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# Use of sweet sheep buttermilk in the manufacture of reduced-fat sheep milk cheese

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ABSTRACT

Semi-hard cheeses were manufactured from sheep milk with protein to fat ratios of 1:1 (A) and 2:1 without (B) or with supplementation (C) with lyophilised sweet sheep buttermilk (SSB) and ripened at 4 or 11 °C. Comparing with B counterpart, the addition of SSB resulted in increased moisture and moisture in non-fat substances content of cheese C and retardation of secondary proteolysis. Phospholipid content of cheese C was 574, 68 or 151 mg 100 $g^{-1}$ fat, cheese or dry matter, respectively, being higher ($P < 0.05$) than those of A and B. SSB supplementation increased hardness and gumminess and decreased adhesiveness and meltability of the cheese. Colour values of cheese C were similar to those of A whereas its flavour score was higher than those of A and B. Overall, the SSB and the decrease of ripening temperature positively affected the characteristics of reduced-fat semi-hard sheep milk cheese.



## 1. Introduction

According to the Codex Alimentarius Commission (FAO/WHO, 2011), cheeses are characterised according to fat content in dry matter (FDM) as "medium fat" (40% > FDM content ≥ 25%), "partially skimmed" (25% > FDM content ≥ 10%) and "skim" (FDM content < 10%). According to the European Union/Regulation No 1924/2006 (EU, 2006), the use of the terms "low fat" (LF) corresponds to fat content ≤ 3% (w/w) and "reduced fat" (RF) to fat content reduction ≥ 30% (w/w). RF and LF cheeses differ from their "full-fat" (FF) counterparts in terms of yield, composition, ripening process, texture and flavour (Banks, 2004; Guinee & McSweeney, 2006; Mohamed, 2015). They are often less acceptable by consumers due to defects in sensory properties (deficient flavour, bitterness, excess acidity), textural properties (increased firmness, rubberiness and dryness, decreased cohesiveness) and functional properties (increased viscosity and melting time, decreased flowability). To overcome the aforementioned defects, various specific technological interventions have been suggested, such as homogenisation, ultrafiltration, pre-acidification, high temperature pasteurisation or supplementation with fat mimetics or substitutes. Moreover, peptidolytic adjunct cultures, curd washing, low to moderate scalding temperatures and/or modified salting conditions can be applied for the treatment of RF or LF cheese curd (Banks, 2004; Guinee & McSweeney, 2006; Mohamed, 2015).

In this respect, the fortification of cheese milk with buttermilk (BM) for the improvement of yield and properties of RF and LF cheese has been studied (Dewettinck et al., 2008; Hickey et al., 2018). BM is the liquid by-product collected during churning of cream in butter production with gross composition similar to that of skim milk due to the transfer of the water-soluble components of cream, i.e., proteins, lactose, minerals. Acid BM derives from biologically-acidified cream and sweet BM derives from non-acidified cream. Additionally, a large portion of milk fat globule membrane (MFGM) is released into BM, imparting significant functional and bioactive properties of great value for the food industry (Conway, Gauthier, & Pouliot, 2014; Dewettinck et al., 2008; Hickey et al., 2018). MFGM functionality is attributed to specific membrane proteins, mostly glycoproteins, which account for nearly 19% of total sweet BM proteins and to amphiphilic polar lipids, namely phospholipids (PL) and sphingolipids, which account for 1.2–2.1% of dry matter. BM exhibits high emulsifying, low foaming and high water-holding capacity (Britten, Lamothe, & Robitaille, 2008; Dewettinck et al., 2008; Hickey et al., 2018). Potential biofunctional properties of BM include promotion of nervous system functionality, lowering of cholesterol levels and antioxidant, antistress, antiinflammatory, anticancer, antibacterial and antirotavirus activities, while it is also a good source of minerals and vitamins (Bahrami, Ahmadi, Beigmohammadi, & Hosseini, 2015; Conway et al., 2014; Dewettinck et al., 2008).

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Sweet and acid BM have been utilised in many forms, i.e., liquid, condensed, ultra-filtered or powdered during cheesemaking of various cheese types and varieties. Sweet BM addition to cheese milk has been reported to increase water retention in curd (Mistry, Metzger, & Maubois, 1996) and moisture content in various cheese varieties (Bahrami et al., 2015; Borges et al., 2020; Govindasamy-Lucey et al., 2007; Govindasamy-Lucey, Lin, Jaeggi, Johnson, & Lucey, 2006; Hickey et al., 2017; Poduval & Mistry, 1999) or cheese models (Morin, Pouliot, & Britten, 2008). Similarly, sweet BM powder addition to curd increased the moisture content in mature cheeses (Hickey et al., 2017). Moreover, cheese milk supplementation with sweet BM also increased actual (Bahrami et al., 2015; Morin et al., 2008) and moisture-adjusted cheese yields (Govindasamy-Lucey et al, 2006, 2007; Mistry et al., 1996). Most researchers have concluded that sweet BM incorporation softens cheese (Bahrami et al., 2015; Borges et al., 2020; Govindasamy-Lucey et al, 2006, 2007; Hickey et al., 2018; Romeih, Moe, & Skeie, 2012; Skeie et al., 2013) and improves its textural characteristics (Mistry et al., 1996; Poduval & Mistry, 1999). However, the utilisation of BM increased firmness in processed cheeses (El Sayed et al., 2010; Raval & Mistry, 1999) and decreased sensory evaluation ratings for texture in FF cream cheese (Bahrami et al., 2015). Raval and Mistry (1999) reported better emulsification in processed cheeses by the use of BM. According to Romeih et al. (2012) fat globules were more homogeneously distributed in protein matrix in LF Cheddar when cheese milk was mixed with sweet BM powder. Furthermore, BM incorporation decreased free oil and meltability in cheeses (Govindasamy-Lucey et al, 2006, 2007; Poduval & Mistry, 1999; Raval & Mistry, 1999). There are contradictory findings for the effect of BM on cheese organoleptic properties (Bahrami et al., 2015; Borges et al., 2020; El Sayed et al., 2013; Govindasamy-Lucey et al., 2006, 2007; Hickey et al., 2018; Skeie et al., 2013).

To our knowledge, there are no research reports about the incorporation of BM in sheep milk (SM) cheese. SM differs from cow milk regarding fat content, fatty acid profile, milk fat globule (MFG) size and MFGM composition (Moatsou & Sakkas, 2019), all of which affect resulting BM and cheese. Publications on BM from the churning of SM cream are also scarce and most focused on biofunctionality (Hamad, Ismail, & El-Menawy, 2016; Parrón et al., 2017). Recently, a study of the technological potential of sweet sheep BM in various mixtures with FF, RF and LF SM has been published by Sakkas, Spiliopoulos, and Moatsou (2020). Considering all of the above along with the interest in cheese products with improved nutritional profile by the concomitant recycling of dairy by-products, the present study was undertaken. The objective was to study the effect of addition of lyophilised sweet sheep BM to RF sheep cheese milk on the properties and ripening course of semi-hard cheese made there from.

