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MICROBIOLOGICAL PROPERTIES AND CHEMICAL COMPOSITION OF MACEDONIAN TRADITIONAL WHITE BRINED CHEESE

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ABSTRACT

The purpose of this study was to asses the chemical and microbial characteristics of 10 artisanal cheeses made from raw ewe's milk without addition of starters, during maturation. Microbial populations were numerous and diverse with Lactic acid bacteria and *Enterobaceriaceae* as a predominant groups of microorganisms. Pathogenic bacteria were not detected. The pH of the cheeses was within the range of 4.04 – 5.05, the moisture content within 46.97 – 51.58%, total protein from 18 – 21.37%, fat content from 26 - 30% and NaCl from 4.38 – 5.43%.

Key words: traditional, ewe's milk cheese, pathogen bacteria, lactic acid bacteria

INTRODUCTION

Dairy products made from locally produced raw milk are still a very important part of daily diet. The characteristics of these products are different from one to another region depending on the local indigenous microflora, which in turns reflects the climate conditions of the area.

Macedonian white cheese is a brined cheese variety with a soft or semihard texture. The main flavor characteristic is the salty acid taste. The cheese is rindless, white coloured and close textured. It is generally made from cow's milk, ewe's milk and less from goat milk, prepared in cubes and ripened for 3 months. Traditionally, this type of cheese have been manufactured by local farmers on a small scale for decades using raw milk and traditional techniques passed down from generation to generation using only basic equipment. Commercial starter cultures are not used in the production and instead, the cheese maker relies on the lactic acid bacteria (LAB) naturally present in the raw unpasteurized milk as adventitious contaminants.

Cheese is chemically, microbiologically and enzymatically a complex and dynamic system. This makes the process of cheese ripening highly complex. One of the characteristics of white-brined cheeses is their high salt content, and this probably accords with the fact that they are traditionally manufactured in countries with hot climates. it is important to note that the composition of different white brined cheeses varies within a broad interval due to the differences in the composition of raw milk, processing parameters and ripening conditions (22).

Bacterial biodiversity arising from the raw milk and environmental contamination (from farm and production practices) constitute the principal source of the microorganisms which are necessary for the development of the typical features (taste, flavor, consistency). The microbial diversity originating from environmental exposure during cheese manufacture and maturation and the initial natural

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diversity of the microbiota present in the raw milk all play a role in fermentation processes and are important in the final development of traditional dairy products (1).

Microbiological quality of white-brined cheeses can be influenced by numerous factors, including the quality of the milk, the use of pasteurization or thermization, various technological parameters and the level and type(s) of microbial contamination that occur throughout the manufacture and storage of the cheese. White-brined cheeses are matured for long periods in brine, and thus the dominant microflora make a significant contribution to the maturation process and, to a degree, regulate the quality of the final product (2).

When cheese is produced following traditional procedures from raw milk, the environmental microflora plays a fundamental role in fermentation and is one of the most important parameters affecting the cheese quality. In addition, the biodiversity of bacteria involved in cheese production can be considered a fundamental factor for the maintenance of the typical features of traditional cheese products. Recent investigations have shown that the indigenous microflora of raw milk influence the biochemical characteristics and flavor of cheeses (3).

No information is available on this cheese microflora and its evolution during ripening. The objective of this study was to obtain the initial insight into the biodiversity of the microbial community associated with this cheese during the ripening. The main purpose was to investigate the dynamics of the microflora and to characterize the dominant groups of LAB during the maturation process and pathogen bacteria as a main risk factor in making cheese in a traditional way.

MATERIAL AND METHODS

Collection of samples and sample preparation

Five samples of white brined ewe's milk cheese in the beginning of the ripening (second week) and five samples in the end of the ripening (after 3 months) made by traditional method were collected aseptically from local producers from five different regions of the country. Samples were brought to the laboratory under refrigerated conditions (4-6°C), and analyzed within 24h.

Microbial population counting and chemical analyses

All of the analysed parameters according to the tested matrix and reference methods (12-19) used are given in Table 1.

