



The effect of saline concentration and storage temperature in the quality of Sharri cheese

Mergim Mestani ¹, Xhavit Ramadani ¹, Tahire Maloku Gjergji ², Hajrip Mehmeti ¹, Arsim Ademi ³ and Ibrahim Mehmeti ^{1,3 *}

¹ Faculty of Agriculture and Veterinary, University of Prishtina, Prishtina, Kosovo. ² Faculty of Medicine, University of Prishtina, Prishtina, Kosovo. ³ Norwegian University of Life Sciences, Ås, Norway.

*e-mail: Ibrahimmehmeti@hotmail.com, Ibrahim.mehmeti@uni-pr.edu

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Abstract

Most consumed cheeses in Kosovo are white brined cheeses produced mostly in traditional form. One of the most popular cheeses in this country is Sharri cheese produced in mountains called Sharri between Kosovo, Macedonia and Albania. The aim of this work was to study the effect of brine salinity (3, 6, 9 and 12%) and temperature (8 and 22°C) on microbiological parameters (total viable count, psychrophile and total lactic acid bacteria-LAB) and sensory quality of Sharri cheese. Traditional methods by using plate counts for total viable count and selective media for counting LAB are used. Molecular methods such as 16S rRNA DNA gene sequencing was used to identify LAB. The results show significant decrease in all microbiological parameters starting from total viable count, psychrophile and total LAB by increasing the brine salinity during a processing until a final product. In raw milk diversity of LAB was shown while on cheese the most dominant strain of LAB identified by 16S rRNA gene sequencing was different. In 3 and 6% of salt most dominant strain was *Leuconostoc pseudomesenteroides* (around 60%) followed by *Lactococcus lactis*, and *Enterococcus faecium*, while in higher concentration *Enterococcus faecalis* and *L. garvieae* became more dominant. Cheese stored on 6% and 22°C had higher scores from the panelists. The results show that, controlling the raw milk quality in microbiological aspects before processing is necessary. In same time for making Sharri cheese the recommended concentration of brine solution is 6-9% and 22°C of storage condition. By using this condition we will have under a control total viable counts, no significant changes between raw milk and final product of LAB diversity and no negative effects in human health.

Key words: Cheese, lactic acid bacteria, sensory analysis, texture profile, brine.

Introduction

White brined cheeses are the most consumed cheeses in Kosovo and very popular in many neighboring countries in Western Balkans ^{7, 10, 33, 35, 38}. In Kosovo, one of most popular cheeses is Sharri cheese, which was named after a mountain range called Sharri Mountains, situated in the border of Kosovo with Macedonia and partly with Albania, from where the cheese is originated. Sharri cheese is traditionally produced from ovine, bovine and mixed milk during the spring and summer seasons, and is classified as a hard and/or semi-hard cheese with high salt content and produced without addition of start culture ^{35, 36}. Briefly described, Sharri cheese is produced from unpasteurized milk tempered at approximately 35°C, using rennet. After pre-maturation process (acidification, curding, curd processing and pressing) cheese is left in wooden shelf's for ripening until day 15 (adding approximately 1/3 of the total salt over it). After that day, cheese should be replaced into the metal, glass or plastic jars filled with brine solution and left for further ripening, for another 45 days in brine ^{35, 36}. Hence, cheese is considered as matured after 60 days of all the cheese making process. During this time, brine salt penetrates

into the cheese giving it a characteristic hard consistence, crumbly texture and strong safety flavor ³⁵. The influence of sodium chloride and period of ripening on sensory properties of cheese were also described in other studies ²⁰. Both, brine saline concentration and storage temperature may affect the cheese-making process from the beginning of the process to the final product, as well as its ripening or storage duration ¹⁸. There are advantages and disadvantages of the high brine saline concentration used in the cheese production ^{19, 21}. Traditionally, high saline concentration is used as a preservative and is added into cheese to control bacterial growth, enzyme activity and to improve cheese flavor ^{2, 6, 19}. High brine saline concentration is reported to block enormously the growth of pathogenic bacteria, such as *Staphylococcus* spp, *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli*, in different dairy products ^{16, 39}. On the other hand, use of high brine saline concentration reduces simultaneously the bacteria involved in fermentation, and hence prolongs cheese maturation process. In addition, consumption of high amounts of salt is reported to

