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Effect of somatic cell count of cow's milk on the lipolysis and fatty acid profile of farmer cheese

[1]Ivanov, G. Y., [2]Bilgücü, E., [1]*Balabanova, T. G. and [3]Ivanova, I. V.

[1]*Department of Milk and Milk Products, Technological Faculty, University of Food Technologies, 4002 Plovdiv, Bulgaria*
[2]*Department of Milk, Çanakkale Onsekiz Mart University - Biga Vocational High School, 17020 Biga, Çanakkale, Turkey*
[3]*Department of Analytical Chemistry, Technological Faculty, University of Food Technologies, 4002 Plovdiv, Bulgaria*

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Abstract

The objective of the present work was to investigate the effect of somatic cell count (SCC) of raw cow's milk on the lipolysis and oxidative processes in farmer cheese. The farmer cheese samples were produced from three different batches of raw cow's milk of low (about 100,000 cells/mL, batch L), medium (between 500,000 and 600,000 cells/mL, batch M), and high (above 1,500,000 cells/mL, batch H) SCC. The farmer cheese samples were aged and cold-stored at 4 ± 1°C for three and ten months, respectively. Lipolysis in the farmer cheese samples was evaluated by monitoring the changes in cheese fatty acid values and peroxide values, as well as the changes in the fatty acid profile. Results indicated intensive lipolysis during aging and cold storage of batch H; increased concentrations of short-chain fatty acids as well as a higher percentage of saturated fatty acids were observed. It can thus be concluded that the accelerated lipolysis in farmer cheese samples made from raw cow's milk with high SCC could cause some quality defects, and reduce cheeses' shelf life.

Introduction

The quality of raw milk is important towards the quality of dairy end-products as well as human health. Numerous benefits can be obtained by improving raw milk quality; for example, microbial degradation of milk can be prevented thereby prolonging the milk shelf life and protecting human health. In addition, keeping animal diseases such as mastitis under control would help to prevent reduction in milk yield. Somatic cells occur as a result of inflammatory response to an intramammary infection. They are part of the natural defence mechanism including lymphocytes, macrophages, polymorphonuclear cells, and some epithelial cells. Therefore, somatic cell count (SCC) is considered as an important criterion for evaluating milk quality and overall udder health, and is often used to distinguish between infected and uninfected quarters. There is a general agreement between infection status and the inflammatory response to this infection as measured by an increased SCC. Typically, the SCC in milk from a healthy mammary gland is less than 100,000 cells/mL, whereas bacterial infection can lead to levels above 1,000,000 cells/mL (Bytyqi *et al.,* 2010).

Many studies have been done to evaluate the effect of SCC on the yield and quality of milk and dairy products, particularly cheese (Mona *et al.,* 2011; Le Maréchal *et al.,* 2011; Richoux *et al.,* 2014; Saha *et al.,* 2020). Increased SCC is associated with the retarded coagulation of milk and worsened curd firming during cheese-making (Andreatta *et al.,* 2007; Vianna *et al.,* 2008). Mikulec *et al.* (2012) reported a yield loss and quality deficiencies in cheese produced from milk with high SCC. Lipolysis in cheese has been found to be clearly dependent on SCC, which may have important consequences on cheese flavour (Chen *et al.,* 2010). The differences in milk SCC levels result from diverse changes in the free fatty acid (FFA) profiles (Sánchez-Macías *et al.,* 2013). Chen *et al.* (2010) found that the individual and total FFA increased significantly during ripening, regardless of the SCC levels. After 120 days of ripening, semi-soft cheese made with low-SCC milk had a higher total FFA than that made with medium- and high-SCC milk. Pavia (2000) also reported that the total FFA in Manchego cheese was correlated with age during the three-month ripening period. However, Jaeggi *et al.* (2003) reported that the total FFA did not change significantly in sheep's milk hard cheese during the first six months of aging.

Information regarding the influence of SCC

*Corresponding author.
Email: tbg_georgieva@yahoo.com

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on lipolysis in different types of cheese is still insufficient. Therefore, the aim of the present work was to investigate the effect of SCC from raw cow's milk on the lipolysis and fatty acid profile of farmer cheese.

