CHAPTER 1

Characteristics of Traditional Cheeses Produced in the Republic of North Macedonia

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Abstract: Traditional cheeses are specific products of the Republic of North Macedonia, mainly produced in small-scale farms or farm houses located in high mountains and rural areas. Some of the best-known cheese types are produced in almost all regions in North Macedonia: kashkaval, white brined cheese and beaten cheese (*"bieno sirenje"*). These types of cheese can also be found in other Balkan countries, only with different tastes and properties. Kashkaval is a type of hard yellow cheese with a natural rind and it belongs to the Pasta filata cheese group. Traditionally, it is made from sheep milk. White brined cheese is the most commonly consumed type of cheese that can be produced from sheep, cow and goat milk. Beaten cheese is a type of yellow hard cheese and the scalding procedure is a crucial step in its production. The great diversity in the manufacturing procedures results with variations in the physical, chemical and microbiological composition, as well as with variations in the proteolysis, the texture, and volatiles. In this chapter, the properties of cheese, the cheese-making technology and the artisan culture of the traditional cheese varieties in the Republic of North Macedonia are discussed.

Keywords: Beaten cheese (*"bieno sirenje"*), Kashkaval cheese, Proteolysis, Volatiles, White brined cheese.

INTRODUCTION

Beaten Cheese (*"bieno sirenje"*), Kashkaval from the Bistra mountain and White

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Brined Cheese ("*belo salamureno sirenje"*) are our typical trademark products which vary in appearance, aroma, flavour, and texture. Artisanal cheeses are differentiated according to their strong bonds with the territory of their origin and so they represent a historical and cultural blueprint of the community they are produced by. The production of these cheeses takes place in limited geographical areas with the use of know-how techniques transferred from generation to generation and the use of milk that has undergone no treatment after milking. From milking to the end of the ripening process, these types of cheese pass through different surroundings where a variety of microorganisms have the opportunity to grow and develop. Raw milk is an example of an environment comprised of many complex communities and different taxonomical groups of microorganisms with approximately 300 species of bacteria, 70 species of yeast, and 40 species of moulds. The conducted research showed that traditionally made cheeses have unique properties in terms of palatable pleasure, richness, and diversity, as well as protection against pathogens. Undoubtedly, these properties are achieved due to the presence of unique indigenous microbiota, especially because of the use of raw milk, combined with the specific skills of the cheese-makers that result in the creation of their general properties and quality. Each traditional cheese originates from a complex system with its own biodiversity components such as environment, micro and macro climate, pasture, autochthonous breeds, use of untreated milk and its natural microbiota, natural coagulants, cheese-making procedures, ancient traditional tools and equipment, and natural ripening environment.

WHITE BRINED CHEESE

Macedonian white cheese is a brined variety of cheese with either soft or semi-hard texture. White colored, rindless, with close texture and salty acid to pickled flavour are the main sensory properties of this type of cheese. This cheese is most commonly made from cow milk, sheep milk and, less commonly, from goat milk, prepared in blocks, and ripened in brine for a period of 90 days (Fig. **9**). Traditionally, this type of cheese has been produced for decades by local farmers on a small-scale, using raw milk and traditional techniques handed down from generation to generation using only elementary equipment. Instead of using commercial starter cultures, artisan cheese-makers rely on the naturally present indigenous microorganisms in the raw non-pasteurized milk and the adventitious contaminants from the soil, the equipment, the surfaces, and the environment in general. According to Davis (1976) and Phelan (1993), the production of white brined cheese has mostly expanded in the regions of the Mediterranean and Balkan Peninsulas. The production range of white brined cheese is very wide, and it is therefore very difficult to determine the whereabouts of its first production. Depending on the region provenance where it is produced, several modified methods, numerous varieties and sub-varieties of this type of cheese have been created. According to international standards, white cheeses in brine belong to the group of semi-solid and soft cheeses, with or without maturing phase. According to the Codex Standard for cheese A-6, 2000, this cheese has a distinctive white to yellowish colour, no crust, and it is ripened and stored in brine until repackaging or sale. According to the wide areas of origin of this type of cheeses in different countries on the Balkan Peninsula, they are included in the group of autochthonous dairy products, which, depending on the region, are characterized by a certain specificity in technology, quality, and name of the cheese. According to technology, this type of cheese is the so-called "Feta" in Greece (Anifamtakis, 1986), it is known as "White brined cheese" in Bulgaria (Dimov, 1963), and it is called "White soft cheese" in Macedonia (according to Srbinovska *et al.*, 1994). Dilanyan (1967) believes that the basic property and characteristic of the white brined cheese is the brine, which is the environment where the process of maturing and storage of the product takes place. Therefore, the duration of keeping the cheese in brine affects its taste and quality.

Production of Traditional White Brined Cheese

Sheep milk produced by autochthonous sheep breeds is exclusively used for the production of sheep white brined cheese (local sheep breeds, Pramenka, and crossbreeds). Accordingly, the milk used for the production of cheese should be of normal chemical composition in accordance with the Rulebook on the special requirements for safety and hygiene and the manner and procedure for performing official controls of milk and dairy products (Official Gazette of the Republic of Macedonia, No. 26, 21.2.2012), as well as the Rulebook on the requirements for the quality of raw milk, the quality standards for consumer milk, dairy products and the use of their names, the quality and activity of starter cultures, curdling and other specific substances and the manner of their use, the manner of additional labelling of the milk and dairy products, as well as the permitted deviation of weight in relation to the declared (Official Gazette of the Republic of Macedonia, No. 96/2011). The criteria for the chemical composition of raw sheep milk are the following parameters: minimum fat 6.09 (average 6.0-9.0%) and minimum proteins 5.2 (average 5.3-5.8%). The average of the chemical composition of raw sheep milk is given in Table **1** (Levkov, 2013; Talevski *et al.*, 2009)

Table 1. Chemical composition of raw sheep milk.

Parameters	Levkov, 2013	Talevski *et al.*, 2009
Total dry matter, %	18.85	18.07
Proteins, %	4.55	5.89
Fat, %	10.09	7.07
Lactose, %	4.09	4.37
Dry matter without fat, %	10.09	11.0

Relatively, from the results, the research conducted by Levkov (2013) and Televski *et al.*, (2009), and the criteria provided in the Rulebook on raw sheep milk, it can be seen that the values for certain parameters are in accordance with the criteria of the Rulebook, but with certain deviations. That is, the values of all examined parameters of Talevski *et al.*, 2009, (Table **1**) are in accordance with the constitutional criteria of the Rulebook and in accordance with Simos *et al.* (1996), whereby normally sheep milk contains 6-9% milk fat, 4-7% protein, 4-6% lactose, and 17-21% dry matter. While according to Levkov (2013), there is a greater deviation of the increase in the value of milk fat (10.9%) and the value of milk proteins are below the prescribed (4.55%) (Table **1**). These obtained values of the chemical composition of raw sheep milk, according to Levkov (2013), are considered to be the result of the use of raw sheep milk at the end of lactation of the sheep, which is a practice among our producers, especially in the production of traditional authentic white cheese. The production of Macedonian White Brined Cheese (MWBC) depends on the hydrolysis of lactose by lactic acid bacteria to produce lactic acid (Sulejmani, 2010). The breakdown of the degradation of lactose in the curd has a major effect on the quality of the ripened cheese; for example, excessive lactic acid in cheese curd leads to a low pH, strong acidic harsh taste, and a brittle structure. Sulejmani (2010) studied the optimization of the production process of Macedonian white brined cow milk cheese, in which case the influence of the starter culture, the optimal temperature of milk curdling, and the optimum time of curd cutting on the quality and yield of the cheese were determined. The highest level of acidity (°SH), protein, and fat at day 1 of ripening (mean ± SE) were observed in the MWBC cheeses with a starter culture of 24.13%, 14.56% and 25.68%, respectively. The flavour and the total sensory scores were significantly influenced by the use of the starter culture, processing technology, and temperature of curdling. Cheeses made using the freeze-dried culture F–DVS YF–3331, curdling at 39°C MWCB2 had a more satisfactory appearance, texture, odour, and quality than other differently treated Macedonian white cheeses (Sulejmani *et al.,* 2011).

The Cheese-making Process

Immediately after milking, which is done twice a day in the pen, the raw sheep milk is processed and transported to the cheese-makers, where it is filtered again and the production of cheese starts immediately. In the case when the milk is processed once a day, it should be cooled to a temperature below 6°C. For the production of traditional cheese, raw milk is used in the presence of natural microbiota. This means that pasteurization of the milk is not performed. For the production of white brined sheep cheese, the milk should have appropriate acidity and it is best to be from 9 to 10°SH, according to Srbinovska (2013).

Curdling

The drained sheep milk is placed in wooden barrels (Fig. **2**) with appropriate milking temperature and curdled, after cooling, *i.e.* T= 26-28°C. The duration of the curdling depends on the weather conditions, meaning that when the outdoor temperature is above 30°C the curdling lasts for 1 hour, while under 10°C the process is prolonged for 2 hours. The curdling is carried out at that temperature by adding a cheese enzyme (chymosin). Earlier, the rennet from the stomach of young lambs was used, while they were still fed with milk, that is while the enzyme chymosin was produced, which is the primary means for milk coagulation. The extracted rennet from the stomach of young lambs was dried in the sun, covered with the lamb's abomasum (the fourth compartment of the lamb's stomach where rennet is secreted) to prevent it from becoming contaminated by external factors and flies. Once dried, it was ground and then, in the form of powder, it was used as rennet. This way of rennet production was replaced by the production of various rennets of different origins, derived from certain microorganisms through synthetic ways. For the traditional way of production of these autochthonous white cheeses, the cheese enzyme used most commonly or recently is an organic production of 100% chymosin. Due to the high coagulation intensity of this type of rennets, they are added in amounts as small as 0.02%. It is recommended to use pure chymosin rennet, obtained synthetically, which is closest to the traditional in terms of intensity and activity of the enzyme, and because this enzyme has a precisely determined place of action and the production of creation of peptide chains of the same length, without the possibility of the appearance of bitter taste of cheese (Mateva, 2004).

In the production facility for renneting the milk, in addition to using wooden barrels (Fig. **2**), some cheese-makers use shallow wooden troughs (Fig. **1**) with dimensions 1m x 0.20-0.35m x 3-4m. Before putting the milk into these troughs, a cheesecloth or plastic foil is placed inside, in order to avoid major losses, especially for milk fats. When the curdling (coagulation) begins, the primary coagulation is measured, which usually begins in 10-15 minutes (once the cheese mass starts to detach from the vessel's wall), while the full coagulation is usually after 1 hour, depending on the temperature and weather conditions. The curds are considered curdled after obtaining a medium firm consistency, with porcelain cross-section and sweet taste, and the whey becomes yellowish-green (Kapac 1988).

Processing and Forming

The formed curd is bound on both sides with the cheesecloth (Fig. **3**), where the whey is separated. On the cheese mass above the cheesecloth, wooden weights are placed on both sides of the curd (Fig. **4**), so that it is nicely outspread and can be evenly divided. When the surface layer of the curd is cooled, it is turned over. After the curd rests for 3-4 minutes and is self-pouring from its own weight, the curd is cut into blocks with a large blade and is moved to a perforated frame, under which is a cheesecloth. Thus, through the cloth and the openings of the frame, the residual whey of the curd is separated, and the process of its separation or syneresis is finished. The separated whey is clear, and yellow and green in colour.

Fig. (1). Wooden troughs for renneting.

Fig. (2). Wooden barrels for renneting.

Fig. (3). The curd is bound with the cheesecloth.

Fig. (4). Added tools - wooden weights.

Pressing

After the self-pressing process has been completed from the very weight of the curd, some masters also practice the load-bearing phase in order to separate the remaining whey. In this stage of pressing under load, the temperature in the room is very important, and it should not be higher than 18°C, because higher temperature can strengthen the separation of the whey, which would cause strong decomposition of the milk sugar (lactose) and getting sour cheese with a larger number of cavities, considered as a cheese defect (Srbinovska, 2013). Pressing the load is carried out gradually so that it does not lead to forming a crust on the mass and its decomposition.