cause different healthy problems and negative effects to the consumers such as hypertension and other cardiovascular problems¹², kidney stones and osteoporosis^{22, 26, 27}. To avoid health risks, the World Health Organization has recommended decreasing of sodium chloride content in all types of food⁴⁴. Temperature also affects the microflora of cheese and other dairy products and hence their characteristics³⁴, although no such information is available for Sharri cheese. The purpose of this research was to study the effect of brine salinity (at 3, 6, 9 and 12% of sodium chloride) and temperature (8 and 22°C) on microbiological parameters and sensory quality of Sharri cheese.

Material and Methods

Cheese making: Full-fat homogenized and unpasteurized bovine milk was purchased from a local dairy farmer (Dragash, Kosovo). Sharri cheese was manufactured according to a protocol described previously³⁶, with some modifications (Fig. 1). Briefly described, sixty litres of milk were filtered before entering into the processing, tempered into the clean tank, and heated up to 35°C. Using a microbiological starter of traditional whey, milk was coagulated for 30 min. After that, the coagulated curd was cut into 1 dm³ (about 1 kg) and left for approximately 5 min, before transferring each part of it into the separate cheesecloths and left for other 15 min to drain at 40°C, and then pressed by 10 kg of weight for 10 h. The total ripening process lasted 60 days. The first 15 days, cheese was stored in the room temperature, and then it was transported to the laboratory, and randomly assigned to four different brine solutions (3, 6, 9, and 12%, wt/wt solution) and stored at two different temperatures (8°C and 22°C) up to the day 60.

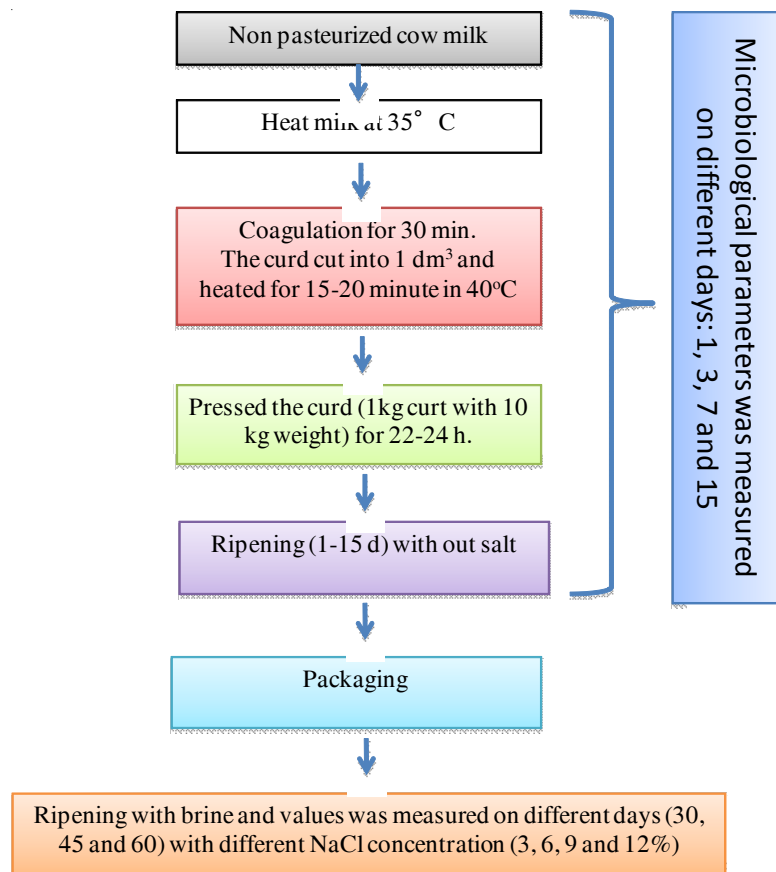


Figure 1. Technological diagram for the Sharri cheese.

Samples of raw milk before entering the cheese making process and cheese samples at day 16 and day 60 were taken for determination of total viable count, psychrophile bacteria, and enumeration and identification of lactic acid bacteria. All analyses were carried out in triplicate.

