

Relationship between somatic cell count in goat milk and mature Kashkaval cheese parameters

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Abstract. It is challenging to ensure Kashkaval cheese consistent quality during the production process which is directly correlated to the somatic cell count (SCC) and bacterial presence. This is one of the most popular and widely discussed topic areas in the dairy industry. SCC is used to limit the inflammatory process and to predict the health status of the animal's mammary glands. The objective of this study was to evaluate the quality characteristics of mature Kashkaval cheese was produced from goat milk with different SCC (below 1,200 thous cells mL⁻¹ - group I (low), above 1,750 thous cells mL⁻¹ - group II (high) and up to 1,600 thous cells mL⁻¹ - group III (medium)) and samples were evaluated on the 1st and 60th day of ripening by chemical, microbiological and sensory profile. The results showed a significant relation ($P < 0.05$) between the levels of SCC and Kashkaval cheese water content during ripening. For all analysed samples, the total lactic acid bacterial count was the highest between the 15th and 45th day of ripening and reached values up to 6.0 log cfu g⁻¹. Pathogenic microorganisms (*Listeria monocytogenes*, *Coagulase-positive staphylococci*) and coliforms were not detected. The highest number of psychrotrophic microorganisms was observed in Kashkaval samples with high SCC. The sensory evaluation revealed a higher score for cheese samples with low and medium SCC in comparison to the cheese sample with a high SCC.

Key words: cheese, chemical profile, microbiological profile, quality, ripening, sensory profile.

INTRODUCTION

In recent years, there is a steady increase in the development of dairy goat farms in Bulgaria (Stankov, 2020). Goat milk provides many specific benefits (Zhou et al., 2016; Metodieva et al., 2018; Sousa et al., 2019). It is rich in proteins with high biological quality, as well as carbohydrates, vitamins, minerals and milk fat with specific composition of medium-chain fatty acids and bioactive compounds which determines its nutritional and functional properties (Zenebe et al., 2014; Bergilos-Meca et al., 2015; Clark & García, 2017; Verruck et al., 2019).

The intensive dairy goat breeding and the increased animal productivity are associated with an increase in inflammatory processes of the mammary gland (Gouveia et al., 2015; Tančin et al., 2017). Mastitis is one of the most common diseases in lactating animals which main indicator of appearance is the elevated somatic cell count (SCC) in

milk (Abebe et al., 2016; Franceschi et al., 2020). The SCC is accepted as a criterion for udder health (Stocco et al., 2019a, Pisanu et al., 2020) and is used worldwide to describe the hygiene control implemented in milk production (Hanuš et al., 2018). According to the European legislation (Regulation 853/2004), the total number of microorganisms in milk from other species than cow's milk is up to 1,500 thous cfu mL⁻¹, but the total number of somatic cells is not mentioned.

Goat milk can be used successfully for production of different kinds of dairy products such as – set or stirred yoghurt, cheese, beverages, cheeses etc. (Aryana & Olson, 2017; Pal et al., 2017; Fazilah et al., 2018; Miller & Lu, 2019; Sepe & Argüello, 2019). Marcinkoniene & Ciprova (2020) show a clear relation between the charge of SCC in goat milk and its suitability for cheese production. Kashkaval cheese is produced primarily in Bulgaria, but the same type of cheeses, called pasta–filata cheeses include a wide range of cheese variants which are also produced in Central and Southeast Europe (Medved'ová et al., 2020). The specific characteristics of Kashkaval cheese are those shared by all hard cheeses: low cooking temperature, cheddarization, and stretching of the cheese curd in hot brine. Factors related to the quality of goat milk, the technological steps in the cheese–making process, and the ripening period are of essential importance in the formation of flavour, aroma compounds and texture of Kashkaval cheese. There is still not enough information about the microbiological quality of goat milk, and its relation on cheese quality.

The aim of this study was to evaluate the changes of Kashkaval characteristics, produced from goat milk with different levels of SCC, during ripening.

