

Introduction

Buttermilk is the aqueous by-product of butter making, collected during the dairy cream churning. It is considered a high-value by-product due to its high content of milk fat globule membrane material. In cheesemaking it is mainly used to increase water retention and moisture content of reduced- and low-fat cheeses and improve their properties. To our knowledge, there are no research reports about the incorporation of buttermilk in sheep milk cheese manufacture.

The objective of the present work was to study the effect of the enrichment of reduced-fat sheep cheese milk with sweet buttermilk from sheep cream on semi-hard cheese manufactured from them.

Materials and Methods

CHEESEMAKING

The main elements of the applied technology were: moderate temperatures during cheesemaking, curd-washing, packaging at 6 days and ripening at two different temperatures.

- Three cheese types were manufactured from sheep milk in triplicate experiments, coded as follows:

A. full fat (FF) cheeses from cheese milk standardized to protein-to-fat ratio (P/F) 0.99 ± 0.051

B. reduced fat (RF) cheeses from cheese milk with P/F 2.09 ± 0.058

C. RF cheeses from cheese milk with P/F 2.00 ± 0.05 supplemented with lyophilized sweet sheep buttermilk (SSB), at 1% w/w and stirred for one hour before milk pasteurization

- For the manufacture of SSB, non-acidified sheep milk cream pasteurized at 85 °C for 5 min and kept overnight at <6 °C was churned.

- After pasteurization of milk at <68 °C and cooling to 32 °C, a commercial starter mixture was added to milk.

- Rennet was added to milk and curd formation took place at 32 °C.

- Curd cutting into 1-1.5 cubes was performed and followed by stirring at 32 °C for 15 min.

- Curd-washing removing and scalding at 33-38 °C for 30 min took place.

- Rectangular molds were used for curd shaping and a weight approximately equal to curd weight was used to press for 40 min.

- Fresh cheese was cut into pieces approximately 12 x 12 x 6 cm and 700-800 g weight and stayed at 11 °C overnight.

- Cheese pieces were salted in brine containing 22 % (w/v) NaCl and 0.3 % CaCl₂ at 11 °C for 1.5-2.1 h, depending on their size and weight.

- All cheeses remained at 11 °C for 6 days, with regular overturning, before packing in plastic film under vacuum.

- Half cheeses of each trial were ripened at 4 °C and coded as “4 °C”, while the other half remained at 11 °C and coded as “11 °C”- until 8 weeks after cheesemaking.

- Ripening was followed by storage of all cheeses at 4 °C until 16 weeks after cheesemaking.

- Whole cheese samples of 700-800 g were taken at 1, 4, 8 and 16 weeks.

MILK, CREAM, BUTTERMILK AND WHEY ANALYSES

- Fat content was determined according to the Gerber method (ISO/IDF, 2008).

- Gross composition of milk, buttermilk and whey was determined by Milkoscan-FT 120

- pH was estimated by a pHmeter.

CHEESE ANALYSES

- pH was estimated by a pHmeter

- Gross composition was determined by FoodScan-Dairy NIR

- Total solids content was determined according to IDF (2004).

- Salt content was determined according to IDF potentiometric titration method (2006a).

- Ash content was determined at 4 weeks according to IDF (2007).

- Total nitrogen and protein content were determined by means of the Kjeldahl method (ISO/IDF, 2014).

- Textural profile analysis was performed by means of the double bite test (Kaminariades & Stachtariades, 2000), using a Shimadzu Testing Instrument AGS-500 NG.

- Cheese color (L*, a*, b* values) was measured at 16 and 24 weeks by a Miniscan XE Chromameter.

- Meltability was evaluated by means of the Schreiber test (Park, Rosenau, & Peleg, 1984), with some modifications.

- Nitrogen fractions (WSN, TCA-SN) were assessed by means of the Kjeldahl method (ISO/IDF, 2014) according to Nega and Moatsou (2012).

