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Research Article
Influence of Brine Concentration and Ripening Temperature on Quality of Sharri Cheese

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Abstract

Background and Objective: Sharri cheese is considered as one of the most popular brined cheeses in Kosovo produced on the highlands of the Sharri mountains. The modest mountain conditions and non-standardized production often lead to a non-standardized quality of this cheese. The purpose of this study was to determine the physical and chemical changes, as well as the changes in the total number of bacteria in Sharri cheese during ripening in different brine solutions and temperatures. **Materials and Methods:** Salting of Sharri cheeses during manufacture was carried out with different concentrations of brine (3, 6, 9 and 12% NaCl) and different ripening temperatures (8 and 22°C). Analyses of physical and chemical parameters such as protein, fat, dry matter, acidity and pH as well as analyses of the total number of bacteria were performed using traditional methods in different periods of ripening (on days 1, 3, 7, 15, 30, 45 and 60, respectively). The one-way analysis of variance, ANOVA model was used to test the effect of different treatments, while Tukey's HSD was applied to the test for significant difference at a level of significance 0.05 (5%) among the different treatments (salt and temperature). **Results:** The results obtained showed significantly increased (5%) values of protein content (26.5-29.1%), dry matter (56.8-59.9%) and total acidity (36.1-45.3%), of the cheese during ripening. However, no significant changes were seen in pH level or fat content during ripening time. The total bacterial count in the first part of ripening process showed a significant decreased from first day 86×10^7-16×10^7 CFU mL^{-1} in day fifteenth, indicating a turbulent process of fermentation. It was also found that increased salt concentration (from 3% up to 12% NaCl) of the brine caused a decrease in the total number of bacteria in the cheese. **Conclusion:** The study demonstrates the need to determine the adequate concentration of brine solution and temperature for Sharri cheese production.

Key words: Sharri cheese, brine solution, protein content, fat content, dry matter, total acidity, total viable count

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sharri cheese is one of the most famous salt cheeses in Kosovo. The characteristics of this product are the specific technology and relatively high salt content. Cheese is a healthy food product for humans and contains proteins, fats, carbohydrates, amino acids and minerals (particularly calcium), all of which are essential for human nutrition[1]. The quality of raw milk, equipment for cheese making, type of starter culture and storage conditions such as brine cheese concentration and storage temperature, are the major factors that determine cheese quality[2]. In Mediterranean and Balkan countries, different brined cheeses with different names are produced, including Beyazpeynir (Turkey), Feta cheese (Greece), Bjalo salamureno sirene (Bulgaria), Domiati (Egypt), Teleme (Greece, Romania, Turkey), Iranian White (Iran) and Beli sir u kriškama (former Yugoslavia)[3]. In Kosovo, large quantities of different cheese types have been produced traditionally from the milk of cows, sheeps, goats or mixed milk. One of the most famous traditional cheeses in Kosovo is Sharri cheese, which is produced in the Sharri mountains on the border between Macedonia, Kosovo and Albania[4,5]. This artisan cheese is produced traditionally in households from raw milk without the addition of a starter culture. Sharri cheese is a typical indigenous dairy product in Kosovo which allows the use of a slightly higher concentration of salt during processing. The technology of Sharri cheese production is not yet standardized. Small or mid-scale dairies located in the Sharri Mountains are trying to use modern processing methods during production which are based on traditional technology with minimal differences between them. Sharri cheese produced from raw milk is mainly consumed after ripening for 30-45 days in brine solution. The specificity of this technology is that after coagulation, the curd is left for 15 days for ripening without brine solution. It is a hard type of cheese with a high fat content and spongy appearance, with a great diversity in production and unequal non-standard quality[4,6].

