Research Article

Received: 2 December 2012 Revised: 23 January 2013 Accepted article published: 7 February 2013 Published online in Wiley Online Library: 25 March 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6092

Influence of autochthonous adjunct cultures on ripening parameters of Argentinean goat's milk cheeses

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Abstract

BACKGROUND: Argentinean semi-hard goat's cheeses manufactured with and without the addition of autochthonous adjunct cultures of *Lactobacillus plantarum* ETC17, *Lactobacillus rhamnosus* ETC14 and *Enterococcus faecium* ETC3 were analysed to evaluate the effect of these strains on ripening parameters.

RESULTS: Gross composition was similar among cheeses. Microbiological analysis indicated that lactic acid bacteria added to cheeses reached high levels. None of the strains assayed affected the primary proteolysis. Overall, *E. faecium* had a clearer effect on the peptide and lipolysis profiles of cheeses. Analysis of the volatile fraction of cheeses indicated that the levels of several compounds involved in the overall flavour of goat's cheeses were affected by the presence of *E. faecium*. This could explain the differences detected in the global perception of cheeses made with this strain compared with control cheeses.

CONCLUSION: The present work represents a first contribution to knowledge of the ripening process of Argentinean goat's cheeses made with the addition of autochthonous adjunct cultures. The results suggest that *E. faecium* ETC3 showed a significant effect during ripening, which was reflected both in the profiles of proteolysis, lipolysis and volatile compounds and in the global sensory perception of cheeses.
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Keywords: autochthonous adjunct cultures; goat's milk cheeses; ripening parameters

INTRODUCTION

Argentina has about 5 million goats, which are mainly used for meat, milk and hair production.[1] The northwestern region of the country has the largest number of animals and accounts for 63% of total milk production.[2] Goat's milk is either processed for drinking or incorporated in cheese, powdered milk and ice cream.

The production and processing of goat's milk are of vital economic and social importance in the northwestern region of Argentina. Goat's cheese production has increased greatly since the 1990s. Although some goat's milk cheeses are produced under artisan conditions, most are manufactured on an industrial scale. Commonly, cheese makers employ the same starters used in the manufacture of cow's milk cheeses, regardless of the particular composition of goat's milk, which is at least partially responsible for the typical sensory attributes of these cheeses. Besides, for hygiene reasons, most cheeses are made from pasteurised milk. This thermal process inactivates both enzymes and native microbiota present in raw milk.[3] Therefore one way of preserving its appreciated organoleptic properties is the addition of adjunct cultures in cheese manufacture, in particular the use of indigenous cultures. Several studies have demonstrated that cheeses made with adjunct cultures have a more intense or better overall flavour.[4–7] Cheese unique flavour is a consequence of the delicate balance between a large number of compounds formed through complex biochemical events, including processes such as proteolysis and lipolysis, metabolism of lactose, lactate and citrate and catabolism of amino acids and free fatty acids.[8]

In recent years, much attention has been focused on traditional dairy products manufactured at farm level because of their distinctive sensory characteristics, which are often correlated with the enzymatic activities of autochthonous and specialised micro-organisms derived from milk and the environment. Among them, enterococci have been recognised as an essential part of the natural microbial population of many goat's dairy products, where they can sometimes prevail over lactobacilli and lactococci.[9–12] Previous studies in our laboratory led to the isolation and characterisation of autochthonous strains from regional milk and cheeses based on acidification rate, enzymatic activity (especially esterase activity) and non-pathogenicity.[11,13–15] These strains were tested in goat's cheese models in order to study the effect of different stages of the manufacturing process on their viability.[16,17]

To date, only limited information is available on microbiological and physicochemical aspects of Argentinean goat's milk cheeses.

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Furthermore, there are no published works concerning the effect of adjunct cultures on ripening parameters of goat's cheeses.

Taking into account the enzymatic activities of the strains assayed previously, the objective of this study was to evaluate the influence of autochthonous strains of *Lactobacillus plantarum* ETC17, *Lactobacillus rhamnosus* ETC14 and *Enterococcus faecium* ETC3 on the compositional and microbiological characteristics and proteolysis, lipolysis and volatile profiles of Argentinean semi-hard goat's cheeses.

MATERIALS AND METHODS

Starter cultures

Experimental cheeses were prepared using a mixed lyophilised commercial culture of *Streptococcus thermophilus* (60%), *Lactobacillus bulgaricus* (20%) and *Lactobacillus helveticus* (20%) (all of type DVS, Chr. Hansen, Buenos Aires, Argentina) inoculated at 10 mL L^{-1} milk as primary starter and the addition of one of the strains *L. plantarum* ETC17 (EP), *L. rhamnosus* ETC14 (ER) and *E. faecium* ETC3 (EF) inoculated at 2.5 mL L^{-1} milk as adjunct culture. These three strains were previously activated in de Man/Rogosa/Sharpe (MRS) broth and then multiplied in milk. Their concentrations ranged between 8.1 and 8.5 log colony-forming units (CFU) mL^{-1}. Respective control cheeses (CP, CR and CF) were made using only the starter culture.

Cheese making

Raw goat's milk (Saanen breed) provided by Experimental Station INTA EEA Santa Cruz (Catamarca, Argentina) was refrigerated and transported at 4 °C to the pilot plant of Instituto de Lactología Industrial (Santa Fe, Argentina) during a lactation period of 6 months. Semi-hard cheeses were made according to a standard process. Each cheese-making day, 40 L of raw milk was batch pasteurised at 65 °C for 20 min. After cooling to 39 °C, $CaCl_2$ (Merck, Darmstadt, Germany) was added to a final concentration of 0.2 g kg^{-1}. The starter culture, previously suspended in sterile milk, was then inoculated and the milk was divided into two vats of 20 L each for the simultaneous manufacture of control and experimental (with added strains) cheeses.

Milk coagulation was achieved by adding 0.014 g L^{-1} chymosin (Maxiren 150, Paris, France) to the vats. When the curd reached the appropriate strength, it was cut to corn grain size. The mixture of curd particles and whey was then gently stirred and heated to 47 °C at 1 °C min^{-1}. After the curd grains had reached an adequate moisture level for semi-hard cheeses, the stirring was stopped and the whey was drained and discarded. The curd was placed in cylindrical moulds (9 cm height, 10 cm diameter) and pressed (0.2–0.3 kg cm^{-2}) for 24 h.

Cheeses were salted by immersion in brine (200 g kg^{-1} NaCl solution, pH 5.4) at 12 °C for 7 h. Ripening was carried out at 12 °C and 85% relative humidity for 60 days. Each cheese weighed approximately 700 g.