Materials and methods

Materials

Milk samples

Raw cow's milk samples were obtained between October 2016 and March 2017 from three different regions (Tokatkırı, Yeniçiftlik, Çelikgürü) from Biga district of Çanakkale province, Turkey. For the purposes of the experimental cheese-making, three different batches of raw cow's milk were selected with low (about 100,000 cells/mL - batch L), medium (between 500,000 and 600,000 cells/mL - batch M), and high (above 1,000,000 cells/mL - batch H) SCC. Raw cow's milk batches (1,000 L/batch) representing different SCC were collected from three different farms (from 70 and 80 animals of Holstein breed) affiliated with the Dairy Producer Associations in Biga district of Çanakkale province, Turkey. The samples (five from each batch per day) were transported to the laboratory of Çanakkale Onsekiz Mart University - Biga Vocational High School, Turkey at 4°C. The SCC, total viable count (TVC), and chemical composition of the raw cow's milk samples were measured. All raw cow's milk analyses were carried out in triplicate.

Farmer cheese samples

The farmer cheese samples were produced from raw cow's milk (1,000 L/batch) with different SCC; batches L, M, and H at the pilot dairy processing plant of Çanakkale Onsekiz Mart University - Biga High School, Turkey. Platform tests (dry matter, fat, acidity, and antibiotics) were carried out, and pasteurisation was applied for 15 min at 68°C. The milk was cooled down to coagulation temperature of 35 - 36°C. A starter culture consisting of 70% *Streptococcus thermophilus*, 30% *Lactobacillus bulgaricus*, and cheese rennet were then added. After 90 min of coagulation, the curd was sliced into nut-sized curd grains, and a portion of the whey was removed. After 5 min of curd ventilation, the curd and remaining whey were heated at 41 - 42°C for 15 min. At the end of heating, the raw cheese was pressed for 2 - 3 h without applying pressure. The young cheeses were then removed from the moulds, and salted in a 16% NaCl solution for 24 h at 15 - 18°C. Following salting, the young cheese was taken out, dried, and packed in polyamide/polyethylene foil under vacuum at 90 - 99.8 Pa. The average cheese yield was 14% (14 kg of cheese from 100 L of milk), and did not differ significantly ($p > 0.05$) among the three batches. Ripening took place in these packages at 4 ± 1°C and relative humidity of 75 - 80% for three months. After that, the cheese samples were subjected to cold storage at 4 ± 1°C for another seven months (to a total of ten months from the start of production).

Determination of SCC, TVC, and chemical composition of raw milk

A Bactocount IBCm (Bentley Instruments, USA) device was used for the SCC determination.

The TVC was determined by using a Plate Count Agar medium according to ISO (2013). The inoculated Petri dishes were incubated at 30°C for 48 to 72 h, and the colony forming units (CFU) were counted following incubation.

The milk fat, protein, lactose, and total solid content of the milk samples were measured by using Infrared Milk Analyser 150 (Bentley Instruments, USA). The instrument was calibrated with certified reference milk samples from Italy Accredited Dairy Laboratories.

Physicochemical analysis of farmer cheese

The fat contents of the farmer cheese samples were determined according to the Gerber-Van Gulik method (BDS, 1989). The dry matters and water contents were determined by heating the farmer cheese samples at 105°C to constant weight. The sodium chloride contents of the farmer cheese samples were determined according to BDS (1982). The pH values of the farmer cheese samples were determined using a pH meter. The lactic acid contents of the cheese samples were determined by the titration method according to BDS (1980).

Fatty acid composition of farmer cheese fat

The fatty acid composition of the farmer cheese fat was determined following the procedures described by Bligh and Dyer (1959). The methylation of fats was performed according to BDS (2002). The methyl esters were analysed on a Shimadzu GC - 17A gas chromatograph (Shimadzu Corp., Kyoto, Japan).

Acid value of farmer cheese fat

The farmer cheese fat extraction was performed following the procedures described by Bligh and Dyer (1959). The acid value of farmer cheese fat was determined by the titrimetric method

according to BDS (2009).

Peroxide value of farmer cheese fat

The farmer cheese fat extraction was performed following the procedures described by Bligh and Dyer (1959). The peroxide value of farmer cheese fat was determined by the iodometric method according to Swedish Standard (2008).