- Cheese peptide profiling was studied by means of HPLC (Nega & Moatsou, 2012).

- Ca, Na, K and Mg contents were determined in the cheese ash fraction at 4 weeks by the atomic absorption spectrometric method and the P content by molecular absorption spectrometry (Zoidou, Plakas, Giannopoulou, Kotoula, & Moatsou, 2015).

- The extraction of cheese fat and the phospholipid quantification were carried out at 4 and 16 weeks as described by Gassi, Famelart, & Lopez (2008), with some modifications.

- The organoleptic evaluation of matured cheeses was performed at 8 and 16 weeks by a panel of six experienced laboratory staff members. The appearance, texture, and flavor evaluation was performed on a scale of 0 to 10 points and the total % organoleptic score was estimated by summing the appearance score, the texture score multiplied by 4 and the flavor score multiplied by 5).

- Statgraphics Centurion XVI was used for the statistical analysis (ANOVA, LSD method, multi-variable analysis).

Acknowledgments

We thank Mr. Theodoros Paschos for the technical assistance in the experimental cheesemakings.

References

- Ali, A. H. (2019). Current knowledge of buttermilk: Composition, applications in the food industry, nutritional and beneficial health characteristics. *International Journal of Dairy Technology*, 72(2), 169-182.
- Gassi, J. Y., Famelart, M. H., & Lopez, C. (2008). Heat treatment of cream affects the physicochemical properties of sweet buttermilk. *Dairy Science and Technology*, 88, 369-385.
- Hickey, C. D., O'Sullivan, M. G., Davis, J., Scholz, D., Kilcawley, K. N., Wilkinson, M. G., & Sheehan, J. J. (2018). The effect of buttermilk or buttermilk powder addition on functionality, textural, sensory and volatile characteristics of Cheddar-style cheese. *Food Research International*, 103, 468-477.
- ISO/IDF. (2004). Cheese and processed cheese – Determination of the total solids content (Reference method). IDF Standard No 4. Brussels, Belgium: International Dairy Federation.
- ISO/IDF. (2006a). Cheese and processed cheese products – Determination of chloride content – Potentiometric titration method. IDF Standard No 88. Brussels, Belgium: International Dairy Federation.
- ISO/IDF. (2006b). Milk – Determination of total phosphorus content – Method using molecular absorption spectrometry. IDF Standard No 42. Brussels, Belgium: International Dairy Federation.
- ISO/IDF. (2007). Milk and milk products – Determination of calcium, sodium, potassium and magnesium contents – Atomic absorption spectrometric method. IDF Standard No 119. Brussels, Belgium: International Dairy Federation.
- ISO/IDF. (2008). Milk-Determination of fat content. IDF Standard No 226. Brussels, Belgium: International Dairy Federation.
- ISO/IDF. (2010). Cheese and processed cheese products – Determination of total phosphorus content – Molecular absorption spectrometric method. IDF Standard No 33. Brussels, Belgium: International Dairy Federation.
- ISO/IDF. (2014). Milk and milk products – Determination of nitrogen content – Part 1: Kjeldahl principle and crude protein calculation. IDF Standard No 20-1. Brussels, Belgium: International Dairy Federation.
- Kaminariades, S., & Stachtariades, S. (2000). Production of processed cheese using: kasseri cheese and processed cheese analogues incorporating whey protein concentrate and soybean oil. *International Journal of Dairy Technology*, 53(2), 69-74.
- Konrad, G., Kleinschmidt, T., & Lorenz, C. (2013). Ultrafiltration of whey buttermilk to obtain a phospholipid concentrate. *International Dairy Journal*, 30, 39-44.
- McDowell, A. K. R. (1958). Phospholipids in New Zealand dairy products. *Journal of Dairy Science*, 25, 192-202.
- Nega, A., & Moatsou, G. (2012). Proteolysis and related enzymatic activities in ten Greek cheese varieties. *Dairy Science & Technology*, 92, 57-73.
- Park, J., Rosenau, J. R., & Peleg, M. (1984). Comparison of four procedures of cheese meltability evaluation. *Journal of Food Science*, 49, 1158-1162.
- Vanderghem, C., Bodson, P., Danthine, S., Paquot, M., Deroanne, C., & Blecker, C. (2010). Milk fat globule membrane and buttermilks: from composition to valorization. *Biotechnology, Agronomy, Society and Environment*, 14(3), 485-500.
- Zoidou, E., Plakas, N., Giannopoulou, D., Kotoula, M., & Moatsou, G. (2015). Effect of supplementation of brine with calcium on the Feta cheese ripening. *International Journal of Dairy Technology*, 68(3), 420-426.