Table 1. Overview of the parameters and methods used in the study

No.	Parameter	Matrix	Reference method
1.	*Enterobacteriaceae*	Cheese	ISO 21528-2:2004
2.	*Listeria monocytogenes*	Cheese	ISO 11290-1:1996
3.	*Escherichia coli*	Cheese	ISO 16649-3:2005
4.	*Staphylococcus aureus*	Cheese	ISO 6888-1:1999
5.	Yeasts and moulds	Cheese	ISO 21527-2
7.	pH	Cheese	pH meter Sartorius
8.	Protein content	Cheese	ISO 8968:1 2001
9.	*Fat content*	Cheese	ISO 16266:2006
10.	Moisture content	Cheese	Mitrovic, 1954
11.	Sodium chloride content	Cheese	Titrimetric method

Lactobacilli were grown on Rogosa agar (RA, Sigma Aldrich) after 5 days at 30°C incubated anaerobically (Gas-Pack anaerobic system, Biomerieux, France). Gram positive, catalase negative cocci on M17 agar, after incubation at 30°C for 48h. Cycloheximide was added (100 mg^{-1}) to prevent the growth of yeasts in M17 agar (Sigma Aldrich). Enterococci were grown on kanamycin esculine azide agar (KAA, Fluka) at 37°C 24h. Presumptive leuconostoc on De Man, Rogosa,

Sharpe (MRS agar, Sigma Aldrich) with 30 μg/ml $^{-1}$ vancomycin.

After the incubation period, the plates containing between 10 and 300 colonies were selected for enumeration. The number of colonies grown on each medium was expressed as log cfu/g^{-1}. The cells from Rogosa and M17 agar were Gram-stained and the catalase activity was determined. All determinations were conducted two times.

RESULTS AND DISCUSSION

In our research ten cheese samples from five different regions have been analyzed. Five were tested at the second week of the ripening (day 10) and five in the end of ripening after three months (day 90). The changes of different microbial groups investigated during the maturation period are shown in the Fig. 1 and Fig. 2.

The chemical composition of all of the cheeses was generally within the range typical for white brined cheese. Macedonian white brined cheese may be characterized as a soft (50 - 60 %) moisture, high fat cheese (25-30%), protein (12–21%) high salt (3–5%) content and the final pH range of 4.20 – 5.05. Decreases in total solid content of brined cheeses throughout ripening generally originate from water soluble proteins and peptides passed from cheese matrix to brine. Increase in salt content during ripening could be attributed to the higher water content because salt penetrates the cheese matrix in water (6).

Table 2. Chemical composition of Macedonian white brined cheese

sample	day	pH	Moisture %	Protein%	Fat%	NaCl%
A	10	6.69	62.01	12.43	25.00	4.09
	90	5.05	50.00	18.21	30.00	4.60
B	10	6.38	59.03	12.41	26.50	3.51
	90	4.04	51.58	19.02	28.00	5.43
C	10	6.20	55.96	14.00	26.00	3.15
	90	4.45	47.83	20.01	27.50	4.38
D	10	6.23	56.10	14.40	26.00	4.09
	90	4.20	46.97	21.37	28.00	5.20
E	10	6.40	55.24	14.45	25.50	4.00
	90	4.25	47.52	21.50	27.00	5.10

The parameters for proteins, moisture content and pH are similar with the parameters determined in Teleme cheese and Feta cheese (20, 21). The salt content of our brined cheese appears to be same as it was found in Feta cheese 3.5-5.0 (21). pH values in the end of the ripening were found to be from 4.04 - 5.05. Tayar (23) stated that, in white cheeses produced in three different plants with traditional methods pH was found to be between 4.38-5.94. The fat content, the pH, moisture and the salt content were found to be the same as in Sjenicki cheese. The protein content had slightly lower levels than it was in found in our study (24). According to the obtained chemical results we can conclude that Macedonian white brined cheese does not greatly differ from the brined cheeses in the region.

In the beginning of the ripening high level of contamination with *Enterobacteriaceae* from 7.24 log cfu/g and *E. coli* 8.69 log cfu/g occurred because of the use of raw milk and artisanal rennet as well as high moisture and low salt content. All above factors mentioned could promote the growth of these bacteria. Furthermore during the maturation process the number of *Enterobacteriaceae* was reduced to 3.07 and 1.77 log cfu/g and in some cheese samples they were not present at all. *E. coli* was not detected in the mature cheese. Similar results were obtained in Feta cheese where number of *E. coli* at day 4 reached 5.3 log cfu/g, after significantly declined and in the end of ripening were not detected. Coagulase positive staphylococci were present at day 10 in some cheese samples within the range from 2.0 – 4.77 log cfu/g but at day 90 they were not detected in none of the samples (11). In addition, even though staphylococci tolerate salt, their growth is not favored by the cheese environment, because

of the combined inhibitory effect of the pH and NaCl concentration (5). Listeria was not detected in the cheese samples.