Statistical analysis

Statistical analyses were carried out on the averages of the triplicate results. The data were analysed using the analysis of variance (one-way ANOVA) method with a significant level of $p \leq 0.05$ (Draper and Smith, 1998). Duncan's multiple comparison test (SPSS) with a significant difference at $p \leq 0.05$ was used to compare the sample means. Significant differences of less than 0.05 between the means were considered statistically significant (Kenward, 1987). All statistical procedures were computed using Microsoft Excel 5.0 software.

Results and discussion

Physicochemical analysis of farmer cheese samples

Table 1 shows the cross composition of raw cow's milk and farmer cheese samples. The contents of the main milk constituents (milk fat, proteins, and lactose) in the raw cow's milk of batches L, M, and H were similar ($p > 0.05$).

No statistically significant ($p > 0.05$) differences were found in the total viable counts of the raw cow's milk of batches L, M, and H. This was probably due to the similar hygiene conditions in the milk supplying farms. The pH values of the raw cow's milk of batches L, M, and H ranged between 6.58 ± 0.18 and 6.64 ± 0.19, and they did not differ significantly either ($p > 0.05$).

The three farmer cheese batches were characterised by similar values of dry matter, fat, protein, and NaCl content (Table 1). The SCC of the raw cow's milk did not have a significant ($p > 0.05$) effect on the composition of the farmer cheese. Mazal *et al.* (2007) reported that SCC did not affect the protein and fat contents of Prato cheese or the fat loss to the whey. Chen *et al.* (2010) reported that SCC did not have a significant effect on the total solid, fat, or protein contents of goat's milk, and consequently, the cheese yield. Albenzio *et al.* (2004) established that high SCC resulted from higher pH value of sheep's milk and lower fat content in cheese curd. In the present work, no statistically significant

Table 1. Cross composition of raw cow's milk and farmer cheese samples.

Composition	Batch L	Batch M	Batch H
Milk			
SCC (log cells/mL)	5.00 ± 0.15[a]	5.75 ± 0.12[b]	6.19 ± 0.14[c]
TVC (log CFU/mL)	5.45 ± 1.24	5.44 ± 0.98	5.51 ± 1.32
Dry matter (%)	12.28 ± 0.22	12.41 ± 0.19	12.05 ± 0.19
pH	6.59 ± 0.15	6.58 ± 0.18	6.64 ± 0.19
Proteins (%)	3.32 ± 0.09	3.26 ± 0.11	3.28 ± 0.08
Lactose (%)	4.58 ± 0.15[a]	4.55 ± 0.18[a]	3.82 ± 0.16[b]
Fat (%)	3.69 ± 0.12	3.71 ± 0.15	3.67 ± 0.12
Farmer cheese			
Dry matter (%)	52.52 ± 0.46	52.48 ± 0.38	51.81 ± 0.41
Fat (%)	32.12 ± 0.32	31.67 ± 0.28	32.13 ± 0.45
NaCl (%)	2.15 ± 0.12	2.11 ± 0.09	2.08 ± 0.11
Proteins (%)	18.2 ± 0.27	18.6 ± 0.22	17.9 ± 0.31
pH	5.84 ± 0.05	5.81 ± 0.05	5.85 ± 0.04

Means within same row followed by similar lowercase superscripts do not differ significantly ($p > 0.05$). SCC: somatic cell count, and TVC: total viable count. Farmer cheese produced from raw cow's milk with low (batch L), medium (batch M), and high (batch H) SCC.

($p > 0.05$) differences were established in the pH values between the farmer cheeses produced from batches L, M, and H. This indicated a similar acidification rate during the production of the farmer cheese samples. Jaeggi *et al.* (2003) and Chen *et al.* (2010) also found that cheese pH did not vary significantly with the change in milk SCC.

Effect of raw milk SCC on the lipolysis and oxidative processes in farmer cheese fat during aging and cold storage

Lipolysis during the aging and cold storage of farmer cheese samples was monitored by the changes in acid values of the cheese fat (Figure 1). During the three months of aging, acid values of the farmer cheese samples from batches L and M did not change significantly ($p > 0.05$). For the same period, the values of this indicator increased from 1.07 ± 0.04 to 1.23 ± 0.06 mg KOH/g fat for batch H. At the end of the aging, no statistically significant ($p > 0.05$) differences were established in the acid values of the farmer cheeses from batches L and M. Contrarily, significantly higher values ($p < 0.05$) of this indicator were established for batch H. Sánchez-Macías *et al.* (2013) found that somatic cells had a major effect on lipolysis by increasing the free fatty acid content regardless of whether the milk was raw or pasteurised.

