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Characterisation of biochemical changes during ripening in Argentinean sheep cheeses

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ABSTRACT

In recent years, there has been an increase in the production of sheep milk in our country, which has been used mainly in cheese-making. This production, however, is not well standardised, as there are neither defined protocols nor well-characterised products for this procedure. Previously, different methodologies for making two types of sheep cheeses were developed at our institute. In the present work, the levels of free amino acids, organic acids, free fatty acids and volatile compounds for these sheep cheeses were studied in order to thoroughly characterise them. Therefore, we have produced two types of cheeses: one using a starter of *Streptococcus thermophilus*, in which the curd was cut into 5-mm pieces, washed and then heated to 43 °C (S cheeses); the other one using a mixed starter composed of *S. thermophilus*, *Lactobacillus helveticus* and *Lactobacillus bulgaricus*, in which the curd was cut into smaller pieces, was not washed and was heated to 47 °C (L cheeses). These cheeses were analysed at 2 and 180 days of ripening. Free amino acids (FAAs) and organic acids were studied by HPLC, free fatty acids (FFAs) were quantified by GC and volatile compounds were analysed by SPME-GC-FID/MS. The concentrations of all FAAs were significantly higher in the L cheeses than in the S cheeses, and this was evident from the beginning of the ripening. Both types of cheeses showed similar changes in the concentrations of some FAAs during ripening, but S cheeses were characterised by higher percentages of Phe, Leu and Val, while L cheeses had higher percentages of Pro, Ile, His and Asp. Lactic and citric acid were the most important organic acids present in both types of cheeses. At the end of the ripening, L cheeses presented higher levels of succinic and formic acids, while S cheeses showed a much higher amount of acetic acid. The levels of FFAs increased during ripening, and myristic, palmitic, stearic and oleic acids were the most abundant ones in both types of cheeses. L cheeses showed significantly higher levels of all FFAs at the end of the ripening and presented a greater increase in the percentages of short-chain fatty acids during ripening compared to S cheeses. Regarding volatile compounds, higher levels of aldehydes and ketones characterised the L cheeses, whereas S cheeses had higher proportions of esters and alcohols. Both types of cheeses presented similar areas in the most compounds of acids group, but the levels of butanoic and hexanoic acids were significantly higher in S cheeses than in L cheeses. The results of the present work offer an important contribution to this field: they have provided a better understanding of the changes that occur during the ripening of the two sheep cheeses manufactured with technologies developed in our group. These technologies could also be used by small sheep farm producers located in our region with the aim of increasing the economic yield of their products.

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1. Introduction

The highest production of sheep milk and sheep dairy products is concentrated in the Mediterranean region. Some of the most popular sheep cheeses around the world are Roquefort (France), Feta and Teleme (Greece), Pecorino (Italy), Idiazabal and Manchego (Spain) and Serra da Estrela (Portugal) (Kalantzopoulos, 1993). These cheeses, together with other less popular ones, have previously been characterised in different studies (Raynal-Ljutovac et al., 2008).

However, sheep cheese production in Argentina has not been developed much until now, as sheep have been primarily used for their wool and meat. As a result, sheep cheeses are produced in only a few plants distributed mostly in the provinces of Buenos Aires and Chubut (Mc Cormick and Lynch, 2003). In Santa Fe, the province in which our Instituto de Lactología Industrial is located, the production of sheep cheese is almost nonexistent, but there are some sheep farms that are seeking another way to expand their production possibilities and take advantage of all of their available resources. Therefore, sheep cheese production seems to be a good opportunity. The Escuela de Agricultura, Ganadería y Granja (EAGyG), a secondary school that works in association with our University, has a small sheep herd, which had not been previously exploited for cheese production. Therefore, because we have a lot of experience in cow cheese production, we faced the challenge of developing the technology for sheep cheese production by setting up a small plant at the EAGyG. This small plant would also set an example to other sheep farms of how profitable cheese production could be for them too.

At our Institute, some of the well-known technologies and starters were adapted for the cheese-making of two types of sheep cheeses: S cheeses with a starter of *Streptococcus thermophilus* and L cheeses with a starter of a mixture of *S. thermophilus*, *Lactobacillus helveticus* and *Lactobacillus bulgaricus* (Candioti et al., 2010). Gross composition, pH and proteolysis of these cheeses have been described in a previous work (Candioti et al., 2010). S and L cheeses presented differences regarding pH and secondary proteolysis (soluble fraction in trichloroacetic acid 12% and phosphotungstic acid 2.5%); L cheeses had lower pH and showed more proteolysis than S cheeses. Moreover, a great diminution of the lactobacilli in the starter was observed in L cheeses during ripening, and these cheeses were characterised by a more intense flavour than S cheeses. It is important to mention that the milk used for this study was from Pampinta ewes, a native breed from Frisia and Corriedale, which was developed by the Instituto Nacional de Tecnología Agropecuaria (INTA), Anguil, La Pampa, Argentina, an institute that has extensive expertise in the management of sheep herds. By means of this study, we obtained valuable information for an initial characterisation of Argentinean sheep cheeses, as there were no data previously published about them.

Important biochemical events such as proteolysis, lipolysis and glycolysis occur in the cheese matrix during ripening, which are responsible for the final characteristics of the taste, aroma and texture of the product. These enzymatic processes must occur in a coordinated way in order to give a unique and well-appreciated flavour characteristic to each type of cheese (Fox and McSweeney, 2004). The FAAs and FFAs produced during proteolysis and lipolysis may contribute directly to the cheese flavour, but their most important contribution is made indirectly, via the production of substrates for further catabolic reactions in which large amounts of volatile compounds are produced (Marilley and Casey, 2004).

In the present work, we describe in detail the characterisation of two different Argentinean sheep cheeses by studying the changes in free amino acids, organic acids, free fatty acids and volatile compounds during ripening.

2. Materials and methods

2.1. Cheese-making

Raw sheep milk, provided by INTA (Instituto Nacional de Tecnología Agropecuaria) (Anguil, La Pampa, Argentina) was refrigerated and transported at 4 °C to the pilot plant of Instituto de Lactología Industrial (INLAIN) during all of the sheep milking period (October to March). On each cheese-making day, 40 L of sheep milk was batch pasteurised at 65 °C for 20 min, and cooled down to 36 °C. Then calcium chloride (Merck, Darmstadt, Germany) was added to a final concentration of 0.02% (w/v). After that, the milk was divided into two aliquots of 20 L each, one destined for the making of S cheeses and the other for L cheeses. A lyophilised culture of *S. thermophilus* (Chr. Hansen) was added as the primary starter for S cheeses to reach a final concentration of 10^6 CFU mL^{-1} in the cheese milk. Then, 15 min later, 0.014 g L^{-1} of chymosin (Maxiren® 150, France) was added, and when the curd reached the appropriate strength, this batch was cut into 5-mm pieces. The curd was washed with hot water, which replaced 10% of the whey. After that, the mixture was heated to 43 °C, and later, the curd was finally moulded.