MATERIALS AND METHODS

Milk collection: Each sample of bulk milk (from 19 goats) was taken at the morning milking once a month, each month, throughout the whole-lactation period from 'Plovdiv 1' - farm, located in the village of Orizare, municipality of Plovdiv, Bulgaria. The bulk milk samples were collected at early (March - May), middle (June - August) and late (September - November) lactation, from the breed Bulgarian White Dairy Goat. According to the values of SCC, milk samples were categorized into three groups, as follows: group I goat milk collected in May with below 1,200 thous cells mL⁻¹ SCC (low); group II goat milk collected in August with above 1,750 thous cells mL⁻¹ SCC (high); group III goat milk collected in November with up to 1,600 thous cells mL⁻¹ SCC (medium).

Cheese preparation: Goat milk samples, from the three groups, were processed into Kashkaval cheese according to a traditional cheese-making technology (Kozhev & Kozhev, 2009) as follows: KL - Kashkaval cheese produced from group I goat milk; KM - Kashkaval cheese produced from group III goat milk; KH - Kashkaval cheese produced from group II goat milk. The milk was further clarified (at 35–45 °C), thermized (at 63 ± 1 °C for 15–20 s), cooled (at 33 ± 1 °C) and a starter culture in amount of 0.35% as well as calcium dichloride solution (50%) in amount of 30 mL per 100 L of milk (previously diluted in water in 1:10 ratio) and rennet (previously diluted in water in 1:10 ratio) in such amount that the coagulation started 10 ± 2 min after enzyme addition were added. A set coagulum was formed after 45 min. The obtained coagulum was cut at two stages - into 5–6 cm grains and after 5–6 min into 6–7 mm grains. The curd was stirred and the grains were stabilized for 5–6 min. They were further heated (at 41–42 °C for

50 ± 5 min). They were drained (separated whey titratable acidity increased by 3–4 °Th). The curd was collected for pressing (5–6 min at pressure 6 atm, 2 kg weight for 1 kg curd) and cut in 50–60 cm parallelepiped slices. The cheddaring process took place (40–60 min, until pH of the curd reached 5.20–5.25) and the cheddared curd was milled (slices with width of 2–3 mm) and salted in a hot water solution (at 70 °C and 14% salt content). The curd was formed in 0.5 kg mould and stabilized the forms by 4–5 turning. The stabilized fresh cheese (at 8–10 °C for 12–16 h) was unmould and further dried (at 6–8 °C for 2–3 days), packed and ripened (at 6–7 °C and relative humidity 70–75% for 60 days).

The chemical composition of the milk samples (dry matter, solid non-fat, milk fat, proteins, lactose, and minerals) as well as density and freezing point were determined by Lactoscan SFP Options Milk Analyzer (Milkotronic Ltd., Bulgaria). Titratable acidity was determined according to Thorner's method (BS 1111:1980) and pH was measured by a pH meter (model MS 2000, Mycrosist, Plovdiv, Bulgaria). Screening for residual antibiotics in milk was performed with BetaStar® S Combo rapid tests (Chr. Hansen, Denmark). The microbiological analysis of raw goat's milk for SCC was determined by Ekoscope-FPS1 (Bultech 2000 Ltd, Bulgaria) and the total bacterial count (TBC) was determined by (ISO 4833-1:2013).

Cheese samples were analysed on the 1st and 60th day of ripening for the following chemical parameters: water content and dry matter (BS 1109:1989); fat content (ISO 3433:2008); total nitrogen by the Kjeldahl method (BS EN ISO 8968-1:2001); sodium chloride content (BS 8274:1982); titratable acidity (BS 1111:1980) and potentiometric pH measurement. Cheese samples were evaluated for the following microbiological indices: total lactic acid bacteria count (IDF Standard 117B:1997) and verified by (IDF Standard 122C:1996); psychrotrophic microorganisms (ISO 17410:2019); yeasts and moulds (BS EN ISO 6611:2006); coliforms (ISO 4831:2006); *L. monocytogenes* (BS EN ISO 11290-1:2017); *coagulase-positive Staphylococci* (BS EN ISO 6888-1:2005). The sensory evaluation of the cheese samples was carried out by (BS 15612:1983) according to the following parameters - taste and aroma, appearance, texture, cut surface and color.