Two trials for each type of experimental cheese (E) with its corresponding control cheese (C) were made on different fabrication days, as summarised in the following schedule:

	Cheese-making trials					
	L. plantarum		*L. rhamnosus*		*E. faecium*	
Cheese	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Experimental (E)	EP1	EP2	ER1	ER2	EF1	EF2
Control (C)	CP1	CP2	CR1	CR2	CF1	CF2

Microbiological analysis

Counts of lactic acid bacteria (LAB) and undesirable micro-organisms were conducted during the course of ripening (3, 30 and 60 days) by plating with different culture media for fungi and yeasts (Yeast Extract Glucose Chloramphenicol Agar, 5 days at 30 °C), total coliforms (VRBA, 24 h at 30 °C), Enterobacteriaceae (McConkey agar, 24 h at 37 °C), mesophilic lactobacilli and lactococci (MRS and M17 agar, 72 h at 30 °C), thermophilic lactobacilli and lactococci (MRS and M17 agar, 72 h at 42 °C) and enterococci (KF agar, 72 h at 42 °C). Values of both trials for each type of cheese were averaged.

Global composition

The global composition of cheeses was determined at the end of ripening (60 days): pH by the potentiometric method, fat by the Van Gulik method,[18] dry matter by the method of oven drying to constant weight[19] and protein by the Kjeldahl method.[20]

For control and experimental cheeses the reported results were average values of the two trials.

Electrophoresis

The primary proteolysis was assessed by urea polyacrylamide gel electrophoresis (PAGE) after 3 and 60 days. Samples were prepared by casein precipitation at pH 4.6 and purified. The insoluble residue was analysed in a Mini-Protean II cube (Bio-Rad Laboratories, Hercules, California, CA, USA) using the method of Andrews.[21] The acrylamide concentration was 75 g kg^{-1}. Proteins were stained with Coomassie Blue G-250.

Peptide profile analysis

Peptide profiles of cheeses after 60 days of ripening were analysed by reverse phase high-performance liquid chromatography (RP-HPLC). The HPLC equipment consisted of a quaternary pump, an online degasser and a UV–visible detector (all Series 200, Perkin Elmer, Norwalk, CT, USA). An interface module connected to a computer was used for the acquisition of chromatographic data with Turbochrom® software (Perkin Elmer). A 220 mm × 4.6 mm Aquapore OD-300 C18, 5 µm, 300 Å analytical column (Perkin Elmer) was employed. Water-soluble extracts were obtained, filtered through 0.45 µm membranes (Millex, Millipore, São Paulo, Brazil) and injected into the chromatograph. Separation was achieved under an increasing linear gradient of acetonitrile in water over 107 min. Detection was performed at 214 nm, the column temperature was 40 °C and the flow rate was 1 mL min^{-1} in accordance with Hynes *et al.*[22]

Lipid profile analysis

The extraction of fat matter from cheeses ripened for 60 days, the isolation of free fatty acids (FFA, $C_{4:0}$–$C_{18:2}$) and their conversion to ethyl esters and the chromatographic analysis were performed following the conditions described by Perotti *et al.*,[23] with some modifications according to Vélez *et al.*[24] A GC-9000 gas chromatograph (Perkin Elmer Corp., Waltham, MA, USA) equipped

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with a split/splitless injector and a flame ionisation detector (FID) was employed. The FFA esters were separated on a PE-Wax fused silica capillary column (60 m length × 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were 250 and 300 °C respectively. The hydrogen carrier gas flow rate was 2 mL min^{-1} and the split ratio 1:50. The oven temperature was initially held at 75 °C for 4 min, then raised to 150 °C at 10 °C min^{-1}, held at 150 °C for 3 min, raised to 230 °C at 10 °C min^{-1} and finally maintained at 230 °C for 15 min. Quantification was performed using the internal standardisation technique, with enantic ($C_{7:0}$) and margaric ($C_{17:0}$) acids (Sigma Aldrich, St Louis, MO, USA) added to the cheese samples at the extraction step as internal standards. The FID out-signal was recorded and the chromatograms were processed using Turbochrom Version 4 software (Perkin Elmer Corp.). Analyses were performed in duplicate and results were expressed as mg FFA kg^{-1} cheese.

Volatile compound analysis

Volatile compounds from cheeses ripened for 60 days were isolated by solid phase microextraction (SPME) according to the procedure of Wolf *et al.*[25] Briefly, 5 g samples of grated cheese were placed in 30 mL glass vials, which were then hermetically sealed with aluminium seals and butylteflon septa. The vials were thermostatted in a water bath at 40 ± 1 °C for 10 min, then a 50/30 μm Stable-Flex DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA) was inserted into the headspace for 15 min. During sampling, the vials were held at 40 ± 1 °C.

Chromatography analysis was carried out with the same equipment and column that were used for lipolysis profile analysis. The compounds retained on the fibre were thermally desorbed at 250 °C for 5 min in the injection port (splitless mode). The injector was provided with a 0.75 mm i.d. inlet liner (Supelco) in order to achieve sharper peaks. The column temperature was programmed as follows: 45 °C (4 min), then 8 °C min^{-1} to 150 °C (3 min) and finally 10 °C min^{-1} to 250 °C (5 min). The detector temperature was 290 °C. Hydrogen was employed as carrier gas at a flow rate of 2 mL min^{-1}.

Peaks were tentatively identified by comparing their retention times with those of authentic standards (Sigma Aldrich, Milan, Italy) when available. A more reliable identification was performed by calculating linear retention index (LRI) values. For this purpose, a series of *n*-alkanes (Sigma Aldrich, Bellefonte, PA, USA) was injected under the same chromatographic conditions as for samples and the values were obtained according to the expression proposed by Van den Dool and Kratz.[26] LRI values were compared with published data.

In order to confirm the compounds identified by GC-FID, the samples were also analysed by mass spectrometry (MS) using a Varian CP-3800 (Agilente, California, USA) gas chromatograph coupled with a Saturn 2000 ion trap mass detector (Agilent, California, USA). The eluted compounds were separated on a Vf-5ht column (Agilent, California, USA) (30 m × 0.25 mm, 0.10 μm). The chromatographic conditions employed were the same as those described for GC-FID analysis. The MS operating conditions were a transfer line temperature of 250 °C, electron impact (EI) ionisation mode, an ionisation voltage of 70 eV, a mass acquisition range from 40 to 350 amu and a scan rate 0.5 scans s^{-1}. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. Mass spectra obtained for each compound were compared with the NIST-98 library database.

Compounds positively identified by GC-FID were integrated and peak areas were reported as arbitrary units. Analyses were performed in duplicate.

Sensory analysis

Sensory analysis was carried out at the end of ripening (60 days) by the triangle difference test.[27] Twelve panellists were selected who tasted two cheese series each (24 responses), making a comparison between the experimental cheeses (EP, ER and EF) and their corresponding controls (CP, CR and CF). Panellists each received three randomly numbered portions of cheese, two the same and one different, and were asked to indicate the different sample according to their global sensory perception. The test significance table[27] was used to detect significant differences, whereby, for 24 responses, with a significance level $P < 0.05$, 13 correct answers are required to obtain significant differences.