For L cheeses, a mix of lyophilised cultures of *S. thermophilus* (60%), *L. helveticus* (20%) and *L. bulgaricus* (20%) (all of Chr. Hansen) was used as the primary starter and was added to reach a final concentration of 10^6 CFU mL^{-1}. This cheese-making process differed from the one used for S cheeses in the following ways: the curd was not washed and was cut into smaller pieces, and, finally, the heat treatment was at 47 °C.

Both S and L cheeses were pressed during 18 h, brined in 20% w/v, pH 5.4 brine for 7 h. Cheeses were ripened at 12 °C and at 80% relative humidity for 6 months. They were sampled at the beginning of the ripening (at 2 days) and at 180 days of ripening. Four cheese replicates were made on different fabrication days, with different milk obtained throughout the whole sheep milking period. In addition, on each fabrication day, four cheeses of approximately 700 g each for each type of cheese were made, using the same milk, that is, four S cheeses and four L cheeses. On each sampling day, a different cheese of each type (S and L cheeses) was sampled, and approximately 100 g of cheese was taken, finely grated and frozen (−20 °C) for the analysis of free amino acids, free fatty acids and organic acids. For the analysis of volatile compounds, the cheese samples were sliced as wedges, wrapped in aluminium foil and stored at −20 °C until analysis.

2.2. Free amino acids

A pre-column derivatization method using 6-aminoquinolyl-N-hydroxy-succinimidyl carbamate (AQC) followed by high-performance liquid chromatography was used for the FAAs analysis in cheese samples. For that, the Chemistry Package of the Waters AccQ·Tag® Amino Analysis Method (Waters Corporation, Mildford, MA, USA) was employed, which comprises the reagent kit for the derivatization reaction, the column, a mixture of amino acid standard, sample tubes and the eluents. The HPLC equipment consisted of a quaternary pump, an on-line degasser and UV/VIS detector, all Series 200 (Perkin Elmer, Norwalk, CT, USA). An interface module connected to a computer was used for acquisition of chromatographic data with the software Turbochrom® (Perkin Elmer, Norwalk, CT, USA). A 3.9 mm × 150 mm Nova-Pak™ C_{18}, 4 μm column (Waters Corporation, Mildford, MA, USA) specifically certified for use with the AccQ·Tag Method and a 15 mm × 3.2 mm, 7 μm guard column (Perkin Elmer, Norwalk, CT, USA) were used. Sample preparation, derivatization reaction and chromatographic separation were performed according to Bergamini et al. (2009).

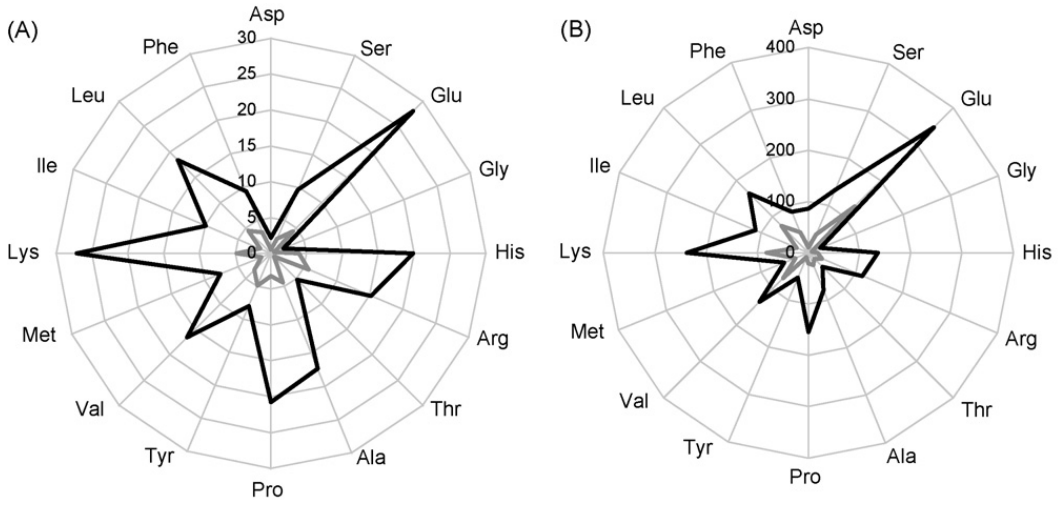

Fig. 1. Levels of free amino acids (mg/100 g) in S (——) and L (– –) cheeses at 2 (A) and 180 (B) days of ripening. Values are means of four cheese replicates manufactured in different days with different milk.

2.3. Organic acids

Organic acids were analysed by high-performance liquid chromatography (HPLC). The HPLC equipment was the same used for free amino acids analysis, previously described.

Analysis was performed isocratically at 65 °C with 10 mM H_2SO_4 and at a flow rate of 0.6 mL min^{-1} on a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm, Hercules, CA, USA). Water-soluble extracts of the cheeses were obtained by blending 5 g of cheese and 15 mL of distilled water with mortar and pestle, then warmed up to 40 °C and maintained for 1 h. The suspension was centrifuged at 3000 × *g* – 30 min and filtered through fast flow filter paper. The filtered solution was adjusted to a final volume of 25 mL. Samples were filtered through 0.45 μm membranes (Millex, Millipore, São Paulo, Brazil), and 60 μL was injected into the HPLC chromatograph. Detection was performed at 210 nm (Zeppa et al., 2001).

2.4. Free fatty acids

Extraction of cheeses lipids, isolation of FFAs, derivatization to ethylic esters, and determination of their concentrations by gas–liquid chromatography were performed according to Perotti et al. (2005) with some modifications. A Perkin Elmer model GC-9000 series gas chromatograph (Perkin Elmer Corp. Waltham, MA, USA) equipped with a flame ionization detector (FID), and with a split/splitless injector was used. The FFAs were separated on a fused-silica capillary column (PE-Wax, 60 m × 0.25 mm) coated with a bonded polyethylene glycol stationary phase (0.25 μm layer thickness). The injector temperature was 250 °C, with manual injection system and the split ratio was fixed at 1:50. After a 4 min hold at 75 °C, the oven temperature was programmed from 75 to 150 °C at a rate of 10 °C/min, held at 150 °C for 3 min, and then increased to 230 °C at a rate of 10 °C/min and held at 230 °C for 15 min. The FID temperature was 300 °C. The flow rate of the carrier gas (nitrogen) was 3 mL min^{-1}. The quantification ($C_{6:0}$ to $C_{18:2}$) was performed using the internal standardisation technique, with enantic ($C_{7:0}$) and margaric acids ($C_{17:0}$) (Sigma–Aldrich, St. Louis, USA) as internal standards added to the cheese sample at the extraction step. The FID outsignal was recorded and the chromatograms were processed using Turbochrom v. 4. Software (Perkin Elmer Corp. Waltham, MA, USA). The results were expressed as μmol of FFAs per g of cheese.

2.5. Volatile compounds

Prior to the volatile analysis, the samples were cut in cubes and finely grated and homogenised using a 600 W food processor.