Figure 1 shows an increase in acid values during cold storage (from the 3rd to the 10th month) of the test samples. This trend was most pronounced in batch H farmer cheese. At the end of the cold storage (10th month), the acid values of batches L, M, and H farmer cheeses reached 1.50 ± 0.08, 1.52 ± 0.09, and 2.87 ± 0.08 mg KOH/g fat, respectively. No statistically significant ($p > 0.05$) differences were established in the acid values of the farmer cheeses from batches L and M, whereas the acid value of batch H farmer cheese was significantly higher ($p < 0.05$). These results indicated an accelerated lipolysis during the aging and cold storage of the farmer cheeses made from high-SCC milk. The slight increase in the acid values of the farmer cheeses from batches L and M suggested retarded lipolysis during their aging and cold storage. The reason for this was the lower activity of the intracellular lipases and esterases produced by the starter culture lactobacilli. Lipolysis only occurs when the membrane of the fat globules is broken and accessible to the enzyme (Sánchez-Macías *et al.,* 2013). According to Le Roux *et al.* (2003), somatic cells contain intracellular lysosomal enzymes which pass into the milk, where they cause an increase of proteolysis and lipolysis in milk or cheese (Albenzio *et al.,* 2004). Intensive

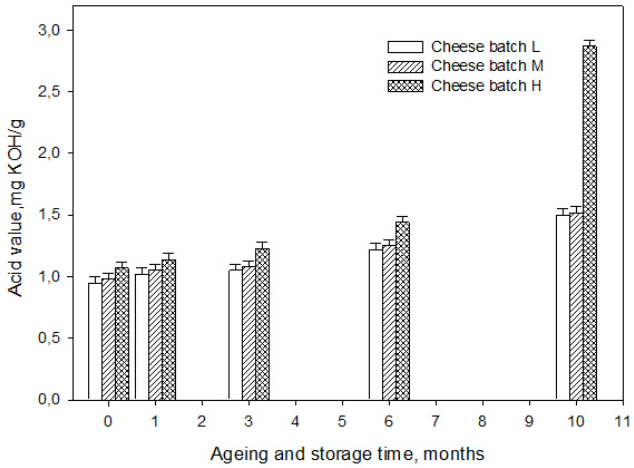

Figure 1. Changes in cheese fatty acid values during the aging and cold storage of farmer cheese produced from raw cow's milk with low (L), medium (M), and high (H) somatic cell count (SCC).

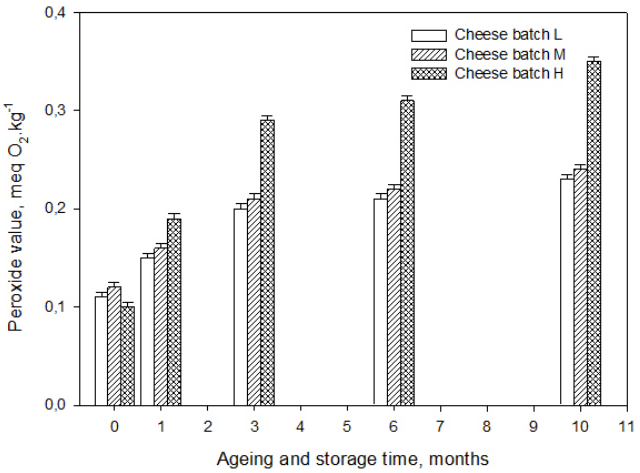

Figure 2. Changes in cheese fat peroxide values during the aging and cold storage of farmer cheese produced from raw cow's milk with low (L), medium (M), and high (H) somatic cell count (SCC).

lipolysis in the cheeses made from milk with high SCC (batch H) was probably due to the activity of the lipases contained in the somatic cells (Gargouri *et al.,* 2008). Milk SCC is suspected to be involved in the lipolysis of the fat globule triglycerides, and thus in the release of free fatty acids (Ma *et al.,* 2000; Santos *et al.,* 2003; Gargouri *et al.,* 2008). Moreover, lipases can resist pasteurisation, hence, pasteurised high-SCC dairy products are more likely to develop off-flavours than low-SCC ones. Accelerated lipolysis during the aging of Cantal cheeses produced from high-SCC milk has been reported by Agabriel *et al.* (2004).

