.78
2 salting	8.76	8.62	8.48	7.41	4.27
1 day brining	8.43	8.40	8.24	6.77	3.81
10 day brining	7.60	7.93	7.50	5.70	3.67
30 day brining	7.45	7.03	6.71	5.30	3.27
45 day brining	7.05	6.70	6.45	5.17	3.31

Total viable counts (TVC) in the milk from two farmhouses were higher then the standards for raw ewe's milk given in regulative of Republic of Macedonia. It is very likely that the high counts of TVC is result from the poor hygienic conditions during milking, storage of milk at temperature higher then 15 ^{0}C and poor sanitation of diary equipment. The presence of this group in high number in raw milk was also noticed during manufacturing of other traditional cheeses (Kakurinov *et al*. [3], Levkov and Kakurinov [4], Aygun *et al*. [5], Pešić-Mikulec and Jovanović [6] and Volken de Souza *et al*. [7]). After curdling, the number of TVC in curd, as well as other investigated groups of microorganisms increased due to their physical entrapment and their multiplication during manufacturing process. After steaming of curd the number of TVC was unchanged. The temperature measured in curd after steaming (44-47.6 ^{0}C) has no inhibitory effect for TVC (Levkov and Kakurinov [4]). Such results are not in concordance with the results of Kakurinov *et al*. [3] who has found that TVC decreased after machine steaming of Kumanovo yellow cheese curd (variety of beaten cheese). It is supposed that unchanged TVC number is a consequence of the differences in thermal treatment of curd. In general, the temperature of steaming is not constant and high enough to have inhibitory effect on TVC growing. During the dry ripening, the total viable counts increased for 1.62-2.14 log units reaching their maximum values. It is evident that the physico-chemical parameters such as pH, salinity, temperature have low influence over microbial growth (García Fontán *et al*. [8]). After dry salting and brining of cheese TVC tend to decline, but in the final stages of brining their number is still high (7.05-7.09 log cfu/g). Such results were expected for traditional cheeses made from ewe's raw milk during the summer period, that were ripened and stored in high temperature conditions (>20 ^{0}C) and produced and handled in poor sanitary conditions (Psoni *et al*. [9], Nikolaou *et al*. [10] and Hatzikamari *et al*. [11]).

The number of coliforms in the cheese was high throughout the entire examined period. The high prevalence of coliform bacteria in milk (5.37-5.80 log cfu/mL) and in cheese afterwards is remarkable in both farmhouses. These values are considerably higher then the standards for food safety in Republic of Macedonia. Such high counts in milk and later in cheese suggest contamination occurred during milking and cheese processing, inadequate storage of milk, as well as additional contamination with dairy equipment (Volken de Souza *et al*. [7] and Manolopoulou *et al*. [12]). Occurrence of high counts of coliforms in the beaten cheese is not unusual since the milk with lower quality is usually used for in cheese processing (Kakurinov *et al*. [3], Levkov and Kakurinov [4], Kakurinov [13]). After curd formation the number of coliform bacteria and other examined groups of microorganisms decreased. After curds steaming the coliform counts in the second farm house decreased for 0.37 log units, while in the first farmhouse the coliform counts was increased for 0.8 log units. It was found that the steaming temperature is not inhibitory for coliform bacteria (Levkov and Kakurinov [4], Spano *et al*. [14]). However, these results are not in concordance with those of Kakurinov *et al*. [3], because in Kumanovo yellow cheese coliform bacteria disappeared after steaming process. During the dry ripening, the increase of the colifrom bacteria counts was comparable with the counts of coliform in Orinotyri (Prodromou *et al*. [15]), San Simón (García Fontán *et al*. [8]), Batzos (Psoni *et al*. [9]) and Tetila (Menéndez *et al*. [16]) cheeses. The prevalence of coliforms in beaten cheese compared to Calabrian raw milk cheeses (Micari *et al*. [17]) and Gorcola cheese samples (Kamber and Úelik [18]) is higher. However, decline of the coliform counts for 0.15 and 0.74 log units is evident after first and second day of dry ripening respectively. An opposite trend in the quantity of coliform bacteria and other investigated microorganisms was determined. When the counts of other investigated microorganisms show maximum values, then the coliform counts decreased. The lower number of coliform bacteria probably is a result of the higher prevalence of LAB as competitive microorganisms in this phase and low the pH values (5.11-5.17) in cheese (Hatzikamari *et al*. [11]). During the dry salting, the colifrom counts increased, probably because of additional contamination (Kakurinov [13]). During the period of brining the gradually decrease of colifrom counts was evident. However, this group of microorganisms did not disappear from cheese as in Anevato cheese (Hatzikamari *et al*. [11]). Because this group of microorganisms is used as one of the indicators for food quality, high abundance of their population in ripe cheeses could cause problems for selling the products (Psoni *et al*. [9]). Regulation of further proliferation of coliform bacteria can be achieved because of synergistic activity of several factor of inhibition: lower pH, increased concentration of NaCl, antibacterial activity of LAB and lower temperature during ripening and maintaining (Prodromou *et al*. [15]).

Analyzes for detection of presence of *Salmonella* in milk and cheese was also carried out. However, the investigations reveal that *Salmonella* was absent in all analyzed samples.