A SPME holder manual equipped with a 1 cm × 50/30 μm Stable-Flex DVB/CAR/PDMS (Supelco, Bellafonte, PA, USA) was used to isolate and concentrate the volatile compounds. Five grams of grated cheese was weighed in a 30 mL glass vial and hermetically sealed with aluminium seal and butylteflon septa. The vials were thermostatised at 40 ± 1 °C for 10 min and then the fiber was exposed to the headspace for 15 min. Volatile compounds adsorbed on the fiber were immediately thermally desorbed in the injector port of a GC at 250 °C for 5 min (splitless mode).

A GC-FID (Perkin Elmer Model 9000) coupled to a flame ionization detector (FID) was used to obtain information of peak areas (arbitrary units) and compare the profile of different cheeses. Separation was carried out with the same column employed in the free fatty acid analyses (PE-Wax). The column temperature was programmed as follows: 45 °C (4 min), 5 °C/min to 150 °C (3 min), 10 °C/min to 250 °C (5 min). Nitrogen was used as carrier gas.

A GC coupled to an ion trap mass spectrometer (GC–MS Shimadzu QP-5000) was used to identify the compounds under the same chromatographic conditions and with the use of the same column as the CG-FID analysis. Mass spectra were obtained with 70 eV electron impact ionization (EI mode). The mass range used was 42–300 *m*/*z* (scan rate 250 amu/seg). The identification of volatile compounds was performed by matching mass spectra with Nist-62 library of standard compounds. Both mass-spectrometric identifications and chromatographic peaks of FID were further confirmed by comparing retention times with reference standards (Sigma–Aldrich, Italy) or bibliographical data (Mallia et al., 2005; Ziino et al., 2005; Povolo et al., 2007; Wolf et al., 2008). All of the analyses were conducted in duplicate.

2.6. Statistical analysis

The data analysis was carried out with SPSS 10.0 (SPSS Inc., Chicago, IL, USA). The results of FAAs, organic acids, FFAs and volatile compounds were compared by one-way analysis of variance. In addition, principal component analysis (PCA) was applied to FAA, expressed as percentages of total FAA, with standardisation to a mean of zero and their original variances (covariance matrix).

3. Results and discussion

3.1. Free amino acids

FAAs are the end products of proteolysis, and they directly influence the background of the cheese flavour. However, their main impact is indirect, as they act as precursor of compounds of flavour and aroma via their catabolism by the cheese microflora (Marilley and Casey, 2004).

The ANOVA of the total and individual amount of FAAs revealed significant differences ($P \leq 0.05$) between both types of cheeses. The total FAAs in the S cheeses was 49 and 627 mg 100 g^{-1} at 2 and 180 days of ripening, respectively.

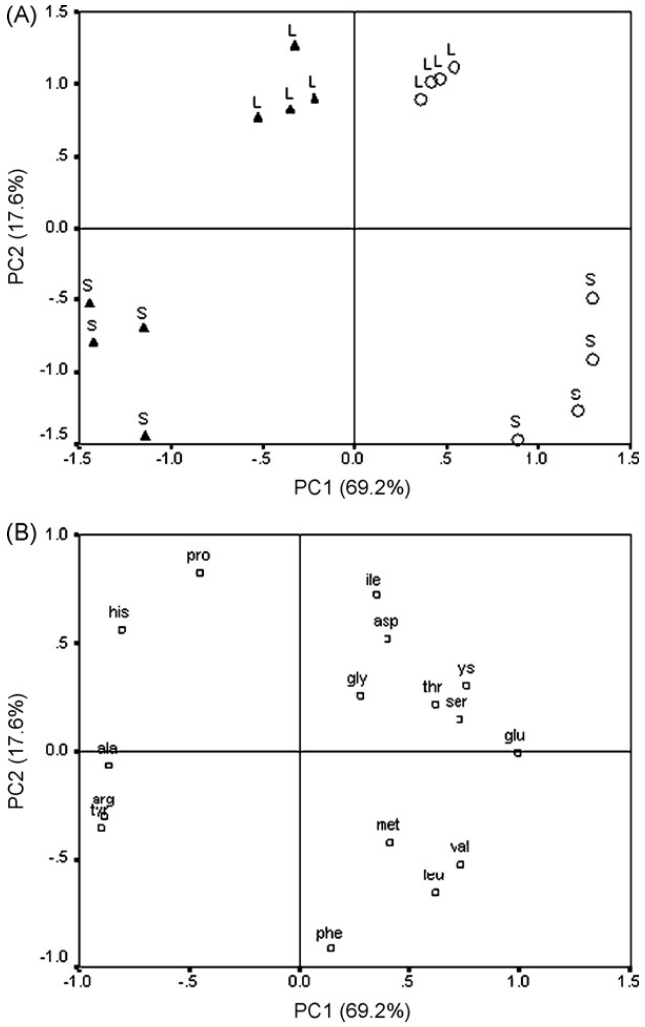

Fig. 2. Principal component analysis of the relative concentration of individual free amino acids (percentage of the total FAAs in each cheese) in S and L cheeses. (A) Score plot of PC1 vs. PC2 of samples at 2 (▲) and 180 (○) days of ripening. (B) Loading plot of PC1 vs. PC2.

The L cheese levels were 217 and 1949 mg 100 g^{-1} at 2 and 180 days of ripening, respectively, that is, approximately 4-fold higher than in S cheeses. Furthermore, the individual level of each amino acid in the L cheeses was between 2- and 8-fold higher than in S cheeses, at 2 as well as at 180 days of ripening (Fig. 1A and B). These results indicate that the depth of proteolysis was much higher in L than in S cheeses and that this was evident from the beginning of the ripening.

On the other hand, PCA was applied on the relative concentration of each FAA. They were expressed as percentages of the total FAAs in each cheese sample to detect whether there were changes in these profiles. Three principal components were extracted, which explained 93.8% of the total variance. A very clear separation of the samples was observed in the score plot of PC1 vs. PC2 (Fig. 2A): separate groups were formed for each type of cheese and for each aging period. PC1, which explained 69.2% of the total variance, grouped the cheeses mainly according to their ripening time, with cheeses at 2 days of ripening scoring negatively and cheeses at the end of the ripening scoring positively on this axis. During ripening, S and L cheeses were characterised by increases in the percentages of Glu, Lys, Ser, and Thr and diminutions in the percentages of His, Ala, Arg and Tyr (Fig. 2A and B). However, PC2, which accounted for 17.6% of the total variance, clustered cheeses based on the starter used, with S cheeses scoring negatively and L cheeses scoring positively. S cheeses were characterised by higher percentages of Phe, Leu and Val, while L cheeses showed higher percentages of Pro, Ile, His and Asp (Fig. 2A and B). Clustering along PC3 did not reveal any further valuable information. Scores of S cheeses at 2 and 180 days had more distance between them along PC1 in the score plot, in comparison with samples of L cheeses at the beginning and at the end of the ripening. These observations suggest that the change in the relative concentration of each FAA during ripening was more pronounced on S cheeses than on L cheeses. These results could be due to a higher variability of the enzymatic pool involved in the proteolysis during ripening in S cheeses.