Sodium chloride plays an important role in cheese ripeness, surface stabilization, husk formation, consistency, structure taste, flavour and the killing of pathogenic bacteria[7]. The salt concentration in different types of cheese varies widely, ranging from 0.7% in some types of Swiss cheeses to 8% in cheese with brine[8-11]. Almost all the sodium in the diet comes from processed foods, to which all types of cheeses belong[12]. Although cheese is a product with high nutritional value, it is perceived that it contains high levels of fat and sodium chloride[13,14]. There is strong evidence that the consumption of high levels of sodium chloride has been linked to health complications, such as stomach cancer, kidney stones, diabetes and osteoporosis[11,15-17]. Moreover, an excess of sodium chloride in the body is associated with high blood pressure and increases the risk of heart attack[18,19]. The World Health Organization recommended a daily intake of sodium of about 2.4 g[20], which demonstrates the other benefits of trying to decrease the salt concentration in Sharri cheese. This shows that decreasing the level of salt will increase the health benefits. Consequently, this study investigated the possibility of preparing cheese from cows milk collected from the rural area of the Sharri mountains, Kosovo, using lower salt concentrations and different ripening temperatures in order to lower the salt level in cheese due to the effect of salt on human health, without affecting the physical and chemical contents or attributes of the cheese's sensory quality during ripening and preserving in an attempt to obtain a dairy cheese that is typical of the traditional Sharri product. Changes in the chemical and microbiological composition caused by the addition or reduction of salt concentration were also evaluated. The aim of this paper was to investigate the effect of sodium chloride concentration and storage temperature during ripening on the chemical composition and total viable counts (TVCs) of Sharri cheese.

MATERIALS AND METHODS

Cheese making: The traditional technology of Sharri cheese is not standardized and includes main technological operations such as collection of fresh milk, clotting, draining and ripening in brine solution for at least 45 days. The procedure of Sharri cheese making is not fully standardized yet, leaving the possibility for farmers of the Sharri mountains to implement the production procedures slightly differently from each other. During the period March-June, 2014, the cheese was produced from cow's milk from the Dragash area. Figure 1 describes the Sharri cheese making procedure.

Cheese manufacturing and sampling: Sharri cheese was produced in the "EkoSharri" dairy, which is located in the village of Buzez in the Dragash municipality, according to traditional procedures with some slight modifications. In brief, 60 L of fresh, unpasteurized full-fat milk were tempered into a clean tank. Milk was filtered and heated to 35°C and rennet was added. After ~90 min, the curd was cut into grains (1 cm) and stirred for 15 min at 35°C to facilitate the removal of whey from the curd. The cheese curd mass was heated again at 44°C for 15 min and was transferred into the cheesecloth and pressed for 6 h to remove the whey. After that, the cheese was placed on wooden shelves for the first storage of ripening without brine. The second stage of ripening started at day 15