## 2. Materials and methods

### 2.1. Cheesemaking

Raw whole SM and skimmed SM were used for the standardisation of cheese milk. Three different cheeses were manufactured in triplicate within three consecutive weeks coded as follows: A, full-fat (FF) cheese; B, reduced-fat (RF) cheese; C, RF cheese supplemented with lyophilised sweet sheep buttermilk (SSB) at a level of 1% (w/w). The respective mean composition of cheese milk A, B and C was as follows: protein, 5.05 ± 0.08%, 5.09 ± 0.05% and 5.34 ± 0.06%, respectively; fat, 5.12 ± 0.18%, 2.44 ± 0.08% and 2.67 ± 0.04%, respectively; mean protein to fat (P/F) ratios, 0.99 ± 0.05, 2.09 ± 0.06 and 2.00 ± 0.05, respectively. For the manufacture of SSB, non-acidified SM cream pasteurised at 85 °C for 5 min and kept overnight at <6 °C was churned and the resulting BM was lyophilised. SSB was added to cheese milk C and stirred for 1 h before milk pasteurisation for sufficient hydration. Forty kg of standardised cheese milk pasteurised at 68 °C/10 min was used for each cheesemaking trial. After subsequent cooling to 32 °C, cheese milk was inoculated with a commercial starter mixture of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Streptococcus thermophilus*, *Lactobacillus helveticus* and *Lactobacillus lactis* at the level indicated by the manufacturer (CHOOSIT AlpD, Danisco, DuPont Nutrition & Biosciences, Copenhagen, Denmark). Four grams of rennet (Naturen Extra 1125 NB, 1115 IMCU $g^{-1}$, Chr. Hansen, Hørsholm, Denmark) per 100 kg milk was added and curd formation took place at 32 °C. Curd cutting into 1–1.5 cm cubes, stirring at 32 °C for 15 min, curd-washing by 25% whey removal and scalding at 33–38 °C for 30 min were performed. Curd was moulded in rectangular moulds and pressed for 40 min with a weight approximately equal to curd weight. Then, fresh cheese was cut into pieces of approximately 12 × 12 × 6 cm. After overnight hold at 11 °C, cheese pieces were salted in brine containing 22% (w/v) NaCl and 0.3% $CaCl_2$ at 11 °C for 1.86 ± 0.15 h; the estimation of salting time of each cheese piece was based on its exact dimensions. All cheeses were ripened at 11 °C for one week, turned over at regular intervals and then they were packed under vacuum in commercial polyamide/polyethylene film of low oxygen and moisture permeability appropriate for cheese. Half of the cheeses from each trial were ripened at 4 °C while the other half remained at 11 °C up to 8 weeks. After ripening, all the cheeses were stored at 4 °C up to 16 weeks. Cheese samples, i.e., a whole cheese weighing approximately 750 g, were taken at 1, 4, 8 and 16 weeks.

### 2.2. Milk, cream, BM and whey analyses

Fat content of milk and BM was determined according to the Gerber method (ISO/IDF, 2008) and fat content of cream was determined using a specially designed “Koehler” butyrometer and analysing a mixture of cream and distilled water at 1:1 ratio. Gross composition of milk, BM and whey was determined by Milkoscan-FT120 (Foss, Hillerød, Denmark) and pH by means of a pH meter.

### 2.3. Cheese analyses

#### 2.3.1. Physicochemical analyses

Cheese pH and composition were evaluated at all sampling time points. Cheeses were analysed for gross composition by FoodScan-Dairy Near Infrared (NIR) analyser (Foss). Total solid content of cheeses (TS) was determined in triplicate according to ISO/IDF (2004). Salt content of cheeses was determined in duplicate using potentiometric titration (ISO/IDF, 2006). Ash content of cheeses was determined at 4 weeks in quadruplicate according to ISO/IDF (2007). Total nitrogen (TN) and protein contents were determined at 1, 4, 8 and 16 weeks in triplicate by the Kjeldahl method (ISO/IDF, 2014).

#### 2.3.2. Proteolysis

Nitrogen fractions, WSN, water soluble nitrogen fraction and TCA-SN, 12% trichloroacetic acid soluble nitrogen fraction, were assessed at 1, 4, 8 and 16 weeks in duplicate by the Kjeldahl method (ISO/IDF, 2014). WSN was further analysed by reversed-phase high performance liquid chromatography (RP-HPLC) according to Nega and Moatsou (2012). Particular fractions of the RP-HPLC profiles of WSN were used as indices, considering that the main part of proteolysis products are roughly eluted as follows: (i) 10–40 min (f10–40), hydrophilic small-size peptides and most free amino acids, (ii) 40–70 min (f40–70), medium to small-size peptides and

primary proteolysis products such as plasmin- and residual chymosin-derived peptides, (iii) 70–100 min (f70–100), hydrophobic and/or large peptides and whey proteins (Nega & Moatsou, 2012). The N contents of WSN and 12% TCA-soluble fraction were expressed as percentages of TN of cheese, whereas the areas of parts of RP-HPLC profiles of WSN were expressed as percentages of the total profile area to overcome the impact of differences in the gross composition of cheese.

#### 2.3.3. Biofunctional properties

*2.3.3.1. Mineral fraction.* Calcium, sodium, potassium and magnesium contents in the cheese ash fraction at 4 weeks were determined by the atomic absorption spectrometric (AAS) method and the phosphorus content by molecular absorption spectrometry (MAS) as described by Zoidou, Plakas, Giannopoulou, Kotoula, and Moatsou (2015). In brief, the stock dilution for AAS analyses was 40 mg of cheese ash in one mL of 25% (v/v) $HNO_3$ made up to 100 mL with ultrapure water. Further dilutions of various quantities of the stock dilution were performed in ultrapure water after the addition of 10 mL of 1% (v/v) $LaCl_3$ and the determinations were done in duplicate. For the MAS method the stock dilution was 100 mg of cheese ash in 2.5 mL of HCl (36 g $L^{-1}$) made up to 100 mL with ultrapure water. Two millilitres of a molybdate/ascorbic acid solution was added to the final dilution and boiling in a water bath for 15 min and subsequent cooling were carried out prior to spectrometric determination.

*2.3.3.2. Phospholipid fraction.* The extraction of cheese fat and the PL quantification were carried out at 4 and 16 weeks in triplicate as described by Gassi, Famelart, and Lopez (2008), with some modifications. In brief, 12 mL of distilled water were added to 3 g of cheese and the mixture was vortexed for 3 min. Afterwards, 7.5 mL of hexane were added, vortexed for 1 min, 5 mL of isopropanol were added, the final mixture was vortexed for 1 min and centrifuged at 1000×*g* for 5 min at 20 °C. The clear upper layer was aspirated and re-extraction of the bottom layer was performed. The two collected upper layers were combined and organic solvent was removed under vacuum at 50 °C for 15 min using an IKA RV 10 rotary evaporator (IKA-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) and holding for 1 h at 102 °C in an oven. Finally, determination of P content in the extracted fat was carried out by digestion of fat in Kjeldahl flasks and molecular absorption spectrometry using a UV/VIS Spectrometer Perkin Elmer Lambda 20 (Gassi et al., 2008; ISO/IDF, 2010). The PL content in cheese fat was estimated by multiplying the P content in fat by 25.44 (Konrad, Kleinschmidt, & Lorenz, 2013).

#### 2.3.4. Textural and physical properties

The parameters of texture profile analysis were estimated by the first and second bite force–distance curves (Gunasekaran & Ak, 2003), using a Shimadzu Testing Instrument AGS-500 NG (Shimadzu Corporation, Kyoto, Japan) at 15 °C. Cheese colour (L*, a*, b* values) was measured at 16 weeks in duplicate by a Miniscan XE Chromameter (Hunterlab, Reston, USA). Meltability was evaluated at 4, 8 and 16 weeks in duplicate by means of the Schreiber test as described by Park, Rosenau, and Peleg (1984), with some modifications. Briefly, cheeses were sliced into discs of 0.5 cm height and 4.1 cm diameter. Discs were placed on an aluminium foil sheet in an oven and heated at 200 °C for 5 min. After cooling for 30 min at room temperature, cheese expansion was measured through six equidistant lines starting from cheese disc centre and mean cheese expansion was calculated. A scale of 0–10 units was used for cheese meltability evaluation; 1 cm of mean cheese expansion corresponded to 2 meltability units.

#### 2.3.5. Organoleptic evaluation

The organoleptic evaluation of matured cheeses was performed by a modified scoring method (ISO/IDF, 1997) at 8 and 16 weeks by a panel of six experienced laboratory staff members. Randomly presented cheeses were evaluated for appearance, texture, and flavour on a scale of 0–10 points. The total % organoleptic score was estimated by summing the appearance score, the texture score multiplied by 4 and the flavour score multiplied by 5.

### 2.4. Statistical analysis

Statgraphics Centurion XVI (Manugistics, Inc., Rockville, MA, USA) was used for the statistical analysis of the data. The effects of cheese milk type, ripening temperature and ripening/storage time and their interactions were assessed by multifactor analysis of variance (ANOVA). LSD method and a significance value of 0.05 were utilised for testing the significant differences between means.