Cutting into Blocks

After finishing the pressing process and the separation of whey (syneresis), the curd, which should be 12 cm in size, is cut into blocks (Fig. **6**). The blocks are 11-12 x 11-12 cm in size because the diameter of the container is 22 cm. The salt, *i.e.* the brine, is prepared beforehand, and the concentration measured with a salinometer should be 18%. The brine is prepared in the production facility from sea salt and water used from natural resources. Troughs filled with pre-prepared souse are used for salting the brine and the cheese blocks are put inside (Fig. **5**). On the upper side of the surface layer, the cheese blocks above the brine are salted with coarse sea salt (which should meet the microbiological and hygienic requirements for its quality). Then, it remains in the brine for 12 hours, and during that time, the cheese blocks are overturned and salted on the surface layer to make the salt equal. By salting the cheese, the concentration and acidity of the brine changes. Each salting results in separation of the whey, which increases the acidity. On the other hand, with the reduction of salt in the cheese, the concentration of salt changes. Therefore, it is necessary to continuously monitor the concentration of salt and acidity of the brine (salt), which should be 8°SH (20^0T), and if it is higher, there will be a process of slow salting (Srbinovska, 2013). Therefore, the temperature of the brine should be 15 °C, while in warm weather, it should be up to 10°C. Ripening white brined cheese should contain 3-3.5% of salt. To achieve such salting, salt consumption in every 100 L of sheep milk should be about 3 kg.

Fig. (5). Salting in brine.

Fig. (6). Cutting the curd in blocks.

Packaging and Ripening

The packaging of white brined cheese is carried out in tin cans (Fig. **8**), and if there is a larger quantity, it is packed in wooden barrels and vats. The cheese blocks are lined up in barrels, and initially, on the bottom of the barrel, coarse sea salt is thrown, then cheese, then salt again, then cheese again, and so on until the barrel is filled (Fig. **7**). While arranging the cheese, regardless of the type of packaging used, it is important to keep an eye out so that there is no space left between the cheese blocks and they would not deform. Then, brine is poured, where the concentration of brine is 18%, but according to Kapac (1988), it should be 22-23%. The acidity of the brine where the cheese ripens should be 24-40°SH (60-100^{0}T). If the acidity is lower, conditions for developing rotting bacteria are created, while higher acidity prevents the ripening and the cheese gets a crushing consistency. The quantity of brine in the barrels should be 10-15% of the total amount of cheese and it should be controlled, it should be clear with a pleasant smell and no mild liquid. Packed cheese in barrels is carried to a special room for ripening, with controlled temperature and humidity conditions for that purpose. Most often the ripening in the pens is done in a special room, an excavated underground basement with good insulation. The walls are made of stone and they are tinned and thatched for better insulation. The ripening is carried out at a temperature of 15-17°C, with humidity of 75-80%, and in the duration of 2 to 3 months. According to Kapac (1988), the ripening of white brined cheese is carried out at a lower temperature of 13-15°C for 30 to 45 days.

Fig. (7). Stacking in cans.

Fig. (8). Ripening in cans.

A major highlight of the white brined sheep cheese ripening process is the formation of milk acid, which begins with the pressing stage of the cheese so that after 15-20 days of ripening, the amount of milk sugar (lactose) is reduced to a minimum or is not present at all. Therefore, the quality of this cheese depends on the dynamics of ripening, which, if faster or slower, inhibits the development of the desired lactic acid bacteria and the creation of their enzymes (ferments). According to Penev (1972), the basic indicator is the pH value which, at the end of the ripening period, should be between 5.8 and 6.0 in order to achieve this acidity of cheese and proper dynamics during the ripening. The same authors consider that for the first ten days, the ripening should be carried out at a temperature of 15-18°C, and afterwards, the cheese should be carried to basements with a lower temperature regime of 10-15°C, until the final stage of ripening.

Fig. (9). White brined cheese.

Ripening and Cheese Yield

Until a certain fermentation stage, the cheese ripens in a warehouse at a temperature below 10°C, where the process continues, which consists of supplementing the brine (as needed). The yield of sheep's white cheese is different, depending on the sheep's lactation period. For the production of 1 kg white brined sheep cheese, an average of 3.3 L sheep milk is needed.

Chemical Composition of White Brined Cheese

The average chemical composition of the traditional white brined cheese is presented in Table **2**. The research by Mojsova (2013), regarding the use of the same raw material (sheep milk), is comparable to Kapac's (1988). While regarding the final product – white brined cheese, the results of Kostova-Mojsova (2013) and Kapac (1988), for the production of traditionally white brined cheese, are comparatively in line with small deviations in the industrial production of white brined cow cheese, according to Mateva (2012). According to Mojsova (2013), in traditional white sheep cheese, the lowest pH value was 4.04, and the highest value was 5.05. The obtained results were consistent with the results of

Turantas *et al.*, (1989) which, for the Turkish white cheese, received pH values of 4.11-5.65.

Table 2. Chemical composition of white brined cheese.

Parameters, %	Kostova-Mojsova, 2013 Traditional Sheep Cheese			Kapac, 1988 Traditional Sheep Cheese	Mateva, 2012 Industrial Cow Cheese		
	average	*min*	*max.*	*average*	*average*	*min*	*max.*
Moisture	49.05	46.97	51.58	52.95	50.43	48.91	52.54
Dry matter	49.98	48.85	53.07	47.05	49.57	47.46	51.09
Fat	28.50	28.00	30.00	26.42	24.02	22.14	25.12
Fat in dry matter	57.62	52.40	60.00	56.21	48.47	45.31	51.87
Nonfat dry matter (NFDM)	21.48	18.85	25.07	20.63	25.55	25.32	25.97
Moisture in NFDM	70.25	64.73	73.07	71.96	66.37	65.31	69.70
Proteins	19.65	18.21	21.37	23.79	17.26	14.42	19.24
Soluble Nitrogen	0.8458	0.6700	0.9213	/	0.2978	0.2677	0.3211
Ripening coefficient	27.19	19.64	31.65	/	11.28	10.67	11.85
Salt	4.90	4.38	5.43	3.26	3.75	3.05	4.01
PH	4.46	4.04	5.05	4.65	4.51	4.26	4.61
Titratable Acidity ^{0}T	216.25	200.00	240.00	220-260	222.21	216.93	225.15

Titratable acidity in fresh cheese ranged from 100-120°T, while in mature cheese, the lowest measured titration acidity was 200°T, and the highest value obtained for titratable acidity was 240°T. The derived results completely coincide with the results obtained for Sjenicko cheese, obtained from sheep milk, where titratable acidity in mature cheese ranged from 143.85-244.32°T (Ruzic-Muslic *et al.*, 2011). According to the moisture content in nonfat dry matter (NFDM) and rheological properties, this type of cheese (Codex standard for cheese, CODEX STAN 283-197) is classified as soft cheese ripened in brine, since this parameter is above 67%, *i.e.* it ranges from 64.73% to 73.07% and is in accordance with the trials of Kapac (1988), with representation of 71.96%, (Srbinovska 2013). According to Mateva (2012), the results for NFDM in cow cheese obtained by industrial production are slightly lower than 67%, *i.e.* by an average of 66.37%, which is the result of using cow milk and other production methods. The content of dry matter in cheese is the most important parameter that affects the ripening process. As a rule, cheeses with a higher moisture content go through a faster ripening process. Therefore, processing the crude as a technological operation aims to regulate the moisture content of the cheese. According to the results from the analyses carried out by the producers themselves and from literature data

(Mojsova, 2013; Mateva, 2012; Kapac, 1988) it can be concluded that the dry matter ranges from 47.05% to 53.07%. The values for fat in dry matter in traditional cheeses examined by Anifantakis (1998) ranged from 49.38-58.05%, which are somewhat lower compared to our results. On the other hand, Ruzic-Muslic *et al.* (2011) published higher values than our lowest value, more precisely with Sjenicko cheese - the values for MCM ranged from 53.32 to 62.50%. Milk fat ranged from 27.51% to 30%, compared to Kapac, where there was low content of fat 26.42%. Based on the content of milk fat, this type of cheese meets the criteria of CODEX STAN 283-197 and belongs to the group of full-cream cheeses. The obtained values coincide with the values for Sjenicko cheese where fats ranged from 24.50-29.50% (Ruzic-Muslic *et al.*, 2011). The results, according to Mateva (2012), for the value of milk fat are justifiably lower, because it is industrially produced white brined cow cheese. According to Mojsova (2013), the lowest values for total protein content are from 18.21% to 23.79%, which is the average according to Kapac (1988). The values for total proteins, according to Mateva (2012), are in accordance with the values of the previous authors. As a basic indicator to determine the coefficient and dynamics of ripening, larger variations in this parameter can be seen between traditional and industrial white brined cheeses. From the results of the value of the ripening coefficient, we can see a large difference of 11.28% (Mateva 2013) to 27.19 (Mojsova 2013) in the final ripening stage of white brined cheese. The lower value of the ripening coefficient in industrial cow cheese is due to the lower intensity of proteolytic decomposition, and at the same time, due to the lower value for soluble nitrogen in this type of white brined cheese. The data for the value of 11.28% (Mateva 2012) for the ripening coefficient in the final stage of ripening are somewhat smaller and in relation to the studies of Maćej (1989), who found a ripening coefficient of 12.63% in Sjenicko cheese. According to authors Maćej (1989) and Jovanović (2001), who compared certain parameters in white brined cheeses produced traditionally and industrially, in relation to cheeses produced on the basis of co-aggregates, they found insignificant differences in the content of dry matter and percentage of milk fat, while in relation to the total nitrogen content, they found an increase of 50% for traditional cheeses compared to industrially produced cheeses and on the basis of co-aggregates. From here arises and is standardized the research in which, according to Stević (1962), biochemical transformations in the ripening process can be divided into primary (basic) ripening – in all types of cheese with special emphasis in solid and semi-hard cheeses with formed crust, and secondary (additional) ripening which is characteristic for cheeses with soft consistency and without crust. Typical for softer cheeses are the changes that occur on their surface, forming compounds that give it a spicy taste and smell. These compounds have the ability to diffuse inside the cheese mass during ripening and form the specific aroma of cheese.

Microbiological Properties of White Brined Cheese

Bacterial biodiversity coming from untreated milk and the surrounding contamination constitute the primary source of microorganisms essential for the peculiar taste, unique flavour and typical consistency and appearance of this traditional cheese. Indigenous microbiota from the raw milk and microorganisms originating from environmental exposure during the cheese-making process are also involved in the fermentation process, the biochemical properties and the final development of the cheese product (Kostova-Mojsova, 2013). Microorganisms, including bacteria, yeasts, and moulds are present in the whole process of ripening and make their contribution to it through their metabolic and enzymatic activity. The quality of the cheese largely depends on the composition of microbiota. When cheese is traditionally made, environmental microbiota plays a fundamental role in the fermentation process and is one of the most essential parameters affecting the quality of the cheese (Demaringny *et al.*, 1997). Microbial populations are numerous and diverse. Microorganisms vary in terms of their abundance and diversity during the cheese ripening process. The major microbial groups, isolated from white brined cheese in the first days of the ripening process were *Lactococcus, Lactobacillus, Enterobacteriaceae, Leuconostoc, Enterococcus,* and yeasts. As the process evolved, these populations changed and at the end of the ripening process, the most prevalent ones were *Lactobacillus* and *Lactococcus* and yeasts groups (Mojsova *et al.*, 2013). 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 in higher numbers at day 10, ranging from 5.44 to 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. **10**). These results indicate that lactococci constituted the predominant bacterial group in the beginning of the ripening period. At the end of the ripening, lactobacilli were the most prevalent group and their number was within the range of 5.35 – 7.43 log cfu/g. The decline in lactococci was probably due to the inhibitory action of the low pH and high salt in moisture values in the maturing cheese (Vafopoulou-Mastrogianaki *et al.*, 1990). The presumptive *Leuconostoc* at day 10 were found within the range from 4.11 – 6.50 log cfu/g. The obtained results are slightly higher than the ones reported by Manolopoulou *et al.* (2003). At the end of the ripening process, at day 90, there was only one cheese sample where *Leuconostoc* was detected. These groups of microorganisms may influence the ripening process through the production of lactic acid, the decrease in oxidation/reduction potential and their proteolytic and lipolytic activities (Steele, 1995). Enterococci were also found in high values at the beginning of the ripening process (from log 2.6 – 6.03 cfu/g) (Mojsova *et al.*, 2013). These high numbers were in accordance with counts of enterococci found in Turkish white cheese (Oner *et al.*, 2006) where the mean log was 5.34 cfu/g. At day 90, they rapidly decreased and enterococci were not detected in some cheese samples (Fig. **10**). The variations in their number in different types of cheeses depend on the type of cheese, the initial contamination of the milk, their survival and growth under certain circumstances in the production and ripening processes (Macedo *et al.*, 1995; Litopoulou-Tzantaki 1990). Enterococci may influence the ripening process due to their proteolytic activity, citrate activity, and lipolytic activity, as well as the production of diacetyl and other aromatic compounds (Kostova-Mojsova 2013; Folquie *et al.*, 2006). The most prevalent species of *Lactobacillus* at the initial point of the ripening process were: *Lb. plantarum, Lb acidophilus, Lb. curvatus, Lb. lidneri, Lb. helveticus, Lb. salivarius. Lb. delbrueckii* subsp. *delbrueckii*. The most prevalent species of Lactobacillus at the final point of the ripening were: *Lb. paracasei* subsp. *paracasei*, *Lb plantarum, Lb. brevis, Lb. fermentum, (*Kostova-Mojsova 2013*).* Among the *Leuconostoc* genus, the most frequently detected species were *Leuconostoc mesenteroides* subsp. *mesenteroides dextranicum, Leuconostoc lactis,* and *Leuconostoc mesenteroides* subsp. *cremoris*.