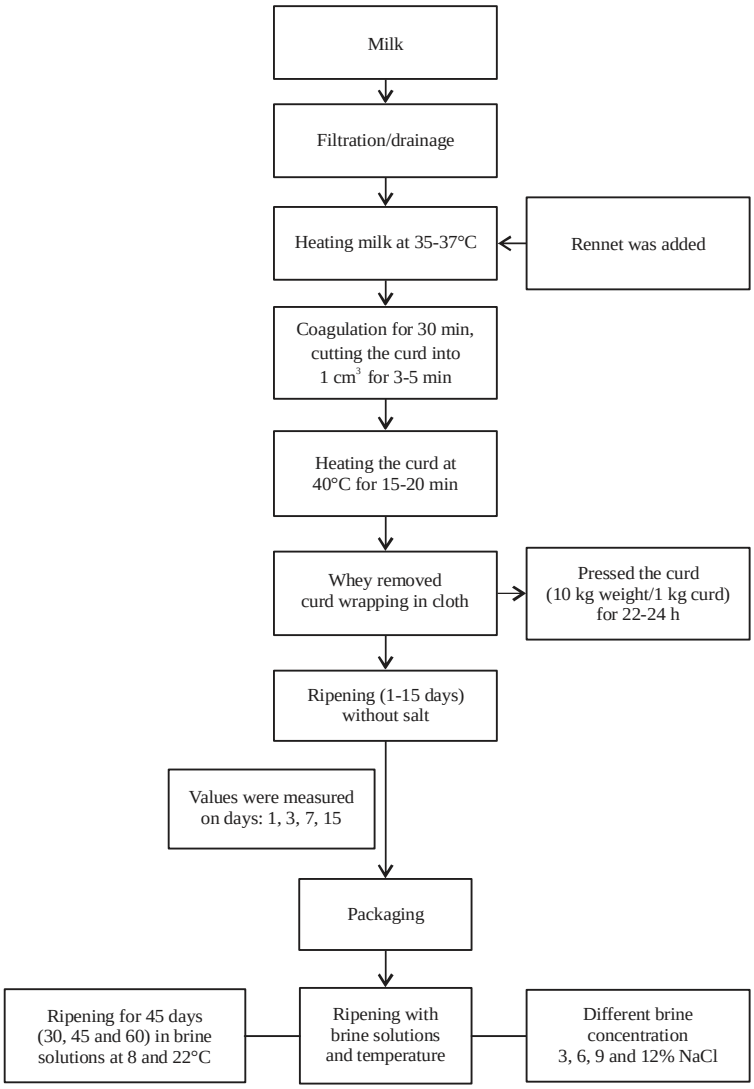

Fig. 1: Production flow chart of artisanal Sharri cheese type

in brine solution. On day 16, the cheese was cut into ~200 g pieces and separated into jars containing four different sodium chloride, ≥99% used for analytical grade (Sigma Aldrich, St. Louis, United States) solutions (3, 6, 9 and 12% NaCl). The samples for assessment were obtained from the milk before processing and from the cheese on days 1, 3, 7, 15, 30, 45 and 60, respectively (Fig. 1). All the samples were collected and analysed in triplicate.

Microbiological analysis: Total 10 g Sharri cheese samples (without rind) were placed into a sterile Stomacher bag with 90 mL of sterile 2% (w/v) tri-sodium citrate solution ≥98% used for analytical grade (Sigma Aldrich, St. Louis, United States) and blended for 5 min in a Stomacher (IUL instruments, Danimark) at room temperature. Serial dilutions were made using Ringers solution and microorganisms were grown on plate count agar (PCA) with final pH 7.0±0.2 (25°C) used for microbiological grade (Sigma Aldrich, St. Louis, United States) to determine TVCs and incubated at 30°C for 72 h. In parallel, TVCs for raw milk were determined and California mastitis tests were performed according to the protocol described previously [21]. Only raw milk with TVCs within ranges stipulated by EU regulations and that were negative for the mastitis test were used for further processing in cheese production.

Physical and chemical analysis: The physicochemical parameters of the cheese samples (dry matter, moisture, salt, total fat, pH, titrable acidity (°SH) and mineral content) were analysed in the Faculty of Agriculture and Veterinary, University of Prishtina, whereas, protein content was analysed

in the Faculty of Agriculture and Food Sciences, University of Sarajevo. The physicochemical composition of each cheese sample was determined using the following standard methods. Dry matter was determined by drying the samples at 102±1°C according to the ISO : IDF Standard[22] and water content (%) was calculated. Total fat content was evaluated by the acido-butyrometric method using sulfuric acid 99% used for analytical grade (Sigma Aldrich, St. Louis, United States) and ethanol 95% used for analytical grade (Sigma Aldrich, St. Louis, United States) described previously by ISO[23]. Mineral content (ash) was measured gravimetrically after burning samples at 600°C[24]. The Kjeldahl procedure[25] was used to determine the total nitrogen (N) content (Buchi, K-360) in the cheese. Salt was measured by using digital sodium chloride refractometer (HI-96821-Official Methods of Analysis of AOAC International). The pH was measured with a pH meter PHM 82 Standard (Radiometer, Copenhagen, Denmark). Titratable acidity was measured by using the titrimetric method described by Soxhlet-Henkel (°SH)[26].

Statistical analyses: The data were analysed using the R statistical program version 3.0.1. The one-way analysis of variance (ANOVA) model was used to test the effect of different treatments, while Tukey's HSD at a level of significance of 0.05 (5%) was applied to the test for significant difference among the different treatments (salt and temperature)[27].

RESULTS

Changes in physicochemical composition and level of TVCs in raw and processed milk during the entire cheese making process, up to day 60 when the final product of Sharri cheese was achieved, started during the first stage of ripening (at days 1, 3, 7 and 15) and the second stage of ripening, with different brine solutions (at days 30, 45 and 60). According to the Ministry of Agriculture, Forestry and Rural Development (MAFRD) of Kosovo, Administrative Instruction MA-No. 20/2006 for quality standards and grade of fresh milk in Kosovo, the level of TVCs should be set at 5×10^5 CFU mL^{-1} as the upper limit, while EU regulations are stricter ($<10^5$ CFU mL^{-1})[28,29].