Statistical analysis

Global composition, microbial count, lipolysis and volatile compound profile data were subjected to one-way analysis of variance (ANOVA) using Minitab® Release 14.1 statistical software (Pennsylvania, USA) to detect differences between control and experimental cheeses. Tukey's multiple comparison test was applied when significant differences ($P < 0.05$) in mean values were detected.

Chromatographic data of peptide profiles were analysed by multivariate methods, including a fuzzy approach for data preprocessing.[28] After that, principal component analysis (PCA) was applied to reduce the dimensionality of peptide profiles using SPSS 10.0 software (SPSS Inc., Chicago, Il, USA).

RESULTS AND DISCUSSION

Microbiological analysis

Microbiological analysis results are given in Table 1. Microbial counts showed a growth of LAB in all cheeses to between 6 and 9 log CFU g^{-1}. As expected, LAB growth was higher in cheeses with added adjunct cultures than in control cheeses. Enterobacteriaceae and total coliforms generally showed counts below the maximum allowed by Argentinean legislation,[29] which establishes for cheeses of medium humidity (540–640 g kg^{-1} dry matter) a maximum of 2.7 units log g^{-1} for Enterobacteriaceae and 3.7 units log g^{-1} for total coliforms. Overall, experimental and control cheeses did not show differences in these groups ($P < 0.05$). Mesophilic lactobacilli showed greater development ($P < 0.05$) in experimental cheeses compared with control cheeses at the end of ripening. In relation to thermophilic lactobacilli counts, significant differences were only found in cheeses with *L. rhamnosus*. There were no significant differences in mesophilic and thermophilic lactococci between experimental and control cheeses. Enterococci showed the only significant differences at all maturation times between experimental and respective control cheeses, indicating that the addition of *E. faecium* ETC3 allowed better development of this micro-organism group.

Global composition

Cheeses with and without adjunct cultures did not show significant differences ($P < 0.05$) in global composition for pH, dry matter, protein and fat at the end of ripening (Table 2). Dry matter and fat values were within the range established by Argentinean legislation for semi-hard cheeses.[29]

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Table 1. Micro-organism counts (log CFU g^{-1}) during ripening of goat's cheeses made with and without adjunct cultures

Ripening day	Type of cheese[a]	Entero-bacteriaceae	Coliforms	Fungi and yeasts	Total mesophilic	Enterococci	Mesophilic lactobacilli	Thermophilic lactobacilli	Mesophilic lactococci	Thermophilic lactococci
3	EP	2.8 ± 0.9a	3.0 ± 0.3a	2.9 ± 1.0a	8.6 ± 0.3a	2.8 ± 0.4a	8.2 ± 0.1a	8.0 ± 0.2a	8.5 ± 0.1a	8.5 ± 0.2a
	CP	3.6 ± 0.6a	3.8 ± 0.1a	3.4 ± 1.6a	8.0 ± 1.2a	3.7 ± 1.4a	6.4 ± 0.5b	6.4 ± 0.1b	8.2 ± 0.6a	8.2 ± 0.4a
	ER	3.0 ± 0.5a	2.9 ± 1.0a	2.3 ± 0.4a	8.8 ± 0.9a	3.6 ± 1.9a	8.7 ± 0.8a	8.5 ± 0.3a	8.8 ± 0.8a	9.0 ± 0.7a
	CR	3.8 ± 0.2a	2.7 ± 0.8a	3.4 ± 1.6a	7.4 ± 0.1a	4.0 ± 1.9a	7.2 ± 0.2a	7.1 ± 0.2b	8.7 ± 0.5a	8.7 ± 0.5a
	EF	3.1 ± 1.3a	2.9 ± 1.0a	2.5 ± 0.8a	8.6 ± 0.7a	6.6 ± 0.6a	7.7 ± 0.5a	7.0 ± 0.4a	8.4 ± 0.6a	8.2 ± 0.8a
	CF	2.6 ± 0.7a	2.7 ± 0.9a	2.0 ± 0.0a	8.6 ± 0.6a	4.0 ± 1.2b	8.0 ± 0.4a	6.9 ± 0.4a	8.3 ± 0.6a	7.6 ± 0.4a
30	EP	3.6 ± 0.4a	2.7 ± 0.9a	3.1 ± 1.2a	9.1 ± 0.2a	2.8 ± 0.8a	8.3 ± 0.8a	8.1 ± 0.5a	8.8 ± 0.4a	9.1 ± 0.0b
	CP	4.5 ± 1.5a	4.9 ± 1.0a	3.7 ± 2.0a	8.4 ± 0.4b	3.5 ± 0.7a	7.3 ± 0.6a	7.1 ± 0.6a	8.3 ± 0.6a	7.9 ± 0.4a
	ER	ND	ND	2.1 ± 1.2a	8.0 ± 0.0a	3.0 ± 0.2a	7.9 ± 0.0a	6.4 ± 0.3a	8.2 ± 0.2a	8.2 ± 0.2a
	CR	ND	ND	4.7 ± 0.b	6.8 ± 0.1b	3.5 ± 0.2a	6.3 ± 0.0b	6.5 ± 0.5a	7.0 ± 0.1b	7.0 ± 0.0b
	EF	2.3 ± 0.4a	2.4 ± 0.4a	2.5 ± 1.0a	9.0 ± 0.6a	6.8 ± 1.1a	8.3 ± 0.4a	6.5 ± 0.8a	8.7 ± 0.8a	7.7 ± 0.0a
	CF	3.5 ± 0.5a	3.1 ± 1.3a	4.0 ± 0.9a	8.1 ± 0.8a	4.4 ± 1.2b	7.5 ± 0.2a	6.4 ± 1.4a	7.4 ± 0.7a	7.1 ± 0.6a
60	EP	2.6 ± 0.7a	2.6 ± 0.7a	2.9 ± 0.8a	8.2 ± 1.3a	3.6 ± 1.9a	7.9 ± 0.3a	8.0 ± 1.4a	8.2 ± 1.1a	8.1 ± 1.2a
	CP	2.7 ± 0.8a	2.7 ± 0.8a	2.7 ± 0.4a	8.0 ± 0.6a	2.9 ± 1.1a	6.9 ± 0.1b	6.7 ± 0.5a	8.2 ± 0.6a	8.4 ± 0.4a
	ER	2.0 ± 0.5a	2.1 ± 0.4a	2.2 ± 0.2a	8.5 ± 0.2a	4.3 ± 1.5a	8.4 ± 0.3a	7.8 ± 0.1a	8.6 ± 0.2a	8.5 ± 0.2a
	CR	2.7 ± 0.8a	2.7 ± 0.9a	3.8 ± 1.2a	7.6 ± 0.9a	3.2 ± 1.4a	6.8 ± 0.2b	6.4 ± 0.4b	7.8 ± 0.7a	7.3 ± 0.2a
	EF	2.2 ± 0.5a	2.5 ± 0.4a	3.2 ± 1.4a	8.6 ± 1.0a	6.8 ± 0.6a	8.1 ± 0.5a	6.9 ± 0.2a	9.2 ± 0.7a	9.0 ± 0.8a
	CF	2.4 ± 0.4a	2.4 ± 0.5a	3.8 ± 1.3a	6.3 ± 1.5a	4.4 ± 0.5b	6.2 ± 0.6b	6.7 ± 0.8a	6.2 ± 0.5b	8.0 ± 1.3a

Values are mean of two trials for each type of cheese. ND, not detected (<2 log CFU g^{-1}). Different letters in each microbial group and at the same ripening time indicate statistical differences ($P < 0.05$) between experimental cheeses and their respective controls.
[a] Experimental cheeses with adjunct cultures of *L. plantarum* (EP), *L. rhamnosus* (ER) and *E. faecium* (EF). Control cheeses without adjunct cultures (CP, CR and CF respectively).