## 3. Results and discussion

### 3.1. Cheese composition

Higher fat loss into whey from cheese C (0.91 ± 0.16%) as compared with B, was observed (0.42 ± 0.08%). Higher fat losses in cheese whey when cheese milk was fortified with BM were also reported by Mistry et al. (1996) for RF Cheddar and by Morin et al. (2008) for model cheeses. According to the former, the increase of fat loss is attributed to the higher numbers of small MFG in BM-fortified cheese, while Morin et al. (2008) suggested that fat loss was enhanced by the softening of paracasein network due to BM addition. The potential of heat-denatured WP and the PL of the added BM to associate with CN micelles (Govindasamy-Lucey et al., 2006) could result in a less continuous and a weaker paracasein network and in a subsequent higher fat loss in cheese whey.

Composition of cheeses (Table 1) was affected only by the cheese milk type and cheese age. The evolution of moisture and pH are shown in detail in Fig. 1. RF cheese B can be considered as control for SSB-supplemented RF cheese C. The fat content of cheese C was lower compared with B confirming thus the higher fat content of the respective whey as mentioned above.

Moisture content of cheese C was higher compared with that of B (Table 1; Fig. 1). According to our previous findings, syneresis of RF SM rennet curds decreased significantly by the addition of either thermalised or pasteurised sweet cream-derived BM (Sakkas et al., 2020). Similar effect of sweet BM has been reported for cow milk cheeses attributed to the complexation of BM heat-denatured WP with casein (CN) micelles, the association of BM-originated phospholipids (PL) with proteins and the high water-binding ability of denatured WP and PL (Govindasamy-Lucey et al., 2006; Hickey et al., 2017). Moisture in non-fat substances (MNFS) content of cheese C was also higher than that of B and similar to that of A. The increase of MNFS of cheese C is advantageous, since it is suggested as a tool for the improvement of the total quality of RF and LF cheeses (Guinee & McSweeney, 2006).

### 3.2. Proteolysis

All the experimental factors affected the proteolysis indices and significant effects of their interactions were also observed as shown in Table 2. In particular, interaction between cheese age and milk type (A, B, C) was significant for the WSN/TN evolution whereas the interaction between cheese age and ripening temperature affected TCA-SN/TN and RP-HPLC f40–70 and f70–100.

WSN/TN and TCA-SN/TN in all cheese types increased during ripening and storage and the increase was higher for cheeses

**Table 1**
Gross physicochemical composition of cheese types A (full fat), B (reduced fat) and C (reduced fat supplemented with lyophilised sweet sheep buttermilk) during 16 weeks of ripening/storage.[a]

| Cheese | Temperature (°C) | Weeks | | | |
|---|---|---|---|---|---|
| | | 1 | 4 | 8 | 16 |
| Fat | | | | | |
| A | 4 | 22.09 ± 0.991$^{B}$ | 21.64 ± 1.482$^{B}$ | 22.53 ± 0.436$^{C}$ | 23.11 ± 0.990$^{B}$ |
| | 11 | | 21.62 ± 0.559$^{B}$ | 22.50 ± 0.665$^{B}$ | 22.96 ± 0.290$^{B}$ |
| B | 4 | 12.41 ± 0.930$^{A}$ | 12.19 ± 0.683$^{A}$ | 12.49 ± 0.621$^{B}$ | 12.97 ± 0.888$^{A}$ |
| | 11 | | 12.05 ± 0.460$^{A}$ | 12.74 ± 0.226$^{A}$ | 12.76 ± 0.622$^{A}$ |
| C | 4 | 11.38 ± 0.522$^{A}$ | 11.34 ± 0.546$^{A}$ | 11.43 ± 0.157$^{A}$ | 11.62 ± 0.441$^{A}$ |
| | 11 | | 11.68 ± 0.521$^{A}$ | 11.82 ± 0.429$^{A}$ | 12.34 ± 0.348$^{A}$ |
| BvC | | | | * | * |
| Moisture in non-fat substances (MNFS) | | | | | |
| A | 4 | 63.31 ± 0.729$^{b}$ | 63.68 ± 1.020$^{b,B}$ | 62.79 ± 0.712$^{a,b,B}$ | 61.55 ± 1.074$^{a,B}$ |
| | 11 | | 63.49 ± 0.001$^{b,B}$ | 63.18 ± 0.423$^{b,C}$ | 61.94 ± 0.040$^{a,B}$ |
| B | 4 | 61.93 ± 0.688$^{b}$ | 61.08 ± 0.576$^{b,A}$ | 60.62 ± 1.189$^{a,b,A}$ | 59.11 ± 1.145$^{a,A}$ |
| | 11 | | 61.77 ± 0.869$^{A}$ | 60.94 ± 0.385$^{A}$ | 60.14 ± 0.706$^{A}$ |
| C | 4 | 62.72 ± 1.184 | 62.94 ± 0.985$^{B}$ | 62.08 ± 0.291$^{a,B}$ | 61.37 ± 0.162$^{B}$ |
| | 11 | | 62.89 ± 0.190$^{A,B}$ | 62.13 ± 0.224$^{B}$ | 61.58 ± 0.719$^{A,B}$ |
| BvC | | | * | * | * |
| Protein | | | | | |
| A | 4 | 19.90 ± 0.321$^{A}$ | 19.51 ± 0.376$^{A}$ | 20.27 ± 0.492$^{A}$ | 20.46 ± 0.428$^{A}$ |
| | 11 | | 19.37 ± 0.044$^{A}$ | 20.10 ± 0.775$^{A}$ | 20.12 ± 0.044$^{A}$ |
| B | 4 | 23.28 ± 0.478$^{a,B}$ | 23.60 ± 0.607$^{a,b,B}$ | 25.08 ± 0.503$^{b,C}$ | 23.64 ± 1.283$^{a,b,B}$ |
| | 11 | | 23.30 ± 0.100$^{a,B}$ | 23.75 ± 0.309$^{a,b,B}$ | 24.40 ± 0.238$^{b,C}$ |
| C | 4 | 22.62 ± 0.185$^{B}$ | 22.90 ± 0.662$^{B}$ | 23.34 ± 0.840$^{B}$ | 23.85 ± 0.575$^{B}$ |
| | 11 | | 23.05 ± 0.706$^{B}$ | 23.75 ± 0.712$^{B}$ | 23.54 ± 0.379$^{B}$ |
| BvC | * | | | | |
| Salt | | | | | |
| A | 4 | 1.82 ± 0.154 | 1.74 ± 0.092$^{A,B}$ | 1.71 ± 0.087$^{A}$ | 1.67 ± 0.098 |
| | 11 | | 1.68 ± 0.103 | 1.82 ± 0.274 | 1.76 ± 0.184 |
| B | 4 | 1.76 ± 0.164 | 1.62 ± 0.135$^{A}$ | 1.87 ± 0.151$^{A,B}$ | 1.78 ± 0.190 |
| | 11 | | 1.88 ± 0.035 | 1.94 ± 0.117 | 1.86 ± 0.224 |
| C | 4 | 1.96 ± 0.076 | 1.91 ± 0.110$^{B}$ | 2.10 ± 0.141$^{B}$ | 1.92 ± 0.141 |
| | 11 | | 1.98 ± 0.161 | 2.14 ± 0.182 | 1.91 ± 0.152 |
| BvC | * | | * | * | |
| Moisture | | | | | |
| A | 4 | 50.53 ± 0.615$^{a,b,A}$ | 51.43 ± 1.669$^{A}$ | 50.16 ± 0.981$^{A}$ | 49.05 ± 1.336$^{A}$ |
| | 11 | | 51.76 ± 0.874$^{a,A}$ | 50.62 ± 0.183$^{a,b,A}$ | 49.40 ± 0.100$^{b,A}$ |
| B | 4 | 55.69 ± 0.999$^{B}$ | 55.16 ± 0.948$^{B}$ | 55.01 ± 1.002$^{B}$ | 53.53 ± 1.290$^{B}$ |
| | 11 | | 55.51 ± 0.002$^{B}$ | 54.72 ± 0.467$^{B}$ | 54.48 ± 0.656$^{B}$ |
| C | 4 | 57.56 ± 1.510$^{B}$ | 57.23 ± 1.714$^{B}$ | 56.55 ± 0.595$^{B}$ | 55.87 ± 0.950$^{B}$ |
| | 11 | | 56.74 ± 0.827$^{B}$ | 56.29 ± 0.625$^{C}$ | 55.66 ± 0.713$^{B}$ |
| BvC | * | | * | * | * |
| Salt-in-moisture (S/M) | | | | | |
| A | 4 | 3.47 ± 0.255 | 3.28 ± 0.070$^{B}$ | 3.31 ± 0.144 | 3.30 ± 0.205 |
| | 11 | | 3.14 ± 0.135 | 3.47 ± 0.516 | 3.44 ± 0.354 |
| B | 4 | 3.07 ± 0.227 | 2.85 ± 0.184$^{A}$ | 3.29 ± 0.202 | 3.21 ± 0.264 |
| | 11 | | 3.27 ± 0.059 | 3.42 ± 0.171 | 3.30 ± 0.346 |
| C | 4 | 3.29 ± 0.182 | 3.23 ± 0.196$^{B}$ | 3.58 ± 0.200 | 3.32 ± 0.198 |
| | 11 | | 3.37 ± 0.242 | 3.66 ± 0.305 | 3.31 ± 0.242 |
| BvC | | | | | |
| Ash | | | | | |
| A | 4 | | 4.23 ± 0.200$^{A}$ | | |
| | 11 | | 4.11 ± 0.105$^{A}$ | | |
| B | 4 | | 4.65 ± 0.091$^{B}$ | | |
| | 11 | | 4.75 ± 0.355$^{B}$ | | |
| C | 4 | | 5.04 ± 0.200$^{C}$ | | |
| | 11 | | 5.04 ± 0.175$^{B}$ | | |
| BvC | | | * | | |