Overall, thermophilic lactobacilli such as *Lactobacillus delbrueckii* and *L. helveticus* have been demonstrated to have much higher proteolytic and peptidolytic activity than *S. thermophilus* strains (Oberg and Broadbent, 1993; Rajagopal and Sandine, 1990). This fact agreed with the results obtained from the strains used in the present work. In addition, the autolysis of starter in cheeses was shown to be an important factor in the flavour development and acceleration of the ripening. In fact, the intracellular enzymes released into the cheese matrix lead to an increase in the secondary proteolysis, resulting in a higher production of small peptides and FAAs (Hannon et al., 2003; Kenny et al., 2006). In the present work, the significant increase in the peptidolysis levels suggests that the diminution of the lactobacilli population (Candioti et al., 2010) was most likely followed by lyses of the lactobacilli strains added. Other authors have also reported similar results in other types of cheeses. Similarly, different autolytic or attenuated strains of *L. helveticus*, added as an adjunct culture, showed a significant increase in the level of FAAs in Cheddar cheese, in comparison with control cheeses made only with lactococci (Madkor et al., 2000; Hannon et al., 2007). Regarding *L. delbrueckii* subsp. *bulgaricus*, Kebary et al. (1997) found an increase of proteolysis in the Kareish cheese due to the addition of an attenuated strain of *L. delbrueckii sp. bulgaricus*, compared to the cheese made with attenuated *S. thermophilus*. Moreover, Oommen et al. (2002) detected a higher proteolysis in Mozzarella cheese containing *L. delbrueckii* subsp. *bulgaricus* than the one found in control cheeses made using only *S. thermophilus*.

3.2. Organic acids

Citric, pyruvic, succinic, lactic, formic and acetic acids were detected in cheeses manufactured in the present work, whereas propionic, butyric and valeric acids were not detected. Isovaleric and isobutyric acids were present in a few cheeses without a reproducible behaviour. The amount of different compounds showed great variability between different replicates. This variability could be due to the character of these compounds because they are intermediates and metabolites in several biochemical processes that occur during cheese ripening. Moreover, milk

Table 1
Levels of organic acids (mg/100 g cheese) in S and L cheeses at 2 and 180 days of ripening. Values are means (±standard deviations) of four cheese replicates manufactured in different days with different milk.

Organic acids	S cheeses		L cheeses	
	2 days	180 days	2 days	180 days
Lactic acid	805.5 ± 65.46^{a}	731.0 ± 167.2^{a}	1387.4 ± 319.2^{b}	920.3 ± 157.8^{a}
Citric acid	123.6 ± 14.8^{a}	143.6 ± 20.2^{a}	143.3 ± 31.1^{a}	143.2 ± 38.8^{a}
Acetic acid	21.2 ± 7.1^{a}	87.9 ± 20.9^{a}	26.1 ± 11.2^{a}	33.7 ± 17.6^{b}
Pyruvic acid	5.48 ± 0.87^{a}	6.5 ± 1.9^{a}	7.9 ± 2.8^{a}	6.7 ± 1.4^{a}
Succinic acid	n.d.[a]	$30.3 \pm 41.5^{a\dagger}$	69.6 ± 15.6^{b}	106.0 ± 40.6^{b}
Formic acid	n.d.	n.d.[a]	n.d.	25.7 ± 9.6^{b}

Means within rows with different superscript (a and b) letters indicate significant differences ($P \leq 0.05$) for the same ripening time.
n.d. = not detected.
† Only two of four cheese replicates presented succinic acid and as a consequence there was a very high standard deviation.

is another important factor in the variability of organic acids in cheeses, especially given the great variability in milk composition during the seasonal production of sheep milk (Lues, 2000). Lactic acid is the major product of lactose catabolism by lactic acid bacteria, and it was the most abundant organic acid in S and L cheeses as well as in other types of cheeses (Buffa et al., 2004; Tormo and Izco, 2004). Although the majority of the citric acid in the milk is lost in the whey, it was the second most abundant organic acid in sheep cheeses manufactured in the present work (Table 1). Similar results were found in Cheddar cheese (Izco et al., 2002), while in other types of cheeses, such as Halloumi cheese (Kaminarides et al., 2007), this acid was not detected.

At 2 days of ripening, significant differences ($P \leq 0.05$) were found for succinic and lactic acids between both types of cheeses. At the end of the ripening, succinic and formic acids showed significantly higher levels in L cheeses, while acetic acid accumulated to higher levels in S cheeses (Table 1). Significant changes ($P \leq 0.05$) during ripening were observed only for acetic acid in S cheeses, which increased at the end of the ripening. In L cheeses, lactic acid decreased significantly during ripening, while formic acid increased significantly ($P \leq 0.05$).

In curd-washed cheeses, the content of residual lactose is lower, which consequently results in a lower level of lactic acid from lactose fermentation (McSweeney and Fox, 2004). In our work, a lower level of lactic acid was found in S cheeses, most likely due to the washing of the curd during the cheese-making. However, the lactic acid concentration in both types of cheeses evened out at the end of the ripening, probably because the lactobacilli in L cheeses had metabolised it, reducing its concentration to levels similar to those found in S cheeses. Succinic acid can be produced from lactate, citrate, isocitrate and amino acids (Dudley and Steele, 2005; Skeie et al., 2008), and certain lactobacilli strains can consume it or produce it (Manolaki et al., 2006). The higher level of succinic acid found in L cheeses suggests that the thermophilic lactobacilli of the starter were able to produce it. On the other hand, formic acid can be produced from the lactate oxidation or from the serine catabolism (Buffa et al., 2004; Skeie et al., 2008). It is known that *S. thermophilus* produces formic acid in yogurt, which is a growth factor for *L. bulgaricus* (Suzuki et al., 1986). However, streptococci in S cheeses did not produce formic acid. Furthermore, several strains of *L. delbrueckii* did not produce a considerable amount of formic acid from lactose in cheeses (Suzuki et al., 1986; Courtin and Rul, 2004), but certain *Lactobacillus* mesophilic strains have shown a production of formic acid from the deamination of serine (Skeie et al., 2008). Therefore, the higher concentration of formic acid in L cheeses could be attributed to the metabolic activity of either the thermophilic lactobacilli of the starter, which can probably produce this acid from serine, or the streptococci of the starter, which can be stimulated by the co-culture with the thermophilic lactobacilli in these cheeses.

3.3. Free fatty acids

Lipolysis releases FFAs contributing directly to cheese flavour, especially short- and intermediate-chain fatty acids, as they have considerably low perception thresholds, giving a particular flavour to the cheeses (Collins et al., 2003). In general, there have only been a few studies on the influence of starter cultures on the FFAs profiles during cheese ripening, and even fewer studies regarding the relation between the extent of starter lysis and the level of lipolysis in cheeses.

The degree of lipolysis represented by the total FFAs values (calculated by the sum of individual FFA) did not differ significantly between S and L cheeses at 2 days of ripening (Table 2). However, after 180 days of ripening the total values varied from 9.7 μmol/g cheese in S cheeses to 15.4 μmol/g cheese in L cheeses. These differences between both types of cheeses were statistically significant ($P \leq 0.05$).