Yeasts and moulds counts, which are environmental contamination indicators were within the interval from 3.64 – 4.99 log cfu/g at day 10 and moulds were detected only in one sample. In the mature cheese at day 90 the population of yeasts have increased and was between 4.30 – 7.00 log cfu/g. Yeast counts in this artisanal cheeses were higher than those reported by other authors who have studied the microbiology of artisanal cheeses made from raw milk without addition of starter cultures (11). The high number of yeasts found in this study may account for the typical organoleptic characteristics of our traditional cheese since recent investigations have shown that some lipolytic and proteolytic enzymes produced by those microorganisms contribute to the development of aroma and flavor compounds (4). At day 90 moulds were found in three samples and could be probably due to contamination.

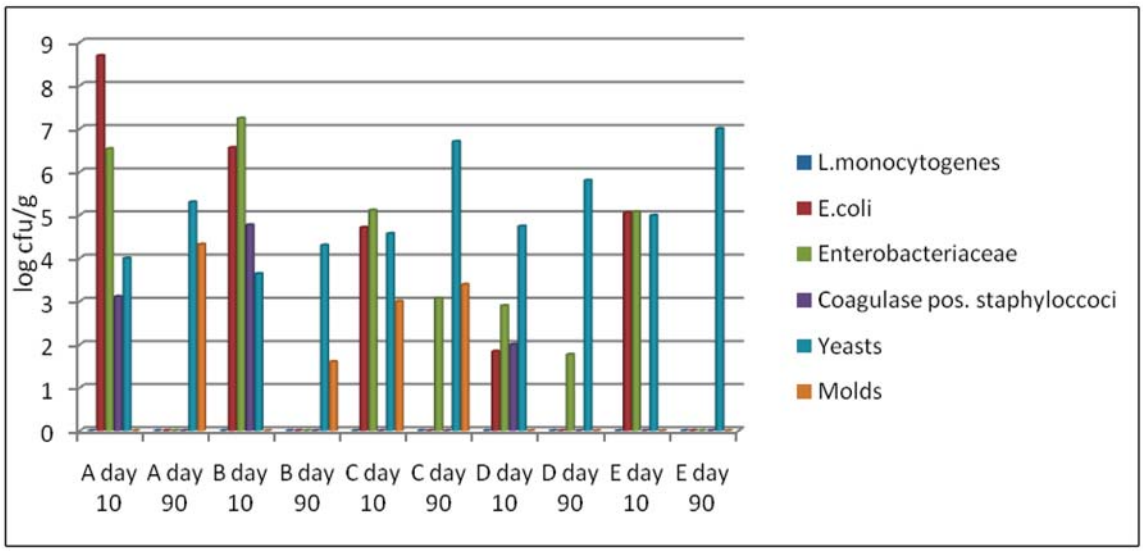

Figure 1. Log counts of microbial groups during cheese ripening

Lactic acid bacteria (LAB) constituted the predominant bacterial group during the ripening process with a population higher than 7 log cfu/g. Among LAB, lactococci were found at day 10 in higher numbers (5.44 – 7.94 log cfu/g) when compared with leuconostoc (4.11 – 6.58 log cfu/g) and lactobacilli from 5.17 in sample A to 6.9 log cfu/g in sample B (Fig. 2). These results indicate that lactococci constitute the predominant bacterial group in the beginning of the ripening period. At the end of the ripening lactobacilli were the most common group and their number was within the range 5.35 – 7.43 log cfu/g. The decline in lactococci is probably due to the inhibitory action of the low pH and high salt in moisture values in the maturing cheese (7). The presumptive leuconostoc at day 10 was found within the range from 4.11 – 6.50 log cfu/g. The obtained results are slightly higher than the one reported by Manolopoulou (11). At the end of the ripening process at day 90, leuconostoc was detected in only one cheese sample. These groups of microorganisms may influence the ripening process through the production of lactic acid, the decrease in the oxidation – reduction potential and their proteolytic and lipolytic activities (10).