[a] Fat and moisture in non-fat substances (MNFS) were estimated by Foodscan. Values (g 100 g$^{-1}$) are means; values followed by superscript lowercase and uppercase letters are significantly different by LSD ($P < 0.05$) between weeks under the same ripening conditions and between cheese types under the same ripening conditions for the same week, respectively, and an asterisk indicates a significant difference ($P < 0.05$) between cheese types B and C for the same week.

ripened at 11 °C compared with those ripened at 4 °C. The WSN/TN, TCA-SN/TN and TCA-SN/WSN ratios were higher in cheeses A and B compared with those in C indicating that SSB supplementation retarded proteolysis. This finding contradicted the higher MNFS content of cheese C compared with that of B. The increase of MNFS promotes proteolysis through upraising free water availability for microbial and enzymatic activity (Guinee & McSweeney, 2006). The higher S/M content of cheese C could have counteracted the effect of MNFS. In addition, the MNFS content correlated negatively (r = −0.411 to −0.609) with the level of all the nitrogen fractions. Govindasamy-Lucey et al. (2006, 2007) found similar TCA-SN contents in moisture-adjusted sweet BM-fortified and control pizza cheeses from partially skim milk that was attributed to the standardised starter and rennet to CN ratios. In accordance to our findings, Hickey et al. (2017) reported a lower pH 4.6-SN by the addition of sweet BM powder and lower free amino acid levels

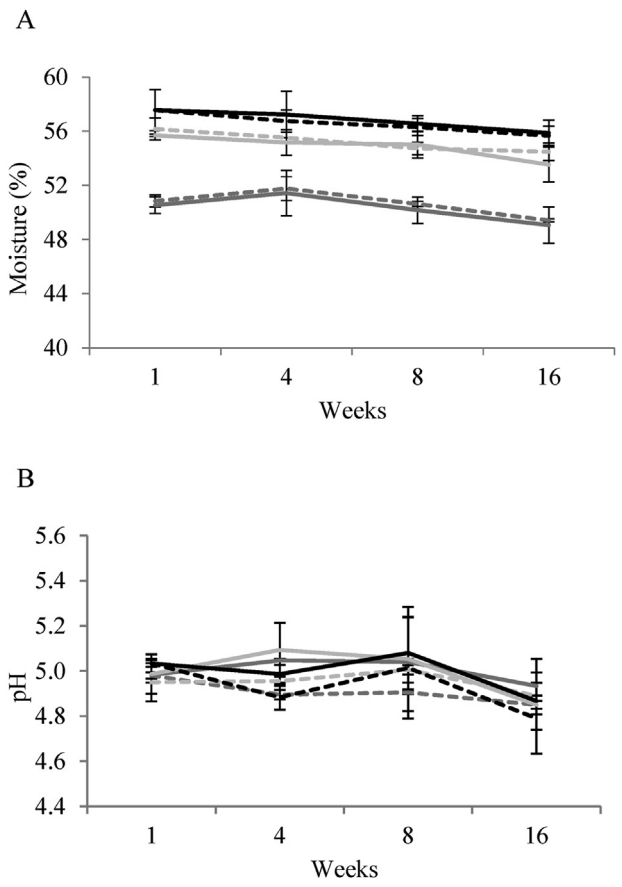


**Fig. 1.** Changes in cheese (A) pH and (B) moisture content (g 100 $g^{-1}$) during 16 weeks of ripening/storage (4 °C and 11 °C): mid-grey lines, full fat cheese; light grey lines, reduced fat cheese; dark grey lines, reduced fat cheese supplemented with lyophilised sweet sheep buttermilk; solid lines, 4 °C; dashed lines, 11 °C.

when the highest (i.e., 10%, w/w) level of BM was used in FF Cheddar.

Interestingly, these phenomena were not observed when liquid BM was utilised. They suggested that the lower extent of primary proteolysis could result from the interactions between curd CN and MFGM material, from the impaired access of the proteolytic enzymes to BM casein due to insufficient rehydration of BM powder or from the hindering of chymosin activity due to higher pH in the 10% BM powder supplemented cheese. In our experiments, pH was not changed by SSB addition and the supplementation of cheese milk with SSB was followed by 1 h stirring prior to pasteurisation for proper hydration. The effect of an association between curd paracasein and MFGM material on primary proteolysis could be a possible explanation for our findings since SSB was manufactured from cream that had undergone a rather severe treatment at 85 °C for 5 min. Subsequently, the lower availability of initial proteolysis products combined with the higher S/M content in cheese C compared with that of B is expected to hinder secondary proteolysis reactions (Hickey et al., 2017), expressed as TCA-SN/TN and TCA-SN/WSN levels. An additional possibility is that the high antioxidant properties of buttermilk (Conway et al., 2014; Wong & Kitts, 2003) might have indirectly affected the biochemical pathways of secondary proteolysis by modifying the redox state in cheese micro-environment.

Significant effect of ripening temperature on the evolution of the fractions in RP-HPLC profiles was rather sporadically observed, i.e., ripening temperature affected f40–70 and f70–100. Moreover, the effect of milk type was significant on the nitrogenous fractions in RP-HPLC f40–70, which was strongly correlated with the N content of the soluble nitrogenous fraction. Finally, the increase of cheese age promoted the accumulation of soluble components in f40–70 and f10–40 and the decrease of the hydrophobic nitrogen fractions in f70–100.

A comparison of proteolysis indices of SSB-supplemented cheese C with the RF counterpart B was carried out using multifactor ANOVA of the indices, excluding the data for FF cheese A. The cheese milk type affected the TCA-SN/TN and f10–40 and f40–70. TCA-SN/TN and nitrogenous fractions in RP-HPLC f10–40 were significantly higher in cheese B, nitrogen fractions in RP-HPLC f40–70 were higher in cheese C and fractions in f70–100 did not differ. Therefore, secondary proteolysis in SSB-supplemented cheese was retarded compared with non-supplemented counterpart B.

### 3.3. Minerals and phospholipids

The cheese milk type affected all minerals, except for potassium content (Fig. 2). Cheese C had higher sodium and magnesium contents compared with B; calcium and phosphorus did not differ significantly. The increase of magnesium in cheese C can be attributed to the supplementation with SSB.

The cheese milk type was the only factor that affected the PL content in fat, cheese and dry matter (Table 3). Opposite to our results, Law, Sharpe, Chapman, and Reiter (1973) reported 50–70% decrease in PL content of FF and LF BM-fortified Cheddar after six months of ripening. The PL content in fat, cheese and dry matter was higher in cheese C than in both A and B apparently due to the abundance of PL in the BM. The PL content of the SSB utilised in the present study was 570 mg 100 $g^{-1}$ powder. Similar findings have been reported by other researchers (El Sayed et al., 2010; Hickey et al., 2017; Morin et al., 2008). The range of PL content in cheese A (181–374 mg 100 $g^{-1}$ fat) was close to the range reported by Rombaut, Dewettinck, and Van Camp (2007) for polar lipid content of Gouda at eight weeks and 36 months and of Cheddar (400–500 mg 100 $g^{-1}$ fat). Moreover, the range of PL content in cheese B (333–467 mg 100 $g^{-1}$ fat) was similar to that of Gouda light at eight weeks estimated as 400 mg 100 $g^{-1}$ fat by the same group.

