Composition of Kashkaval cheese manufactured from different levels of somatic cell counts in sheep milk

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Abstract. The purpose of the present study was to investigate the influence of somatic cell count (SCC) on the composition of Kashkaval cheese. Kashkaval cheese samples were produced from three different batches of sheep milk with low (610 000 cells/ml), medium (770 000 cells/ml), and high (1 310 000 cells/ml) SCC, respectively. The main chemical parameters, such as pH, titratable acidity, moisture content, fat content in the dry matter, protein content, sodium chloride content, and microbiological parameters (lactic acid bacteria count, pathogenic microorganisms, coliforms, psychrotrophic, yeasts and molds) were studied during the ripening and storage periods. No statistically significant ($P<0.05$) changes were found in the values of the chemical parameters during the ripening period. At the beginning of ripening, the total lactic acid bacteria count for all cheese samples was about 4.1 log cfu/g, then increased to 6.2 log cfu/g (at 60 days of ripening) for test samples. The data collected in this study showed a slight decrease in pH values and a gradual increase in the titratable acidity, which was an indication for retarded fermentation during storage at low temperature. The lactic acid bacteria showed good survival, but higher sensitivity was observed in *Lactobacillus* spp. in comparison with *Streptococcus* spp.

1 Introduction

Mastitis is one of the most common diseases in dairy cattle and responsible for major economic losses. A number of authors have found that mastitis reduces the milk yield [1], quality and safety of raw milk [2] and dairy products [3; 4; 5]. The total somatic cell count (SCC) in milk is widely accepted as an indicator of udder health. This indicator is used worldwide for implementing hygienic control in the milk production process [6].

The most popular hard cheese produced in Bulgaria is traditional Kashkaval cheese made from cow, sheep, caprine, buffalo milk, or a mix of them. Furthermore, the main specific organoleptic characteristics of Kashkaval cheese depend on groups of factors related to milk quality, the cheesemaking process, ripening and storage periods. Therefore, those factors having an impact on cheese quality are of essential importance in the formation of flavor, aroma compounds and texture.

A limited number of studies are available in the literature on the influence of the high total somatic cell counts in sheep milk on the qualitative characteristics of dairy products [7]. Therefore, the aim of this study was to evaluate the changes in the chemical composition and microbial properties during ripening and storage of Kashkaval cheese produced from sheep milk with different levels of SCC.

2 Materials and methods

2.1 Sample collection

Individual milk samples of 600 Black-head Pleven dairy sheep breed were pooled at the morning milking with the aim to screen for SCC. Bulk milk samples were collected from March to August. On the basis of the results obtained, milk samples were distributed in three different batches with low (610 000 cells/ml), medium (770 000 cells/ml), and high (1 310 000 cells/ml) SCC, respectively (data are published).

2.2 Cheesemaking and cheese analysis

Samples from the three kinds of sheep milk were processed into Kashkaval cheese, according to BNS 14:2010 [8] as follows:

- SκL - Kashkaval cheese produced from sheep milk with low SCC;
- SκM - Kashkaval cheese produced from sheep milk with medium SCC;
- SκH - Kashkaval cheese produced from sheep milk with high SCC.

Kashkaval cheese samples were analysed in dynamics during of ripening and the storage periods. Chemical analyses were performed for: fat content in the dry matter [9]; sodium chloride content [10]; moisture content and dry matter [11]; total nitrogen by the Kjeldahl method [12], and then the protein content was calculated as the total nitrogen multiplied by 6.38; titratable acidity

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(TA) by the Thorner's method [13], and potentiometric pH measurement: pH meter-7110 WTW (Germany).

Microbial analyses were carried out on total lactic acid bacteria, according to [14]; psychrotrophic microorganisms [15]; coliforms [16], and total yeasts and molds [17]. The complete microbial composition analysis of the Kashkaval samples also included determination of pathogens like *L. monocytogenes* [18] and coagulase-positive *Staphylococci* [19].

2.3 Statistical analysis

The resulting data were processed using the program Microsoft Excel 2010 (ANOVA). The results are presented as mean values ±SD (n=3).

3 Results and Discussion

The changes in the chemical composition of Kashkaval cheese samples during the ripening and storage periods are given in Table 1 and Table 2, respectively.

The changes that occur in the chemical parameters during ripening at 9±1°C for 60 days are of crucial importance for the quality of Kashkaval cheese. The results show that in the study period the moisture content of the SkL, SkM and SkH samples varied between 42.0-44.5%, and the dry matter content between 55.0-57.5%. A similar trend was observed for the indicators proteins, fat content in the dry matter, and salt content, whose values did not undergo significant changes. The results of this study correspond with the results of Hachana et al., [20] where no significant differences were observed in moisture, fat, and total protein contents in mozzarella cheese samples prepared with milk consisting of different levels of SCC. For the purposes of the experiment, the storage process was carried out at 3±1°C for 12 months. During the storage period, the experimental samples of Kashkaval cheese had standard chemical parameters. Despite the fact that Kashkaval cheese was produced with milk containing different SCCs (SkL, SkM and SkH), no statistically significant (P>0.05) changes in the values of the chemical parameters were established in this study. This was probably due to the fact that ripening and storage took place under vacuum in an oxygen-free environment.