Changes in the peroxide values were studied in order to assess oxidation processes in the cheese fat of the test samples during aging and cold storage (Figure 2). It can be seen that the values of this indicator demonstrated a certain increase in all test

Table 2. Fatty acid profile of farmer cheese fat at the end of the cold storage period (10th month).

Fatty acid	Content (g/100 g lipid)		
	Batch L	Batch M	Batch H
Caproic acid (C6:0)	1.95 ± 0.09[a]	1.99 ± 0.11[a]	2.61 ± 0.15[b]
Caprylic acid (C8:0)	2.23 ± 0.11[a]	2.35 ± 0.14[a]	3.07 ± 0.17[b]
Capric acid (C10:0)	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Lauric acid (C12:0)	9.57 ± 0.42[a]	9.90 ± 0.41[a]	11.47 ± 0.44[b]
Tridecylic acid (C13:0)	0.73 ± 0.03[a]	0.78 ± 0.04[a]	0.97 ± 0.06[b]
Myristic acid (C14:0)	0.87 ± 0.04[a]	1.04 ± 0.05[b]	1.14 ± 0.07[b]
Myristoleic acid (C14:1)	0.29 ± 0.02	0.29 ± 0.02	0.32 ± 0.03
Palmitic acid (C16:0)	29.88 ± 0.82[a]	30.94 ± 0.84[ab]	32.31 ± 0.81[b]
Palmitoleic acid (C16:1)	1.67 ± 0.08	1.59 ± 0.07	1.73 ± 0.10
Margaric acid (C17:0)	0.50 ± 0.03[a]	0.48 ± 0.03[a]	0.72 ± 0.04[b]
Heptadecenoic acid (C17:1)	0.49 ± 0.02[a]	0.54 ± 0.02[a]	0.61 ± 0.02[b]
Stearic acid (C18:0)	13.45 ± 0.67	13.49 ± 0.65	13.11 ± 0.61
Oleic acid (C18:1)	31.74 ± 0.96[a]	29.92 ± 0.91[a]	25.84 ± 0.72[b]
Linoleic acid (C18:2)	3.84 ± 0.18	3.63 ± 0.16	3.29 ± 0.17
Linolenic acid (C18:3)	0.38 ± 0.02	0.38 ± 0.02	0.32 ± 0.02
Arachidic (C20:0)	0.83 ± 0.04	0.81 ± 0.04	0.72 ± 0.03
Eicosenoic acid (C20:1)	0.19 ± 0.01	0.20 ± 0.01	0.19 ± 0.01
Arachidonic acid (C20:4)	0.33 ± 0.02	0.36 ± 0.02	0.35 ± 0.02
Behenic acid (C22:0)	0.34 ± 0.02[a]	0.28 ± 0.02[ab]	0.27 ± 0.02[b]
Total fatty acids	**99.40 ± 4.92**	**99.09 ± 4.48**	**99.16 ± 4.55**

Means within same row followed by similar lowercase superscripts do not differ significantly ($p > 0.05$). Farmer cheese produced from raw cow's milk with low (batch L), medium (batch M), and high (batch H) SCC.

Table 3. Percentage ratio of main fatty acid groups in the farmer cheese fat at the end of the cold storage period (10th month).

Fatty acid group	Batch L	Batch M	Batch H
Saturated fatty acids (%)	60.84 ± 2.11[a]	62.75 ± 2.08[ab]	67.07 ± 2.14[b]
Monounsaturated fatty acids (%)	34.59 ± 1.23[a]	32.84 ± 1.15[ab]	28.93 ± 1.04[b]
Polyunsaturated fatty acids (%)	4.58 ± 0.21	4.41 ± 0.18	3.99 ± 0.16
Total unsaturated fatty acids (%)	39.16 ± 1.46[a]	37.25 ± 1.38[ab]	32.93 ± 1.22[b]
Saturated/unsaturated fatty acids (%)	1.55 ± 0.06[a]	1.68 ± 0.06[ab]	2.04 ± 0.08[b]

Means within same row followed by similar lowercase superscripts do not differ significantly ($p > 0.05$). Farmer cheese produced from raw cow's milk with low (batch L), medium (batch M), and high (batch H) SCC.

samples. This trend was most pronounced in the farmer cheese samples made from high-SCC milk (batch H).