The PL content of cheese A was approximately 20% higher compared with that of B counterpart, much lower than the nearly two-fold higher fat content of the former. This contradiction was also depicted in the much higher PL on fat content of cheese B compared with that of FF counterpart A indicating that the fat content is not the only determinant factor for the PL content of cheeses. Two phenomena that take place during centrifugal cream separation can contribute to the high ratio of PL on fat of cheese B, which was manufactured from a mixture of SM with defatted SM at 1:1 ratio. Firstly, the transfer of more than 50% of polar lipids of raw milk into skimmed milk (Britten et al., 2008; Rombaut, Van Camp, & Dewettinck, 2006) and secondly the high amount of small-sized MFG in the lipid fraction of skimmed milk, which are related with more MFGM surface per g fat (Logan et al., 2017; Walstra, Wouters, & Guerts, 2006). Moreover, as mentioned above, the reduced fat of cheese B resulted in a more compact paracasein network comparing with A. Consequently, cheese B could retain more easily the small MFG during syneresis compared with A.

### 3.4. Textural and physical properties

The experimental factors affected cheese hardness, adhesiveness and gumminess, shown in Fig. 3. In general, the average changes of textural parameters were more prominent between one and four weeks. Statistical analysis showed moderate or weak

**Table 2**
Proteolysis indices (mean value ± standard deviation) of cheese types A (full fat), B (reduced fat) and C (reduced fat supplemented with lyophilised sweet sheep buttermilk) during 16 weeks of ripening/storage.[a]

| Cheese | Temperature (°C) | Weeks | | | |
|---|---|---|---|---|---|
| | | 1 | 4 | 8 | 16 |
| % WSN/TN | | | | | |
| A | 4 | 14.65 ± 0.968$^{a}$ | 19.36 ± 1.296$^{b}$ | 25.74 ± 0.960$^{c,B}$ | 29.36 ± 2.061$^{d}$ |
| | 11 | | 21.69 ± 1.103$^{b}$ | 29.25 ± 0.516$^{c}$* | 30.94 ± 0.453$^{c,B}$ |
| B | 4 | 15.23 ± 1.911$^{a}$ | 17.92 ± 1.128$^{b}$ | 21.57 ± 0.214$^{c,A}$ | 29.04 ± 2.131$^{d}$ |
| | 11 | | 19.58 ± 0.849$^{b}$ | 27.17 ± 1.223$^{c}$* | 31.30 ± 1.174$^{d,B}$ |
| C | 4 | 15.67 ± 0.886$^{a}$ | 18.15 ± 1.348$^{a}$ | 23.50 ± 1.743$^{b,A,B}$ | 26.38 ± 1.200$^{c}$ |
| | 11 | | 19.76 ± 1.971$^{b}$ | 24.03 ± 2.377$^{c}$ | 26.43 ± 0.993$^{c,A}$ |
| % TCA-SN/TN | | | | | |
| A | 4 | 3.19 ± 0.318$^{a}$ | 6.68 ± 0.306$^{b,B}$ | 9.08 ± 1.024$^{c}$ | 14.48 ± 0.884$^{d,B}$ |
| | 11 | | 9.38 ± 0.091$^{b,B}$* | 12.28 ± 0.297$^{c}$* | 16.25 ± 1.980$^{d}$ |
| B | 4 | 3.12 ± 0.404$^{a}$ | 6.09 ± 0.282$^{b,A}$ | 7.84 ± 0.467$^{b}$ | 13.47 ± 2.079$^{c,A,B}$ |
| | 11 | | 8.11 ± 0.883$^{b,A,B}$* | 12.58 ± 0.742$^{c}$* | 16.25 ± 1.980$^{d}$ |
| C | 4 | 2.71 ± 0.386$^{a}$ | 5.92 ± 0.225$^{b,A}$ | 8.69 ± 0.855$^{c}$ | 11.74 ± 0.650$^{d,A}$ |
| | 11 | | 7.70 ± 0.299$^{b,B}$* | 10.75 ± 1.744$^{b}$ | 16.25 ± 1.980$^{d}$ |
| RP-HPLC 10–40 min (% area on total profile area) | | | | | |
| A | 4 | 7.49 ± 0.776$^{a}$ | 8.84 ± 1.099$^{a}$ | 9.36 ± 1.304$^{a}$ | 12.06 ± 2.796$^{b}$ |
| | 11 | | 8.76 ± 0.382$^{a}$ | 9.08 ± 0.997$^{a,b}$ | 10.50 ± 0.410$^{b}$ |
| B | 4 | 7.71 ± 0.431$^{a}$ | 8.83 ± 0.292$^{b}$ | 8.87 ± 0.842$^{b}$ | 9.93 ± 0.707$^{c}$ |
| | 11 | | 7.99 ± 0.453$^{a}$* | 8.89 ± 0.679$^{a,b}$ | 10.45 ± 0.650$^{b}$ |
| C | 4 | 7.17 ± 0.269$^{a}$ | 7.76 ± 0.641$^{a,b}$ | 8.22 ± 0.730$^{a,b}$ | 9.04 ± 1.054$^{b}$ |
| | 11 | | 7.80 ± 0.722$^{a,b}$ | 8.52 ± 1.153$^{a,b}$ | 9.49 ± 1.686$^{b}$ |
| RP-HPLC 40–70 min (% area on total profile area) | | | | | |
| A | 4 | 23.64 ± 0.078$^{a,A}$ | 33.08 ± 3.447$^{b}$ | 38.08 ± 3.026$^{c}$ | 40.12 ± 0.911$^{c,A}$ |
| | 11 | | 38.23 ± 0.106$^{b}$ | 41.19 ± 0.276$^{c}$ | 42.16 ± 1.258$^{c}$ |
| B | 4 | 23.45 ± 0.676$^{a,A}$ | 35.02 ± 1.275$^{b}$ | 39.42 ± 2.033$^{c}$ | 41.74 ± 1.364$^{d,A}$ |
| | 11 | | 40.00 ± 0.106$^{b}$ | 42.83 ± 0.686$^{c}$ | 41.37 ± 1.216$^{b,c}$ |
| C | 4 | 24.68 ± 0.746$^{a,B}$ | 36.25 ± 0.999$^{b}$ | 41.04 ± 0.240$^{c}$ | 43.74 ± 0.960$^{d,B}$ |
| | 11 | | 39.74 ± 1.565$^{b}$ | 43.43 ± 2.250$^{c}$ | 44.14 ± 2.532$^{c}$ |
| RP-HPLC 70–100 min (% area on total profile area) | | | | | |
| A | 4 | 54.88 ± 1.011$^{a}$ | 46.64 ± 1.350$^{b}$ | 43.21 ± 0.866$^{c}$ | 38.69 ± 2.835$^{d}$ |
| | 11 | | 42.64 ± 0.721$^{b}$* | 41.02 ± 0.049$^{c}$* | 39.45 ± 0.021$^{d,A}$ |
| B | 4 | 55.63 ± 2.587$^{a}$ | 44.81 ± 0.913$^{b}$ | 42.15 ± 0.796$^{c}$ | 39.18 ± 1.152$^{d}$ |
| | 11 | | 42.96 ± 0.863$^{a}$ | 40.79 ± 0.417$^{a}$ | 40.06 ± 0.467$^{a,B}$ |
| C | 4 | 56.03 ± 1.158$^{a}$ | 44.95 ± 1.556$^{b}$ | 42.15 ± 1.641$^{c}$ | 38.80 ± 0.374$^{d}$ |
| | 11 | | 42.22 ± 1.923$^{b}$ | 40.01 ± 2.349$^{b,c}$ | 37.46 ± 0.734$^{c,B}$* |

[a] Abbreviations are: WSN, water soluble nitrogen fraction; TN, total nitrogen fraction; TCA-SN, 12% trichloroacetic acid soluble nitrogen fraction; RP-HPLC 10–40 min, 40–70 min, 70–100 min, parts of RP-HPLC profiles of WSN, expressed as % area on total profile area. Values followed by superscript lowercase and uppercase letters are significantly different by LSD ($P < 0.05$) between weeks under the same ripening conditions and between cheese types under the same ripening conditions for the same week, respectively, and an asterisk indicates a significant difference ($P < 0.05$) between ripening conditions for the same week.