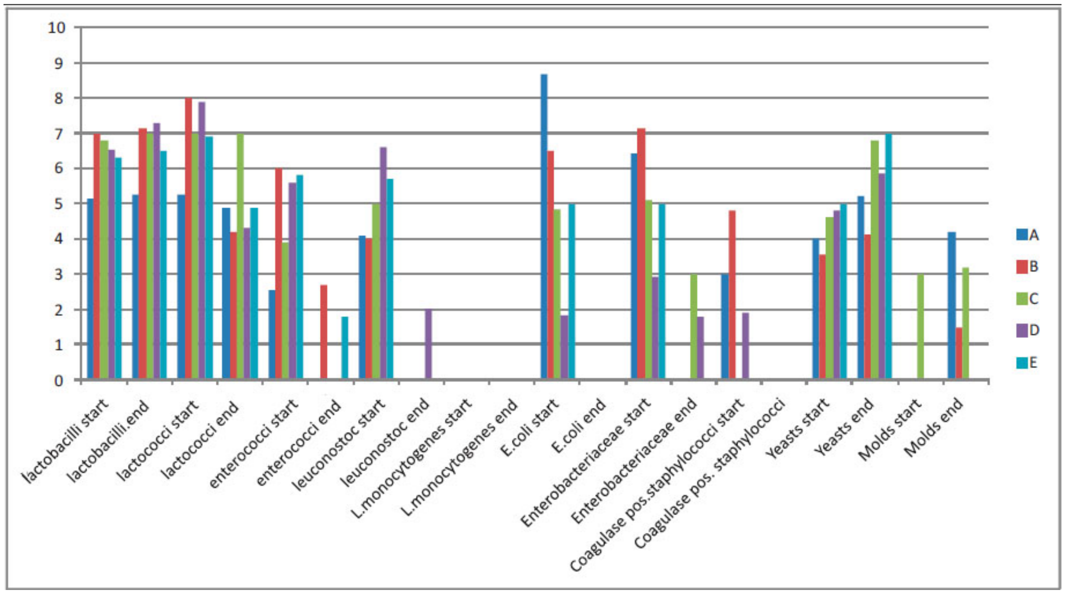

Fig. (10). Log count of microbial groups during ripening of the white brined cheese (beginning and ending point).

Enterococci are part of the lactic acid bacteria (LAB) and present a complex, diverse and important group of bacteria in terms of their interaction with food and humans. Probably because of their usual presence as contaminants of raw milk, thermotolerance, low toxicity and the ability for acidification, enterococci became an essential part of fermented food (Franz *et al.*, 1999). Enterococci are omnipresent bacteria that harbour the digestive tract of humans and animals, and are also part of the environment including the soil, surface waters, plants, and vegetables. Furthermore, they can be found in food, and specifically in cheese (Giraffa, 2002). They are often present in artisanal cheeses made in the southern regions of Europe, mostly from raw sheep or goat milk, where they occur as contaminants from animal faeces, water or milking equipment, and storage tanks. (Folque-Moreno *et al.*, 2006). As an important component of artisanal cultures, they play a key role in the ripening process, (Manolopoulou *et al.*, 2003) giving the authentic taste and aroma (Kostova-Mojsova 2013). According to Mojsova (2016), the most dominant species of enterococci in traditionally made cheeses are *E. faecium* and *E. Faecalis,* and less dominant are *E. durans, E. hirae* and *E. gallinarum.* These results are completely in accordance with the results found in other Mediterranean cheeses (Gelsomino *et al.*, 2001; Aarestrup *et al.*, 2002; Manolopoulou *et al.*, 2003; Foulquie *et al.*, 2006). Enterobacteriaceae are part of the normal microbiota of many dairy products. In fact, contamination of raw milk inhabited with enterobacteriaceae is naturally unavoidable, and even additionally during inappropriate cooling after milking and improper transport of the milk in terms of failing to meet the requirements for cold chain transport, as well as during handling when processing. The growth of enterobacteriaceae can be influenced by the pH value during the process of fermentation, stress factors along the production stages, salt concentration, and the presence of bacteriocins. As a result of all of the abovementioned factors, these groups of microbiota are not commonly present in the final product. At the beginning of the ripening process, a high level of contamination with *Enterobacteriaceae* from 7.24 log cfu/g and *E. coli* 8.69 log cfu/g occurred, which was expected due to the use of raw milk and artisanal rennet, as well as the high moisture and low salt content. All of the factors mentioned above could promote the growth of these bacteria. Furthermore, during the maturation process, the number of *Enterobacteriaceae* was decreased 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 final stage of ripening (Mojsova *et al.*, 2013). Similar results were obtained in Feta cheese, where at day 4, *E. coli* reached 5.3 log cfu/g, after which they are significantly dropped and at the end of the ripening they are not detected at all (Manoloupoulou *et al.*, 2003). According to Kongo (2008), the number of *Enterobacteriaceae* is very important for evaluating the quality of raw milk and hygiene during the process of making, handling, and storage. On the contrary, their presence is very important in creating the flavour, aroma, and texture in some of the traditional cheeses (Dahl *et al.*, 2002). In addition, the presence of *Enterobacteriaceae* in all stages of the cheese production could be related to their role of ripening and creating the typical aroma. (Mojsova *et al.*, 2013). The presence of coagulase positive staphylococci at the beginning of the ripening process was within the range from 3.11-4.77 log cfu/g, and at the end of the production, they were not detected in any of the samples (Mojsova *et al.*, 2013). Even though staphylococci tolerate salt, their growth is not favoured by the cheese environment, because of the combined inhibitory effect of the pH and NaCl concentration (Litopolou-Tzanetaki 1997). In addition, some bacteriocin-producing enterococci isolated from the cheese samples showed inhibitory activity against them (Mojsova *et al.*, 2015).

Although there is evidence of survival of the *L. monocytogenes* in brine, Papageorgiou & Marth (1989) and other authors, after their conducted researches concluded that Listeria can grow and survive in mature Feta cheese, while *L. monocytogenes* was not detected in the Macedonian white brined cheese samples (Mojsova *et al.*, 2013). This data could be explained by the presence of bacteriocin producing enterococci in the cheese that showed the activity predominantly against *L. monocytogenes*. (Mojsova *et al.*, 2015).

Yeasts are inevitable microorganisms in traditional cheeses and they were present through the whole ripening process within the interval from 2.74- 5.30 cfu/g (Mojsova *et al.*, 2013). High yeast numbers can be attributed to their tolerance to low pH values, low water activity, ability to grow at low temperatures, ability to use the lactose and organic acids and tolerance to high concentrations of salt and cleaning agents and sanitation. (Bouton *et al.*, 1998). Although the presence of yeasts may have adverse effects in terms of spoilage of cheese (Pereira-Dias *et al.*, 2000, Minervinu *et al.*, 2001), according to some authors they play an important role in creating the aroma and the nutritional quality as a result of the producing essential amino acids, vitamins, aromatic compounds such as diacetyl, acetaldehyde, methyl ketone, and ethyl alcohol. The increased number of yeasts found in the study of Mojsova *et al.*, (2013), may contribute to the typical organoleptic characteristics of our traditional cheese, since recent examinations have shown that some lipolytic and proteolytic enzymes produced by these microorganisms contribute to the development of the aroma and flavour compounds (Marino *et al.*, 2003). Moulds were found at day 90 in some of the samples and could probably be due to contamination occurred during the ripening process (Mojsova *et al.*, 2013).

TRADITIONAL BEATEN CHEESE

Traditional cheeses are characteristic products of Balkan and Mediterranean countries, one of which is the Republic of Macedonia. Their production is typical of the rural areas, but also of the mountain regions where the livestock is kept and pastured in summer. The consumers' increased interest in traditional cheeses due to their unique organoleptic properties (Montel *et al.*, 2014; Franciosi *et al.*, 2009) is positive from many aspects, such as: preservation of the microbial biodiversity, support to the rural regions' economic development, creation of new jobs, etc. (Zeppa *et al.*, 2004; Talevski 2012). It is considered that the production of traditional Beaten cheese dates back as early as the age of the Ottoman Empire (Sulejmani *et al.*, 2014b), and more precisely – that Beaten cheese was invented by the Vlach sheep breeders under the name Vlashko Cheese (Talevski 2012). According to Micev (1969), Beaten cheese was produced in rural households in the region of Mariovo from raw sheep milk. Over the course of time, its production spread almost on the whole territory of today's Republic of North Macedonia and, besides sheep milk, it has also been produced from cow and goat milk or their mixture. According to Dubrova-Mateva *et al.* (2016), very often for the production of Beaten cheese, milk from the end phase of the lactation is used, since this milk is richer in milk fat. The small changes in the production technology, in combination with climatic and geographic properties, the milk quality and the animals' nutrition (Micari *et al.*, 2007) influence the quality and the properties of the cheese. A common feature of all the variants is the specific production process, namely the stage of churning (beating the cheese, wherefrom comes its name) when the curd is crushed into tiny particles like larger grains of sand in size. Beaten cheese is distinguished by its hard consistency, has a yellowish colour, and when cut, a lot of cavities of different sizes and unequal disposition can be seen. A very important feature of this type of cheese is the high concentration of salt. According to Kapac-Parkačeva (1974), by its physical and chemical properties and the technology of production, the Beaten cheese belongs in the group of hard cheeses, while by the way of ripening and keeping (in brine), it belongs in the group of sour-salty cheeses.

The Cheese-making Process

The milk for making Beaten cheese is drained through a cheesecloth (not obligatory) and poured into a curdling vessel. The curdling is most often done by using an enzymatic rennet with the strength of 1:5000, or the rennet chymosin CHY-MAX (2080 mica/g) at the temperature of the milk of 25–35°C. In the past, for curdling, a home-made rennet was used, obtained from the lamb's stomach (Talevski 2012). The curdling process lasts 30–50 minutes. After that, the curd is submitted to processing (churning or beating) by using a wooden tool (the so-called "kyurkalo") (Fig. **11 a**). The process of churning (beating) is done in three series of 50 strokes (150 strokes in total), and after each series, the curd is left to "rest" for 5–10 minutes. In this process, it may come to the separation of a part of the milk fat, in which case the fat is skimmed and removed from the vessel. When the beating process ends, the curdled mass is warmed up by adding warm water at a temperature of 53-90°C, depending on the particular manner of production. Then the curdled mass is left to stay with warm water for some 5–20 minutes, whereupon the curdled mass slowly settles down at the bottom of the vessel. After that, the water and the whey are removed from the vessel and the curdled mass is shaped into a ball by hand (Fig. **11 b**). The curd is put into a cheesecloth, hung up and left for some 16–20 hours so that the mass can drain and self-press (Fig. **11 c**). After that, the stage of ripening follows, which differs depending on the traditions in the particular region where the cheese is produced. The cheese is laid on a wooden table covered with cloth, to ripen at room temperature for 2–7 days (Fig. **11 d**), or it can be exposed to sun ("sunning") for 2-3 days, wherein the cheese gets waxy yellow colour caused by the milk fat emerged to the surface. When the stage of ripening is finished, the cheese is cut into pieces 5–6 cm thick and it is salted with coarse salt (Fig. **11 e**). The amount of salt depends on the cheesemaker's estimate. After salting, the pieces are put into tin or plastic cans and left to stay for 1-2 days (Fig. **11 f**). Then the cans are filled with brine (20-22%) till the pieces are fully covered, and the cheese is left to ripen for 30-45 days.

Fig. (11). Technology of beaten cheese (Bieno sirenje) production; a) cheese processing (churning of curd); b) shaping of curdled mass; c) curd draining and self-pressing; d) dry ripening; e) preparing for salting with coarse salt; f) cheese stacking in can.