The extent of lipolysis found in these sheep cheeses, with levels of about 2200 and 3500 mg/kg of cheese for S and L cheeses, respectively, is lower than those of other cheese varieties in which this biochemical event plays an important role, such as hard Italian cheeses and surface- and mould-ripening cheeses. However, it is worth mentioning that the levels of lipolysis were similar to those of some varieties of cow cheeses. Similarly, a wide range of total FFAs values, 2800–8000 mg/kg, was reported in Cheddar cheeses (Guinee and McSweeney, 2006; O'Mahony et al., 2006). Walstra et al. (1993) informed similar results for Dutch-type cheeses. Choisy et al. (1997) found lipolysis values of approx. 4000 mg/kg for Gouda cheese. Moreover, a similar degree of lipolysis (approx. 2400 mg/kg) was found in Pategrás cheese, a semi-hard variety of Argen-

Table 2
Levels of free fatty acids (FFAs) (μmol/g cheese) in S and L cheeses at 2 and 180 days of ripening. Values are means (±standard deviations) of four cheese replicates manufactured in different days with different milk.

FFAs	S cheeses		L cheeses	
	2 days	180 days	2 days	180 days
$C_{4:0}$	$0.20 \pm 0.03^{a,A}$	$0.45 \pm 0.06^{a,B}$	$0.21 \pm 0.04^{a,A}$	$0.73 \pm 0.10^{b,B}$
$C_{6:0}$	$0.20 \pm 0.06^{a,A}$	$0.63 \pm 0.11^{a,B}$	$0.20 \pm 0.04^{a,A}$	$1.09 \pm 0.35^{b,B}$
$C_{8:0}$	$0.19 \pm 0.07^{a,A}$	$0.41 \pm 0.06^{a,B}$	$0.19 \pm 0.06^{a,A}$	$0.71 \pm 0.31^{a,B}$
$C_{10:0}$	$0.40 \pm 0.23^{a,A}$	$0.86 \pm 0.15^{a,B}$	$0.42 \pm 0.22^{a,A}$	$1.25 \pm 0.22^{b,B}$
$C_{12:0}$	$0.19 \pm 0.13^{a,A}$	$0.44 \pm 0.10^{a,B}$	$0.19 \pm 0.12^{a,A}$	$0.60 \pm 0.10^{b,B}$
$C_{14:0}$	$0.51 \pm 0.18^{a,A}$	$1.02 \pm 0.38^{a,B}$	$0.70 \pm 0.38^{a,A}$	$1.84 \pm 0.35^{b,B}$
$C_{16:0}$	$1.52 \pm 0.31^{a,A}$	$2.44 \pm 0.56^{a,B}$	$1.82 \pm 0.61^{a,A}$	$3.90 \pm 0.39^{b,B}$
$C_{18:0}$	$0.70 \pm 0.10^{a,A}$	$0.95 \pm 0.18^{a,B}$	$0.86 \pm 0.20^{a,A}$	$1.36 \pm 0.19^{b,B}$
$C_{18:1}$	$1.37 \pm 0.24^{a,A}$	$2.13 \pm 0.55^{a,B}$	$1.53 \pm 0.44^{a,A}$	$3.31 \pm 0.56^{b,B}$
$C_{18:2}$	$0.20 \pm 0.02^{a,A}$	$0.38 \pm 0.11^{a,B}$	$0.23 \pm 0.06^{a,A}$	$0.56 \pm 0.10^{b,B}$
Total	$5.48 \pm 1.25^{a,A}$	$9.70 \pm 1.75^{a,B}$	$6.36 \pm 2.09^{a,A}$	$15.35 \pm 1.51^{b,B}$
SCFA	0.59	1.49	0.61	2.53
MCFA	0.59	1.30	0.61	1.85
LCFA	4.30	6.91	5.14	10.97

$C_{6:0}$: caproic acid, $C_{8:0}$: capric acid, $C_{10:0}$: caprylic acid, $C_{12:0}$: lauric acid, $C_{14:0}$: myristic acid, $C_{16:0}$: palmitic acid, $C_{18:0}$: stearic acid, $C_{18:1}$: oleic acid, $C_{18:2}$: linoleic acid. Total: sum of FFA, SCFA: short-chain fatty acids, $C_{6:0}$–$C_{8:0}$; MCFA: medium-chain fatty acids, $C_{10:0}$–$C_{12:0}$; LCFA: long-chain fatty acids, $C_{14:0}$–$C_{18:2}$.
Means within rows with different superscript letters (a and b) indicate significant differences ($P \leq 0.05$) for the same ripening time.
Means within rows with different superscript letters (A and B) indicate significant differences ($P \leq 0.05$) for the same group of cheese.

tinean cheese (Perotti et al., 2009). Moreover, similar data have been reported for Spanish sheep cheeses. Poveda et al. (2000) found a total FFAs concentration of 2500 and 3600 mg/kg for Manchego cheeses made during two different seasons of the year and at 150 days of ripening. Furthermore, in a study of seasonal variations of FFAs of Spanish PDO ovine milk cheeses, Fernández-García et al. (2006) found a total FFAs content that range between 2000 and 2800 mg/kg for Manchego, 1500–2800 mg/kg for Zamorano, and 2500–3300 for La Serena cheeses. For Idiazabal cheese, Virto et al. (2003) and Hernández et al. (2009) obtained similar values of approximately 3000 mg/kg after 6 months of ripening.

In general, lipolysis during cheese ripening is the result of the action of indigenous and microbial lipases and of lipases provided by certain type of milk coagulants, such as rennet paste. In the present work, the indigenous lipase (lipoprotein lipase, LPL) was probably inactivated during the milk pasteurisation and the curd cooking, whereas the milk coagulant used had no lipolytic activity, and no exogenous lipase was added. Consequently, the moderate level of lipolysis observed was produced either by the lipases released in the cheese matrix from the lactic acid bacteria used as starter or by the non-starter lactic bacteria (NSLAB). *Lactobacillus* spp. has a lower lipolytic activity than other bacteria and moulds.

In both types of cheeses, the different groups of FFAs: short- (SCFA, $C_{6:0}$–$C_{8:0}$), medium- (MCFA, $C_{10:0}$–$C_{12:0}$) and long-chain fatty acids (LCFA, $C_{14:0}$–$C_{18:2}$) increased during the cheese ripening process, with the LCFA group being the most quantitatively significant one (Table 2). In addition, the individual free fatty acid contents also increased significantly ($P \leq 0.05$) throughout storage. Palmitic and oleic acids were the most abundant acids in cheeses, and myristic and stearic acids were the next FFAs in order of decreasing concentration. This trend has also been reported to occur in many cheeses, regardless of the level of lipolysis attained.