Statistical analysis was done by computer processing of the results was performed using Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by LSD method. The results are presented as mean values ± SD ($n = 12$, per every 3-month analysed period which refers to 3 milk samples of each 2 cheese samples were produced of each 2 analysis were made) and were considered as statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

The influence of seasonal variations on the chemical and microbiological composition of raw goat milk for the production of Kashkaval cheese is presented in Table 1.

During the study period, the indicators of dry matter, solid non-fat, proteins and minerals remained almost unchanged, and a similar tendency was observed for the indicator of density. In the summer months of the year, milk fat content reached its lowest value. These results were consistent with the results of Pamukova et al. (2020) for the period May - September 2017, where the percentage of fat in the milk from local goats averaged 3.5%, solids non-fat were about 8.2%, total protein - 3.1%, and the dry

matter - was 11.8%. Comparable results were reported by Paskas et al. (2020) where the fat content of milk reached the lowest values during the summer period, followed by an increase, especially toward the end of lactation. A similar tendency was observed for the concentrations of dry matter and solid non-fat which was in agreement with the data described by Marcinkoniene & Ciprovica (2019). The lowest carbohydrate content recorded in goat milk in August was directly related to the higher somatic cell count in the milk. Kalevska & Kocoski (2013) explained this fact with the proliferation of somatic cells in milk. May be the reason why SCC were elevated (pathological or metabolic process) was a reason for that result. It can be suggested that the seasonal variations on milk is more accentuated than SCC. The freezing point is related to other properties of the milk e.g. dry matter. This indicator was relatively higher during the transitional periods of lactation, which was due to the higher milk fat content. The values of titratable acidity and active acidity remained within the normal limits during the studied period. The results for the variations in the chemical composition of raw goat milk during lactation were in agreement with the results reported by Sulejmani & Hayaloglu (2017). The rapid test did not detect any inhibitors in the milk, which was an important fact for the production of Kashkaval cheese.

Table 1. Chemical and microbiological properties of raw goat milk

Properties	May	August	November
SCC (thous cells mL ⁻¹)*	1,200 ± 1,440 ^a	1,750 ± 1,750 ^b	1,600 ± 1,600 ^c
Dry matter (%)	12.75 ± 0.34	12.00 ± 0.25 ^a	12.63 ± 0.30
Solid non-fat (%)	8.80 ± 0.10	8.50 ± 0.04 ^a	8.60 ± 0.05
Milk fat (%)	3.95 ± 0.05	3.50 ± 0.06 ^a	3.90 ± 0.05
Proteins (%)	3.40 ± 0.10	3.20 ± 0.11	3.30 ± 0.10
Lactose (%)	4.60 ± 0.10	4.45 ± 0.04 ^a	4.60 ± 0.08
Minerals (%)	0.80 ± 0.01	0.80 ± 0.02	0.80 ± 0.01
Density (g mL ⁻¹)	1.029 ± 0.002	1.029 ± 0.001	1.028 ± 0.001
Freezing point (°C)	-0.570 ± -0.001	-0.567 ± -0.001 ^a	-0.569 ± -0.001
Titratable acidity (°Th)	18 ± 1	17 ± 2	17 ± 1
pH	6.78 ± 0.03	6.72 ± 0.03	6.76 ± 0.01
Antibiotics and inhibitors	Negative	Negative	Negative
TBC (thous cfu mL ⁻¹)*	680 ± 250 ^a	910 ± 320 ^b	740 ± 300 ^c

^{a-c} Means with different letters within a row are significantly different ($P < 0.05$); * The term thousand refers only for the first value.