Physical and Chemical Properties of Traditional Beaten Cheese Made of Raw Sheep Milk

As can be seen from Table **3**, the pH value changes during the process of cheese ripening. In the beginning, there is a steady decrease, whereas during the period of ripening in brine (from day 10 to day 45) there is a slight increase. The differences in the pH values between the cheeses made in different households are not statistically significant ($p<0.05$). The differences in the production technology, the milk quality and the season of the year when the cheese is made, have an influence on the active acidity of the cheese which resulted in lower pH values compared to the pH values obtained by Sulejmani *et al.* (2014a, 2014b) and Talevski (2012). The amount of moisture in the cheese decreases during the period of ripening when a statistically significant difference ($p<0.05$) was found. This is due to the unequal amounts of salt added and the speed of its diffusion in the cheese as well as the expulsion of the water from the curdled mass. The results obtained are in line with those obtained by Kapac-Parkačeva *et al.* (1974) and Dimitrovska *et al.* (2017). In the process of ripening, the amount of total proteins and fats gradually increases (Table **3**). The comparative research shows that statistically significant differences ($p<0.05$) in the presence of total proteins and fats have been found not only in the fully ripened cheese but also after the first and the tenth day of ripening in brine. The results obtained on the amounts of proteins and fats in the Beaten cheese are in line with those obtained by Sulejmani *et al.* (2014a, 2014b), Dimitrovska *et al.* (2017) and Talevski (2012). The dynamic of the amounts of fat in dry matter in both variants of Beaten cheese is similar, although certain statistically significant differences ($p<0.05$) were found after 10 days of ripening in brine. Some similarities have been found with the results obtained by Mijačević and Bulajić (2008), Mijačević *et al.* (2005) and Caridi *et al.* (2003). The main feature of the Beaten cheese is the high concentration of salt added in the stage of salting and in the stage of preparing the brine, put by the cheesemakers at a rough estimate. Despite that, the differences in the percentages of salt in the examined samples of beaten cheese are statistically significant ($p<0.05$) only in the stage of ripening, after 30 days (Table **3**). These results are in line with those obtained by Sulejmani *et al.* (2014a, 2014b), Dimitrovska *et al.* (2017), Talevski (2012) and Kapac-Parkačeva *et al.* (1974). The non-standardized way of salting also influences the amount of salt in moisture (S/M) which shows similarities with the results obtained by Sulejmani *et al.* (2014a), but it is higher than the results obtained by Arenas *et al.* (2004). The percentage of ash in the Beaten cheese increases in the process of ripening as a consequence of the increase in the amount of dry matter (Abdel-Razig & Al Gamry, 2009). Statistically significant differences ($p<0.05$) in the amounts of ash between both variants of Beaten cheese were noticed in the stages of ripening in brine, namely, after 10 and 45 days of ripening. The higher amount of ash is also a result of the high concentration of salt in the cheese, whereas the variations in the percentage of ash are most probably due to the higher diffusion of inorganic substances in the brine caused by the higher percentage of moisture in the cheese (Abdalla & Ahmed, 2010). The results obtained regarding the concentrations of salt in this research are similar to those obtained by Kapac-Parkačeva *et al.,* (1974), Sulejmani *et al.* (2014a, 2014b) and Dimitrovska *et al.,* (2017).

Table 3. Physical and chemical properties of Traditional Beaten Cheese ("bieno sirenje").

Ripening Days	House Hold	pH	Titratable Acidity	Moisture %	Proteins %	Fat %	MFDM %	Salt %	S/M %	Ash %
dry ripening	A	5.09-5.13 Av 5.11±0.02	60.00-64.00 Av 62.13±2.01[a]	43.37-47.98 Av 45.54±2.31	26.33-26.51 Av 26.41±0.09	21.82-23.66 Av 22,78±0.92	40.34-43.23 Av 41.84±1.44	nd	nd	2.39-2.47 Av 2,43±0.04
	B	5.13-5.21 Av 5.17±0.04	69.60-75.20 Av 75.20±4.87[b]	45.38-48.12 Av 46.43±1.47	24.29-25.63 Av 24.82±0.71	23.66-28.57 Av 25,62±2.60	45.43-52.30 Av 47.78±3.91	nd	nd	2.21-2.28 Av 2,25±0.03
ripening with salt	A	5.06-5.11 Av 5.09±0.03	62.40-69.60 Av 65.07±3.95[a]	36.48-38.65 Av 37.86±1.19	26.67-29.17 Av 27.69±1.31	28.66-30.86 Av 29,73±1.10	46.56-48.58 Av 47.84±1.11	2.43-4.49 Av 3,75±1.14	6.66-11.61 Av 9.85±2.77	5.13-6.51 Av 5,94±0.72
	B	5.02-5.12 Av 5.08±0.05	77.60-80.00 Av 79.20±1.39[b]	29.69-35.22 Av 33.36±3.18	27.99-29.91 Av 28.93±0.96	28.49-33.58 Av 30,31±2.84	43.95-47.75 Av 45.42±2.04	3.71-3.79 Av 3,76±0.04	10.53-12.76 Av 11.34±1.23	6.39-7.09 6,85±0.39
1 day brining	A	5.09-5.10 Av 5.10±0.01	72.80-76.00 Av 74.77±1.72	37.26-40.31 Av 38.80±1.52	25-96-26.98 Av 26.63±0.58	26.22-28.29 Av 27,09±1.07[a]	43.74-45.09 Av 44.25±0.72	2.87-5.24 Av 3,92±1.20	7.39-14.06 Av 10.17±3.46	6.98-7.87 Av 7,39±0.44
	B	5.05-5.14 Av 5.10±0.05	72.80-87.20 Av 78.13±7.90	36.48-38.42 Av 37.70±1.06	26.37-26.93 Av 26.59±0.29	28.64-30.38 Av 29,35±0.91[b]	45.08-49.16 Av 47.13±2.03	4.36-5.05 Av 4,71±0.34	11.95-13.21 Av 12.48±0.65	6.72-7.20 Av 6,92±0.24
10 days brining	A	5.09-5.17 Av 5.15±0.07	62.70-68.80 Av 66.23±3.16[a]	38.26-40.16 Av 39.08±0.97	24.22-25.90 Av 24.82±0.93	26.31-27.71 Av 26,96±0.70[a]	43.89-44.88 Av 44.25±0.54[a]	5.41-6.68 Av 6,01±0.64	13.90-16.63 Av 15.35±1.35	7.39-7.86 Av 7,56±0.25[a]
	B	5.10-5.16 Av 5.13±0.03	80.00-84.80 Av 81.87±2.57[b]	36.93-38.82 Av 37.91±0.94	24.54-28.09 Av 26.81±1.96	28.38-30.04 Av 29,31±0.85[b]	45.76-48.21 Av 47.20±1.27[b]	5.04-6.27 Av 5,59±0.62	13.26-16.97 Av 14.76±1.95	7.95-8.29 Av 8,13±0.17[b]
30 days brining	A	5.14-5.20 Av 5.17±0.03	67.20-69.60 Av 68.27±1.22[a]	37.39-38.79 Av 37.93±0.74	25.60-28.00 Av 26.81±1.20	27.32-30.07 Av 28,72±1.37	46.14-53.34 Av 49.17±3.73	530-5.86 Av 5,60±0.28[a]	13.66-15.67 Av 14.77±1.02[a]	7.93-8.44 Av 8,25±0.28
	B	4.96-5.18 Av 5.10±0.12	81.60-88.00 Av 85.33±3.33[b]	37.53-39.48 Av 38.38±0.99	24.59-27.96 Av 26.80±1.91	26.61-28.26 Av 27,61±0.88	43.00-46.69 Av 44.82±1.84	6.90-7.34 Av 7,14±0.22[b]	18.16-19.24 Av 18.59±0.57[b]	8.62-8.82 Av 8,69±0.11
45 days brining	A	5.18-5.29 Av 5.22±0.06	74.40-78.40 Av 76.27±2.01	37.08-40.66 Av 38.86±1.79	25.70-26.24 Av 25.90±0.29[a]	26.72-30.34 Av 28,17±1.91	43.61-49.59 Av 46.08±3.12	5.20-6.51 Av 6,07±0.75	14.02-16.76 Av 15.59±1.41	6.99-7.47 Av 7,19±0.25[a]
	B	5.03-5.18 Av 5.13±0.06	73.60-83.20 Av 78.93±4.84	37.00-39.07 Av 38.36±1.18	27.31-28.26 Av 27.78±0.47[b]	26.73-30.83 Av 28,11±2.35	43.82-48.94 Av 45.57±2.91	6.52-6.72 Av 6,62±0.10	16.99-17.62 Av 17.27±0.31	8.92-9.94 Av 9,28±0.57[b]

Average values±SD within the column with no common superscript letters differ ($p<0.05$)
nd – not determined

Microbiological Properties of Beaten Cheese

The ripening of Beaten cheese, like in all other traditional types of cheese, is a result of the interaction between the rennet enzyme, the natural milk enzymes, and the naturally present lactic-acid bacteria. The dynamics of the amounts of the examined groups of micro-organisms in the sheep Beaten cheese produced in two households in the region of Mariovo is shown in Fig. (**12**).

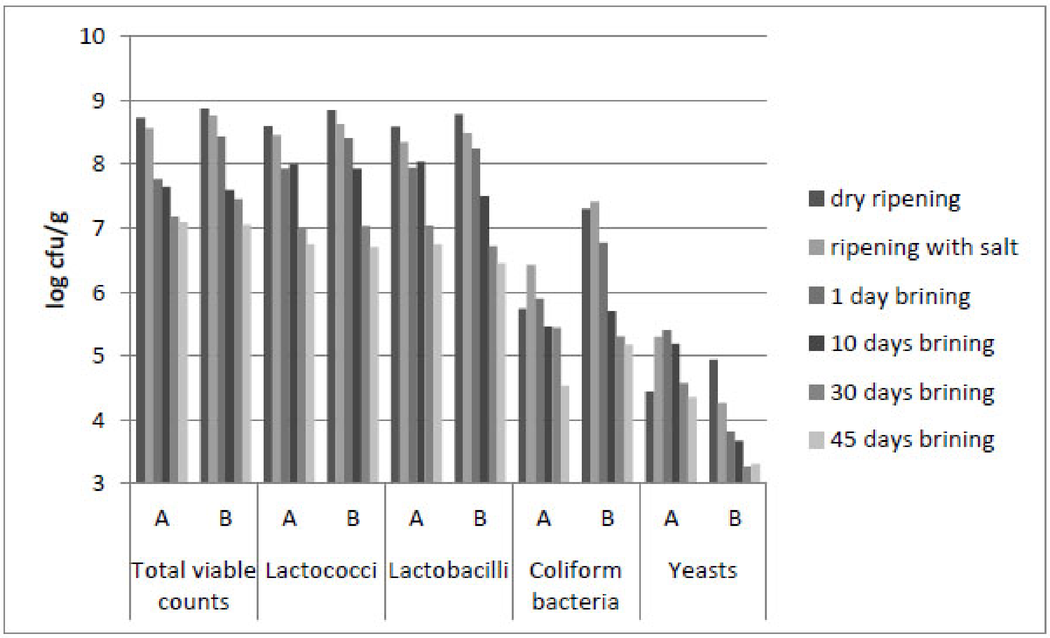

Fig. (12). Dynamic of examined microorganisms in sheep Beaten cheese.