The content of each FFAs group can be seen in Table 3, represented by the percentages of the total FFAs at 2 and 180 days of ripening. In this table, it is possible to observe that the percentages of SCFA and MCFA increased during ripening, while the percentages of LCFA decreased between 2 and 180 days of ripening in both types of cheeses (Table 3). These results may indicate that there is a preferential release of short- and intermediate-chain fatty acids during ripening over that of long-chain fatty acids, which is in agreement with results reported for Terrincho (Pinho et al., 2003) and Idiazabal cheeses (Hernández et al., 2009).

At the beginning of the ripening, the lipolysis profiles of both cheeses were similar. However, at the end of this period, all of the FFAs were significantly higher ($P \leq 0.05$) in L than in S cheeses (Table 2). In addition, L cheeses showed an increase of 6.9% in the percentages of SCFA during ripening, which was higher than the 4.9% increase observed on S cheeses (Table 3). This could be attributed to the higher lipolytic activity of *Lactobacillus* lipases present in L cheeses, which have more specificity towards short-chain fatty acids that are located at the sn-3 position of the triglycerides (MacGibbon and Taylor, 2006). Overall, lipases and esterases from lactic bacteria are intracellular and are only available when released into the cheese matrix

Table 3
Concentrations of short- (SCFA, $C_{6:0}$–$C_{8:0}$), medium- (MCFA, $C_{10:0}$–$C_{12:0}$) and long-chain fatty acids (LCFA, $C_{14:0}$–$C_{18:2}$), expressed as percentages of total FFAs in S and L cheeses at 2 and 180 days of ripening. Values are means of four cheese replicates manufactured in different days with different milk.

Parameters	Age (days)	S cheese	L cheese
SCFA as % of total FFAs	2	10.9	9.7
	180	15.8	16.6
MCFA as % of total FFAs	2	10.1	9.2
	180	13.6	12.0
LCFA as % of total FFAs	2	79.0	81.1
	180	70.6	71.5

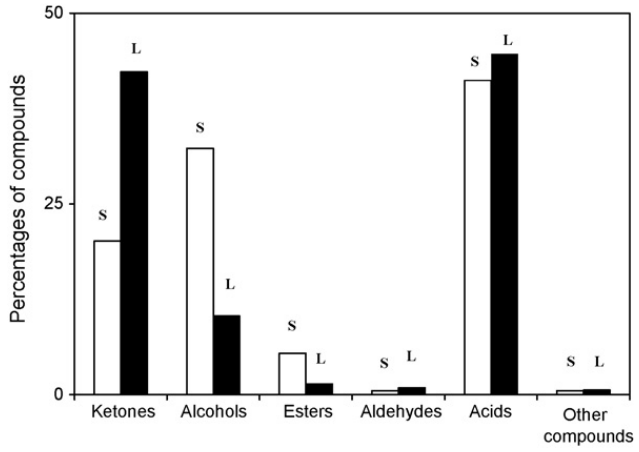

Fig. 3. Relative amount of each group of volatile compounds (ketones, alcohols, esters, aldehydes, acids and other compounds), expressed as percentages of total area, in S and L cheeses. Values are means of four cheese replicates manufactured in different days with different milk.

due to cell lysis. Hannon et al. (2007) found that Cheddar cheeses containing a highly autolytic strain of *L. helveticus* had much higher levels of FFAs than those cheeses made with strains of *Lactococcus lactis* that have a low level of autolysis. This suggests that the lysis of *L. helveticus* could result in an increased lipase/esterase activity in cheese. On the other hand, Katsiari et al. (2009) evaluated the lipolytic characteristic of Greek sheep cheeses made with mesophilic and thermophilic starter cultures (such as *L. lactis*, *S. thermophilus* and *L. bulgaricus*), and they found that the extent of lipolysis was not affected by the starter used, despite the differences found among some FFAs.

3.4. Volatile compounds

A total of 44 volatile compounds were identified by SPME-GC–MS/FID in those cheeses analysed at 180 days of ripening, including mainly ketones, alcohols, esters, aldehydes and fatty acids (Table 4). The peak area values in most of the identified compounds showed statistical differences between S and L cheeses.

Ketones: Eight ketones were identified, and, among them, 2-pentanone + diacetyl (unresolved peak), 2-heptanone and acetoin had the highest area values. Considerable differences were found in the percentages of ketones in both types of cheeses; they represented 20 and 42% of the total area in S and L cheeses, respectively (Fig. 3).

Ketones are usually reported as one of the main fractions of the volatile components in Italian and Spanish cheeses made from sheep milk. In these cheeses, 2-propanone, 2-butanone, 2-pentanone, 2-heptanone, acetoin and diacetyl were found at the highest levels (Izco and Torre, 2000; Larráyoz et al., 2001; Gómez-Ruiz et al., 2002; Coda et al., 2006; Barron et al., 2007).

Methylketones such as 2-pentanone, 2-heptanone and 2-nonanone are related to the lipolytic activity of microflora in cheeses. In the present work, 2-pentanone and 2-heptanone area values were significantly higher ($P \leq 0.05$ and $P \leq 0.10$) in L cheeses (Table 4), which agreed with the more intense lipolysis found in them. Moreover, L cheeses also showed the highest area values of diacetyl, acetoin and 2-butanone. Diacetyl is one of the key odorants in some types of cheeses and is also associated with *buttery* notes (Curioni and Bosset, 2002). Diacetyl can not only be produced by citrate and lactose metabolism but can also be synthesised from aspartic acid (Yvon, 2006). This amino acid, as well as all free amino acids, had a higher concentration in L cheeses. Acetoin is produced from reduction of diacetyl or may be synthesised from pyruvate, lactose or citrate by lactic acid bacteria (Di Cagno et al., 2003). Due to its low perception threshold, the effect of acetoin on the sheep cheese aroma could be very important (Izco and Torre, 2000; Di Cagno et al., 2003).

Alcohols: A total of 14 alcohols were identified in cheese samples. Ethanol had the highest area value in both types of cheeses. Other alcohols such as 1-butanol, 1-hexanol, 2-pentanol, 3-methyl 1-butanol and 2,3-butanediol were also important, considering their area values. The incidence of alcohols on the volatile profile was different between S and L cheeses, accounting for 32 and 10% of the total volatile compounds, respectively (Fig. 3).

Alcohols have been reported to be the main chemical family in some sheep cheeses, while ethanol has quantitatively been shown to be the most important one (Izco and Torre, 2000; Kondyli et al., 2002; Bintsis and Robinson, 2004; Carbonell et al., 2002). In Spanish cheeses (Manchego, Idiazabal and Roncal), the percentages of these compounds ranged from 10 to 60% (Mallia et al., 2005; Barron et al., 2005; Irigoyen et al., 2007).

Significant differences were detected in the area values of some alcohols between S and L cheeses. Regarding secondary alcohols, such as 2-pentanol, 2-heptanol and 2-nonanol, higher area values were found in L cheeses. These secondary alcohols can be produced by enzymatic reduction of the corresponding methylketones, and their presence had been associated with the lipolytic action of the microflora (Collins et al., 2003). In accordance with these results, L cheeses presented the highest lipolysis levels.