Microbiological analyses

Standard plate counting (SPC) of microorganisms and psychrophile bacteria: Total viable counts of microorganisms and psychrophile bacteria were conducted according to standard procedures, determined by International Standards Organization, by using pure plating technique as described previously²⁵. Briefly, 20 g of cheese were placed into sterile Stomacher bag mixed with 180 ml of sterile 2% (w/v) tri-sodium citrate solution and blended for 5 min in a Stomacher (IUL instruments, Danimark) at room temperature. Serial dilution (10 to 10⁻⁸) was made using tri-sodium citrate solutions and 100 µl from each dilution were plated. Mesophilic aerobic bacteria were incubated at 30°C for 72 h in Plate Count Agar (PCA) (Oxoid, Basingstoke, United Kingdom). Psychrophile bacteria counts were determined at 10°C for 96 to 120 h on PCA. LAB were incubated at 30°C for 24-48 h in de Man, Rogosa and Sharpe Agar (MRS) (Oxoid, Basingstoke, United Kingdom). All microbiological analyses were performed in triplicate.

Isolation, enumeration and phenotypic characterization of LAB:

Traditional methods were applied for isolation and identification of LAB species as previously described²⁹. Briefly, Sharri cheese samples were mixed in milk broths and 10-fold serial dilutions were applied. Samples were diluted at different serial dilutions (from 10 up to 10⁻⁸), and 100 µl from each dilution was spread onto M17 agar supplemented with 0.5% w/v glucose (GM17) and MRS agar plates, and incubated at 30°C for 24 to 48 h. Based on the morphological differences single colonies from each experiment were picked up (15-20 colonies from each sample), re-streaked and stored in GM17 containing 15% glycerol at -80°C. The pure cultures were grown at 10, 15 and 45°C in MRS or GM17 and broth was evaluated visually after 24, 48 and 72 h of incubation. Tests for presence of catalase, production of ammonia from arginine, type of fermentation, different salt tolerance and pH were carried out as described previously¹. The isolates were grouped based on biochemical tests and from each % of sodium chloride concentration and temperature 15 strains were used for future phenotypic and genotypic identification.

DNA isolation, PCR, and DNA sequencing:

The DNA was extracted by using the bacterial genomic DNA Kit (Sigma-Aldrich, Missouri USA)²⁹. PCR was done on genomic DNA to amplify 16S rRNA encoding genes, using the universal primers 11F (50-TAACACATGCAAGTCGAACG-30) and 5R (50-GGTACCTTGTTACGACTT-30)^{8,40}. The thermal profile used for PCR was done at 90°C for 1 min, and 35 cycles at 95°C for 15 s, 54°C for 30 s, and 70°C for 90s. PCR products were purified with NucleoSpin Extract II (Macherey-Nagel, Düren, Germany), and

sequenced by ABI prism 377 DNA sequencing system (Applied Bio System, Darmstadt, Germany).

Sensory analysis and evaluation: Twelve trained panelists from the Faculty of Agriculture and Veterinary in Kosovo carried out sensory evaluation of control and treated Sharri cheese (in different sodium chloride concentration and temperature). Profiles of 6 sensory attributes of cheese evaluated by panelists were: appearance (color – from whitish to yellow – and presence of “eyes”), odour (odour intensity), texture (hardness, springiness and friability), flavor (intensity) and taste (salty, sharp and strange tastes). In brief, the sensory evaluation sessions were held at mid-morning; about three hours after breakfast and panelists were supplied with mineral water before each session. In each group, cheeses were presented to each panelist individually, cut in triangular and thickness.

Statistical analyses: The data were analyzed by using R statistical program version 3.0.1. ANOVA model was used to test effect of different treatments, and Tukey’s HSD at level of significance 0.01(1%) was applied to test for significant difference among different treatments (salt and temperature).

Results and Discussion

Enumeration of microorganisms: The characteristics of microbial populations in cheese at the time of processing and storage conditions have a significant influence on shelf life, sensoric quality, bacterial level and cheese making as well as on the other dairy products. Therefore, the total viable counts (TVC), number of psychrophile and lactic acid bacteria (LAB) in Sharri cheese have been enumerated, and results are summarized in Table 1.