The results of the physicochemical parameters of raw milk before processing showed levels of proteins in the amount of 3.83%, milk fat 4.03%, moisture 87.4%, ash content 0.86%, pH value 6.46 and titrable acidity 7.85°SH. Analysed milk composition and quality was within EU limits and limits set by regulation MA 20/2006 and fulfilled conditions for processing and cheese production. In Table 1, the results are presented as the mean and standard deviation for TVCs and physicochemical parameters of Sharri cheese on days 1, 3, 7 and 15 of ripening. The results show increased levels of proteins from the first day of production, increasing from 26-29.1% by day 15. The TVC decreased while the other parameters showed no significant changes (Table 1). However, to our knowledge, the exact concentrations of brine solution and storage temperatures of Sharri cheese are not yet standardised. To understand the effect of brine concentration and storage temperatures on the physicochemical aspects, cheese on the day 15 was transferred to brine solutions with different salt concentrations (3, 6, 9 and 12% of sodium chloride) and stored at two different temperatures (8 and 22°C) until day 60. The results of Sharri cheese stored at 8 and 22°C with different salt concentrations of brine are presented in Table 2.

DISCUSSION

The aim of this study was to determine the total viable count and physicochemical composition of raw milk as a starter material of cheese through to the end product. The results of this research indicate that no significant differences in the physicochemical parameters of the cheese were observed, except the level of proteins, which increased by approximately 3%, rising from 26.5% on the first day to 29% by day 15 (Table 1). It is obviously that the physicochemical parameters are in agreement with other data reported previously for brined cheese[30]. The yellow colour of the cheese appeared to be stronger when the level of mineral content increased and when whey was warmed. At the same time, the yellow colour is an indication of a high fat content in the raw milk[31]. However, the results indicate that by increasing the concentration of the brine solution, the dry matter increased significantly, from approximately 62% in low concentration up

Table 1: Physico-chemical parameters and TVCs of sharri cheese from days 1, 3, 7 and 15 of ripening

Time of ripening (days)	TVC g^{-1}	Dry matter (%)	Moisture (%)	Mineral content (ASH) (%)	Fat (%)	pH	Titratable acidity (°SH)	Protein (%)
1	86×10^7	56.8±1.1	43.2±1.1	4.3±0.6	26.7±0.8	5.6±0.2	36.1±5.7	26.5±0.6
3	99×10^7	58.9±2.0	41.1±2.1	4.9±0.6	28.0±1.3	5.4±0.3	39.9±3.4	27.0±1.63
7	37×10^7	59.2±3.2	40.8±3.3	5.3±0.3	27.3±0.8	5.4±0.3	42.3±5.1	28.8±0.7
15	16×10^7	59.9±2.1	40.1±2.1	5.0±0.4	27.0±1.9	5.5±0.1	45.3±4.9	29.1±1.3

Mean±SD

Table 2: Changes in the physico-chemical characteristics and TVCs of Sharri cheese during ripening on different time point and temperature