During the 3-month aging period, the peroxide values of batches L and M farmer cheeses increased from 0.11 ± 0.02 to 0.20 ± 0.03 meqO_2/kg and from 0.12 ± 0.01 to 0.21 ± 0.02 meqO_2/kg, respectively. At the end of the aging process (3rd month), no statistically significant ($p > 0.05$) differences were established in the peroxide values of batches L and M farmer cheeses. In contrast, batch H farmer cheese had significantly ($p < 0.05$) higher value (0.29 ± 0.03 meqO_2/kg).

Additionally, no statistically significant ($p > 0.05$) changes were found in the peroxide values during the cold storage of the farmer cheese samples from batches L and M. Batch H farmer cheese showed slight increase in the values of this indicator, which reached to 0.35 ± 0.02 meqO_2/kg at the end of the cold storage (10th month). These data indicated intensive oxidation processes in the cheese fat during the aging and cold storage of the farmer cheese sample made from high-SCC milk.

Fatty acid profile of farmer cheese samples

The fatty acid profiles of farmer cheese samples at the end of the cold storage (10th month) are presented in Tables 2 and 3. In all samples, palmitic and oleic fatty acids predominated. Higher concentrations of stearic, lauric, and linoleic fatty acids were also detected. Among the short-chain fatty acids, the percentages of caprylic and caproic acids were the highest. No statistically significant ($p > 0.05$) differences were established in the concentrations of these two fatty acids in the farmer cheeses from batches L and M. A higher ($p < 0.05$) concentration of caprylic and caproic acids was observed in batch H farmer cheese.

Short-chain fatty acids participate in the formation of cheese taste since they are the precursors to the formation of important volatile compounds (Collins *et al.,* 2003). Therefore, their contents in cheese are important for sensory characteristic development. Table 3 shows that the saturated fatty acids accounted between 61 and 67%, and the unsaturated fatty acids accounted between 33 and 39% of the total fatty acid content of the cheese samples. A higher saturated fatty acid percentage was detected in the farmer cheese samples made from high-SCC milk (batch H). This could be attributed to the intensive hydrolytic and oxidation processes occurred in the fat matter of these cheeses. These data are in agreement with the results obtained on the acid and peroxide values of the test samples. Similar results were also reported by Chen *et al.* (2010) who found that semi-soft cheese made from high-SCC milk exhibited higher levels of lipolysis (based on the changes in the free fatty acid content) during ripening than that made from low-SCC milk. D'Amico and Donnely (2009) stated that the increased lipolytic activities in milk with the highest SCC may have contributed to more pronounced lipolysis. According to Auldist *et al.* (1996), cheddar cheeses made from high-SCC milk have been described as having a "lipolytic" or "oxidised" flavour. The authors also found that the milk used to prepare these cheeses had a higher concentration of free fatty acids which can induce rancidity in dairy products. Chen *et al.* (2010) concluded that lipolysis in cheeses was clearly dependent on the somatic cell counts of milk which may have important consequences for cheese flavour.

Conclusion

The results obtained in the present work showed that SCC of raw cow's milk had a significant effect on lipolysis during the ripening and cold storage of farmer cheese. Higher levels of lipolysis were detected in farmer cheese samples made from raw cow's milk with a high SCC. Increased concentrations of short-chain fatty acids such as caprylic and caproic acids, and a higher percentage of saturated fatty acids were found in farmer cheese samples produced from raw cow's milk with a high SCC. The intensive lipolysis during the aging and cold storage of farmer cheese made from raw cow's milk with higher SCC could thus lead to some quality defects and reduction in the shelf life of cheese. Therefore, the appropriate control of SCC in raw cow's milk is important for both cheese quality and storage considerations.

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