According to the EU regulation the TVC should be $<10^5$ cfu/ml, while the Ministry of Agriculture, Forestry and Rural Development of Kosovo (MAFRD) regulation is less strict, with a TVC of 5×10^5 cfu/ml set as the upper limit^{9,24}. Level of TVC in raw milk before processing was analyzed, and only milk with number of bacteria according to EU regulation, has been used for further processing in cheese production. On the day 15 all the microbiological parameters were analyzed. The results of matured Sharri cheese showed level of bacterial counts to be: TVC $8.6 \log_{10}$ CFU g⁻¹, psychrophile bacteria $6.8 \log_{10}$ CFU/ml, and LAB $8.3 \log_{10}$ CFU g⁻¹. Usually, high level of TVC is an indicator of bacterial contamination. In our study the most of bacteria found were LAB, indicating that this group of bacteria was responsible for the fermentation process during cheese maturation. In the similar types of cheese the number of total LAB during the fermentation process

was reported to reach more than $6.8 \log_{10}$ CFU g⁻¹^{3,42}. Sodium chloride is traditionally used as a cheese preservative and is added to improve its flavor, enzymatic activity and for bacterial growth control^{6,19}. However, the use of high amounts of sodium chloride in foods was reported to cause different health problems¹¹. Based on that, World Health Organization has recommended decreasing the level of salt in dairy products (including cheese)⁴³. Sharri cheese manufacturing is not fully standardized yet and homemade cheese makers or industries still use high concentration of salt to control bacterial growth. Traditional cheese makers use to store cheese for maturation in different conditions and temperatures. Some prefer to store it in the room temperature, while the others prefer to do that on cold rooms or cold-water streams.

To understand possible effects of brine salt concentration and storage temperature, after the day 15-cheese samples were divided equally and placed on different brine solution (3, 6, 9 and 12%) and stored in two different temperatures (8°C and 22°C) up to day 60. All microbiological parameters mentioned previously have been measured again in the end of experiment (at day 60). Results showed significant effect of both factors (NaCl % and temperature) in all microbiological parameters (TVC, psychrophile bacteria and LAB) (P<0.01) (Table 1). The total viable counts (TVCs) varied significantly between cheeses stored in two different temperatures, ranging from $6.99 \log_{10}$ to $4.44 \log_{10}$ and 6.48 to $4.29 \log_{10}$, respectively. Similar significant differences were also shown for the other groups of bacteria. However, brine salinity had the main effect in microbiological parameters. Our results are in agreement with results obtained in a previous study¹⁴, showing that sodium chloride controlled the microbiological growth and enzyme activity of cheese. Unfortunately, it is well known that, extended storage of cheese or other dairy products in cold temperatures, as a common practice in dairy sector today, favors the growth of psychrophile bacteria. In general, these groups of bacteria are able to form termo-resistant enzymes (protease, lipases and phospholipases), which can cause spoilage of cheese and other dairy products⁴. In this study, the number of psychrophile bacteria was lower than total viable count. In previous works variable ratios between TVC and psychrophile bacteria have been reported. In some cases number of psychrophile bacteria is even higher than TVC, while in other cases the opposite is true. Higher number of psychrophile bacteria is an indicator that pasteurization of raw milk before processing is required. Nowadays, psychrophile bacteria represent a major problem in dairy industry, by causing cheese spoilage (not a good view for customers) and hence economic losses³⁷. Based on that, special attention is required to control raw milk processing and storage conditions.

Table 1. The level of different groups of microorganisms under different temperature and sodium concentration in cheese.

NaCl (%)	TVC			<i>Psychrophilus</i>			LAB		
	8 °C	22 °C	Mean ¹	8 °C	22 °C	Mean ¹	8 °C	22 °C	Mean ¹
3	6.99	6.48	6.73	5.61	5.12	5.37	6.46	5.40	6.43
6	6.78	6.37	6.58	5.48	4.92	5.20	6.29	5.99	6.14
9	6.25	5.06	5.65	4.41	4.31	4.36	5.92	5.84	5.88
12	4.44	4.29	4.36	3.76	3.29	3.52	5.84	5.37	5.61
Mean ²	5.11	4.55		4.82	4.41		6.13	5.90	

TVC – total viable count, LAB – Lactic acid bacteria, TC – total coliforms. Data are present on \log_{10} .