Results and discussion

Foodscan	A (N=20)			B (N=20)			C (N=24)		
	Parameter	Value	Significance	Value	Significance	Value	Significance	Value	Significance
Foodscan	Fat	22.25 ± 0.146 ^a		12.44 ± 0.146 ^b		11.62 ± 0.131 ^c		25.39 ± 0.163 ^c	
	Protein	23.07 ± 0.182 ^a		26.60 ± 0.182 ^b		25.39 ± 0.163 ^c		55.06 ± 0.190 ^c	
	Moisture	48.95 ± 0.212 ^a		53.39 ± 0.212 ^b		55.06 ± 0.190 ^c		1.38 ± 0.044 ^b	
	Salt	1.28 ± 0.049 ^{ab}		1.20 ± 0.049 ^a		1.38 ± 0.044 ^b		2.44 ± 0.074 ^a	
	TSI	51.05 ± 0.212 ^a		46.61 ± 0.212 ^b		44.94 ± 0.190 ^c		2.586 ± 0.173 ^c	
	S/M	2.54 ± 0.082 ^a		2.21 ± 0.082 ^b		2.44 ± 0.074 ^a		2.19 ± 0.015 ^c	
	FDM	43.57 ± 0.193 ^a		26.67 ± 0.193 ^b		25.86 ± 0.173 ^c		1.99 ± 0.028 ^b	
	NFS	77.75 ± 0.146 ^a		87.56 ± 0.146 ^b		88.38 ± 0.131 ^c		4.96 ± 0.079 ^c	
	MNFS	62.95 ± 0.170 ^a		60.97 ± 0.170 ^b		62.30 ± 0.152 ^c		5.00 ± 0.029	
P/F	1.04 ± 0.016 ^a		2.14 ± 0.016 ^b		2.19 ± 0.015 ^c		4.96 ± 0.026		
Reference methods	Protein	19.94 ± 0.133 ^a		23.76 ± 0.133 ^a		23.21 ± 0.119 ^c			
	Moisture	50.47 ± 0.225 ^a		55.03 ± 0.225 ^b		56.68 ± 0.201 ^c			
	Salt	1.77 ± 0.031 ^a		1.82 ± 0.031 ^a		1.99 ± 0.028 ^b			
	S/M	3.38 ± 0.050 ^a		3.20 ± 0.050 ^b		3.38 ± 0.044 ^a			
	Ash	4.17 ± 0.088 ^a		4.70 ± 0.088 ^b		4.96 ± 0.079 ^c			
pH	4.95 ± 0.029		5.00 ± 0.029		4.96 ± 0.026				

PHYSICO-CHEMICAL PROPERTIES

- The P/F ratio was duplicated in the RF cheeses.
- The experimental factor of cheese milk type affected significantly (P<0.05) all the physicochemical parameters, except for pH.
- Moisture and moisture on non-fat substances (MNFS) were significantly higher (P<0.05) in C cheeses compared to B cheeses.

Table 2. Mean gross physicochemical composition (g 100g⁻¹) and properties of cheese types A (full fat), B (reduced fat) and C (reduced fat supplemented with lyophilized sweet sheep buttermilk) during 16 weeks of ripening/storage. TS: total solids; S/M: salt in moisture; FDM: fat on dry matter; NFS: non fat solids; MNFS: moisture on non-fat substances; P/F: protein-to-fat ratio.