Table 2. Global composition of experimental and control goat cheeses at end of ripening

Parameter	Type of cheese[a]					
	EP	CP	ER	CR	EF	CF
Dry matter (g kg^{-1})	614.2 ± 23.7	606.6 ± 10.9	622.1 ± 3.0	610.2 ± 12.9	628.8 ± 15.3	596.3 ± 22.8
Fat (g kg^{-1})	257.5 ± 10.6	250.0 ± 7.1	240.0 ± 7.5	255.0 ± 7.1	260.0 ± 7.1	265.0 ± 0.0
Protein[b] (g kg^{-1})	312.0 ± 9.9	329.5 ± 14.9	340.4 ± 10.4	335.9 ± 5.9	347.1 ± 14.4	318.4 ± 5.4
pH	4.84 ± 0.57	5.11 ± 0.37	5.12 ± 0.26	5.11 ± 0.33	5.54 ± 0.27	5.43 ± 3.5

Values are mean of two trials for each type of cheese.
[a] Experimental cheeses with adjunct cultures of *L. plantarum* (EP), *L. rhamnosus* (ER) and *E. faecium* (EF). Control cheeses without adjunct cultures (CP, CR and CF respectively).
[b] N × 6.38.

EP and ER cheese dry matter, like that of their controls, increased throughout ripening ($P < 0.05$), while EF cheese dry matter, like that of its control, only increased until day 30 of ripening ($P < 0.05$) (data not shown). The chemical composition of cheeses was similar to that of other semi-hard goat's cheeses.[30,31]

Electrophoresis

The nitrogen fraction insoluble at pH 4.6 was studied by analysing the electrophoretic patterns obtained by urea PAGE. Owing to the similarities found in all control cheeses and in both trials of cheeses with added adjunct cultures, Fig. 1 shows as an example the patterns for control and one trial of experimental cheeses.

As can be seen, these patterns did not show appreciable differences among cheeses, indicating that the strains assayed do not affect the primary proteolysis. It can also be appreciated that β (β_1 and β_2)-caseins were similar between different cheeses and remained almost intact during ripening. Mendia *et al.*[32] and Ferreira *et al.*[33] obtained the same result for Idiazabal and Terrincho cheeses respectively. For other types of cheese, however, different levels of β-casein hydrolysis have been reported. This situation has been mainly attributed to the kind and origin of milk coagulant used and to the activity of plasmin (milk alkaline protease), which increases after heating the curd.[34,35] In our experiences the action of plasmin has been negligible, because the pH of cheeses (5.0–5.4) were somewhat lower than the optimal pH (7.5).[36] On the contrary, αs_1-casein showed moderate hydrolysis because it is easily attacked by chymosin.[37] Effectively, the primary site of chymosin action on αs_1-casein is the bond Phe23–Val24, releasing the peptide αs_1-I-casein,[38] whose presence was detected throughout the ripening process in all cheeses. At the end of ripening, αs_1-casein breakdown was not affected by the addition of any adjunct culture.

Peptide profiles

The peptide profiles of cheeses after 60 days of ripening are shown in Fig. 2. In general, the chromatograms of control cheeses were

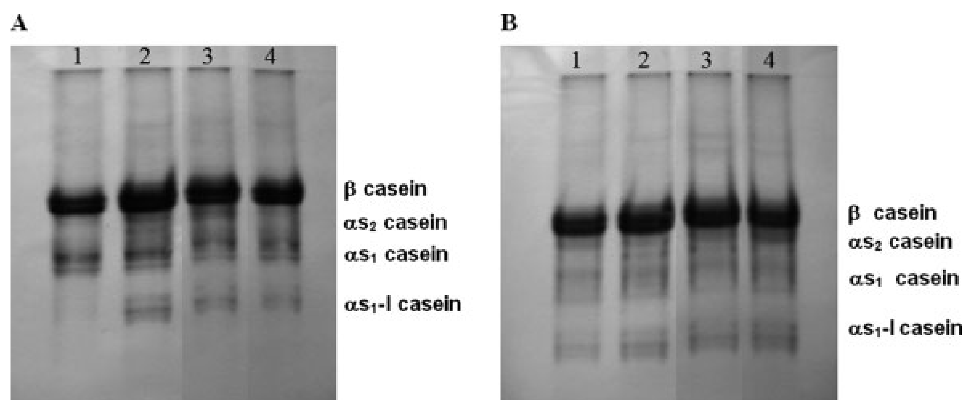

Figure 1. Urea PAGE of insoluble residue at pH 4.6 after (A) 3 and (B) 60 days of ripening: 1, experimental *Lactobacillus plantarum*; 2, experimental *Lactobacillus rhamnosus*; 3, experimental *Enterococcus faecium*; 4, control.

very similar to those of the corresponding experimental cheeses in each trial. However, some differences were detected between experimental and control cheeses. In particular, the *E. faecium* strain produced changes in the initial part of the chromatograms of EF1 and EF2 cheeses in comparison with CF1 and CF2 cheeses respectively. In effect, one peak with a retention time of 7.5 min was lower in EF than in CF cheeses, while another, whose retention time ($\sim$6 min) corresponds to the amino acid tyrosine (Tyr), was higher in EF cheeses. Therefore this adjunct culture showed an influence on the release of this aromatic amino acid (Fig. 2C). On other hand, the peptide profile of CR2 cheese was very different in comparison with the corresponding cheese ER2 and with all other cheeses. Therefore some uncontrolled factor probably influenced the peptide profile of this cheese. Finally, some differences were observed between the chromatograms of cheeses from different trials (Fig. 2B). This variability can be attributed mainly to variation in the milk composition used on each cheese-making day. Similarly, other researchers have also detected a high variation between cheese replicates.[39]

In addition to their visual analysis, peptide profiles were analysed by a multivariate method. In the present work, chromatographic data were divided into 21 classes of retention time using a fuzzy approach for data preprocessing according to Piraino *et al.*[28] The areas of these classes were considered as entry variables for PCA, with standardisation to a mean of zero and their original variances (covariance matrix). All peptide profiles were analysed by this method, with the exception of the CR2 cheese, which showed a very different profile. Two principal components (PCs) were extracted that explained 90.7% of the total variance. In the score plot of PC1 *vs* PC2 (Fig. 3) it was observed that control and experimental cheeses from trial 1 for *L. plantarum* (CP1 and EP1) were grouped separately from the same cheeses of trial 2 (CP2 and EP2). This difference is also observed in the chromatograms, where cheeses from trial 1 for *L. plantarum* showed more chromatographic peaks than the other cheeses (Fig. 2A).