The use of raw goat milk with known microbiological indices such as total bacterial count (TBC) and somatic cell count (SCC) ensured a high-quality cheese. The results indicated that the TBC in the milk samples varied in the range from 680 to 910 thous cfu mL⁻¹ (Table 1). From the onset to the middle of the lactation period, this indicator tended to increase, then decreased again and reached a value of 740 thous cfu mL⁻¹ at the end of the study period. The total number of somatic cells in the studied milk was found to increase with progressing lactation. The SCC at the beginning of lactation was relatively low - 1,200 thous cells mL⁻¹, and reached its highest value during the summer months of the year - 1,750 thous cells mL⁻¹, while at the end of the period it began to decline again, reaching 1,600 thous cells mL⁻¹ (Table 1). The obtained data were in agreement with those reported by Paskas et al. (2020) who stated that SCC and TBC were the most variable indices during lactation and by Marcinkoniene & Ciprovica

(2020) who established that the SCC increased in the second lactation. According to Skeie (2014) the frequency of intramammary infection was due to different factors, such as – lactation phase, environmental condition, month of milking, parity of lactation, etc. In contrast, Sulejmani & Hayaloglu (2017) observed that the value of SCC was lower in summer goat milk. However, Puggioni et al. (2020) concluded that mastitis should be preferably monitored by testing goat milk at the peak of lactation.

The chemical composition of Kashkaval cheese samples during the ripening period is given in Table 2.

Table 2. Chemical composition of Kashkaval cheese during ripening

Properties	KL		KH		KM	
	1 st day	60 th day	1 st day	60 th day	1 st day	60 th day
Water content (%)	44.50 ± 0.24 ^{aA}	43.60 ± 0.23 ^{bA}	46.8 ± 0.22 ^{aB}	45.00 ± 0.23 ^{bB}	44.90 ± 0.23 ^{aA}	43.90 ± 0.25 ^{bA}
Dry matter (%)	55.50 ± 0.33 ^{aA}	56.40 ± 0.24 ^{bA}	53.90 ± 0.20 ^{aB}	54.80 ± 0.24 ^{bB}	55.10 ± 0.22 ^{aA}	56.10 ± 0.20 ^{bA}
NaCl (%)	2.20 ± 0.05 ^{aA}	2.30 ± 0.04 ^{bA}	2.20 ± 0.05 ^{aA}	2.50 ± 0.06 ^{bB}	2.10 ± 0.04 ^{aC}	2.20 ± 0.05 ^{bC}
Fat content (%)	26.00 ± 0.10 ^{aA}	26.50 ± 0.10 ^{bA}	25.00 ± 0.10 ^{aB}	25.50 ± 0.10 ^{bB}	27.00 ± 0.10 ^{aC}	27.50 ± 0.10 ^{bC}
Total protein (%)	27.50 ± 0.05 ^{aA}	26.50 ± 0.04 ^{bA}	25.00 ± 0.05 ^{aB}	24.00 ± 0.04 ^{bB}	26.00 ± 0.04 ^{aC}	25.00 ± 0.05 ^{bC}
Titrate acidity (°Th)	160 ± 2 ^{aA}	169 ± 2 ^{bA}	152 ± 2 ^{aB}	178 ± 1 ^{bB}	155 ± 2 ^{aC}	162 ± 1 ^{bC}
pH	5.10 ± 0.02 ^{aA}	5.02 ± 0.02 ^{bA}	5.20 ± 0.03 ^{aB}	4.95 ± 0.05 ^{bB}	5.20 ± 0.04 ^{aC}	5.03 ± 0.03 ^{bA}

^{a-c} Means with different letters within columns, for the same sample but for a different ripening period, are significantly different ($P < 0.05$); ^{A-C} Means with different letters within columns, for the same ripening period, are significantly different ($P < 0.05$).