TEXTURAL PROFILE ANALYSIS

- Hardness, adhesiveness and gumminess were significantly different (P<0.05) between B and C cheeses.
- Hardness, adhesiveness, gumminess and cohesiveness were significantly different (P<0.05) between 4 °C and 11 °C cheeses. The experimental factor of ripening temperature did not affect significantly (P<0.05) the cheese moisture, but there was a medium positive correlation among nitrogen fraction assessment results and textural characteristics, except for hardness.

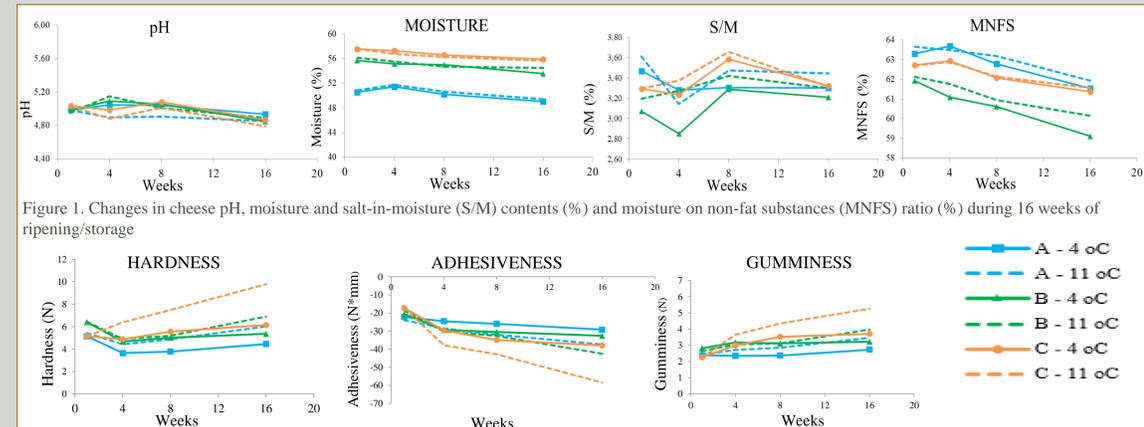


Figure 1. Changes in cheese pH, moisture and salt-in-moisture (S/M) contents (%) and moisture on non-fat substances (MNFS) ratio (%) during 16 weeks of ripening/storage

Figure 2. Changes in textural characteristics of cheeses (hardness, adhesiveness, gumminess) during 16 weeks of ripening/storage

COLOR

Regarding color parameters, C cheeses showed significantly higher L* and significantly lower b* values (P<0.05) than B cheeses and very close to A cheese values. As a result, they were more “bright” and less “yellow” than B cheeses, but their color was did not differ significantly from full fat cheese color.

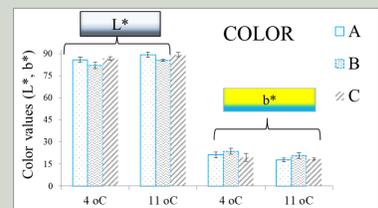


Figure 3. Effect of cheese type (A, B, C) and ripening temperature (4 °C, 11 °C) on cheese color (L* and b* values) at 16 weeks of ripening/storage

NITROGENOUS FRACTIONS

- Kjeldahl analysis of nitrogenous fractions showed that their values were significantly affected (P<0.05) by all the experimental factors.
- WSN/TN and TCA-SN/TN ratios were significantly higher in A and B cheeses compared to C cheeses.
- The above ratios were positively and strongly correlated to cheese age, showing that ripening promoted proteolysis in all cheeses.
- WSN/TN and TCA-SN/TN ratios were significantly higher in 11 °C cheeses compared to those ripened at 4 °C, indicating higher proteolysis rates at 11 °C ripening temperature.