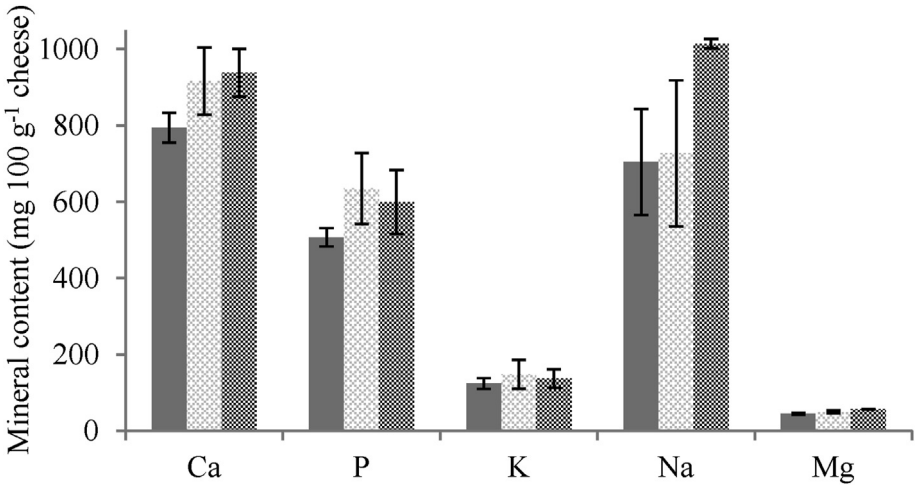


**Fig. 2.** Mineral content of cheeses (mg 100 g$^{-1}$) at 4 weeks of ripening/storage: ■, full fat cheese; ▒, reduced fat cheese; ▓, reduced fat cheese supplemented with lyophilised sweet sheep buttermilk.

correlations between cheese composition and textural characteristics with coefficients (r) ranging from −0.413 to 0.429 ($P < 0.05$). On the other hand, there was strong correlation (r = −0.829 to 0.839, $P < 0.05$) between proteolysis indices and textural parameters except for hardness. Interestingly, the increase of proteolysis level caused either by the ripening time or the elevation of ripening

**Table 3**
Effect of cheese milk type, age and ripening temperature on the phospholipid content of cheese types A (full fat), B (reduced fat) and C (reduced fat supplemented with lyophilised sweet sheep buttermilk) during 16 weeks of ripening/storage.[a]

| Factors | | PL/fat | PL/cheese | PL/DM |
|---|---|---|---|---|
| Cheese milk type | A | 258[a] | 57[b] | 112[a] |
| | B | 384[b] | 47[a] | 99[a] |
| | C | 574[c] | 68[c] | 151[b] |
| | SE | 21 | 3 | 6 |
| | BvC | * | * | * |
| Cheese age | 4 | 417 | 59 | 125 |
| | 16 | 394 | 56 | 116 |
| | SE | 17 | 2 | 5 |
| Ripening temperature | 4 °C | 423 | 59 | 124 |
| | 11 °C | 387 | 55 | 117 |
| | SE | 18 | 3 | 5 |

[a] Abbreviations are: PL, phospholipids; DM, dry matter. Values are mg PL100 $g^{-1}$ fat, cheese or cheese DM; values within the parts of columns that correspond to each experimental factor with different superscript letters are significantly different ($P < 0.05$) and an asterisk denotes significant differences between B and C ($P < 0.05$).

temperature from 4 to 11 °C did not soften cheese. According to Lawrence, Creamer, and Gilles (1987) the lower the ratio of moisture to casein the firmer will be the casein matrix of cheese. In the present study, the ratios of moisture to intact casein at 1, 4, 8 and 16 weeks were 2.99 ± 0.09, 3.27 ± 0.13, 3.33 ± 0.12 and 3.40 ± 0.24, respectively, for cheese A ripened at 4 °C and 2.99 ± 0.09, 3.41 ± 0.00, 3.57 ± 0.18 and 3.56 ± 0.04, respectively, for cheese A ripened at 11 °C. The same ratios for cheese C at 1, 4, 8 and 16 weeks were 3.02 ± 0.12, 3.05 ± 0.13, 3.17 ± 0.17 and 3.19 ± 0.16, respectively, for cheese ripened at 4 °C and 3.02 ± 0.12, 3.07 ± 0.09, 3.13 ± 0.19 and 3.21 ± 0.10, respectively, for cheese ripened at 11 °C. Regarding cheeses A and C, ANOVA showed that there was no significant effect of ripening time within 4–16 weeks and of ripening temperature on this ratio. The ratios of moisture to intact casein at 1, 4, 8 and 16 weeks were 2.85 ± 0.15, 2.85 ± 0.07, 2.80 ± 0.11 and 3.20 ± 0.32, respectively, for cheese B ripened at 4 °C and 2.85 ± 0.15, 2.97 ± 0.02, 3.17 ± 0.12 and 3.25 ± 0.13, respectively, for cheese B ripened at 11 °C. Regarding cheese B, ripening temperature affected this ratio only at 8 weeks and the effect of time was significant from 8 to 16 weeks for cheese ripened at 4 °C and from 4 to 8 weeks for cheese ripened at 11 °C.

Despite the differences in proteolysis and hardness between cheeses ripened at 4 °C and 11 °C, ripening temperature had no significant effects on their compositional parameters. Lawrence et al. (1987) also reported that the increase of storage temperature of Emmental cheese increased proteolysis and increased the firmness and shortness of the cheese body. On average, cheese hardness decreased at four weeks and increased at 16 weeks of ripening. According to Gunasekaran and Ak (2003), there is a similar trend in Cheddar-type cheese, thus softening in the first two weeks and hardening afterwards, while Soodam, Ong, Powell, Kentish, and Grass (2017) reported softening of Cheddar in the first 98 days and its hardening after that point. In both cases the assumption was that the hardening of cheese during the second period might be a proteolysis side-effect, because of a decrease in water activity due to released charged amino and carboxylic acid groups competing for the available water.

Composition parameters in our study differentiated in cheeses A and B through the ripening period and could be linked to their age-related differences in hardness, as it is well known that higher moisture and lower protein contents are related to lower firmness in cheeses (Banks, 2004; Borges et al., 2020; Gunasekaran & Ak, 2003). Moisture and MNFS contents decreased from four to 16 weeks and from eight to 16 weeks, respectively, in cheese A ripened at 11 °C, MNFS content decreased from four to 16 weeks in cheeses