On the other hand, the ethanol and 2,3-butanediol area values were significantly higher ($P \leq 0.05$) in S cheeses. Ethanol is a direct product of lactose or citrate fermentation (Marilley and Casey, 2004), but it can also be produced from alanine via Strecker degradation (Izco and Torre, 2000; Irigoyen et al., 2007). The presence of 2,3-butanediol in cheeses is the result of acetoin reduction by starter bacteria. This compound would seem to accumulate in S cheeses.

Regarding branched-chain alcohols, 3-methyl 1-butanol is an important volatile component in sheep cheeses (Kondyli et al., 2002; Bintsis and Robinson, 2004) and it is produced from leucine catabolism by Strecker degradation (McSweeney and Sousa, 2000; Marilley and Casey, 2004). This compound provides the pleasant aroma of fresh cheese (Curioni and Bosset, 2002; Di Cagno et al., 2003). Statistical differences ($P \leq 0.05$) were detected in 3-methyl 1-butanol and leucine in both types of cheeses, with their values higher in L cheeses than in S cheeses.

Esters: Five esters were identified, being ethyl butanoate the most abundant one. Esters constituted a small proportion of the total area of compounds detected, being a minority group in S and L cheeses (5 and 1%, respectively).

Table 4
Volatile compounds identified in S and L cheeses. Values are mean areas (±standard deviations) of four cheese replicates manufactured in different days with different milk.

	S cheeses	L cheeses	Identification (1)
Ketones			
2-Propanone	$292 \pm 27^{a**}$	$382 \pm 64^{b**}$	MS, ST, R
2-Butanone	$32 \pm 5^{a*}$	$52 \pm 9^{b*}$	MS, ST, R
2-Pentanone + diacetyl	$1023 \pm 262^{a*}$	$3506 \pm 598^{b*}$	MS, ST, R
2-Hexanone	$63 \pm 14^{a*}$	$114 \pm 23^{b*}$	MS, ST, R
2-Heptanone	$587 \pm 142^{a*}$	$2697 \pm 771^{b*}$	MS, ST, R
3-Hydroxy 2-butanone or acetoin	$727 \pm 301^{a*}$	$1511 \pm 232^{b*}$	MS, ST, R
2-Nonanone	$123 \pm 46^{a*}$	$561 \pm 404^{a*}$	MS, ST, R
Alcohols			
2-Propanol	$33 \pm 10^{a**}$	$18 \pm 6^{b**}$	MS, ST, R
Ethanol	$3782 \pm 633^{a*}$	$1433 \pm 426^{b*}$	MS, ST, R
2-Pentanol	$50 \pm 2^{a*}$	$84 \pm 31^{b*}$	MS, ST, R
1-Butanol	$126 \pm 46^{a*}$	$91 \pm 60^{a*}$	MS, ST, R
3-Methyl 1-butanol	$170 \pm 22^{a*}$	$217 \pm 27^{b*}$	MS, ST, R
1-Pentanol	$12 \pm 9^{a*}$	$15 \pm 6^{a*}$	MS, ST, R
2-Heptanol	$26 \pm 4^{a*}$	$46 \pm 13^{b*}$	MS, ST, R
1-Hexanol	$43 \pm 9^{a*}$	$95 \pm 122^{a*}$	MS, ST, R
2-Ethyl 1-hexanol	$10 \pm 7^{a*}$	$6 \pm 5^{a*}$	MS, ST, R
2-Nonanol	0^{a**}	$13 \pm 11^{b**}$	MS, ST, R
2,3-Butanediol	$292 \pm 75^{a*}$	$96 \pm 37^{b*}$	MS, R
1-Octanol	$6 \pm 13^{a*}$	$6 \pm 12^{a*}$	MS, ST, R
Phenyl methanol	$8 \pm 8^{a*}$	$22 \pm 17^{a*}$	MS, R
2-Phenyl ethanol	$5 \pm 6^{a*}$	n.d.^{a*}	MS, R
Esters			
Ethyl acetate	$51 \pm 15^{a*}$	$57 \pm 25^{a*}$	MS, ST, R
Ethyl butanoate	$565 \pm 68^{a*}$	$162 \pm 45^{b*}$	MS, ST, R
Ethyl hexanoate	$89 \pm 25^{a*}$	$9 \pm 1^{b*}$	MS, ST, R
Isopropyl hexanoate	$42 \pm 3^{a*}$	$55 \pm 18^{a*}$	MS, R
Ethyl octanoate	$12 \pm 6^{a*}$	n.d.^{b*}	MS, ST, R
Aldehydes			
Acetaldehyde	$26 \pm 2^{a*}$	$102 \pm 15^{b*}$	MS, R
2-Methyl butanal	$9 \pm 3^{a*}$	$35 \pm 5^{b*}$	MS, R
3-Methyl butanal	$34 \pm 11^{a*}$	$56 \pm 13^{b*}$	MS, ST, R
Acids			
Acetic acid	$1051 \pm 331^{a*}$	$2674 \pm 1660^{a*}$	MS, ST, R
2-Methyl propanoic acid	$64 \pm 25^{a*}$	$70 \pm 38^{a*}$	MS, ST, R
Butyric acid	$2460 \pm 1015^{a*}$	$4084 \pm 217^{b*}$	MS, ST, R
3-Methyl butanoic acid	$538 \pm 248^{a*}$	$521 \pm 536^{a*}$	MS, ST, R
Pentanoic acid	$19 \pm 3^{a*}$	$29 \pm 5^{b*}$	MS, ST, R
Hexanoic acid	$1170 \pm 294^{a**}$	$1569 \pm 281^{b**}$	MS, ST, R
Heptanoic acid	$18 \pm 11^{a*}$	$10 \pm 3^{a*}$	MS, ST, R
Octanoic acid	$175 \pm 30^{a*}$	$207 \pm 37^{a*}$	MS, ST, R
Nonanoic acid	$74 \pm 62^{a*}$	$73 \pm 63^{a*}$	MS, ST, R
Decanoic acid	$33 \pm 11^{a*}$	$46 \pm 12^{a*}$	MS, ST, R
Dodecanoic acid	$23 \pm 3^{a*}$	$24 \pm 7^{a*}$	MS, ST, R
Other compounds			
1,3-Pentadiene	$31 \pm 8^{a*}$	$101 \pm 78^{a*}$	MS, R
m-xylene	$14 \pm 17^{a*}$	$2 \pm 4^{a*}$	MS, R
D-Limonene	$4 \pm 8^{a*}$	$8 \pm 10^{a*}$	MS, ST, R
γ-Hexanolactone	$10 \pm 7^{a*}$	$11 \pm 9^{a*}$	MS, ST, R

Means in the same row followed by different superscript letters (a and b) indicate significant statistical differences.
(1) Methods used for the identification of compounds. MS: spectra comparison using NIST-62 Library; ST: authentic standard injection (Sigma–Aldrich, Italy); R: comparison with published data (Mallia et al., 2005; Ziino et al., 2005; Povolo et al., 2007; Wolf et al., 2008).
n.d. = not detected.
* $P \leq 0.05$.
** $P \leq 0.10$.