Salt (%)	Days	TVC×g^{-1}		Dry matter (%)		Moisture (%)		Mineral content (ash) (%)		Fat (%)		pH		Titrable acidity (°SH)		Salt (NaCl) (%)		Protein (%)	
		8°C	22°C	8°C	22°C	8°C	22°C	8°C	22°C	8°C	22°C	8°C	22°C	8°C	22°C	8°C	22°C	8°C	22°C
3	30	121×10^5	86×10^5	61.9±2.1	62.8±1.6	38.2±2.1	37.2±1.6	5.5±0.2	5.8±0.3	28.8±1.3	27.7±1.8	5.3±0.1	5.2±0.1	51.4±3.3	50.2±1.3	3.1±0.4	3.1±0.2	27.3±0.3	27.8±0.9
	45	106×10^5	52×10^5	62.6±1.0	62.1±1.8	37.4±1.0	38.0±1.8	6.4±0.3	6.3±0.4	27.3±1.5	27.5±1.3	5.4±0.2	5.4±0.3	49.6±1.7	51.1±4.5	4.3±0.3	4.3±0.3	28.9±0.2	29.2±0.5
	60	97×10^5	30×10^5	62.7±1.6	63.5±2.1	37.3±1.6	36.5±2.1	6.5±0.2	6.8±0.3	28.2±0.4	28.2±1.8	5.2±0.1	5.4±0.2	51.0±5.9	50.4±7.4	4.4±0.4	4.3±0.3	28.6±1.4	28.7±1.0
6	30	99×10^5	79×10^5	62.9±1.6	63.2±1.4	37.0±1.6	36.8±1.4	5.7±0.4	5.7±0.2	28.7±1.6	29.3±1.8	5.4±0.3	5.4±0.4	46.5±4.9	50.0±4.1	3.2±0.3	3.0±0.2	28.1±0.1	27.7±0.7
	45	72×10^5	43×10^5	63.2±2.9	63.7±1.9	36.8±2.9	36.3±1.9	6.4±0.4	6.4±0.1	27.7±2.7	27.5±1.5	5.5±0.3	5.3±0.2	50.6±2.2	51.6±3.4	4.5±0.3	4.6±0.2	29.1±0.9	29.1±0.6
	60	60×10^5	23×10^5	62.1±2.5	63.5±2.1	37.9±2.5	36.5±2.1	6.6±0.3	7.3±0.5	27.5±0.8	28.5±1.4	5.3±0.4	5.2±0.1	51.5±5.2	52.4±1.3	4.6±0.2	4.8±0.5	28.7±0.4	28.5±0.4
9	30	32×10^5	35×10^5	63.4±0.9	65.5±1.7	36.6±0.9	34.5±1.7	5.7±0.5	6.0±0.2	28.3±1.3	27.8±1.2	5.3±0.3	5.5±0.2	50.2±2.3	50.1±3.8	3.5±0.5	3.8±0.2	28.5±0.5	29.8±0.5
	45	26×10^5	25×10^5	63.9±1.1	64.4±1.5	36.2±1.1	35.6±1.5	6.7±0.4	6.5±0.2	28.8±2.1	27.5±1.3	5.3±0.1	5.3±0.1	49.4±2.8	51.2±4.2	4.4±0.3	4.1±0.2	28.4±1.2	28.9±1.3
	60	17×10^5	11×10^5	62.9±1.8	62.4±2.0	37.1±1.8	37.6±2.0	7.4±0.3	7.4±1.2	27.8±1.9	28.8±1.4	5.3±0.2	4.3±0.4	49.2±5.0	52.3±3.6	4.5±0.2	4.7±0.3	28.6±1.1	28.1±1.2
12	30	39×10^5	37×10^5	62.8±2.5	63.5±2.1	37.2±2.5	36.5±2.1	6.0±0.3	6.3±0.1	27.2±1.3	28.7±1.6	5.2±0.1	5.4±0.4	50.4±2.8	48.6±4.8	3.8±0.3	3.9±0.1	29.9±0.1	29.1±1.5
	45	31×10^4	24×10^4	63.7±1.0	63.6±2.8	36.3±1.0	36.4±2.8	6.5±0.3	6.5±0.2	28.8±1.3	28.2±0.8	5.3±0.2	5.2±0.1	51.6±2.1	50.4±2.6	4.3±0.3	4.4±0.2	28.1±0.7	28.7±1.2
	60	27×10^3	19×10^3	63.6±1.3	64.9±1.7	36.4±1.3	35.1±1.7	7.5±0.2	7.5±0.5	29.0±2.7	28.8±2.8	5.2±0.1	5.1±0.1	51.6±3.5	52.1±5.4	4.7±0.4	4.8±0.3	27.2±1.4	29.1±1.9