Enterococci were found also in high numbers at day 10 (from log 2.6 – 6.03 cfu/g). This results were similar with the results obtained in the Turkish white cheese (9) where the mean log was 5.34 cfu/g. At day 90 they rapidly decreased in two cheese samples to 2.69 log cfu/g in cheese B, and to 1.9 log cfu/g in cheese E. In cheese A, C and D enterococci were not detected. The presence of a high number of enterococci could be due to poor hygienic practices during the manufacturing process and the resistance of enteroccoci to unfavorable conditions (8). Enterococci may influence the ripening process due to their caseinolytic and lypolitic activity.

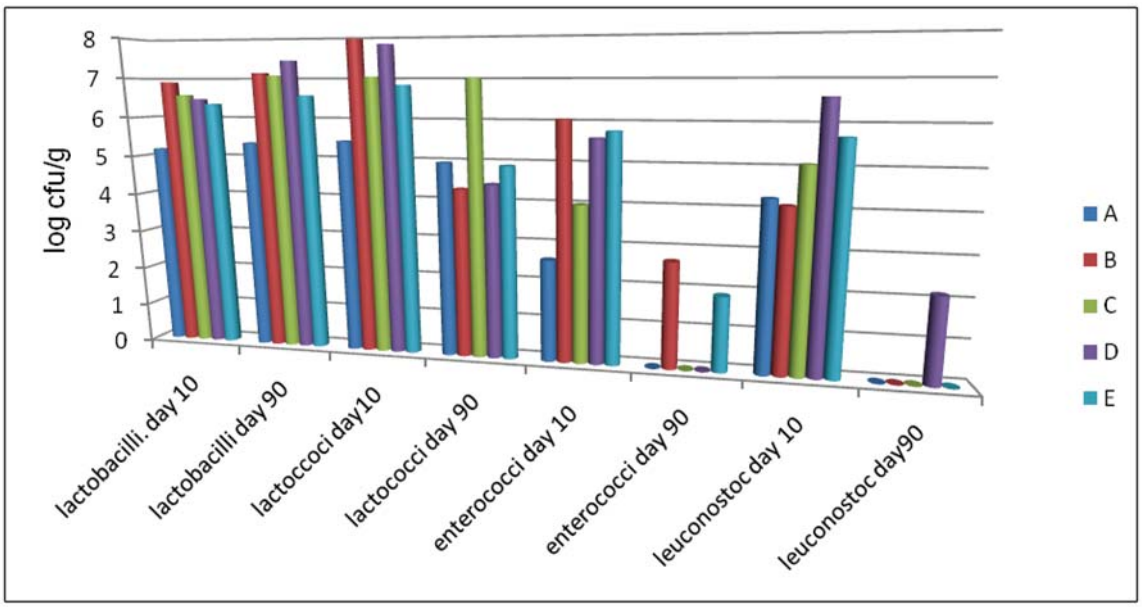

Figure 2. Log counts of the lactic acid bacteria groups during ripening of white brined cheese

Table 3. Microbiological criteria for food safety given in Official Gazette No.78/2008

Parameter	Acceptable limits
Listeria monocytogenes	Absence in 25 g
Escherichia coli	100 cfu/g
Coagulase-positive staphylococci	100 cfu/g

From the obtained microbiological results, Macedonian artisanal cheese could be considered as safe from a hygienic point of view and would be categorized as acceptable according to the Book of Rules for microbiological criteria in food (Official Gazette 78/2008).

It can be concluded that this study provides a new approach to ripening of white brined cheese, which is the most consumed cheese in Macedonia.

Further knowledge of the natural microbial communities present in this artisanal cheese may help to prevent loss of microbial diversity associated with local and regional traditions.