The dynamics of the fermentation process of the Kashkaval samples during ripening and storage are presented in Fig. 1 and Fig. 2, respectively. Fermentation of lactose to lactic acid by the lactic acid bacteria is an essential process that is vigorous in the early stages of the cheesemaking process (biological maturation of milk, curdling, processing, heating, and cheddaring) and continues at a slower rate during ripening and storage. The resultant growth and activity of the lactic acid microflora correlated with the decrease in the active acidity (pH) and increase in the titratable acidity. As the data in Figs. 1a and 1b show, at the beginning of the ripening process the microbial population was in the range of 4.0 - 4.1 log, rising to 2 log by the end of the period. The pH values also decreased at a steady rate by about 0.5, and TA increased by approximately 20 °T.

Table 1 Chemical composition of Kashkaval cheese during ripening

Indicator	SκL		SκM		SκH	
	Ripening period, days					
	1	60	1	60	1	60
MC, %	43.3±1.3	42.6±1.4	44.0±1.4	42.5±1.3	44.5±0.9	44.1±1.5
DM, %	56.7±1.4	57.4±1.3	56.0±1.2	57.5±1.1	55.5±1.0	55.9±1.4
P, %	26.5±0.5	27.0±0.4	27.0±0.4	27.5±0.5	26.0±0.3	26.5±0.4
FDM, %	49.4±0.4	49.6±0.3	51.8±0.4	51.3±0.5	54.0±0.3	53.7±0.4
NaCl, %	2.0±0.1	2.1±0.1	2.1±0.1	2.2±0.1	2.2±0.1	2.3±0.2

MC – moisture content; DM – dry matter; P – protein; FDM – fat in dry matter.

Table 2 Chemical composition of Kashkaval cheese during storage

Indicator	SκL		SκM		SκH	
	Storage time, months					
	1	12	1	12	1	12
MC, %	42.5±1.0	41.9±1.1	42.5±1.2	42.0±1.3	44.0±1.2	43.1±1.1
DM, %	57.5±1.3	58.1±1.2	57.5±1.4	58.0±1.3	56.0±1.0	56.9±1.5
P, %	27.0±0.3	27.1±0.5	27.5±0.4	27.6±0.3	26.5±0.2	26.7±0.4
FDM, %	49.6±0.5	49.5±0.4	51.3±0.3	51.3±0.4	53.7±0.5	53.6±0.4
NaCl, %	2.1±0.1	2.2±0.2	2.2±0.1	2.3±0.2	2.3±0.1	2.4±0.2

MC – moisture content; DM – dry matter; P – protein; FDM – fat in dry matter.