This minority may, however, contribute in a synergistic way to the overall flavour due to their low perception thresholds (Di Cagno et al., 2003; Liu et al., 2004).

The volatile profile of sheep cheeses is characterised by the presence of ethyl esters (mainly ethyl acetate, ethyl butanoate and ethyl hexanoate). They are considered very important by their particular contribution to the aroma (Izco and Torre, 2000; Larráyoz et al., 2001; Di Cagno et al., 2003; Barron et al., 2005; Coda et al., 2006; Irigoyen et al., 2007). The main mechanism of ester biosynthesis by LAB esterases in an aqueous media seems to be alcoholysis or esterification (Liu et al., 2003; Abeijon Mukdsi et al., 2009).

Some investigations suggested that ethanol bioavailability is probably a critical factor in ester formation (Liu et al., 2004).

The area values corresponding to ethyl butanoate, ethyl hexanoate and ethyl octanoate (Table 4) were statistically higher ($P \leq 0.05$) in S than in L cheeses, probably because S cheeses had more ethanol available for ester biosynthesis. It is known that *S. thermophilus* can synthesise significant quantities of esters in aqueous biological systems. In addition, *S. thermophilus* strains have, on average, significantly higher ethyl butanoate-synthesising activity than other LAB (Liu et al., 1998, 2003, 2004).

Aldehydes: Acetaldehyde, 2-methyl butanal and 3-methyl butanal were identified in S and L cheeses, and their percentages were lower than 1% (Fig. 3).

Aldehydes are commonly found in the volatile profiles of sheep cheeses, and, within this group, acetaldehyde and 3-methyl butanal are considered characteristic and important compounds (Izco and Torre, 2000; Larráyoz et al., 2001; Carbonell et al., 2002; Kondyli et al., 2002; Bintsis and Robinson, 2004; Barron et al., 2007). In spite of their low concentrations, some aldehydes have low perception thresholds and may play an important role in cheese aroma (Izco and Torre, 2000; Bintsis and Robinson, 2004).

L cheeses made using a starter culture that contains *Lactobacillus* had higher levels of aldehydes (Table 4). Acetaldehyde is produced during lactose metabolism by lactic acid bacteria but also by the breakdown of threonine (Larráyoz et al., 2001). Branched-chain aldehydes such as 2-methyl butanal and 3-methyl butanal are originated from isoleucine and leucine, respectively, by Strecker degradation (McSweeney and Sousa, 2000; Marilley and Casey, 2004). In Cheddar, Roncal-type and Feta-type cheeses containing thermophilic and mesophilic lactobacilli as adjuncts, a relationship between the aldehyde levels and the precursor amino acid concentrations was found (Bintsis and Robinson, 2004; Irigoyen et al., 2007; Hannon et al., 2007). This fact could also be observed in L cheeses, which presented higher levels of acetaldehyde, 2-methyl butanal, 3-methyl butanal and their precursor amino acids: threonine, leucine and isoleucine, respectively.

Acids: Eleven acids were identified, mainly saturated fatty acids of an even number of carbon atoms from C_2 to C_{12} as well as branched-chain acids. The main ones (taking into account their peak areas) were etanoic, butanoic, hexanoic and 3-methyl butanoic acids.

Acids made up the largest group of aroma components, accounting for 41 and 45% of all volatiles identified in S and L cheeses, respectively (Fig. 3).

Acids play an important role in the aroma development of aged sheep cheeses (Gómez-Ruiz et al., 2002; Barron et al., 2005). High percentages of these compounds have been found in Spanish sheep cheeses (ranging from 50 to 90% of the total area), especially short-chain acids (C_2 to C_{10}) (Mallia et al., 2005; Barron et al., 2005). This last group of acids is responsible for the strong, balanced and piquant flavour that characterises Pecorino cheeses (Coda et al., 2006). Branched-chain fatty acids, mainly 2-methyl butanoic and 3-methyl butanoic acids, are characteristic impact compounds of goat and sheep cheeses (Curioni and Bosset, 2002). 3-Methylbutanoic acid is known to contribute to the *rancid*, *cheesy*, *sweaty* and *putrid* notes of aged sheep cheeses (Curioni and Bosset, 2002; Marilley and Casey, 2004). The presence of branched-chain fatty acids in cheeses is related to the catabolism of branched-chain amino acids such as valine, isoleucine and leucine.

Significant differences ($P \leq 0.05$) were found in the content of butanoic and hexanoic acids between S and L cheeses. These compounds derive from the lipolysis of milk fat by the lipase and esterase activity. In addition to their higher levels of lipolysis, L cheeses had the highest area values of these compounds. 2-Methyl propanoic and 3-methyl butanoic acids had similar area values in both types of cheeses, despite the high concentrations of precursor amino acids (valine and leucine) found in L cheeses.

Other compounds: Some compounds belonging to the terpenes (D-limonene), lactones (γ-hexanolactone) and hydrocarbons (m-xylene and 1,3-pentadiene) families were identified in some analysed samples. The presence of these compounds had previously been reported in sheep cheeses (Larráyoz et al., 2001; Di Cagno et al., 2003; Barron et al., 2005; Coda et al., 2006; Barron et al., 2007). None of these compounds presented statistical differences between S and L cheeses, probably because some of them are not related to the ripening process.

4. Conclusions

Two different Argentinean sheep cheeses manufactured with Pampita sheep milk (a native breed), were well characterised during ripening regarding the production of free amino acids, organic acids, free fatty acids and volatile compounds. S cheeses, made with a starter composed only of streptococci, were characterised by moderate proteolysis and low levels of lypolysis. Higher percentages of Phe, Leu and Val were observed in these cheeses, compared to L cheeses. Moreover, S cheeses showed lower levels of the total area of volatile compounds than L cheeses, with alcohols and esters being the compounds that prevailed in the volatile profile. On the other hand, the addition of thermophilic lactobacilli in the starter of L cheeses (which died and probably lysed during ripening) produced a more noticeable proteolysis, a slightly higher lipolysis, and a greater production of volatile compounds. Therefore, L cheeses accumulated a much higher amount of free amino acids than S cheeses, and their profiles were characterised by higher percentages of Pro, Ile, His and Asp. Moreover, L cheeses showed the highest levels of all free fatty acids at 180 days of ripening, and they also showed a greater increase in the percentages of short-chain fatty acids during ripening, compared to S cheeses. Volatile profiles of L cheeses were characterised as having higher percentages of aldehydes and ketones than those of S cheeses. These results suggest an acceleration of ripening in L cheeses, probably due to the enzymatic activity of *L. helveticus* and *L. delbrueckii*, which is in agreement with the more intense flavour found in these cheeses on previous studies (Candioti et al., 2010). Lactic and citric acids were the most important organic acids in both sheep cheeses.

These results are an important contribution in this area not only because they can provide a better understanding of Argentinean sheep cheeses but also because they can help

in the characterisation and classification of the products manufactured with Pampinta ewes milk, a native sheep breed.

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