From (Fig. **12**), it can be seen that there is a higher presence of total viable bacteria, especially in the stage of dry ripening. The traditional sheep Beaten cheese is most often made of combined milk (obtained from morning and evening milking) and ripens at room temperature (20-24°C), so the higher presence of total viable counts (TVC) is in some way within the expected. According to Levkov & Kakurinov (2012), the sheep milk used for making Beaten cheese contains a higher number of aerobic mesophilic bacteria (6.72-7.10 log cfu/g), and this, in combination with the climatic factors (the cheese is produced in summer) results in the high number of TVC in the cheese (Bonetta *et al.* 2008). A significant decrease in the total viable counts in the examined samples was noticed after the 30^{th} and the 45^{th} day of ripening in brine when their number was in the range of 7.05-7.45 log cfu/g. The examinations conducted by Levkov & Kakurinov (2012) show that the TVC is most probably influenced by the high concentrations of salt and the percentage of salt in moisture which after day 30 and day 45 of ripening ranged in the scope of 5.60-7.14% and 14.77-18.58%, respectively. According to Levkov *et al.* (2014), the negative coefficients of correlation ($r=-0.84$ and $r=-0.80$, $p<0.01$) for NaCl and TVC or S/M and TVC, respectively, indicates the inhibitory effect of these factors. The lower value of the coefficient of correlation between the pH values and TVC ($r=0.61$, $p<0.01$) is explained by the same authors as a consequence of the microorganisms' ability for adaptation to the lower pH values. The higher presence of TVC in the traditional cheeses was also found by Enriquez *et al.* (2016), Gaglio *et al.* (2014) and Şengül (2006). The lactic acid bacteria (LAB), as the most important for the process of ripening (Parente & Kogan 2004; Özkalp *et al.*, 2007) are present in the largest number during the stage of dry ripening of the Beaten cheese. The number of presumptive lactococci in this stage ranges from 8.59 to 8.83 log cfu/g, and of lactobacilli from 8.58 to 8.77 log cfu/g. After the stage of dry salting, a slight decrease of their number has been noticed (8.45–8.62 log cfu/g for lactococci and 8.34–8.48 log cfu/g for lactobacilli), while after 45 days of ripening in brine, the number of lactococci and lactobacilli was 6.70–6.74 log cfu/g and 6.45–6.74 log cfu/g, respectively (Fig. **12**). The obtained results are similar to those found in the examinations done by Andalla & Omer (2017), Picon *et al.* (2016), Mrkonjić-Fuka *et al.* (2013), Levkov *et al.* (2013) and Kirdar & Korsun (2011). From Fig. **12**, it can be seen that the dynamics of the number of lactobacilli is very similar to that of lactococci, though it is well-known that the lactobacilli are more resistant to the unfavourable conditions that occur during the cheese's ripening, such as low pH, reduced amount of loose water, reduced concentration of oxygen, and high concentration of salt (Fitzsimons *et al.*, 2001; Marino *et al.*, 2003). Nevertheless, their number starts decreasing after day 30 and day 45 of the stage of brining. The study done by Levkov *et al.* (2014) shows that the number of LAB is influenced in the largest amount by the high concentration of salt, one of the main characteristics of Beaten cheese, as well as the high percentage of S/M. The authors support these conclusions with the statistical analyses regarding the coefficient of correlation between lactococci or lactobacilli and the percentage of salt or S/M. According to the analyses, the coefficient of correlation between the salt or S/M and the lactococci is $r=-0.83$ or $r=-0.81$ ($p<0.01$), respectively, while regarding lactobacilli, this coefficient is $r=-0.87$ or $r=-0.85$ ($p<0.01$), respectively. The negative coefficient of correlation between lactococci and pH in the cheese ($r=-0.46$, $p<0.05$) indicates that the lower pH value may have an influence on the decrease of the number of lactococci. Anyway, the percentages of salt and salt in moisture have a key role in the process of inhibition of these bacteria's growth. According to the examinations done by Levkov *et al.* (2014, 2017), mainly mesophilic lactic acid bacteria were isolated from the traditional Beaten cheese. Also, variations in their composition were noticed depending on the particular household where the cheese had been made.

The determination of LAB was made on the basis of their morphological, physiological and biochemical characteristics, using API 50 CHL system (BioMérieux) patterns. The largest percentage of the isolated cocci-shaped LAB belongs to the species *Lactococcus lactis* ssp. *lactis* (35%). Of the isolates, 16.7% belongs to the genus *Pediococcus*, and only 1% to *Leuconostoc*. According to Levkov *et al.* (2017), *L. lactis* ssp. *lactis* were isolated from the cheese only in the initial stages of its production (after draining and dry ripening, and after dry salting), while during the stage of ripening in brine, this species was not found in the samples taken. Members of the genus *Pediococcus* sp. were isolated from the cheese in all the stages of its ripening, whereas members of *Leuconostoc* sp. were isolated only from the curd prepared in the household A. Of the isolated lactobacilli, the species *Lactobacillus paracasei* ssp. *paracasei* (22.5%), then *L. planatarum* (15.8%) were present in the largest percentage, while the species *L. brevis* (9.2%) was isolated only from the cheese from the household A. *Lactobacillus paracasei* ssp. *paracasei* and *L. planatarum* were isolated from the curd as well as from the cheese in the stages of ripening, dry salting and ripening in brine, while *L. brevis* was isolated from the cheese in the stages of dry salting and ripening in brine. The lactic-acid bacteria's composition by species during the production of the Beaten cheese, and all the other cheeses produced in a traditional way, is subject to change. The changes in the composition by species occur because of the microorganisms themselves, namely – because of their potential for certain metabolic activities. On the other hand, the expression of this potential depends on the conditions in which the microorganisms live. These conditions are, in the first place, the properties of the milk, then the biochemical changes that occur with the formation of the curd, as well as the technology implemented in the production and the conditions that occur during the process of ripening (Montel *et al.*, 2014). According to the authors, an additional source of microorganisms in the milk and the cheese is the equipment for making the cheese, the environment where the animals are kept (*e.g.* the sheep pens, the rooms for milking), as well as the food, the silage and the hay used for feeding the animals. The lactic-acid bacteria's composition by species in the Beaten cheese corresponds with the data in the literature about traditional cheeses (Terzić-Vidojević *et al.*, 2015; Domingos-Lopes *et al.*, 2017; Li *et al.*, 2017; Enriquez *et al.*, 2016; Hayaloglu *et al.*, 2002). The coliform bacteria in both variants of sheep Beaten cheese were present in a lesser number compared to the lactic-acid bacteria (Fig. **12**). The lower pH value of the cheese during ripening, as well as the increased number of lactic acid bacteria and their higher competitiveness, affect the number of coliform bacteria in relation to its decrease (Manolopoulou *etal.*, 2003; Caridi *et al.*, 2003). It is also evident from Fig. **12** that there is a higher presence of this group of bacteria in the Beaten cheese variant from household B as compared with the variant from household A in the stage of ripening in brine. The number of coliform bacteria in the cheese depends in a large amount on their presence in the milk, the hygiene of the vessels and the personnel engaged in making the cheese, the temperature of ripening and keeping, the season when the production takes place, and the possibility of additional contamination especially when the cheese is produced in poor hygienic conditions (Manolopoulou *et al.*, 2003; Psoni *et al.*, 2003; Bonetta *et al.*, 2008). Although, in the process of ripening in brine, a reduction in the number of coliform bacteria was noticed, nevertheless, after 45 days of ripening their number was higher compared with the data from the examinations done by Turkoglu *et al.* (2003), Kamber & Çelik (2007), Kirdar & Korsun (2011), and Mrkonjić-Fuka *et al.* (2013). The high presence of coliform bacteria was found by Pešić-Mikulec & Jovanović (2017) in Serbian traditional cheeses, as well as by Abdalla & Omer (2107) in the cheese Gybna Bayda. The higher presence of coliform bacteria indicates that the combination of stress factors will not lead to the disappearance of these microorganisms from the Beaten cheese. Anyway, the present coliform bacteria should not be considered pathogenic microorganisms, but their presence can be considered an indicator of contamination with more detrimental bacteria (Arenas *et al.*, 2004). The number of coliform bacteria is, in the largest extent, influenced by the high concentration of salt in the cheese and salt in moisture (Levkov *et al.*, 2014). In support of this conclusion are the statistical analyses of the coefficients of correlation of the salt and of S/M, which are $r=-0.82$ and $r=-0.76$ ($p<0.01$), respectively. The presence of coliform bacteria in high amounts may result in the occurrence of defects in the cheese or it can be detrimental to the consumers' health. In order to eliminate or at least reduce this risk, it is recommendable the ripening of traditional cheeses to last for a longer period of time (60 days). The examinations for the presence of *Salmonella* sp. in sheep Beaten cheese showed results of its absence in all the production stages. Similar are the data reported by Dimitrovska *et al.* (2017) and Talevski (2012), who found no members of *Salmonella* sp. and *Listeria monocytogenes* in traditionally produced ripened cow Beaten cheese. According to Dimitrovska *et al.* (2017), in the samples of cheese produced in five different households, the presence of *Enterobacteriaceae* was found in the scope of 20–2,000 cfu/g, of *E. coli* in the scope of 10–1,200 cfu/g, and of coagulase-positive *Staphylococcus* 36–180 cfu/g. The examinations of cow Beaten cheese done by Talevski (2012) showed very similar results. Namely, in 10 examined traditionally produced cheeses, the presence of *Enterobacteriaceae* was in the range of 260-3,000 cfu/g, of *E. coli* in the range of 0–1,300 cfu/g, and of coagulase-positive *Staphylococcus* 5–110 cfu/g. Yeasts are present in the sheep Beaten cheese during the entire production process. Their presence was also found in the milk used for producing the cheese, in the amount of 4.09-4.38 log cfu/g (Levkov & Kakurinov, 2012). Their presence was higher in the variant produced in household A than in the one produced in household B (Fig. **12**), a phenomenon which, according to Manolopoulou *et al.* (2003), is nothing unusual. According to the authors, the number of yeasts can differ between the cheeses coming from two dairies, but also between the batches coming from the same dairy. This is a result of the variations in the efficiency of pasteurization, the efficiency of the cleaning agents, the additional contamination with yeasts and the hygiene during the production process itself. The analysis of the samples taken in the stage of ripening in brine shows the presence of yeasts in the cheese, which is owed to their ability to grow in extreme conditions such as lower pH, high concentration of salt, and low content of moisture (Alonso-Calleja *et a*.l, 2002; Tarakci *et al.*, 2004). Similar dynamics of the yeasts was found in the research done by Özdemir *et al.* (2010), Pešić-Mikulec & Jovanović (2005), Abdalla & Omer (2017) and Golić *et al.* (2013). The yeasts isolated from the sheep Beaten cheese in this study have been determined on the basis of their morphological, physiological and biochemical properties. The isolates belong to the genus *Trichosporon* sp., *Torulopsis* sp., *Debariomyces* sp., *Rhodotorula* sp., *Pichia* sp., *Kluyveromyces* sp. and *Candida* sp. The isolated yeasts belong to genera most often present in traditional cheeses (Montel *et al.*, 2014). Their composition by species is in line with the results obtained by Bintzis and Papademas (2002), Gardini *et al.* (2006) and Golić *et al.* (2013). In the beaten cheese, moulds have occasionally been found in various phases of the ripening stage, which is a consequence of subsequent pollution caused either from the premises where the cheese is made or the equipment used (Levkov & Kakurinov, 2013). From both variants of cheese, 11 isolates of moulds have been isolated, belonging to the genus: *Aspergillus* sp., *Fusarium* sp., *Alternaria* sp., *Curvularia* sp. and *Penicillium* sp.

TRADITIONAL KASHKAVAL CHEESE

In recent years, we have been witnessing increased consumption of traditional sheep dairy products, especially cheese produced with traditional technology (Santa & Srbinovska, 2017). Cheese made using sheep milk is very popular among consumers, primarily due to its specific sensory characteristics (Kalantzopoulos, 1993). Kashkaval is one of the most popular hard cheeses in many Mediterranean countries and its production dates back to the eleventh and twelfth century (Kindstedt *et al.*, 2004). It belongs in the group of Pasta filata cheeses, which are characterized by a unique texture which is malleable, smooth, fibrous, and sliceable. These qualities arise mainly from the cooking/stretching step which is common to all these varieties, whether they are soft, semi-hard, or hard (Fox, 2017). Pasta filata cheeses have a long tradition in most East-Mediterranean and neighbouring countries as well. In many of them, these cheeses are highly consumed and in some are the second most popular cheese group after white brined cheeses. Kashkaval-type cheeses are the best known

Pasta filata cheeses, made for centuries in this region (Alichanidis & Polychroniadou, 2008). According to Bertozzi & Panari (1993), geographical origin indication and the traditional character of the cheeses are the two most important factors which influence the selection of cheese by the consumers.