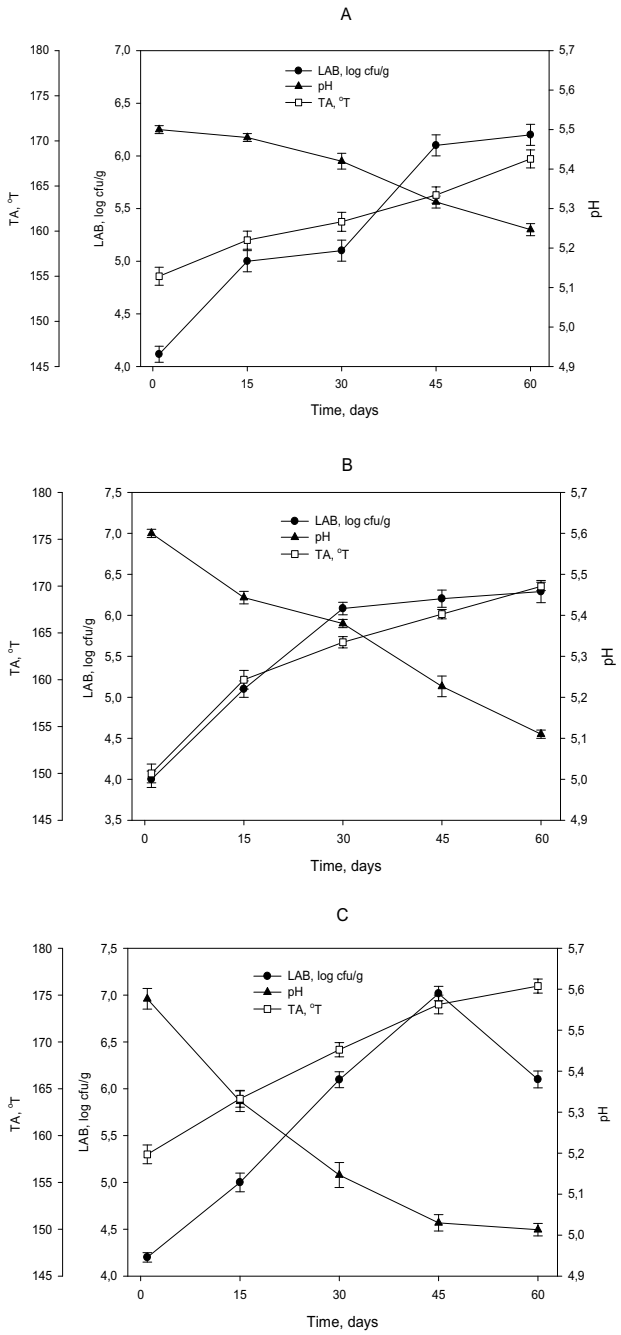

Fig. 1. Fermentation process of SkL (A), SkM (B) and SkH (C) during ripening. TA – titratable acidity; LAB – lactic acid bacteria.

There no significantly larger ($P<0.05$) increase in the starter microflora and TA was observed in the SkH samples (Fig.1c) and lower pH compared to samples SkL and SkM. The increase in the titratable acidity and the decrease in pH in the cheeses during ripening have been reported by a number of other authors [21; 22; 23]. The results of this study are not in agreement with those of Mazal et al., [24] who found that the pH value of milk was not affected by the SCC, therefore, the cheese produced from high-SCC milk presented significantly higher pH values during manufacture and a longer clotting time.

During storage, fermentation is slow due to the slow growth of the lactic acid microflora composed mainly of thermophilic microorganisms. Somalis et al., [25] reported that in the production of hard Greek cheese made from sheep: goat milk mixture not only theorization of the milk at 60-67 °C for 30 s but also stretching the cheese curd in hot brine significantly reduce lactic acid bacteria. Figs. 2 a, b and c show that the increase in the titratable acidity was more intense than pH.

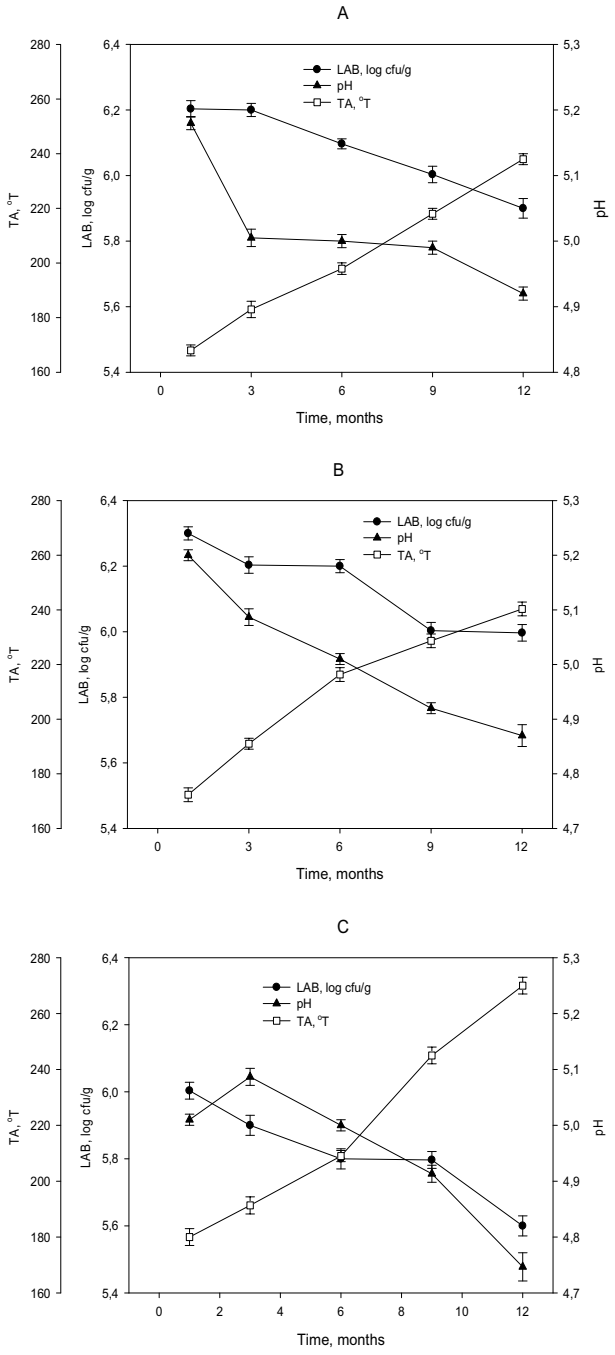

Fig. 2. Fermentation process of SkL (A), SkM (B) and SkH (C) during storage. TA – titratable acidity; LAB – lactic acid bacteria.