Domination of lactic acid bacteria (LAB) over the other investigated groups of microorganisms is evident (Tables 1 and 2). Maximal average values were attained during the dry ripening stadium. During the first day of dry ripening, compare to stage of draining the number of lactococci in first farmhouse increased for 0.29 log units while the number of lactobacilli increased for 0.83 log units. During the first day of dry ripening the

number of lactococci in first farmhouse increased for 0.29 log units while the number of lactobacilli increased for 0.83 log units compared to the stage of draining. In the second farmhouse lactococci during this stage increased for 0.23 log units while lactobacilli increased for 0.15 log units. Maximum values of LAB during the stage of ripening were detected in other traditional cheeses produced from raw milk particularly during the summer period (Pešić-Mikulec and Jovanović [6], Volken de Souza *et al*. [7], García Fontán *et al*. [8], Psoni *et al*. [9], Nikolaou *et al*. [10], Kamber and Úelik [18], Alonso-Calleja *et al*. [19] and Marino *et al*. [20]).

The ratio of lactic acid bacteria shows that lactococci are more prevalent in the milk and curd while the lactobacilli are dominant during the stage of dry ripening and brining. This is likely a result of lactococci ability to ferment lactose and to grow rapidly during the stages of ripening (García Fontán *et al*. [8]). Lactobacilli need different growth conditions then lactococci and therefore appear in the later stages of cheese production. Lactobacilli are able to utilize some of the metabolites produced during the stages of ripening because of lactococi metabolitic activity (lactate, citrate, amino acids) (Volken de Souza *et al*. [7]). After steaming of curd, the number of lactococci in the first farmhouse and the number of lactobacilli in second farmhouse slightly decreased. Similar influence of steaming process over the number of LAB in other varieties of beaten cheese was also noticed (Kakurinov *et al*. [3], Levkov and Kakurinov [4]). Trough the salting and brining stages LAB counts in both farmhouses gradually declined or stabilized. This is probably a result of their ability to survive under lower pH and larger concentration of NaCl (García Fontán *et al*. [8] and Alonso-Calleja *et al*. [19]).

Yeasts often can be significant part of microorganisms in different cheese varieties and can contribute to their aroma and texture development or may be a reason for their spoilage (Corbo *et al*. [23]). Yeasts in cheese can contribute to cheese ripening and aroma developing because they contain proteolytic and lipolytic enzymes (Alonso-Calleja *et al*. [19] and Marino *et al*. [20]). The prevalence of yeasts was determinate in different type of cheeses Cheddar (Ferreira and Vijoen [21]), different variations of Beaten cheese (Kakurinov *et al*. [3]) and Feta (Manolopoulou *et al*. [12]). In this study, the counts of yeasts in both farmhouses were higher then counts determinated for Serrano cheese (Volken de Souza *et al*. [7]), while they are comparable with the results of analyses of fresh white cheese, (Pešić-Mikulec and Jovanović [6]), yellow cheese and beaten cheese made in Kumanovo (Kakurinov *et al*. [3]). The counts of yeasts as well as the counts of other investigated groups of microorganisms increased after the stage of coagulation. After steaming of curd, the number of yeasts in cheese samples from both farmhouses declined. Steaming did not caused disappearing of the yeasts from beaten cheese as was reported by Kakurinov in 1997 for Kumanovo yellow cheese (Kakurinov *et al*. [3]). During the stages of dry ripening yeasts counts increased. Such results are in accordance with the results obtained from investigations of other varieties of beaten cheese (Levkov and Kakurinov [4] and Kakurinov [13]). However, estimated values in this study were slightly higher than the results of yeasts counts analyses in Calabrian raw milk cheeses (Micari *et al*. [17]), but correspond to results of Gorcola cheese (Kamber and Úelik [18]). After cheese, salting in first farmhouse was noticed that the yeast counts increased, similarly as in Anevato cheese (Hatzikamari *et al*. [11]). During cheese brining the counts of yeasts decline, but it is noticeable that yeasts counts in samples from first farm house are constantly higher then the second one. It is very likely that the diary equipment and the environment in the first farmhouse is more contaminated with yeasts. In addition, the incidence of yeasts can vary between dairies as well as between consecutive days in the same diary (Manolopoulou *et al*. [12]). Additional contamination of cheese with yeasts can be caused by brine. Low values of pH, high concentration of NaCl, low moisture content that appears through cheese ripening can be stimulative or selective for yeasts (Viljoen [22] and Corbo *et al*. [23]).

Moulds are not continuously present through the whole cheese production process. They were noticed during brining in both farmhouses. Their appearance is incidentally in a range of 100-13000 cfu/g in cheese samples from the first farmhouse and 200 cfu/g or 300 cfu/g in the cheese samples from second farmhouse. Incidentally, appearance of moulds is a result of additional contamination with mould spores (Kakurinov [13]). During cheese production in first farmhouse, moulds were evident on the wall where the cheese was manufactured and stored. The hygiene of rooms for milking and for milk storage as well as for cheese production, ripening and cheese storage is very important.

4. Conclusions

- The obtained results suggest that LAB are dominant microorganisms in the beaten cheese and are responsible for cheese production.
- The high numbers of undesirable microorganisms may affect the cheese quality. Therefore, it is important to increase the hygienic quality of milk and cheese. This can be obtained by improving the hygienic conditions through milking, milk storage and cheese making.
- It is necessary to improve the sanitation of equipment and tools for cheese making. The high

number of coliforms in milk and cheese is not only a result of contamination but it is also a result of the influence of storage temperature.

– The present results could be useful as information for improving and promoting of beaten cheese made in farmhouse on traditional way.

5. References

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