One of the traditional cheeses with a long history is the kashkaval produced on the Bistra mountain in Macedonia. The flora in this country is among the richest, not only in the Balkans but also on the entire European continent (Niketić, 2014). The Bistra mountain belongs to the group of mountains which constitute the most interesting mountain-pasture area of the Balkan Peninsula, with the most beautiful hilly pastures (Srbinovska, 2013). The dominating plants in this region are from the family of Poaceae grasses with 48.84%, then leguminous plants with 11.63%, aromatic plants with 18.60% and other species with 20.93% (Micevski, 1994). The specific climatic conditions, the hilly mountain, as well as the sheep breeding tradition are altogether excellent preconditions for the production of cheese in this region. For the production of cheese on the Bistra mountain, the milk originates from local breeds, which are adapted to the specific geographical and climatic conditions (Santa & Srbinovska, 2017). Although traditional cheeses are recognized as important for cheese producers, they are losing the battle against the industry and they might become extinct (Mijacevic *et al.*, 2005). In Macedonia, the number of traditional cheese producers is continuously decreasing (Santa & Srbinovska, 2014). Furthermore, there is not much information in the literature about the Macedonian kashkaval. Traditional kashkaval is produced in only two regions in Macedonia - Galichnik and Lazaropole, both located on the Bistra mountain. In the region where it is produced, local sheep breeds (Pramenka and cross breed) are dominant and characterized by low milk productivity which is 60-80 litres during lactation. In the summer period, sheep graze on pastures at 1500m above sea level. The kashkaval from this region has a long history (Fig. **13**). The produced cheese was sold in all of the markets in the region and abroad. In 1927, this cheese was even exported to the US market, and this was documented in the Yugoslavian newspapers as a significant event.

The Cheese-making Process

The production process of the traditional kashkaval for both regions where it is produced is shown in the flowchart (Fig. **14**), (Santa & Srbinovska, 2014; Santa, 2015). Some of the steps are different between the two types of cheese, depending on the region.

Fig. (13). Old facility for production of kashkaval on the Bistra mountain.

Raw sheep milk
↓
Clarification - filtration
↓
Renneting (at 33-37 °C, coagulation complete in 45 min)
↓
Cutting and stirring the coagulum
↓
Whey draining
↓
Wrapping the curd with cheese cloth
↓
Pressing (3-6 hours)
↓
Cutting the curd in blocks
↓
Curd ripening (cheddaring) Galichnik- 18h 12-15C, Lazaropole 3h-30°C
↓
Cutting the curd (“baskija”) in pieces (62-65°SH and pH 5,2-5,4)
size of the pieces (20x5-10x0,5-1 cm)
↓
Stretching in hot brine, plasticization (5%NaCl, 72-75°C, 5 min)
↓
Hand shaping, dry salting and moulding
↓
Removing the moulds (after 24 hours)
↓
Ripening (3months, RH 75-80%)
Galichnik – 12-15 °C, Lazaropole 15-18°C
↓
Dry salting, cleaning with cloth, wheels rotation
↓
Expose the cheese to the sun (10-15 min for each side)
↓
Packaging
↓
Storage (5 ºC)

Fig. (14). Flow chart for traditional kashkaval production.

Milk Reception and Renneting

After milking on the farm, raw milk is transported to the production facility, where, again, it is filtered. In cases when the milk is processed once a day, it is cooled to a temperature lower than 6°C. The renneting is carried out by adding powdered chymosin in an amount which enables coagulation of the milk for 45-60 minutes at 33-38°C and the initial coagulation should occur 10 minutes after adding the enzyme. In cases when the milk is cooled, a second heating treatment is performed. This is usually done when a certain amount of milk is heated at a temperature of 65 to 75°C, and then the heated milk is well mixed with the remaining amount of milk. The cheesemaker assesses the curd if it has enough firmness and elasticity and then starts the production. The curd is cut and stirred for around 30 min with a special wooden tool called "krstach" and then rested for about 10 minutes (Fig. **15**). Once the required size of the curd is uniform and given some time to rest, the curd falls to the bottom and the whey is separated on the surface and drained from the vat (Fig. **16**). Then the curd is placed on the cheesecloth on the table, and the whey is drained from the table.

Fig. (15). Cutting and stirring the coagulum with "krstach".

Fig. (16). Draining of the whey from the vat.

Then the fresh curd is chopped by hand and this is very quick (5 min) so that the curd does not cool. The aim of the chopping is to remove the remaining whey from the curd. The curd is wrapped in a cheesecloth and pressed using 12-15 kg per 1 kg of curd. The duration of the pressing process is 3-6 hours, depending on the room temperature. The pressed curd is called "baskija" or "teleme" which is usually in blocks of 25 cm width (Fig. **17**). For the cheese from Lazaropole, the cheesemaker places hot water over the pressed "baskija" so it does not cool.

Fig. (17). Baskija.

Curd Ripening (Cheddaring)

During the pressing, the whey continues to be drained both from the surface and from the inside of the "baskija". This whey contains a higher percentage of milk fat. After the process of pressing, the curd ("baskija") is cut into smaller pieces weighing 5-8 kg, with width and height of 10-15 cm and length of 25-30 cm. The curd pieces are covered with a cheesecloth and a polyethylene cloth and the process of curd ripening called "cheddaring" starts. The process is usually extended at lower temperatures (not lower than 10°C) for around 20 hours (Galichnik), or at warm temperatures of 30-35°C for 3-4 hours.

Stretching the Curd in Hot Brine (Plasticization)

Cheddared curd contains small holes, an elastic consistency, a light yellow colour and a pleasant smell with optimum pH value between 5.2-5.4 or 62-66 °SH. According to the cheese maker's decision, the production continues when a small piece of cheese is soaked in hot brine and stretched well. Then the curd ("baskija") is sliced into pieces (20 × 5-10 × 0.5-1 cm) (Fig. **18**) and placed in a wooden basket by immersing the basket in the hot brine (5% NaCl, 73-75 °C) for about 5 min (Fig. **19**). The cheesemaker manually conducts the mixing and stretching of the curd, and according to Kosikowski (1982), this is the hardest and most intense work required in the cheese-making process. The most characteristic result of mixing and moulding is the removal of some water from the fresh curd and the transformation of the rough, short-textured curd into a smooth, malleable, long-grained, satiny plastic mass.

Fig. (18). Baskija sliced into pieces.

Fig. (19). Stretching the baskija.

Moulding

The cheese dough is placed on the table and kneaded carefully for about 10-15 minutes until a compact elastic mass is obtained and air bubbles are eliminated.

According to Kindstedt *et al.* (2004), texturing has an additional advantage, *i.e.* a pasteurizing effect which suppresses undesirable microbial growth and encourages desirable fermentation and ripening, resulting in high-quality cheese. In the process of kneading the curd (around 5-10 minutes), the cheesemaker applies dry salt. During moulding, the cheese is carefully closed like a mushroom and a small piece of cheese called “navel” (“papok”) is pulled out in order for the dough to completely fill the mould (Fig. **20** and Fig. **21**).

Fig. (20). Molding the cheese.

Fig. (21). Cheese in mold.

Ripening

In order to remove the air from the cheese, the cheesemaker carefully pinches the cheese with metal needles of 2 to 3 mm diameter. Within 24 hours, the mould with cheese has been turned over several times and then it is removed from the cheese. The cheese is dry salted several times (5-7 times) during the period of 20-30 days. The dynamic of the salting depends on the cheesemaker.

Ten days after the final salting, each wheel is brushed in warm water (35-40 ° C) (Fig. **22**) and then dried for seven to eight days (Simonovska, 2018). The drying temperature should not exceed 22 ° C, and the optimum temperature is 15-18 °C. One of the specific characteristics of the Bistra mountain kashkaval is that the cheese is exposed to the sun. The process of "sunning" takes around 10-15 minutes on each side where the surface softens and fat globules appear on the cheese (Figs. **21**, **23**) (Simonovska, 2018). The cheese is placed on the shelves for ripening one over another to a maximum of four wheels and they are each rotated periodically (Fig. **24**). The ripening period is for the duration of three months at a temperature of 12-15 °C (Galichnik) and 15-18°C (Lazaropole). The ripened kashkaval acquires the shape of a flattened cylinder with a weight of 3-3.5kg, 5-6kg or 8-9 kg. It is usually packed in vacuum polymer foil.

Fig. (22). Washing the cheese.

Fig. (23). Expose the cheese to sun.

Fig. (24). Cheese ripening.

Physical and Chemical Properties of the Traditional Kashkaval

The cheese quality depends on its composition, especially the moisture and dry matter, salt, pH, and the percentage of fat in dry matter (Fox, 2000). The dynamics of the physicochemical characteristics of the traditional kashkaval from Galicnik during the ripening were reported by Santa & Srbinovska (2017). In Tables **4** and **5**, the average results for both kashkaval cheeses are presented, for the regions of Galichnik and Lazaropole. The ripening of cheese resulted in the decrease of the pH value and the increase of protein, fat, ash and NaCl. The difference in the fat content of both cheeses occurs due to the difference in the composition of the raw material and the processing of the cheese itself. In the ripening process, there is a significant reduction in moisture content. The results of the dynamics of moisture during the cheese ripening and storage show that on the 180th and 360th day, there was a significant decrease in the moisture content. Similar results were reported by Niro and *et al.* (2014) in examining the cheese Caciocavallo, where the moisture content on the day of production was 45.4%, while on the 60th day of ripening it decreased to 39.8%. Hattem *et al.* (2012), in their study on the hard cheese Ras made from unpasteurized cow milk, reported lower results on the moisture content which ranged from 40.28% on the first day to 31.26% after the 6th month of ripening. The difference in results is due to the different origin of milk.

Table 4. Physicochemical characteristics of the kashkaval from Galichnik (A) and Lazaropole (B).

Time (days)	Moisture (%)		Fat (%)		Protein (%)	
	A	B	A	B	A	B
1	46,48e[1]	45,81b	20,75a[1*]	25,38a*	11.88a[1*]	18,78a*
30	43.8de	41,13b	21,75ab*	27,25ab*	12.06a*	23,77b*
60	39,21cd*	32,68a*	24,38bc*	32,25c*	21.03b*	25,83bc*
100	36,64bc	32,63a	27,88cd	30,00b	23,05bc*	26,65cd*
180	32,92ab	31,20a	29,00d*	33,00c*	25,50cd	27,62cd
360	27,54a	27,98a	31,40d	32,50c	28.41 d	28,39d

[1]Means in the same column with different letters are significantly different ($p < 0.05$)
*Means in the same period for different variants are significantly different ($p < 0.05$)

Table 5. Physicochemical characteristics of kashkaval from Galichnik (A) and Lazaropole (B).

Time (days)	NaCl (%)		Ash (%)		Acidity (pH)	
	A	B	A	B	A	B
1	1,20a[1]	0,79a	3,54a[1]	3,57a	5,47b[1]	5,61b
30	1.29a	1,45ab	4,01ab	4,50ab	5,42ab	5,26a
60	1,48ab	1,84abc	4,24abc	5,23bc	5,35ab*	5,14a*
100	2,02abc	2,17bc	4, 85bcd	5,27bc	5,34ab	5,16a
180	2,44 bc	2,84cd	5,43cd	6,49cd	5,28ab	5,15a
360	2,90c	3,94d	5,81d*	7,62d*	5,26a	5,29a

[1]Means in the same column with different letters are significantly different ($p < 0.05$)
*Means in the same period for different variants are significantly different ($p < 0.05$)

The results on the fat content correspond with previous studies on kashkaval from different regions. According to Kindstedt *et al.* (2004), the content of milk fat in Bulgarian cheese is 30%, in Greek cheese it is 33.8%, and in Turkish cheese it is from 25.5% to 31.4%, in Egyptian cheese it is from 21.1% to 26.3%, while the content of fat in the countries of former Yugoslavia ranged from 27 to 32%. Also, Mijačević *et al.* (2005) in their study on Pirotski kashkaval got similar results for the fat content which ranged from 28.8% to 31.1%.

Furthermore, Gobbeti *et al.* (2002), examining the dynamics of the chemical composition during ripening of the Caciocavallo Pugliese produced from cow milk, reported that the fat content was 24%, which is an expected lower value than the one in sheep cheese. Similar results for the fat content in cheese were detected in the Pecorino cheese (Coda *et al.*, 2006) and traditional Italian cheeses (Di Cagno *et al.*, 2007). Dry salting, which is a part of the technology process of kashkaval, leads to a higher loss of water and the cheese where dry salting is used is usually characterized by greater firmness (Jovanović, 2001). The degree and the method of salting is different for different cheeses and it depends on the technology process, the salting technique, the consumer's preference, etc. Similar findings for salt content were reported by Kindstedt *et al.* (2004) for the kashkaval from the former Yugoslavian area. It is the cheese-maker's professional skills that ensure the various phases in the cheese making process are carried out correctly. He relies on his years of experience and training when making these decisions (De Roest, 2000).