Mean±SD

to 64% (Table 2). According to the results obtained, dry matter increased significantly after day 15. This is probably as a result of syneresis and osmosis[8,30,32,33]. Brine concentration helps in cheese consistency and controlling the microflora of cheese[8,34]. The level of moisture decreases by approximately 1% between days 30 and 60 and the pH decreased significantly (from 5.5-4.3) during the ripening process (Table 2). Usually, the reduction of moisture content occurred due to the migration of whey from cheese during these days of the ripening process[8,34]. Its low water content categorizes Sharri cheese into the group of hard cheeses (maximum 39% moisture), similar to Cheddar and Colby cheese, which are internally ripened by bacterial fermentation or Swiss Emmental or Gouda, which are internally ripened by a combination of lactic acid bacteria and CO_2 production, resulting in holes or "Eyes"[8]. These results are in agreement with the classification scheme of the Codex Alimentarius, which classifies this type of cheese in the group of hard cheeses[35]. Salt penetration into the cheese, moisture loss during brining and the influence of brine concentration on cheese physicochemical properties were shown in other studies[36,37], indicating that the concentration of the brine solution may influence chemical composition, proteolysis development and rheological characteristics of cheese[38,39]. The titratable acidity increased continually during the storage period. The mineral content of the cheese directly correlated with its amount in the milk and the effects on rheological characteristics of the final product. The protein level increased significantly from the first day until the end of the ripening stage, which is in accordance with a previous study[5]. The microflora of the raw milk is diverse and most of the microorganisms present play a key role in the fermentation processes. The obtained results show that TVC increased between day 1 and 3 of ripening, while level of dry matter decreased until day 15. The significant decrease (5%) in the TVC after 15 day of ripening is an indication of the effect of the brine solution on Sharri cheese. Increased TVC during milk processing from day 1-15 (from 86×10^7-16×10^7) is an indication of the activation of fermentation bacteria called lactic acid bacteria (LAB). An earlier study showed that, in general, milk quality and in particular, TVC and the presence of *Staphylococcus aureus* in raw milk were above the required limits[40] and mastitis infections in cows was frequently present[21,41]. According the obtained results here regarding the raw milk composition before processing, it was ascertained that this milk was within the limits set by regulations and with a high enough quality for consumption and processing. Moreover, these results are in accordance with those reported in a previous study by Huppertz *et al.*[33] for required milk

parameters before processing, indicating that the raw milk from this study is ready for further processing. Good quality of raw milk is an indication of having a good product[31]. In addition to fermentation, LAB play important roles in improving food value, digestion of lactose, controlling infections in the intestines, controlling certain types of cancer and controlling serum cholesterol levels[42]. The high diversity of LAB in Kosovan raw milk was previously reported by Mehmeti *et al.*[43] and seems to be activated during the first days of cheese making, as the TVC of raw milk was very low compared with days 1, 3, 7 and 15. In the previous study[27], we reported a high diversity of LAB. This is an indication that the process of fermentation takes place and that different types of LAB are involved in the process. This is a limitation of raw milk. If raw milk is of good quality and within the limits in terms of microbiological and physical aspects, the quality of the product is good. However, if the raw milk is outside of limits, the products will not be good. Nevertheless, different groups of bacteria have been shown to survive under different stress response conditions. It has been previously reported that these groups of bacteria are able to survive in sodium chloride up to 6.5%[44,45]. Usually, due to relatively poor sanitary conditions and primitive production technologies, pathogenic bacteria can be present in the cheese[46]. Traditional cheese markers often use a high concentration of brine salt with the aim of reducing the presence of pathogens in the product. In medical terms, salt manifests several negative effects, causing several symptoms and diseases such as hypertension and other cardiovascular problems, kidney stones and osteoporosis[10,11,16,18]. The brine concentration for the same types of cheese has been previously shown to reach up to 12-18%[47,48]. Some of the producers eliminate the high concentration of salt by using pasteurized milk. The advantage of pasteurized milk is the elimination of pathogenic bacteria; however, the disadvantage of using pasteurized milk is that it causes changes in the microflora of the product.

CONCLUSION

High concentrations of brine decrease the TVC. By using a strong brine solution, the farmer is trying to protect this cheese from contamination and other problems which may occur in the future. This study demonstrates that, 9% of brine solution (no need more) is normal for Sharri cheese and no changes on physicochemical and microbiological parameters. By increasing salt concentration, it will show significant changes in the physicochemical and microbiological parameters during ripening.

SIGNIFICANCE STATEMENT

This study discovers the effect of different brine solutions (3, 6, 9 and 12% NaCl), temperature (8 and 22 °C) in physical and chemical aspects, as well as in the microbiological aspects especially in total viable counts of bacteria in Sharri cheese which can be beneficial to improve the quality of Sharri cheese. This study will help the researcher to uncover the critical parts which are missing in this cheese that so far many researchers were not able to explore. Using this study it will be able to get a better understanding to make a good combination which it will improve the quality of this type of cheese.

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