Total counts and identification of LAB from cheese samples: LAB species are mostly used on dairy products fermentation as a starter culture during maturation process⁴¹. Therefore, in our study level of cheese LAB on the day 15 was higher ($8.3 \log_{10} \text{CFU g}^{-1}$) but decreased significantly ($P < 0.01$) in the end of maturation process after cheese storing on different brine salt concentration for 45 days, ranging from 6.4 to $5.37 \log_{10} \text{CFU g}^{-1}$ (from the lowest to the highest concentration of salt, respectively). Different species are included on the LAB group. To identify the present species inside this group we have used five to ten colonies (623 isolates), during different experimental conditions. The classification has been performed based on the morphological characteristics and different dilutions. The preliminary identification of LAB isolates was done based on biochemical test (cell morphology, catalase activity, Gram staining, and growing on different growth condition: temperature, pH and percentage of sodium chloride). The results showed that all 623 isolates were Gram positive and catalase negative and showed different pattern based on the growth conditions (data not shown). Finally, 15 isolates from each treatment combination group of brine saline concentration and temperature (total 120 isolates) and 20 isolates before brine treatment were identified based on 16S rRNA gene sequencing. The predominant LAB species on the day 15 were *Leuconostoc* (9 out of 20 isolates), while the other species present were: lactococci (7 out of 20 isolates), enterococci (3 out of 20 isolates) and lactobacilli (1 out of 20 isolates) (Table 2). Our results about LAB species present in cheese are consistent with findings from other studies on similar types of cheese from Western Balkan countries^{17, 41}. Interestingly, after storing cheese in brine saline for 45 days the presence of LAB species was changed, and varied on different brine solutions. In low concentration of brine saline (3%) the most dominant species was *Leuconostoc pseudomesenteroides* (7 out of 15 isolates) and *Lactococcus lactis* (5 out of 5 isolates), while by increasing brine saline concentration *Enterococcus faecalis* (5 out of 15) and *Lactococcus garvieae* (5 out of 15 isolates)

became more dominant. In brine solutions with 6 and 9 % salt mixed lactic acid bacteria species were observed. It is known that, the presence of mixed LAB plays a positive effect on cheese quality. Lactobacilli and lactococci are the main contributors for cheese maturation, as they are able to participate on acidification and proteolytic activity as well as on the other enzymatic processes during cheese ripening. *Leuconostoc* and enterococci produce a typical flavor, texture and aroma component^{5, 13, 31}. Interestingly, on 12% brine saline concentration the only LAB species present were *Lactococcus garvieae* and *Enterococcus faecalis* (Table 2), while the other groups of bacteria found in lower brine saline concentration (like *Lactobacillus* and *Leuconostoc*) were not found, indicating that high concentration of brine saline changes the total microflora of Sharri cheese. Diversity of LAB was also reported for other cheese varieties similar to the Sharri cheese in the region, like in Zlatar cheese from Serbia⁴², Feta cheese from Greece²⁸ and Beaten cheese from Macedonia²³. In previous study in Sicilian cheese called Ragusano temperature was shown not to have a significant effect on cheese fermentation process¹⁵. In Feta cheese cold storage was shown to prevent further fermentation²⁸. This is an indication of differences on the effect of storage temperature on fermentation process among cheese types.

Sensory evaluation: Sensory evaluation based on acceptability scores of Sharri cheese obtained from 11 panelists is displayed in Table 3. The cheese ripened in the highest (12%) brine saline concentration had the best score for overall acceptability. However, cheese stored in 12% brine saline concentration was significantly different in overall acceptability only from cheese stored in 3%, but not from that stored in 6 and 9% (scored 3.08, 2.12, 2.56 and 2.93, respectively). In addition, storage temperature was not shown to affect the overall acceptability significantly. It is well known that people from Balkan countries, Middle East and countries bordering Mediterranean Sea are used to eat dairy products with

Table 2. Distribution of lactic acid bacteria in artisanal Sharri cheese under different brine concentration and temperature.