On the other hand, all control cheeses (with the exception of CP1) were grouped together, with negative scores for PC2 and positive scores for PC1. In addition, the experimental cheese with *L. plantarum* of trial 2 (EP2) was included in this group. Finally, both experimental cheeses with *E. faecium* were grouped separately from their control cheeses along PC1 and PC2, which showed an influence of this adjunct culture on the peptide profiles. A similar tendency was observed for experimental cheeses with *L. rhamnosus*, above all for ER2.

It is generally accepted that mesophilic lactobacilli have a more limited proteolytic activity than thermophilic species because, unlike the latter, they grow well in milk only when it is supplemented with amino acids and peptides.[40] Gilbert *et al.*[41] demonstrated a lower caseinolytic activity of one *Lactobacillus casei* strain with regard to three strains of thermophilic lactobacilli. Similarly, Madkor *et al.*[42] found a greater increase in the level of total free amino acids in fat-reduced Cheddar cheese by the addition of attenuated strains of *L. helveticus* compared with the influence of attenuated strains of *L. casei*. Therefore the proteolytic activity of thermophilic lactobacilli, which were used as primary starter in the present work, could mask the influence of adjunct mesophilic lactobacilli. Various researchers detected little or no influence on the peptide profiles of different strains of *Lactobacillus* spp., while others observed a significant influence. These results indicate that the metabolic activity is strain-dependent.[43–46] In our study we detected only a slight influence of *L. rhamnosus* on the peptide profiles of goat's cheeses. In the case of *L. plantarum*, there was great variability in the peptide profiles between the two trials.

On other hand, the addition of *E. faecium* PR88 as adjunct culture in a Cheddar cheese made with *Lactococcus lactis* as primary starter showed an influence on secondary proteolysis as determined by an increase in most free amino acids.[4] In addition, Sarantinopoulos *et al.*[47] and Centeno *et al.*[9] detected a significant influence of various *Enterococcus* strains on peptide profiles. In our study we detected an increase in a peak, corresponding to Tyr, in the chromatogram of cheeses made with *E. faecium*, and a differentiation between control and experimental cheeses was also observed in the multivariate analysis. Therefore it would be interesting to study the influence of this strain on other amino acids.

Lipolysis

FFA from $C_{4:0}$ to $C_{18:2}$ were quantified in cheeses made with the addition of adjunct cultures and in their respective control cheeses. The lipolysis degree was determined as the total FFA content obtained by summing individual FFA concentrations. This ripening index ranged from approximately 1.2 to 2.2 g kg^{-1} cheese. These results are in agreement with those described for other types of goat's milk cheese. Poveda and Cabezas[48] found total FFA values between 2.4 and 6.7 g kg^{-1} in Spanish goat cheeses after 2 months of ripening, while Atasoy and Türkoglu[49] reported values of 0.72 and 0.99 g kg^{-1} for pasteurised and raw goat milk cheeses respectively after 90 days of ripening. Meanwhile, Franco *et al.*[50] obtained a value of 3.5 g kg^{-1} for Babia-Laciana cheese

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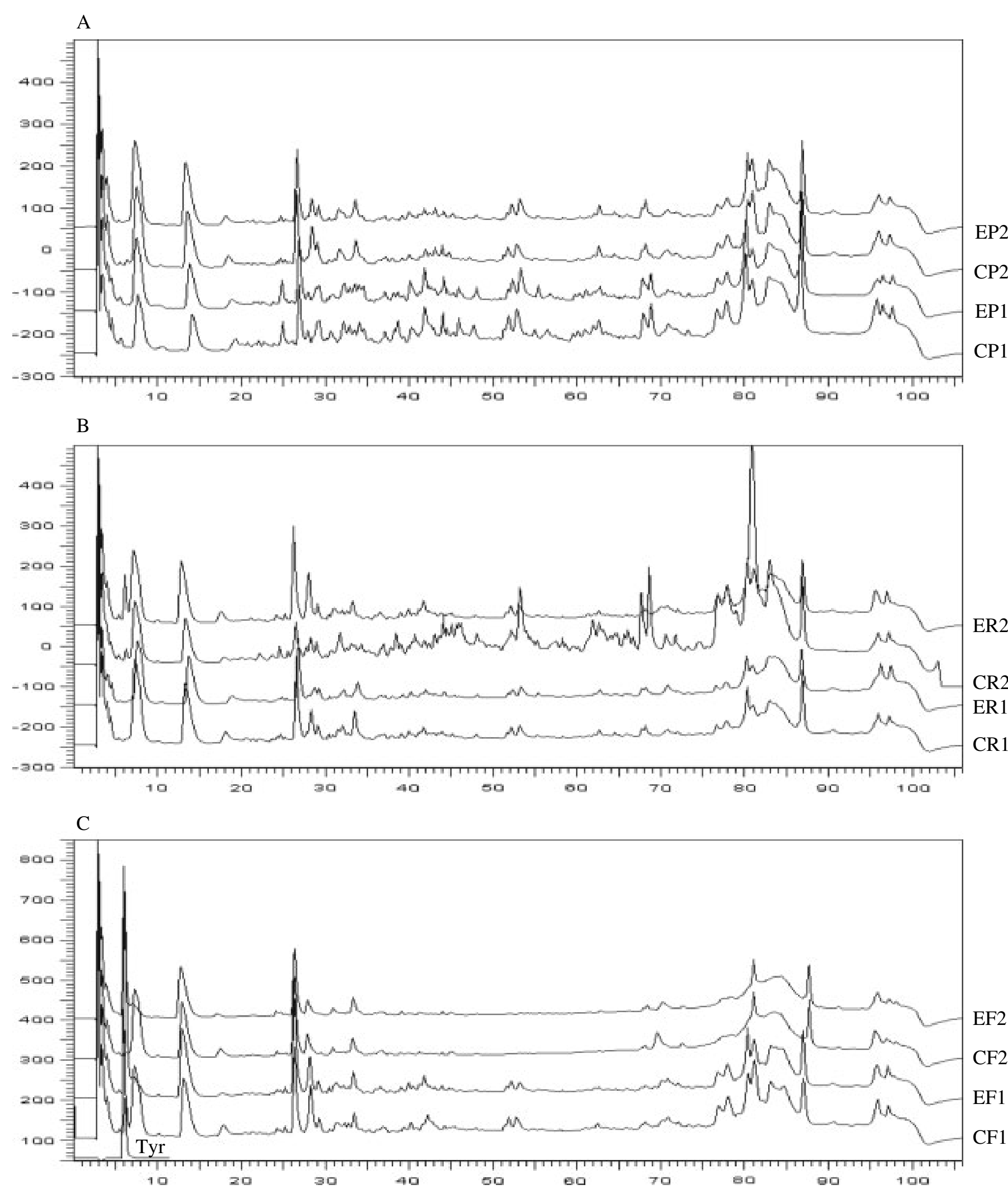