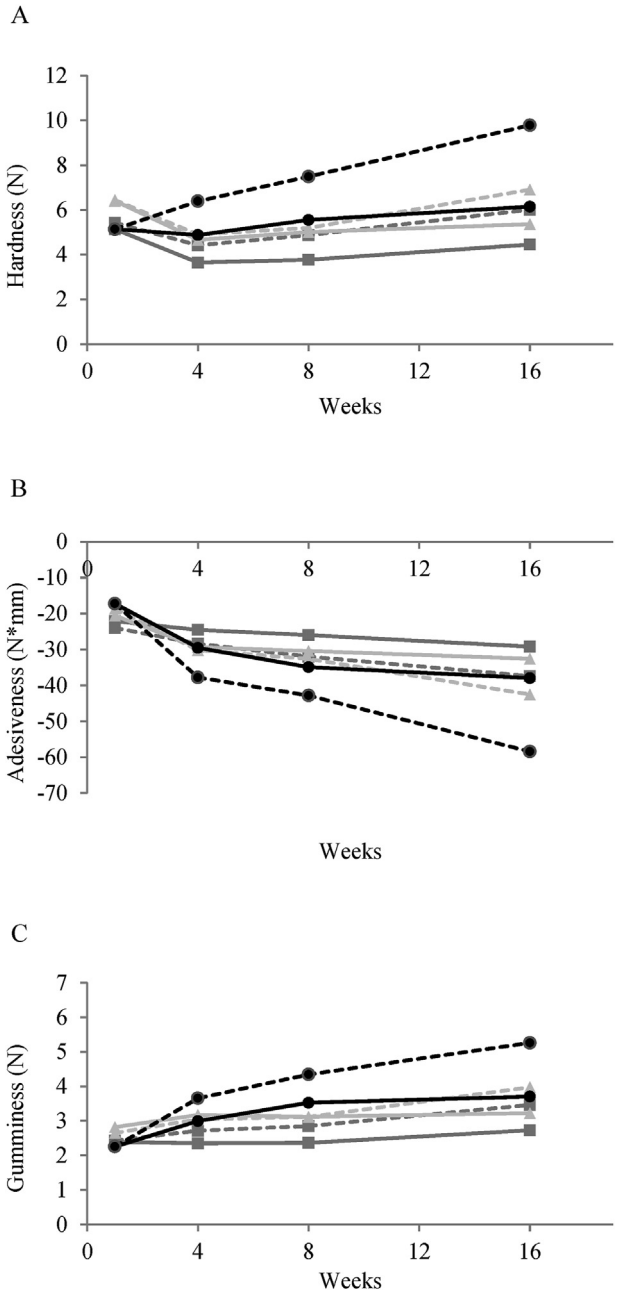


**Fig. 3.** Changes in texture properties of cheeses (A, hardness; B, adhesiveness; C, gumminess) during 16 weeks of ripening/storage (4 °C and 11 °C): ■, full fat cheese; ▲, reduced fat cheese; ●, reduced fat cheese supplemented with lyophilised sweet sheep buttermilk; solid lines, 4 °C; dashed lines 11 °C.

A and B ripened at 4 °C and protein content increased from one to eight weeks and from four to 16 weeks in cheese B ripened at 4 °C and 11 °C, respectively. However, there were no significant effects of time on moisture content, other compositional parameters or pH of cheese C ripened at 4 °C or 11 °C. Taking into account that proteolysis has little effect on cheese texture when moisture content and pH remain unchanged (Walstra, Vouters, & Geurts, 2006), the increase in hardness of cheese C during ripening at 11 °C may be associated with the nature of the protein matrix, which is of great importance for the development of cheese texture (Gunasekaran & Ak, 2003). The addition of BM enriched cheese C with high amounts of PL, which possess great emulsifying properties and could affect

cheese firmness (Hickey, Auty, Wilkinson, & Sheehan, 2015). The high amounts of PL combined with the high abundance of small size of MFG in defatted SM, as previously mentioned, could enhance emulsification phenomena in cheese, thus increasing its hardness.

The supplementation of cheese milk C with SSB resulted in higher hardness, gumminess and lower adhesiveness compared with control counterpart B. Adhesiveness is the work necessary to overcome the attractive forces between the surface of the food and surface of other materials with which the food comes in contact, while gumminess is the energy needed to disintegrate a semisolid food until it is ready for swallowing. Both these parameters are masticatory properties perceived during chewing (Gunasekaran & Ak, 2003). Addition of sweet BM could soften cheese through increase in water retention and subsequent increase of moisture content, which make cheese microstructure more porous (Hickey et al., 2018) or/and through the lubricating action of the incorporated MFGM fragments into the protein matrix (Romeih et al., 2012). However, this effect has not been always confirmed. Hickey et al. (2018) did not find any change in Cheddar cheese firmness by the addition of sweet BM powder to curd. Raval and Mistry (1999) reported increase in processed cheese hardness by the addition of ultra-filtered sweet BM, while El Sayed et al. (2010) observed increased hardness when acid BM precipitate was added to processed cheese. Apart from fat and moisture, calcium is also associated with cheese hardness (Hickey et al., 2015; Mohamed, 2015). The calcium content expressed in cheese dry matter was higher in cheese C (21.8 ± 2.70 mg $g^{-1}$) compared with that of cheese B (20.2 ± 1.69 mg $g^{-1}$). Furthermore, the higher hardness of cheese C is also in accordance with its higher S/M and salt contents compared with that of cheese B. The increase of S/M content retards proteolysis and promotes aggregation and subsequent inaccessibility of β-CN by chymosin (Lawrence et al., 1987). Moreover, salt enhances the emulsification of fat thus increasing cheese firmness (Gunasekaran & Ak, 2003).

As mentioned previously, textural parameters other than hardness were affected strongly (r = −0.829 to 0.839) by the experimental factors and proteolysis. Cohesiveness was affected by cheese age and ripening temperature, being higher after week 4 and ripening at 4 °C. Cheese milk type and ripening temperature did not affect elasticity and chewiness, which decreased at week 4, remaining unchanged thereafter. Cohesiveness and gumminess correlated positively with WSN/TN, TCA-SN/TN and RP-HPLC f40−70 and negatively with RP-HPLC f70−100. The positive correlation of cohesiveness with RP-HPLC f10−40 indicated that the degradation of CN resulted in a more uniform distribution of CN particles in the cheese matrix. Gumminess followed the same trend as it is interrelated to cohesiveness, being the product of cohesiveness over hardness. Adhesiveness, elasticity and chewiness were correlated negatively with WSN/TN, TCA-SN/TN, RP-HPLC f10−40 min and f40−70 min parts and positively with RP-HPLC f70−100 min part. Therefore, the degradation of CN impeded cheese elasticity, chewiness that is the product of elasticity over gumminess and adhesiveness. Cohesiveness was not affected by the addition of BM while gumminess increased. The latter is the product of hardness over cohesiveness and its increase was due to the respective increase in hardness.

In general, fat reduction decreased cheese meltability (Fig. 4). According to Banks (2004), the reduction of free oil that is liberated during heating and the high para-casein percentage of RF and LF cheeses result in poor flowability. The cheese age did not affect meltability, while cheeses ripened at 4 °C exhibited higher values in comparison with 11 °C counterparts. Meltability values were lower in cheese C compared with B, that is, the supplementation with SSB induced a further significant decrease of this property. This finding is consistent with the findings of most relevant studies (e.g., Govindasamy-Lucey et al., 2006; Poduval & Mistry, 1999; Raval & Mistry, 1999). There are many possible explanations for the lower meltability of cheese C compared with counterpart B. It can be related to the effect of SSB on the paracasein matrix, that is, cross-linking of MFGM material and of heat-denatured WP in BM with paracasein (Govindasamy-Lucey et al., 2006; Hickey et al., 2018; Raval & Mistry, 1999). The increased moisture content caused by SSB supplementation can also impair cheese meltability (Govindasamy-Lucey et al., 2006; Poduval & Mistry, 1999; Raval & Mistry, 1999), while SSB fatty acids apparently do not affect meltability due to their low melting points (Poduval & Mistry, 1999).

Although cheese meltability has been associated to proteolysis, the statistical analysis did not reveal strong correlations between our meltability and proteolysis results; the former was positively correlated with RP-HPLC f10−40 (r = 0.398) and negatively with RP-HPLC f40−70 min (r = −0.418). In fact, previous reports are not consistent. Guinee, Auty, and Fenelon (2000) and Guinee, Feeney, Auty, and Fox (2002) reported that the increase of cheese soluble

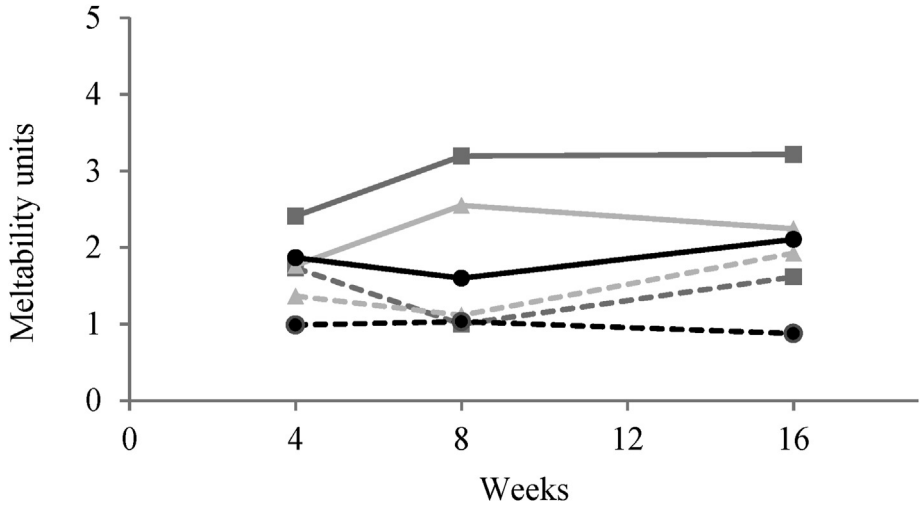


**Fig. 4.** Changes in meltability of cheeses during 16 weeks of ripening/storage (4 °C and 11 °C): ■, full fat cheese; ▲, reduced fat cheese; ●, reduced fat cheese supplemented with lyophilised sweet sheep buttermilk; solid lines, 4 °C; dashed lines 11 °C.