The reduction in pH was in the range of 0.3 while the increase in the titratable acidity was by 70 °T in the SkL and SkM samples. In sample SkH it is noteworthy that during the study period the titratable acidity increased by 90 °T, while pH changed by only 0.3 units. This is

probably due to the buffer capacity of ripe Kashkaval cheese. There were no statistically significant (P<0.05) changes in the number of lactic acid bacteria in the SkL and SkM samples, compared to their lower number in SkH.

The changes in the nonstarter microflora in samples of Kashkaval cheese from sheep milk during ripening and storage are given in Table 2. It was found that during the ripening period the number of psychrotrophic microorganisms increased by about 1 log in the SkL and SkM samples, and by 2 log in SkH. The more vigorous growth of this type of microorganisms was most likely due to the retarded lactic acid process, which correlated with the higher titratable acidity values and lower pH value. Furthermore, it was found that the inability of the starter microorganisms to grow and adapt to the medium during ripening also substantially contributed to the growth of psychrotrophs. Tripaldi et al., [26] reported that stretching the cheddared cheese curds in a saline solution resulted in low contamination of fresh cheese with pathogenic and hygiene marker microorganisms and no presence of *Enterobacteriaceae* or coliforms were found.

During storage, the number of psychrotrophic microorganisms increased by about 2 log in the three samples of Kashkaval cheese, with the highest value in the SkH sample. The growth of this type of microorganisms was favored by the fact that storage was carried out at temperatures optimal for their development. According to Farkye, [27] psychrotrophs (of the genera *Pseudomonas*, *Aeromonas* and *Acinetobacter*) are undesirable microorganisms in cheese, as they may contaminate the cheese via the technological equipment or post-processing contamination might occur leading to inferior quality of the final product, deviation of cheese colour and/or texture, or reduced shelf life.

In the experimental samples of Kashkaval cheese, no coli bacteria, molds, yeasts or pathogenic microorganisms were detected during the ripening and storage periods. The reported low levels of adventitious microorganisms in the samples were the result of good manufacturing and hygiene practices followed throughout the cheesemaking process. The absence of undesirable adventitious microflora in the unripened product is an important prerequisite for the proper course of the ripening process. A study by Baruzzi et al., [28] found that storing mozzarella cheese at 4.0±1.0°C significantly increased the number of nonstarter microorganisms. The authors found that cheeses provide a good environment for the development of microorganisms, and the high moisture content and storage temperature above 1°C favour the growth of their population. Pappa et al., [29] reported that in fresh cheese the amount of detected yeasts was below 100 cfu/g but with the progress of the ripening process their amount increased and at the end of the study period reached a value not higher than 3 log cfu/g. However, the established values for molds remained negligibly low <50 cfu/g.

4 Conclusion

The results of this study have led to the conclusion that during ripening at 9±1°C for 60 days and storage at 3±1°C for 12 months somatic cell counts did not affect the chemical parameters (such as moisture content, dry matter, fat content in the dry matter, total protein and salt content) of Kashkaval cheese samples. During the same period SkH cheese samples had a significantly lower lactic acid bacteria count and pH values, and higher titratable acidity compared to SkL and SkM samples. The number of psychrotrophic microorganisms in Kashkaval cheese from sheep milk with high SCC (SkH) was highest compared with SkL and SkM samples. No coli bacteria, molds, yeasts or pathogenic microorganisms were detected in all samples during the ripening and storage periods. Further studies are needed to determine the influence of SCC on the dynamics of proteolysis and lipolysis during ripening and storage, and the sensory acceptability of the final cheese product.

Acknowledgements: This study was carried out with the financial support of the Scientific Investigation Section Project 03/20-H, Science Fund, University of Food Technologies, Plovdiv, Bulgaria.

Table 2. Survival of nonstarter microflora in Kashkaval from sheep milk during ripening and storage

Nonstarter microflora, Log cfu/g	SkL		SkM		SkH		SkL		SkM		SkH	
	Ripening period, days						Storage period, months					
	1	60	1	60	1	60	1	12	1	12	1	12
Psychrotrophic microorganisms	3.1±0.1	4.3±0.4	3.2±0.3	4.4±0.3	3.2±0.2	5.3±0.3	4.3±0.4	6.3±0.2	4.4±0.3	6.4±0.4	5.3±0.3	7.2±0.2
Yeast and mold	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Coliforms	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Coagulase-positive staphylococci	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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