Free Fatty Acid Profile of Traditional Kashkaval

Lipids in foods may undergo hydrolytic or oxidative degradation. Although some lipolysis occurs in most or all cheeses, it is most extensive in some hard Italian varieties and in blue cheese (McSweeney, 2004). While short-chain fatty acids contribute directly to the cheese flavour, free fatty acids (FFAs) also contribute indirectly to the cheese flavour by acting as precursors for the production of volatile flavour compounds through a series of reactions collectively known as the metabolism of fatty acids. Lipolysis in kashkaval cheese is not very intense. According to Kozev (2006), the kashkaval made from cow milk has low fat hydrolysis and thus does not have a very intensive taste and aroma. More evident and intensive hydrolysis in cheese is noticed in the kashkaval from sheep milk. Cheeses made from raw milk or heat-treated milk in sub-pasteurisation conditions tend to contain higher amounts of FFAs, because the indigenous milk lipase remains intact or is not entirely inactivated, respectively. The major FFA acids present in the kashkaval from the Bistra mountain are palmitic (C16:0), oleic (C18:1) and myristic acid (C14:0) (Table **6**).

The content of butyric acid (C4:0) corresponds to the results obtained by Gobbetti *et al.* (2002) in the Caciocavallo Pugliese cheese. The results of our research are similar to the results of the research by Woo *et al.* (1984) on the concentration of butyric acid (C4:0) in Parmesan and Provolone. A similar fatty acid profile was reported by Ergönül *et al.* (2011) on Haloumi cheese.

Table 6. Free fatty acid profile of kashkaval (n=4), g•100g^{-1} of the cheese (kashkaval) from Galichnik (A) and Lazaropole (B).

FFA g•100g^{-1}	A	B	FFA g•100g^{-1}	A	B
C4:0	2.09±1.98	3.32±0.06	**C17:0**	0.51±0.04	0,61±0,1
C6:0	/	2.66±0.29	**C17:1**	0.22±0.01	0,67±0,06
C8:0	0.83±0.18	1.45±0.29	**C18:0**	7.68±2.43	8,79±0,67
C10:0	6.46±0.60	5.08±0.46	**C18:1 c**	24.89±1.05	23,60±0,32
C11:0	/	0.28±0.05	**C18:1 t**	/	/
C12:0	5.20±1.28	4.87±0.59	**C18:2 t**	0.47±0.05	0,58±0,05
C13:0	/	0.13±0.03	**C18:2 c**	2.12±0.99	2,33±0,07
C14:0	11.01±0.69	10.82±0.46	**C20:0**	0.44±0.10	0,05±0,11
C14:1	0.26±0.10	0.30±0,08	**C18:3 n-6**	0.92±0.29	0,62±0,05
C15:0	1.35±0.13	0.40±0,07	**CLA**	4.13±0.74	3,28±0,34
C15:1	0.28	/	**C18:3 n-3**	0.85±0.22	1,11±0.11
C16:0	30.57±4.17	26.71±1,36	**C21:0**	1.66±0.08	/
C16:1	/	2.27±0,12	**C20:4 n-6**	0.38±0.01	0,34±0.57

PROTEOLYSIS AND VOLATILES OF MACEDONIAN TRADITIONAL CHEESES

Kashkaval is a Pasta-filata type of cheese of large popularity after the white-brined cheese (Belo sirenje) in Macedonia (Sulejmani *et al*., 2014a). Its production dates back to the 11th and 12th century, however, historical references suggest that it has an even older tradition (Santa & Srbinovska, 2014). Sulejmani *et al.* (2016) evaluated the differences in the heat treatment effect (60, 70 and 90 °C for 5 min) in several biochemical and technological properties of the Kashkaval cheese. The chemical composition of 1 day old Kashkaval cheeses ranged between 0.2–0.3% for titratable acidity (as g of lactic acid per 100 g of cheese), 58.2–60.8% for total solids, 23.9–25.2% for protein, 29.7–31.3% for fat, and 0.7%–0.9% for salt. The use of a different stretching temperature significantly affects the pH, the soluble nitrogen fractions and the colour parameters of the cheeses ($P < 0.05$) (Table **7**). Urea-PAGE patterns of the water–insoluble fractions of the cheeses after 30, 60, 90 and 120 days of ripening are presented in Table **7**. During the ripening of the Kashkaval cheese, as1-casein (f24-199) and c-caseins were produced early, indicating chymosin and fairly high plasmin activity (Kindstedt 1993). However, it was seen that the as1-casein hydrolysis pattern was higher than that of b-casein during all stages of ripening. As a consequence of as1-casein hydrolysis, the bands corresponding to as1-I-casein (as1–casein f102–199) were observed after 60 days of ripening in K60 and K70 cheeses. Other bands characterized by slower mobilities than as1-I-casein (as1-CN f24–199) are shown in 120 days-old K60 and K70 cheeses (Fig. **25**). These may be produced by the actions of the residual coagulant and the native milk proteinases.

Table 7. The levels of pH, water-soluble nitrogen (WSN), 12% Trichloroacetic acid-soluble nitrogen (TCA-SN), total free amino acids (TFAA) of Kashkaval cheeses stretched at different temperatures (60, 70 and 90 °C).

	Cheeses	**1**	**60**	**90**	**120**
pH	K60	5.21±0.01^{a}	5.09±0.02^{aA}	5.14±0.02^{A}	5.18±0.01^{A}
	K70	5.29±0.04^{ab}	5.08±0.01^{aA}	5.24±0.02^{B}	5.15±0.03^{AB}
	K90	5.43±0.03^{b}	5.29±0.10^{aB}	5.17±0.09^{AB}	5.21±0.07^{A}
WSN	K60	3.95±0.10^{ab}	7.79±0.32^{aA}	9.54±0.05^{cB}	10.69±0.02^{bC}
(% of TN)	K70	3.99±0.22^{b}	7.55±1.04^{aAB}	9.11±0.12^{bAB}	10.47±0.04^{bB}
	K90	2.73±0.56^{a}	5.38±1.34^{aA}	6.39±0.06^{aA}	9.00±0.08^{aB}
TCA-SN	K60	1.73±0.17^{a}	4.46±0.21^{bB}	5.41±0.03^{cBC}	6.55±0.56^{bC}
(% of TN)	K70	1.48±0.02^{a}	4.11±0.67^{abAB}	4.94±0.06^{bB}	6.51±0.02^{bC}
	K90	1.39±0.09^{a}	2.38±0.13^{aB}	3.00±0.03^{aC}	4.43±0.04^{aD}
TFAA	K60	NA	4.37±1.51^{bAB}	4.61±1.10^{bB}	3.53±0.04^{cAB}
mg Leu/g	K70	NA	2.69±0.24^{abB}	3.45±0.20^{abC}	2.50±0.01^{bB}
	K90	NA	0.96±0.05^{aAB}	1.11±0.13^{aBC}	1.35±0.05^{aC}

$^{a-c}$Means ± SD with different letters were significantly different among cheese samples within the column of the similar ripening period (P < 0.05). $^{A-D}$ Means ± SD with different letters were significantly different among storage periods within the rows of each cell (P < 0.05). NA: Not analyzed. (*Adapted from Sulejmani et al. 2016*)

After 30 days of ripening, K60 and K70 cheeses exhibited pronounced proteolysis which resulted as 1-I-casein peptide (as1-CN f110-199) (Sulejmani *et al.,* 2016). From c-caseins (the polypeptides generated by the activity of plasmin), the c3- (f108–209) and c1- (f29–209) caseins were present in the highest amounts, attended by c2-casein b-(f106–209). Also, Sulejmani *et al.* (2016) studied the residual coagulant activity in the Kashkaval cheese, ranged between 7.1 and 71.6%. During ripening, K60 and K90 cheeses showed the highest and lowest residual coagulant activity, respectively. The mean residual coagulant activity of Kashkaval cheeses were higher than other types of cheeses reported in the literature (Roa, Lopez, & Mendiola 1999). Similarly, Boudjellad *et al*. (1994) observed a higher amount of residual coagulant activity (86%) in a Swiss type of cheese.

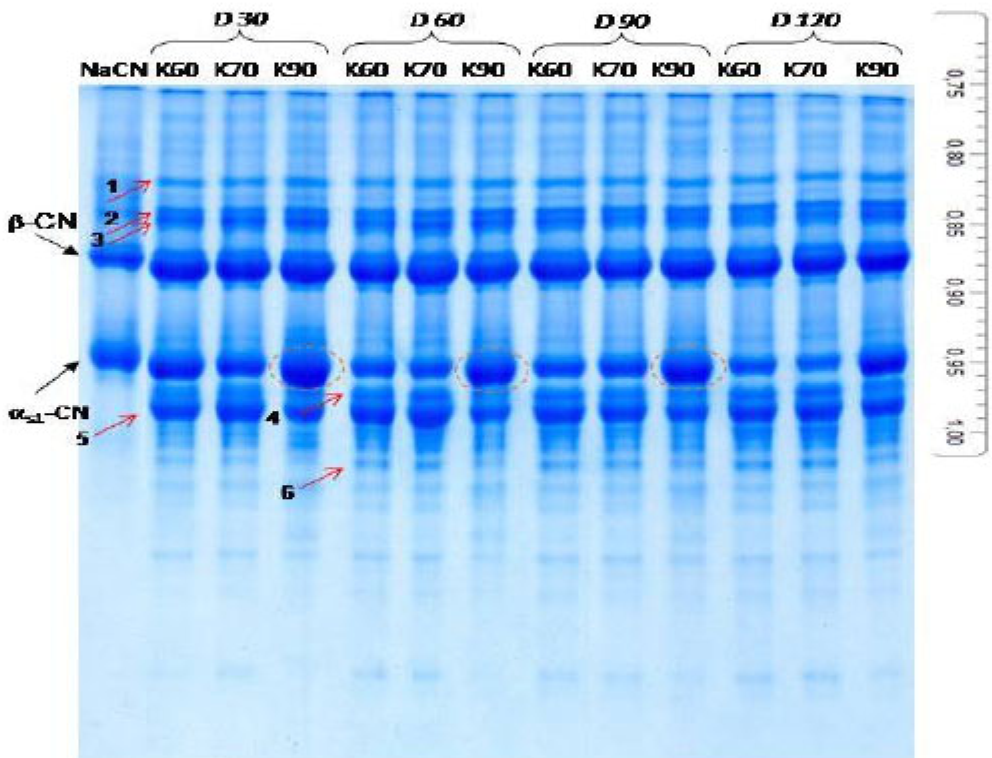

Fig. (25). Urea-polyacrylamide gel electrophoretograms (left) of the water-insoluble fractions of Kashkaval cheeses made using different stretching temperatures (60, 70 and 90 _C) during 120 days of ripening. Casein bands, 1: c2-CN (b f 106–209), 2: c1-CN (b f 29–209), 3: c3-CN (b f108–209), 4: as1-CN (f24-199), 5: as1–CN (f 102–199), 6: as1-CN (f 110–199). (*Adapted from Sulejmani et al. 2016).*

Sulejmani *et al.* (2016) emphasized that the volatile compounds present in the volatile fraction of Kashkaval cheeses consisted of 8 acids, 13 esters, 11 alcohols, 24 hydrocarbons, 7 ketones, and 3 other groups. Acids and esters composed the main chemical classes (mean concentration of 57.1% and 26.8% w/w of total volatile compounds, respectively). Statistically significant differences for thirty-eight and thirty-two volatile compounds were determined in the terms of storage and type of production, respectively. As most of the volatile compounds increased, a few decreased and new compounds were formed during maturation. Compared with the 1st day, an important increase in the total amount of volatile compounds was found after 120 days of ripening. Carboxylic acids are one of the principal volatile groups in Kashkaval as all sampling times and stretching temperatures influenced the volatile concentration (Sulejmani *et al.*, 2016). Butanoic acid was the most important acid found in the headspace of Kashkaval cheese. The most noticeable differences between the Kashkaval cheeses were in the concentration of butanoic acid, which was identified at higher levels in K60 cheese samples. However, the content of acetic acid significantly increased ($P < 0.05$) after 60 days of ripening and it was the second main compound isolated in the headspace of Kashkaval cheeses scalded at 90 °C. Esters were the second most abundant volatile group isolated in the headspace of Kashkaval, with a percentage of 26–28% of total volatiles. Among the esters, 7 ethyl esters, 5 methyl esters and 1 methyl propyl ester were detected. Lower levels of heat treatment of the curd on the first day in K60 cheeses increased the levels of total FAAs, which are one of the precursors of ethanol by Strecker degradation (Sulejmani *et al.*, 2016). Hydrocarbons were the largest group of volatiles (24 compounds) identified in the headspace of Kashkaval cheeses. These compounds do not directly make a considerable contribution to the aroma of Kashkaval cheese, as these compounds have high threshold values.