nitrogen or calcium content are related to low flowability while Hickey et al. (2018) associated meltability reduction with the lower extent of primary proteolysis in BM-fortified cheese. Interestingly, similar flowability has been estimated for different cheese varieties with soluble nitrogen ranging from 2.9 to 17.2% of total N (Guinee, Pudja, Miocinovic, Wiley & Mullins, 2015). Considering all the above, we suggest that along with the lower secondary proteolysis of cheese C and the changes in the paracasein structure caused by the SSB addition, also the calcium content should be considered as factors for the lower flowability of cheese C. Calcium contents of cheese A, B and C, were significantly differentiated from each other while no other experimental factor exhibited a significant effect.

The increase of ripening temperature increased the brightness/lightness L* values of cheeses and decreased the yellowness b*. Cheese C exhibited higher L* and lower b* values than RF counterpart B but very close to that of A. Therefore, the SSB addition in RF cheese resulted in a colour more similar to FF counterpart. Since lightness is related to the number and size of particles, the above-mentioned complexation or incorporation of SSB components or particles in the paracasein matrix should be considered. The aforementioned cross-linking of inserted MFGM material and heat-denatured WP with paracasein increase the size of particles and density fluctuations in the paracasein matrix, increasing thus the light scattering (Pastorino, Dave, Oberg, & McMahon, 2002). El Sayed et al. (2010) detected differences in cheese colour by the use of high levels (i.e., 30%) of BM concentrate in the manufacture of processed cheese spread, while Borges et al. (2020) did not detect significant differences in cheese colour by the addition of ultra-filtrated BM in RF cheese.

### 3.5. Organoleptic evaluation

All cheeses were acceptable regarding appearance, flavour and texture and obtained a score higher than 80% (Fig. 5). After eight weeks, bitter, metallic taste and sticky texture in cheese were reported, which were not detected at 16 weeks. Scores were higher at 16 weeks in terms of texture, flavour and total scores than those at eight weeks. Flavour and total organoleptic scores of cheeses at eight and 16 weeks were higher in cheeses ripened at 4 °C than those of cheeses ripened at 11 °C.

RF cheese B did not differ in sensory properties from its FF counterpart (A); therefore, fat reduction did not change organoleptic acceptance. Cheese C exhibited higher flavour scores compared with both A and B counterparts. MFGM enzymes and components originated from SSB, in combination with moisture binding, could have contributed to proteolysis and lipolysis and acted as substrates for starter bacteria, thus resulting in flavour improvement (Dewettinck et al., 2008). Additionally, Dewettinck et al. (2008) have reported that the enlarged MFGM surface provided by SSB addition can increase the contact area of cheese fat with mouth receptors and thus facilitates the absorption of aroma substances.

Cheese C had higher total organoleptic score than cheese A. The retardation of proteolysis observed when ripening took place at 4 °C coincided with improvement of the organoleptic score. Nevertheless, the flavour scores were not significantly correlated with proteolysis indices. Cheese ripening is a complicated phenomenon in which several biochemical reactions take place, i.e., glycolysis, lipolysis and proteolysis, as well as interactions and catabolism of their final products. Ripening at higher temperature will accelerate all the reactions involved, including non-desired reactions yielding unpleasant or non-balanced flavour (Fox & Cogan, 2004). Additionally, the differentiated textural properties between cheeses ripened at 4 °C and 11 °C could have an effect on the perception of cheese flavour in mouth, since the flavour perception is related to the cheese consistency (Walstra et al., 2006) and the release rate of flavour substances through mastication (Banks, 2004).

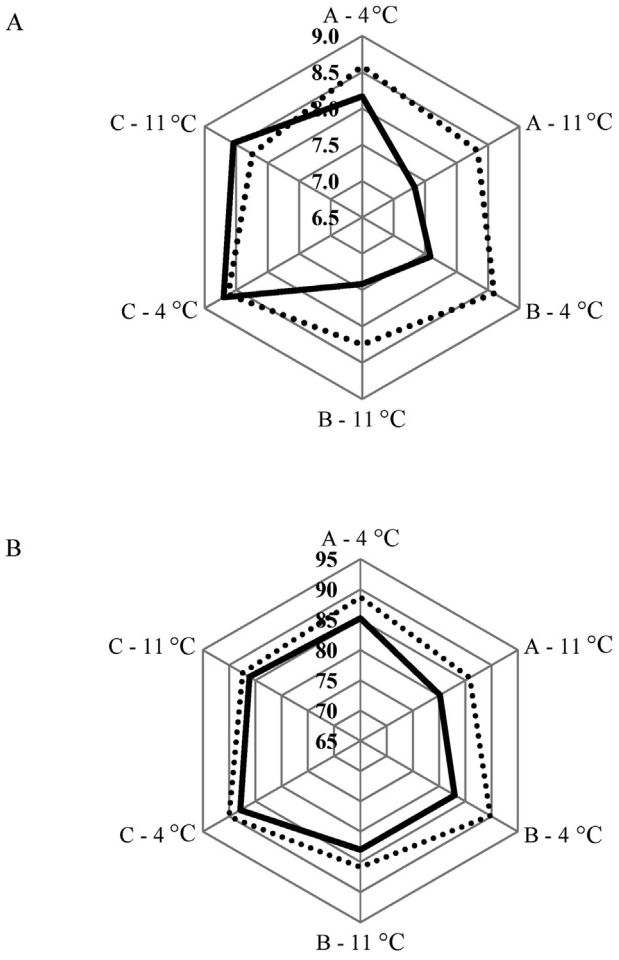


**Fig. 5.** Evaluation of (A) flavour (1–10) and (B) total % organoleptic score of cheeses at 8 weeks (solid line) and 16 weeks (dotted line) of ripening/storage at 4 °C and 11 °C: A, full fat cheese; B, reduced fat cheese; C, reduced fat cheese supplemented with lyophilised sweet sheep buttermilk.

Appearance score was lower in cheese C compared with those of A and B and it was correlated negatively ($r = -0.503$) with colour parameter of "lightness" L*. Borges et al. (2020) observed improved sensory attributes in BM-supplemented RF cheese when compared with the RF counterpart and similar sensory scores when compared with the FF counterpart. Flavour improvement and increase in overall acceptability were reported by El Sayed et al. (2010) after addition of up to 30% (w/w) BM concentrate in processed cheese spread. However, other researchers have reported flavour deterioration by the addition of sweet BM in LF (Skeie et al., 2013) and FF cheeses (Bahrami et al., 2015; Hickey et al., 2018).

## 4. Conclusions

The current study investigated the effect of the enrichment of reduced-fat (RF) sheep cheese milk with sweet sheep buttermilk (SSB) on the resultant semi-hard cheese. SSB powder addition in cheese milk at a level of 1% improved particular properties of RF cheese in comparison with non-supplemented counterpart. This intervention can be used for the development of sheep milk cheeses with improved nutritional and functional characteristics (e.g., reduced fat, enhanced PL concentration, controlled

meltability) in the context of circular, sustainable cheese-making practices.

Further research is needed to investigate the sheep buttermilk preparation and detailed composition, the fortification conditions of sheep cheese milk and the optimum cheesemaking and ripening steps.

## Credit author statement

**Lambros Sakkas**: Formal analysis, Investigation, Writing-Original draft preparation.

**Efrosini Alatini**: Formal analysis, Investigation.

**Golfo Moatsou**: Conceptualization, Methodology, Resources, Writing-Reviewing & Editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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