Figure 2. Peptide profiles of control and experimental cheeses: A, experimental (EP1 and EP2) and control (CP1 and CP2) cheeses with and without adjunct of *Lactobacillus plantarum* respectively; B, experimental (ER1 and ER2) and control (CR1 and CR2) cheeses with and without adjunct of *Lactobacillus rhamnosus* respectively; C, experimental (EF1 and EF2) and control (CF1 and CF2) cheeses with and without adjunct of *Enterococcus faecium* respectively. The lower chromatogram in C corresponds to tyrosine (Tyr).

after 60 days of ripening, and Delgado *et al*.[51] reported a value of approximately 6.7 g kg^{-1} after 60 days of ripening for Ibores cheese made from raw milk. On the other hand, considerably higher lipolysis levels were found for artisanal Majorero cheese, namely 24 g kg^{-1} after 60 days of ripening[52] and 16 g kg^{-1} after 150 days of ripening.[53] The wide range of reported values can be attributed to various factors such as type of milk (raw or pasteurised) used, primary starter strains employed, presence of adjunct cultures, type of coagulant and technology used for cheese manufacture and duration of cheese-ripening period.

No significant differences were found between experimental cheeses and their corresponding controls. However, variability in the lipolytic patterns among replicate trials of cheese making was observed. In particular, cheese-making trials with *E. faecium* and their controls differed in degree of lipolysis in comparison with other cheese-making trials. Some authors have pointed that differences in the initial level of lipolysis of fresh milk,[54] changes in composition at the fat/protein interface and accessibility of the fat substrate to lipolytic enzymes[53] can be among the sources of this variation.

The present study of the FFA profile of cheeses indicated that the most abundant acids were oleic ($C_{18:1}$) and palmitic ($C_{16:0}$), followed by stearic ($C_{18:0}$) and myristic ($C_{14:0}$) and to a lesser extent by capric ($C_{10:0}$), representing together about 85% of the total FFA content. A similar pattern has been reported in goat's cheeses of different origins.[48,53]

Table 3 shows the molar percentages (mol%) of short-chain (SCFA, $C_{4:0}-C_{8:0}$), medium-chain (MCFA, $C_{10:0}-C_{14:0}$) and long-chain (LCFA, $C_{16:0}-C_{18:2}$) fatty acids at the end of ripening. In spite of the quantitative importance of the MCFA and LCFA

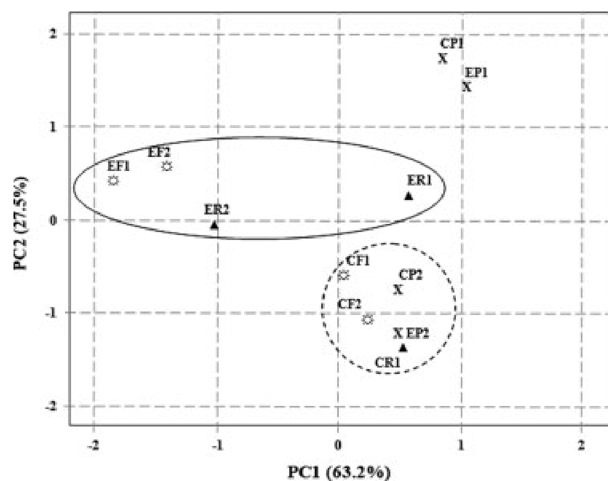

Figure 3. Principal component score plot (PC1 *vs* PC2) of peptide profiles of cheeses after 60 days of ripening. Full ellipse encloses experimental cheeses with *Enterococcus faecium* and *Lactobacillus rhamnosus*. Broken ellipse encloses all control cheeses (except CP1) and experimental cheese with *Lactobacillus plantarum* from trial 2.

Table 3. Free fatty acid (FFA) contents (mol%) of control and experimental cheeses at end of ripening

FFA group[a]	Type of cheese[b]	*L. plantarum* T1	*L. plantarum* T2	*L. rhamnosus* T1	*L. rhamnosus* T2	*E. faecium* T1	*E. faecium* T2
SCFA	C	13.6	14.0	13.9	13.1	12.7b	12.3b
	E	14.8	14.9	14.7	13.8	16.9a	17.6a
MCFA	C	22.1	19.2	21.4	20.0	17.7	18.6
	E	20.6	20.5	19.8	21.5	16.9	17.2
LCFA	C	64.4	67.5	64.6	67.2	69.0	68.9
	E	64.6	65.2	65.2	65.2	67.1	65.1

Values are mean of duplicate analyses. T1 and T2, replicate cheese-making trials.
[a] SCFA, short-chain fatty acids; MCFA, medium-chain fatty acids; LCFA, long-chain fatty acids.
[b] C, control cheeses without adjunct cultures; E, experimental cheeses with adjunct cultures.

groups, they are not the main contributors to cheese flavour, since the SCFA group has a greater impact on flavour. Some interesting differences were noted in mol% SCFA between control and experimental cheeses at the end of ripening. Cheeses made with *E. faecium* had higher values of mol% SCFA ($P < 0.05$) than their respective control cheeses. Butyric and caproic acids were the main short-chain FFA in EF1 and EF2 cheeses in comparison with CF1 and CF2 cheeses. Cheeses made with *L. plantarum* (EP1 and EP2) and *L. rhamnosus* (ER1 and ER2) did not show differences from their corresponding controls.

This could be associated with the activity of different lipases/esterases from the microflora that predominate in cheeses. Native lipase of milk (lipoprotein lipase) would not produce an important effect in the cheeses studied, because the heat treatment applied to the milk would almost totally inactivate this enzyme. In addition, the coagulant employed is not lipolytic and exogenous lipases are not included in cheese manufacture. Hence the addition of *E. faecium* as adjunct culture in goat's cheeses influenced the production of FFA in the cheese matrix. In fact, the results obtained by Oliszewski *et al.*[15] showed that *E. faecium* ETC3 has high esterase activity, releasing butyric and caproic acids.

The information reported in relation to the effect of inclusion of adjunct cultures on cheese lipolysis is variable, depending on strains used, cheese-making technology and duration of ripening process, among other factors. In fact, inter-species and strain differences in esterase activity have been detected in 15 species of LAB isolated from Cheddar cheese.[54–56] Kondyli *et al.*[5] found higher levels of total FFA in cheeses with added *L. rhamnosus* compared with control cheeses, probably due to the slight lipolytic activity of the strain. Similar results were obtained by Di Cagno *et al.*,[57] who reported that all cheeses with adjunct cultures (*L. rhamnosus*, *L. casei*, *Lactobacillus paracasei* and *Lactobacillus curvatus*) had a higher concentration of FFA than the control cheese. Likewise, an important lipolysis level was developed in cheeses with *Enterococcus* strains.