The quality characteristics of Kashkaval cheese was largely determined by the water content, as it affected by the metabolic activity of lactic acid bacteria from the starter culture during ripening. The number of somatic cells reflected on some chemical parameters of the final product. In the KH samples, a more intensive process of acid formation was observed due to the higher percentage of water content in the end of the ripening period, also connected with the accelerated physicochemical and biochemical processes. The dynamics in the development of acidity predetermined the increased hydrophilic properties of the milk protein, as a result of which the KH sample had a higher water content compared to the KL and KM samples. The results for the dynamic of water content during cheese ripening were comparable to the conclusions of Ivanov et al. (2018) and Mateva et al. (2020). A similar tendency was established by Niro et al. (2014) who found that for a period of 60th days of ripening, the values of the water content of Caciocavallo cheese decreased by 5.7%. During ripening, the salt content in all samples slightly increased. The intensity of the salting process was closely related to the water content of the samples and the acid formation. The salt content increased by 0.1% for samples KL and KM, and by 0.3% for KH. Similar results about NaCl content in Kashar cheese were also observed by Kavak (2020). According to Marinova et al. (2016) the physicochemical parameters of Kashkaval cheese from cow milk did not

change significantly during the process of ripening. Almost similar pH values and salt content to the present study, but lower dry matter was reported for Kashar cheese ripened up to 60 days (Temizkan et al., 2014). At the same time, closer fat content but a higher protein value was found in Caciocavallo cheese (Perna et al., 2015). Cheeses obtained from milk with high SCC were with increased recovery of lactose in the curd and water retention (Stocco et al., 2019b). Our study revealed that the pH decreased during ripening in contrast to the results reported by Darnay et al. (2019) where the pH values increased slightly and remained higher than pH 5.0 during ripening. The fat content in cheese samples remained practically almost constant throughout ripening, without any significant differences being observed ($P > 0.05$). The chemical composition, microbial properties and types of milk, cheese-making steps and ripening conditions may well explain the above differences. Maybe not SCC directly influenced cheese parameters but milk composition changes, and SCC was just related indicator to this different outcome.

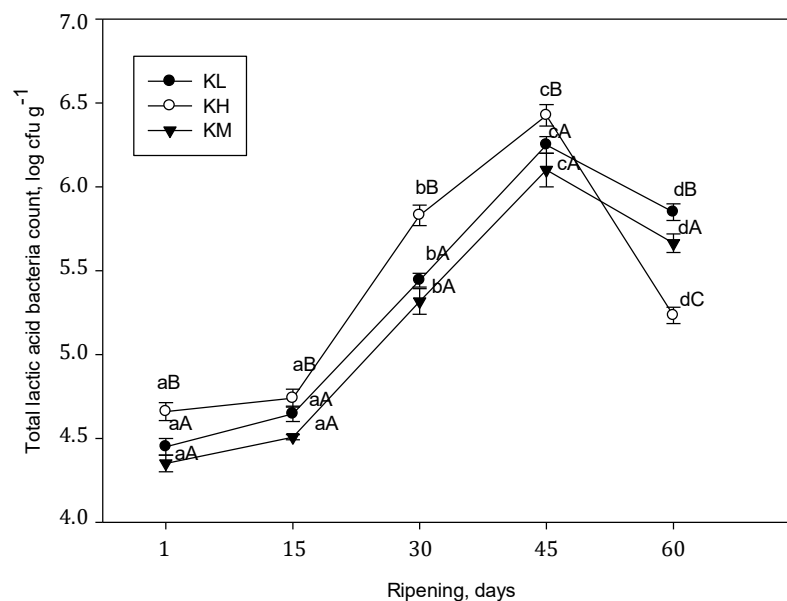


Figure 1. Change of total lactic acid bacteria count in Kashkaval cheese from goat milk during ripening.

^{a-d} Means with different letters within a range are significantly different ($P < 0.05$); ^{A-C} Means with different letters within different series are significantly different ($P < 0.05$).