PROTEOLYSIS AND VOLATILES OF BEATEN CHEESE

Beaten cheese ("bieno sirenje") is a traditional autochthonous type of cheese produced in Macedonia and other Balkan countries with a relatively high nutritional value and performance features (Sulejmani *et al.*, 2014a). The traditional cheese-making procedure is still used in farms and villages. The scalding procedure is a crucial step in the production of Beaten cheese and it substantially affects the physical, biochemical and sensory properties of the cheese (Dubrova Mateva *et al.*, 2008).

Raw sheep milk is used in the production of Beaten cheese by the traditional method, however, milk is pasteurized in large-scale dairies. The raw/pasteurized sheep milk used for the manufacture of Beaten cheese was coagulated by using animal rennet (Chr. Hansen) at a level of 20 mL/100 L. The milk was allowed to coagulate at 34°C for 45 min. Following the coagulation, cheese curd was mechanically stirred and beaten 40 to 50 times with a special metal tackle (horizontally and vertically knitted metal wires) for about 5 min, and then left for 30 min to facilitate the drainage of whey. The traditional cheese curd is usually stirred and beaten manually with a wooden stick until a homogenous compact structure is obtained. After the drainage, the curd was cooked in hot water between 70°C to 90°C for 20 min. Following the cooking, the curd was again beaten for 2 to 5 min. and then left to rest for 15 to 20 min. Following the rest period, the curd was molded into balls, which were then each transferred to a cotton cloth to hang by their own weight or by a weight pressure of 3 kg/kg for about 24 h. Then, the curd was pre-ripened for 3 days at 35°C or in sunlight for the traditional way of production. During this period, the curd gained a specific yellowish colour due to the proportional increase of β-carotene among casein micelles (Fig. **26**). Then, the cheese loaf was cut in pieces 30 to 40 cm long and 4 to 5 cm wide. The cheese pieces were salted by the dry-salting method for 24 h and then followed by the brine salting. The pieces were then collected and packed in plastic barrels containing brine with a concentration of 18 to 20% (wt/vol) NaCl for long-term storage. Sulejmani *et al.* (2014a) reported that the total FAA contents were the highest in the industrial Beaten cheeses, probably due to the lower scalding temperature used in industrial production compared with the traditional method. The level of total FAA reached 1.48 mg of Leu/g of cheese on day 180 in industrial cheeses. However, it was 1.18 mg of Leu/g of cheese for traditional cheeses during the same sampling time. The level of total FAA in Beaten cheeses showed a similar trend to the soluble nitrogen fractions, including WSN-SN, during ripening, as reported by other researchers (Andic *et al.*, 2010). Some differences were observed during the ripening for the fractions of peptides, which were eluted from industrial cheeses at higher levels than the ones eluted from traditional cheeses (Sulejmani *et al.*, 2014a). Increases were observed in the concentrations of peptides that were mainly eluted between min 20 and 40 from the industrial cheeses (Fig. **27**). In the chromatogram, between min 60 and 66, the peak heights in traditional cheeses were generally much higher than in industrial cheeses. In the case of traditional cheeses, the particular peaks with retention time between min 22 to min 26 appeared and varied strongly, depending on the type of cheese and ripening process. However, the pattern and extent of proteolysis varied due to differences in the manufacturing practices (industrial and traditional methods), which caused differences in the moisture content and residual coagulant activity. Peaks that appeared at a retention time of min 60 to min 69 corresponded to large hydrophobic peptides, which are strongly correlated with the bitterness of cheeses (Bumberger & Belitz, 1993), and were higher mostly in industrial cheeses. Sulejmani *et al.* (2014b) extensively investigated the volatile compounds in beaten cheeses obtained from different local retailers in Macedonia, in 2012 (Table **8**). These compounds belonged to different chemical families including acids, ketones, esters, and alcohol. A total of 11 acid compounds were detected, and the main acidic compounds in Beaten cheese were butanoic, hexanoic, octanoic and decanoic acids. The principal acid within this group was butyric and its concentration was about 59.5 mg/kg per cheese. Butyric acid is produced principally by the catabolism of lactic acid due to butyric acid fermentation. The reduction of salt in dry matter (SDM) in Beaten cheese is claimed to significantly increase the susceptibility to butyric acid fermentation, which was also reported for Gouda cheese (Van den Berg *et al.* 1986).

Ketones were the second most abundant compounds isolated in beaten cheeses. In total, 11 ketones were identified; 2-butanone, 2-pentanone, and acetoin were the most abundant ketones in Beaten cheese samples. Acetoin originates from the metabolism of citrate as a reduction of diacetyl by the action of lactic acid bacteria, and they are very important compounds for dairy products. Also, they

Fig. (26). Beaten sheep milk cheese with visible holes (*Adapted from Sulejmani et al., 2014b).*

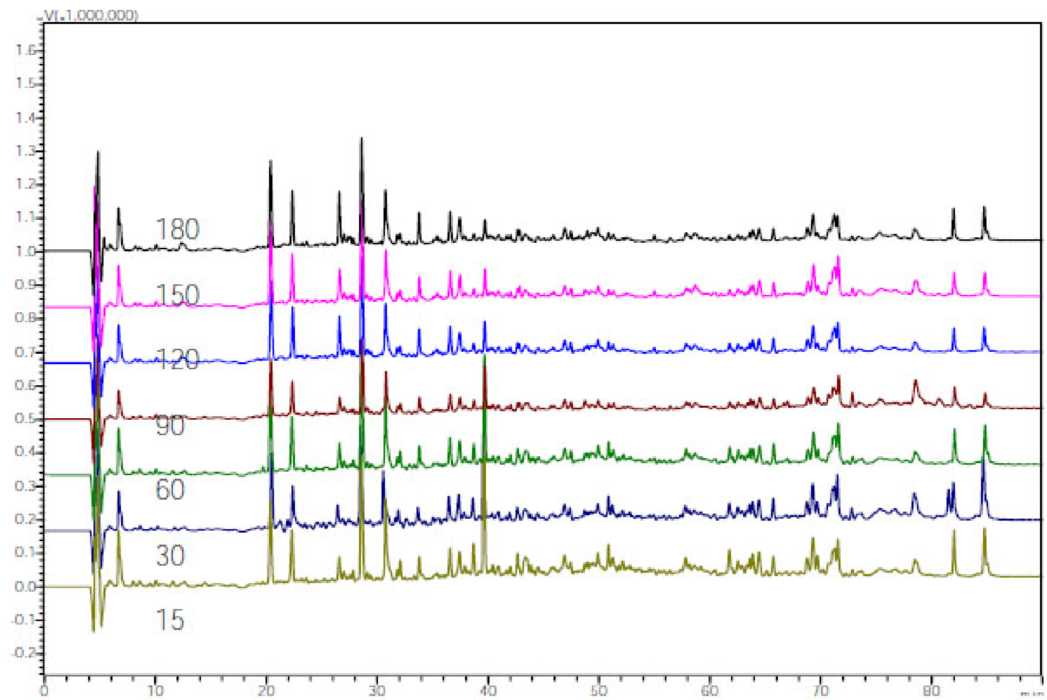

Fig. (27). Reversed-phase HPLC profiles of the water-soluble fraction of industrial beaten sheep milk cheese during ripening. Detection was at 214 nm. mAU = milliabsorbance units. *Adapted from Sulejmani et al. (2014a).*

have very particular odours and low perception thresholds (McSweeney & Fox, 2004). Concentration of acetoin detected in Beaten cheeses was about 650 μg/kg cheese. A total of 16 ester compounds with a total concentration of 2731 µg/kg were identified in Beaten cheese. Ethyl butyrate (1505 µg/kg), ethyl caproate (806 µg/kg) and ethyl octanoate (121 µg/kg) were the most abundant esters in Beaten cheeses. Sulejmani & Hayaloglu (2018) extensively investigated the effect of heat treatment of cheese milk on the biochemical and volatile properties of white brined cheese made using goat milk, during its maturation. The raw goat milk (GR) cheeses were characterised by a higher amount (78%) of total volatile compounds than the pasteurized goat milk (GP) cheeses, during maturation. Compared to day 1, an important decrease in the total amount of volatile compounds (except ketones and alcohols) was found after 120 days of maturation.

Table 8. Volatile compounds (µg/kg cheese) in beaten sheep milk cheeses (*n=12*).

	Aroma Characteristics	**RI**	**Mean± SE**
Acids			
Butyric acid	Cheesy	838	59484±17165
3-Methyl butanoic acid	Cheesy, sour	891	3641±983
2-Methyl butanoic acid	Acidic, dirty,	923	410±48
Hexanoic acid	Sweaty, sour	1016	27618±7453
Octanoic acid	Fatty, waxy	1192	13704±3097
Nonanoic acid	Fatty, waxy	1267	11±2
Decanoic acid	Rancid, sour	1369	6619±1426
Dodecanoic acid	Fatty	1558	256±74
Myristic acid	Waxy, soapy	1757	4±1
Oleic acid	Fatty, vegetable oil	1939	46±12
Palmitic acid	Waxy, soapy	1958	206±31
Total acids			***111999***
Ketones			
Acetone	Solvent	<600	371±135
2-Butanone	Etheral, fruity	<600	1419±695
2-Pentanone	Sweet, banana-like	679	1227±304
Acetoin	Buttery, creamy	708	650±177
2-Hexanone	Etheral	791	52±12
2-Heptanone	Fruity, spicy	893	563±179
8-Nonen-2-one	Fruity baked	1084	41±1
2-Nonanone	Dirty, earthy	1092	36±18

	Aroma Characteristics	**RI**	**Mean± SE**
Acids			
Delta octalactone	Coconut, creamy	1288	3±1
2-Undecanone	Fruity, creamy cheese	1292	2±1
Deltadecalactone	Sweet, coconut	1501	7±1
Total ketones			***4371***
Esters			
Ethyl acetate	Fruity, grape	613	30±8
Ethyl butyrate	Fruity, apple	802	1505±383
Isoamyl acetate	Banana	884	45±10
Methyl caproate	Fruity, pineapple	928	51±5
Ethyl caproate	Fruity, green banana	998	806±195
Ethyl heptanoate	Pineapple, fruity	1096	10±5
Methyl octanoate	Fruity	1123	15±5
Ethyl octanoate	Fruity	1194	121±51
Phenylethyl acetate	Rosey	1260	22±0
Propyl octanoate	Coconut	1285	5±2
Isoamylbutyrate	Fruity, melon	1058	69±3
Butanoic acid butyl ester	Sweet, fatty	1373	6±1
Ethyl decanoate	Waxy, fruity	1392	36±9
Ethyl palmitate	Faty, waxy, balsam	1590	6±0
Ethyl laurate	Waxy, soapy	1612	3±1
Ethyl myristate	Sweet, waxy	1791	1±0
Total esters			2731
Alcohols			
1-Butanol	Fusel oil	653	784±276
Isoamylalcohol	Whiskey, fruity	738	599±98
2,3-Butanediol	Buttery, creamy	787	104±50
2-Heptenol	Green grass	896	96±37
2,6,-Dimethyl-4-heptanol	Fermented, yeasty	953	175±58
1-Octanol	Waxy, green	1073	12±4
Phenylethylalcohol	Rosey	1116	46±12
Alpha terpineol	Floral	1424	7±0
Total alcohols			***1823***

	Aroma Characteristics	RI	Mean± SE
Acids			
Terpenes			
Alpha pinene	Turpentine	936	56±17
3,7-Dimethyl-octa-1,6-diene	-	948	83±18
(Z)-3,7 Ddimethyl-2-octene	-	971	61±23
Carene	Citrus	996	2043±420
Phellandrene	Citrus, terpenic	1003	332±102
Carvomenthene	-	1023	80±24
p-Cymene	Terpy, woody	1026	60±6
D-Limonene	Limon, citrus	1031	1±0
Gamma terpinene	Terpy, lime-like	1062	1±0
Copaene	Woody	1385	7±1
Trans caryophyllene	Spicy	1432	4±1
Delta cadinene	Thyme herbal	1530	1±0
Total terpenes			***2729***

MS, mass spectral identification using NIST 07 and Wiley 7 library; RI, identification using alkane series (C10–C26) by calculation of retention indices at the same chromatographic conditions. S.E: Standard error. ; n, number samples occurred the corresponding compounds. RI.Retention index based on P-5 MS column. Adapted from Sulejmani *et al.* (2014b).

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

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