Volatile compounds

A total of 38 volatile compounds were detected in the headspace of cheeses analysed by SPME, which belonged to the chemical families of ketones (seven), alcohols (12), esters (four), aldehydes (three), acids (eight) and other compounds (four). All compounds identified in the present work have been previously reported in goat's cheeses of various origins.[58–63]

Table 4 lists the 38 volatile compounds along with their mean area values (± standard deviation) and LRI values. The latter were in concordance with published data.[64–66]

Ketones

Among ketones, methyl ketones (C_3–C_9), 2,3-butanedione (or diacetyl) and 3-hydroxy-2-butanone (or acetoin) were mainly detected. These compounds are commonly found at relatively high concentrations in goat's cheeses.[58–60,62,67] Owing to their low threshold values and characteristic notes (fruity, floral, buttery and mushroom),[68] their role in the flavour of some varieties of goat's milk cheese is considered very important.[59,60,62,63]

For both trials of *L. plantarum* and *L. rhamnosus*, a clear effect of these adjunct cultures on the production of 2-propanone, 2,3-butanedione and 3-hydroxy-2-butanone was observed, with higher area values in experimental cheeses than in control cheeses. On the contrary, the addition of *E. faecium* does not appear to affect the ketone content. In fact, the higher area values for 2-heptanone and 2-nonanone were found in control cheeses.

The production of diacetyl and its reduction product acetoin is mainly associated with pyruvate, lactose or citrate metabolism by some LAB starters.[8] It is also known that certain species of non-starter LAB isolated from milk and cheese have the capacity to metabolise citrate. Several studies have demonstrated that *L. rhamnosus* and *L. plantarum* strains are capable of using citrate by producing, among other compounds, acetoin and diacetyl.[39,69] Randazzo *et al.*[7] reported high levels of acetoin in ewe's milk cheeses made with a mix of adjunct cultures containing, among other species, one strain of *L. rhamnosus*.

Methyl ketones such as 2-heptanone and 2-nonanone are compounds derived from fatty acid catabolism.[70] Other methyl ketones such as 2-propanone are usually formed by the oxidation of butyric acid or can be synthesised in the mammary gland and from there pass to the milk,[60] whereas 2-butanone is derived from 2,3-butanediol by adventitious bacterial activity.[71]

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Table 4. Volatile compounds identified in control (C) and experimental (E) cheeses made without and with adjunct cultures (*Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Enterococcus faecium*) respectively

		L. plantarum ETC17						*L. rhamnosus* ETC14						*E. faecium* ETC3					
		Trial 1			Trial 2			Trial 1			Trial 2			Trial 1			Trial 2		
Compound	LRI[a]	CP1	EP1	*P*	CP2	EP2	*P*	CR1	ER1	*P*	CR2	ER2	*P*	CF1	EF1	*P*	CF2	EF2	*P*
Ketones																			
2-Propanone	811	265 ± 8	333 ± 18	*	275 ± 18	395 ± 4	*	167 ± 3	283 ± 10	*	221 ± 8	317 ± 10	*	588 ± 28	623 ± 20	NS	258 ± 31	320 ± 12	NS
2-Butanone	902	24 ± 0.9	26 ± 0.4	NS	33 ± 1	32 ± 2	NS	202 ± 10	146 ± 7	*	65 ± 4	31 ± 0.2	*	55 ± 2	59 ± 6	NS	39 ± 2	28 ± 5	NS
2,3-Butanedione	974	719 ± 32	1389 ± 28	*	1465 ± 38	1739 ± 29	*	813 ± 19	980 ± 37	*	697 ± 14	1202 ± 52	*	4932 ± 75	5103 ± 47	NS	1364 ± 73	1332 ± 28	NS
2-Hexanone	1079	41 ± 0.5	44 ± 7	NS	86 ± 7	83 ± 7	NS	29 ± 4	28 ± 0.7	NS	43 ± 10	66 ± 3	NS	143 ± 2	126 ± 10	NS	71 ± 7	63 ± 7	NS
2-Heptanone	1180	115 ± 4	193 ± 3	*	426 ± 28	552 ± 6	*	523 ± 22	553 ± 32	NS	369 ± 23	493 ± 11	*	4390 ± 15	3649 ± 45	*	1444 ± 23	971 ± 12	*
3-Hydroxy-2-butanone	1291	4691 ± 213	5740 ± 68	*	2638 ± 18	3547 ± 37	*	601 ± 23	1054 ± 75	*	1586 ± 61	3400 ± 52	*	1431 ± 31	1191 ± 33	*	736 ± 4	721 ± 19	NS
2-Nonanone	1391	17 ± 0.8	28 ± 0.1	*	34 ± 5	22 ± 1	NS	31 ± 0.9	42 ± 2	*	68 ± 9	80 ± 2	NS	1373 ± 41	646 ± 17	*	402 ± 2	218 ± 7	*
Alcohols																			
2-Propanol	923	ND	ND	NS	15 ± 2	9 ± 2	NS	8 ± 2	6 ± 0.5	NS	12 ± 7	22 ± 1	NS	ND	ND	NS	6 ± 0.9	10 ± 1	NS
Ethanol	934	112 ± 8	469 ± 6	*	2046 ± 29	2160 ± 22	*	379 ± 11	393 ± 18	NS	4861 ± 41	3083 ± 23	*	2082 ± 64	2518 ± 33	*	705 ± 7	780 ± 19	*
2-Butanol	1003	43 ± 0.5	42 ± 3	NS	16 ± 1	14 ± 0.3	NS	11 ± 0.5	10 ± 0.6	NS	92 ± 1	105 ± 8	NS	55 ± 2	59 ± 6	NS	59 ± 2	53 ± 4	NS
2-Methyl-1-propanol	1085	27 ± 0.2	27 ± 4	NS	ND	21 ± 0.4	*	ND	ND	NS	183 ± 12	118 ± 1	*	34 ± 7	48 ± 7	NS	39 ± 7	33 ± 4	NS
2-Pentanol	1121	12 ± 4	12 ± 0.1	NS	44 ± 0.1	41 ± 2	NS	35 ± 0.4	31 ± 2	NS	133 ± 12	63 ± 2	*	48 ± 8	143 ± 14	*	71 ± 4	129 ± 6	*
1-Butanol	1150	49 ± 10	17 ± 0.3	*	64 ± 3	39 ± 0.8	*	99 ± 6	69 ± 2	*	139 ± 1	35 ± 0.6	*	43 ± 3	55 ± 3	NS	26 ± 0.8	23 ± 2	NS
3-Methyl-1-butanol	1210	148 ± 2	78 ± 1	*	128 ± 2	96 ± 5	*	75 ± 3	55 ± 2	NS	1679 ± 47	591 ± 0.9	*	180 ± 8	150 ± 7	NS	65 ± 0.1	94 ± 0.6	*
1-Pentanol	1252	23 ± 6	ND	*	17 ± 2	15 ± 0.3	NS	ND	ND	NS	39 ± 9	39 ± 11	NS	29 ± 0.6	30 ± 4	NS	22 ± 4	22 ± 3	NS
2-Heptanol	1323	21 ± 1	ND	*	29 ± 3	19 ± 1	*	19 ± 0.9	30 ± 0.5	*	12 ± 0.4	28 ± 4	*	122 ± 2	73 ± 2	*	56 ± 6	72 ± 0.2	NS
1-Hexanol	1357	35 ± 1	40 ± 2	NS	21 ± 0.8	18 ± 0.4	NS	14 ± 1	7 ± 0.4	*	756 ± 28	146 ± 4	*	90 ± 1	115 ± 3	*	95 ± 2	100 ± 0.6	NS
2-Nonanol	1522	ND	ND	NS	14 ± 2	ND	*	ND	ND	NS	42 ± 3	14 ± 0.1	*	164 ± 8	124 ± 7	*	78 ± 4	47 ± 7	*
2,3-Butanediol	1546	289 ± 27	207 ± 5	*	48 ± 5	79 ± 4	*	151 ± 6	70 ± 5	*	222 ± 7	172 ± 12	*	75 ± 4	63 ± 2	NS	ND	ND	NS
Esters																			
Ethyl acetate	882	7 ± 0.1	42 ± 3	*	25 ± 2	38 ± 2	*	22 ± 0.4	5 ± 0.2	*	303 ± 31	126 ± 2	*	27 ± 2	55 ± 2	*	13 ± 1	49 ± 0.1	*
Ethyl butanoate	1034	125 ± 5	172 ± 13	*	169 ± 10	225 ± 14	*	93 ± 4	103 ± 7	NS	259 ± 0.6	139 ± 0.5	*	162 ± 2	182 ± 5	*	50 ± 2	75 ± 2	*
Ethyl hexanoate	1236	37 ± 2	33 ± 1	NS	33 ± 5	32 ± 2	NS	69 ± 0.9	73 ± 1	NS	16 ± 1	19 ± 4	NS	13 ± 0.6	9 ± 0.8	*	ND	ND	NS
Isoamyl butanoate	1265	21 ± 3	22 ± 5	NS	42 ± 4	41 ± 1	NS	38 ± 0.7	33 ± 3	NS	21 ± 2	27 ± 3	NS	57 ± 4	62 ± 1	NS	ND	ND	NS
Aldehydes																			
Acetaldehyde	668	100 ± 11	75 ± 8	NS	143 ± 4	108 ± 10	*	ND	ND	NS	54 ± 3	69 ± 2	*	101 ± 5a	106 ± 6a	NS	140 ± 38	98 ± 4	NS