Fig. 1 illustrates the dynamics in the development of lactic acid microflora during ripening of the studied samples of Kashkaval cheese from goat milk during ripening. The results showed that at the beginning of the ripening period the total number of lactic acid bacteria in samples KL and KM was approximately $4.4 \log \text{cfu g}^{-1}$, and in KH it was $4.6 \log \text{cfu g}^{-1}$. These lower values of the total number of lactic acid bacteria in the three Kashkaval cheese samples were due to the hot salting technological process, leading to a significant reduction of the population of these microorganisms. In all samples, an increase in the amount of lactic acid microflora was recorded after 15 days of ripening. This was a result of the longer time required for the starter culture adaptation to the ripening temperature. In samples KL and KM, a gradual increase in the bacterial population was observed between the 15th and 45th days, but at the end of the studied period its levels decreased and reached a value of approximately $5.6 \log \text{cfu g}^{-1}$. A more

intensive process of acid formation and a higher initial number of lactic acid bacteria were found in the KH samples. Thus, the reported higher values for water content in the KH samples (Table 1) correlated with the increase in the hydrophilicity of milk protein, leading to accelerated ripening process and faster accumulation of bacterial biomass between the 15th and 45th days of ripening. These results were similar to the data obtained by Sulejmani & Hayaloglu (2016) who studied the same influence on Turkish Kashkaval-type cheese Kashar. Our results were in agreement with Talevski et al. (2017) who concluded that a slight decrease in the active acidity after the 30th day could be noticed due to the presence of residual lactose fermented by active lactic acid bacteria at the ripening temperature from 10–12 °C. The count of lactic acid bacteria decreased after 45 days of maturation probably because of the depleted amount of lactose and the low pH.

The results about the change in nonstarter microflora in samples of Kashkaval cheese from goat milk during ripening are given in Table 3.

Table 3. Survival of nonsarter microflora in Kashkaval from goat milk during ripening

Nonstarter microflora (log cfu g ⁻¹)	KL		KH		KM	
	1 st day	60 th day	1 st day	60 th day	1 st day	60 th day
Psychrotrophic microorganisms	2.04 ± 0.30 ^{aA}	3.36 ± 1.00 ^{bA}	3.32 ± 1.18 ^{aB}	4.53 ± 1.65 ^{bB}	2.11 ± 0.70 ^{aC}	3.32 ± 1.11 ^{bC}
Yeasts and moulds	< 1	< 1	< 1	< 1	< 1	< 1
Coliforms	ND	ND	ND	ND	ND	ND
<i>Listeria monocytogenes</i>	ND	ND	ND	ND	ND	ND
<i>Coagulase-positive staphylococci</i>	ND	ND	ND	ND	ND	ND

^{a-b} Means with different letters within columns, for the same sample but for a different ripening period, are significantly different ($P < 0.05$); ^{A-C} Means with different letters within columns, for the same ripening period, are significantly different ($P < 0.05$).

It was found that during ripening the number of psychrotrophic microorganisms increased significantly ($P < 0.05$) in the three samples of Kashkaval cheese. The data in Table 2 showed that the higher levels of titratable acidity and the lower pH values favoured the development of psychrotrophic microorganisms. According to Faccia et al. (2013) *Pseudomonas* spp. counts were similar to those commonly found in bovine mozzarella cheese with high moisture content. The presence of moulds and yeasts in the tested cheese samples was significantly low ($P > 0.05$), which suggested that their growth was inhibited during the ripening period. Our findings coincided with the results described by Pappa et al. (2020) who found low levels of moulds and yeast in samples of Kashkaval cheese produced in the mountainous region of Pindus, Greece. No coliform bacteria, coagulase-positive staphylococci or listeria monocytogenes were detected in this study. Mastromatteo et al. (2014) concluded that the right control of the growth and activity of these microorganisms during cheese ripening were essentially important because they caused the quality alteration of cheese. This was in accordance with the hygienic quality of Fior di latte cheese from goat milk stated by Faccia et al. (2015). These findings may be awarded to the good microbiological quality of the raw goat milk and the thermal effect of the stretching process, which had a preservative effect on Kashkaval cheese (Pappa et al., 2019; Samelis et al., 2019). According to the results obtained in this study, it can be concluded that the combination of good quality goat milk (samples KL and KM), which was subjected to thermisation and subsequent heat

treatment of the curd during the stretching process at 72 °C possibly had a positive preservative effect on Kashkaval cheese.