REFERENCES

1. Garabal JI. (2007), Biodiversity and the survival of autochthonous fermented products, Int Microbiol, 10, 1-3.
2. Bintsis, T., Papademas P. (2002). Microbiological quality of white brined cheeses: a review. International Journal of dairy Technology, 55 (3) : 113–120.
3. Demarigny, Y., Beuvier, E., Buchin, S., Pochet, S. and Grappin, R. (1997) Influence of raw milk microflora on the characteristics of Swiss-type cheeses. II. Biochemical and sensory characteristics. Lait 77, 151-167.
4. Marino M, Maifreni M and Rondinini G. (2003) Microbiological characterization of artisanal Montasio cheese: analysis of its indigenous lactic acid bacteria.FEMS Microbiology Letters 229 133-140.
5. Litopoulou-Tzanetaki, E., (1977) Staphylococci and micrococci in Kefalotyri cheese. Milchwissenshaft 32, 211-214
6. Hayaloglu, A.A.: (2003) Influence of the strains of Lactococcus used as a starter on the characteristics and ripening properties of Turkish white cheese. PhD Dissertation. Cukurova University, Adana, Turkey 2003.

7. Vafopoulou-Mastrogianaki A, Litopoulou-Tzanetaki E and Tzanetakis N (1990) Effect of Pediococcus pentosaceus on ripening changes of Feta cheese. Microbiologie-Alimnetis-Nutrition 8 53-62.

8. Moreno M R, Sarantinopoulos P, Tsakalidou E and De-Vuyst L (2006) The role and application of *Enterococci* in food and health. International Journal of Food Microbiology 106 1-24.

9. Oner Z, Karahan A G and Aloglu H (2006) Changes in the microbiological and chemical characteristics of an artisanal Turkish white cheese during ripening. Food Science and Technology 39 449-454.

10. Steele, J.L., (1995) Contribution of lactic acid bacteria to cheese ripening. In: Malin, E.L., Gibbs, M., Skinner, F.A. (Eds.), Chemistry of structure – Function Relationship in cheese. Academic Press, London, UK, pp. 65-79.

11. Manolopoulou E, Sarantinopoulos P, Zoidou E, Aktypis A, Moschopoulou E, Kandarakis IG, Anifantakis EM (2003). Evaluation of microbial population during traditional Feta cheese manufacture and ripening. Int. J. Food Microbiol., 82: 153-161.

12. ISO 11290-1:1996Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and enumeration of Listeria monocytogenes -- Part 1: Detection method

13. ISO 21528-2:2004 Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of Enterobacteriaceae -- Part 2: Colony-count method

14. ISO 16649-2:2001 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli. Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide

15. ISO 6888-1:1999 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) -- Part 1: Technique using Baird-Parker agar medium

16. ISO 21527-2:2008 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 2: Colony count technique in products with water activity less than or equal to 0,95

17. Mitrovic M., (1954), Prirucnik laboratoriskih (hemiskih) metoda za ispitivanje zivotnih namirnica, Medicinska knjiga, Beograd-Zagreb.

18. ISO 8968-1:2001 (IDF 20-1: 2001)Milk -- Determination of nitrogen content -- Part 1: Kjeldahl method

19. Litopoulou-Tzanetaki E., Tzanetakis N., Vafopoulou-Mastrojiannaki A., (1993) Effect of type of lactic starter on microbiological, chemical and sensory characteristics of Feta cheese. Food Microb., 1031–34.

20. Alichanidis, E., Polychroniadou, A., Tzanetakis, N. & Vafopoulou, A. (1981) Teleme cheese from deep-frozen curd. Journal of Dairy Science, 64, 732–739.

21. Abd El-Salam M.H., Alichanidis E., (2004) Cheese varieties ripened in brine (3rd ed.). In: P.F. Fox, P.L.H. McSweeney, T.M. Cogan, T.P. Guinee, Editors, Cheese: chemistry, physics and microbiology Vol. 2, Elsevier, Amsterdam, pp. 227-249.

22. Hayaloglu, A.A., Guven, M., Fox, P.F. (2002): Microbiological, biochemical and technological properties of Turkish White cheese, "Beyaz Peynir". International Dairy Journal 12, 635-648.

23. Tayar, M., (1995). Beyaz peynirlerin olgunlaşma süresince kimyasal ve mikrobiyolojik özelliklerindeki değişmeler. Gıda. 20 (2) 97-101.

24. Macej, O., Jovanovic, S., Barac, M.(2004): Biotehnologija u stocarstvu, vol. 20(1-2), 109-117.