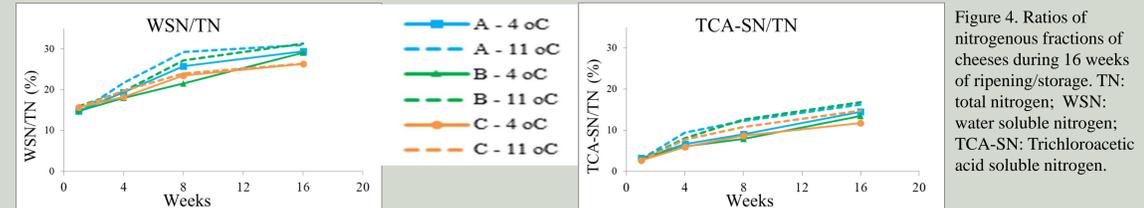


Figure 4. Ratios of nitrogenous fractions of cheeses during 16 weeks of ripening/storage. TN: total nitrogen; WSN: water soluble nitrogen; TCA-SN: Trichloroacetic acid soluble nitrogen.

PHOSPHOLIPID CONTENT

The cheese milk type was the only factor that affected the phospholipid content in fat, cheese and dry matter, which was significantly higher (P<0.05) in C cheeses

comparing to both A and B cheeses.

Parameter	Cheese milk type			Weeks of ripening/storage		Ripening temperature	
	A (N=10)	B (N=10)	C (N=12)	4 (N=16)	16 (N=16)	4 °C (N=18)	11 °C (N=14)
g PL 100g ⁻¹ fat	258 ± 21 ^a	384 ± 21 ^b	574 ± 19 ^c	417 ± 17	394 ± 17	423 ± 15	387 ± 18
g PL 100g ⁻¹ cheese	57 ± 3 ^a	47 ± 3 ^b	68 ± 3 ^c	59 ± 2	56 ± 2	59 ± 2	55 ± 3
g PL 100g ⁻¹ DM (dry)	112 ± 6 ^a	99 ± 6 ^b	151 ± 6 ^b	125 ± 5	116 ± 5	124 ± 5	117 ± 5

ORGANOLEPTIC EVALUATION

- Cheeses C exhibited significantly higher flavor scores compared to both A and B counterparts. Regarding the total organoleptic score, they exhibited higher –but not statistically significant- values than cheese B and significantly higher values compared to cheese A.
- Flavor and total organoleptic scores were significantly higher (P<0.05) in cheeses ripened at 4 °C.

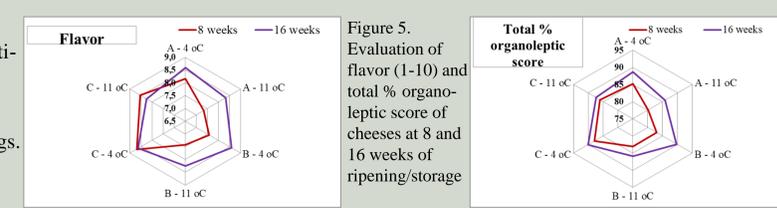


Figure 5. Evaluation of flavor (1-10) and total % organoleptic score of cheeses at 8 and 16 weeks of ripening/storage

Conclusions

- Compared to control full-fat cheese, the duplication of protein to fat ratio in the reduced fat cheeses did not affect their stability and organoleptic scores.
- The enrichment with SSB increased significantly (P<0.05) cheese moisture and MNFS of reduced fat cheese, without affecting pH.
- Textural analysis parameters of SSB reduced-fat cheese were significantly different (P<0.05) compared to reduced-fat counterpart.
- SSB addition elevated the phospholipid content and affected positively the cheese color parameters and organoleptic scores.
- Finally, the use of SSB combined with ripening under packaging and decrease of ripening temperature affected positively the characteristics of sheep milk semi-hard reduced-fat cheese