The sensory profiles of Kashkaval samples from goat milk were evaluated at the end of the ripening process and are presented in Fig. 2.

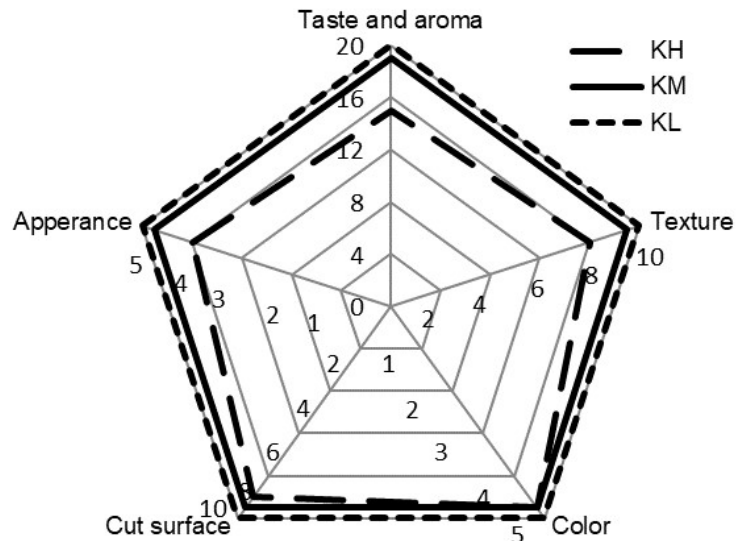


Figure 2. Sensory evaluation of Kashkaval cheese from goat milk after ripening.

Samples KL and KM were characterized by better flavour and aroma, specific for the respective type of cheese, while the KH samples were given lower sensory scores. According to Chen et al. (2010) high SCC ($1,000 \text{ thous cells mL}^{-1} < \text{SCC} < 1,500 \text{ thous cells mL}^{-1}$) in goat milk seemed to affect the sensory characteristics and quality of matured cheeses. As a result, KL and KM had a smooth, homogeneous structure, uniform creamy-yellow colour, dense and elastic consistency, distinct aroma of mature Kashkaval cheese, specific for the type of milk from which it was produced. The lower scores awarded to the indicators flavour and aroma, cut surface and consistency were a result of the intensity with which the biochemical reactions occurred during the ripening period in the KH samples. At the end of the study period, the KH samples had a very strong specific aroma, slightly pungent taste, soft texture and rich amber colour. These data were in agreement with the findings of Semjon et al. (2019), who associated the modification of the elasticity of cheese due to a more pronounced lactic acid bacteria activity and resulting proteolysis. Bezerra et al. (2020) also reported that a high SCC in raw milk had a significant effect on the quality of pasteurized milk and Coalho cheese as it presented lower sensory acceptance.

CONCLUSIONS

The results showed that the high number of somatic cells was related to some chemical parameters such as fat and lactose content, as well as the freezing point. The high number of somatic cells, in combination with the increased total number of microorganisms, led to increased water content in the samples of Kashkaval produced from the correspondent milk which made it worse compared to the other variants. Reduced active acidity favoured the development of a great number of psychrotrophic

microorganisms in the sample with high number somatic cells. Significant differences in the sensory profile of the samples containing high levels of somatic cell count were observed. Its appearance, texture, taste and aroma were deteriorated. However, further work is required to evaluate the relation between SCC and the level of proteolysis and lipolysis during the ripening and storage periods.

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