Species	Before salting	8°C				22°C			
		3%	6%	9%	12%	3%	6%	9%	12%
<i>Enterococcus</i> spp.	3	3	3	6	7	2	3	6	6
<i>Enterococcus faecalis</i>	1	0	0	5	6	0	1	5	6
<i>Enterococcus faecium</i>	2	3	3	1	1	2	2	1	0
<i>Lactococcus</i> spp.	7	5	5	3	6	5	5	4	7
<i>Lactococcus lactis</i>	7	5	4	2	1	5	4	2	2
<i>Lactococcus garvieae</i>	0	0	1	1	5	0	1	2	5
<i>Leuconostoc</i> spp.	9	6	5	3	1	7	4	3	1
<i>Leuconostoc pseudomesenteroides</i>	9	6	5	3	1	7	4	2	1
<i>Leuconostoc mesenteroides</i>	0	0	0	0	0	0	0	1	0
<i>Lactobacillus</i> spp.	1	1	2	3	1	1	3	2	1
<i>Lactobacillus sakei</i>	1	1	1	2	1	1	2	1	0
<i>Lactobacillus curvatus</i>	0	0	1	1	0	0	1	1	1

Table 3. Sensory evaluation of Sharri cheese.

Temperature (°C)	NaCl (%)	External appearance	Internal appearance	Flavor	Taste	Texture	Average	%
8	3	2.55	2.5	2.38	2.29	1.4	2.22	44.48
	6	2.91	2.75	2.54	2.36	2.13	2.54	50.76
	9	3.64	3.08	2.92	2.64	2.47	2.95	59.00
	12	3.64	3.42	3.00	2.71	2.33	3.02	60.40
22	3	2.18	2.17	2.08	2.21	1.40	2.01	40.16
	6	3.09	2.75	2.46	2.57	2.00	2.57	51.48
	9	3.64	3.08	2.92	2.64	2.27	2.91	58.20
	12	3.82	3.17	3.08	3.00	2.67	3.15	62.96

higher percentage of salt (up to 18%)³⁰. For example, a Turkish cheese called Beyaz cheese, one of the most preferable cheeses in Turkey, contains 12 g NaCl in 100 g of cheese (12 %). However, less salty brine (6 - 8%) have been reported for Feta cheese from Greece²⁸, and Feta cheese from Serbia (up to 8%)⁴². If we want to decrease the brine saline concentration, because of possible health risks for consumers by use of high saline concentration, we may need to consider replacing NaCl salt by other chemicals less dangerous for human health, in order to keep unchanged the cheese taste for the consumers.

Increase of the brine saline concentration was reported to change the properties of cheese. It is well known that in Feta cheese, higher percentage of brine saline increases cheese hardness and pH values, while the moisture content is decreased³². Comparison of results from the sensory evaluation and LAB diversity showed that 6 and 9% brine saline concentration to be best, in order to keep unchangeable the cheese taste, which is in accordance with results from other studies for similar type of the cheeses in Western Balkan and Mediterranean countries. Texture of the cheese treated with 3% brine saline concentration was significantly different from cheeses with higher concentration of brine saline. This is in accordance with previous findings, that sodium chloride increases cheese hardness.

Conclusions

The level of TVC, psychrophile bacteria and LAB were all decreased significantly by increasing the level of saline concentration. In the cheeses stored on 3, 6 and 9% saline concentration, mixed species of LAB (including: lactococci, lactobacilli, enterococci and leuconostoc) were found, while on 12% the dominant LAB species were *Enterococcus faecalis* and *Lactococcus garvieae*. On sensory evaluation the best scores for flavor were for 6, 9 and 12% brine saline concentration. To avoid the healthy problems and save the traditional cheese making the sensory evaluation results coupled with microbiological results suggest that Sharri cheese could be successfully manufactured using 6% brine instead of higher concentration currently used (12 % or higher) and stored on room temperature. Due to the negative

effect of sodium chloride it would be interesting to replace the sodium chloride with other compounds and then to study the possible changes on microbiological flora and sensory quality of Sharri cheese.

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