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Table 4. *(Continued)*

		L. plantarum ETC17						*L. rhamnosus* ETC14						*E. faecium* ETC3					
		Trial 1			Trial 2			Trial 1			Trial 2			Trial 1			Trial 2		
Compound	LRI[a]	CP1	EP1	*P*	CP2	EP2	*P*	CR1	ER1	*P*	CR2	ER2	*P*	CF1	EF1	*P*	CF2	EF2	*P*
2-Methylbutanal	909	8 ± 1	6 ± 0.7	NS	26 ± 1	22 ± 0.2	*	19 ± 2	20 ± 0.6	NS	6 ± 0.3	5 ± 0.1	NS	29 ± 1a	32 ± 0.3a	NS	31 ± 4	32 ± 1	NS
3-Methylbutanal	914	27 ± 0.2	22 ± 0.9	*	68 ± 3	57 ± 0.8	*	45 ± 0.2	52 ± 2	NS	32 ± 1	38 ± 3	NS	59 ± 2a	77 ± 1b	NS	75 ± 3	92 ± 2	*
Acids																			
Ethanoic	1465	3488 ± 59	4955 ± 308	*	989 ± 67	2653 ± 89	*	1457 ± 81	881 ± 56	*	947 ± 79	867 ± 51	NS	567 ± 5	477 ± 40	NS	428 ± 67	407 ± 25	NS
2-Methylpropanoic	1583	920 ± 20	718 ± 9	*	95 ± 9	70 ± 0.9	NS	284 ± 3	168 ± 2	*	179 ± 69	552 ± 25	*	21 ± 0.4	26 ± 5	NS	ND	ND	NS
Butanoic	1640	879 ± 31	1075 ± 9	*	1188 ± 82	1534 ± 12	*	1559 ± 44	1895 ± 18	*	590 ± 13	740 ± 26	*	1569 ± 29	1839 ± 15	*	1010 ± 42	1248 ± 63	*
3-Methylbutanoic	1682	1886 ± 32	2540 ± 89	*	260 ± 13	393 ± 7	*	1144 ± 26	710 ± 15	*	299 ± 19	281 ± 12	NS	94 ± 2	80 ± 5	NS	62 ± 3	59 ± 13	NS
Hexanoic	1861	341 ± 4	386 ± 7	*	625 ± 10	755 ± 12	*	608 ± 24	745 ± 29	*	353 ± 13	435 ± 19	*	486 ± 25	613 ± 28	*	365 ± 15	458 ± 14	*
Octanoic	2073	63 ± 3	81 ± 4	*	64 ± 1	95 ± 3	*	112 ± 8	90 ± 5	NS	111 ± 19	102 ± 5	NS	149 ± 0.6	169 ± 24	NS	137 ± 12	108 ± 24	NS
Nonanoic	2183	ND	ND	NS	107 ± 13	13 ± 2	*	11 ± 2	18 ± 0.5	*	ND	ND	NS	15 ± 8	22 ± 2	NS	17 ± 6	11 ± 0.5	NS
Decanoic	2286	35 ± 0.6	41 ± 2	NS	31 ± 5	20 ± 2	NS	44 ± 3	42 ± 0.5	NS	44 ± 7	50 ± 2	NS	57 ± 10	62 ± 2	NS	73 ± 14	68 ± 28	NS
Other compounds																			
p-Xylene	1139	ND	ND	NS	15 ± 1	12 ± 0.8	NS	ND	ND	NS	18 ± 12	26 ± 0.6	NS	26 ± 4	17 ± 3	NS	ND	ND	NS
m-Xylene	1142	11 ± 2	8 ± 3	NS	27 ± 7	22 ± 2	NS	21 ± 0.2	26 ± 5	NS	73 ± 3	86 ± 10	NS	86 ± 9	72 ± 3	NS	11 ± 2	8 ± 3	NS
β-Myrcene	1160	14 ± 3	17 ± 1	NS	38 ± 7	41 ± 0.2	NS	27 ± 2	26 ± 0.4	NS	7 ± 2	13 ± 0.5	NS	18 ± 3	24 ± 0.7	NS	9 ± 0.1	8 ± 0.7	NS
D-Limonene	1189	463 ± 32	427 ± 10	NS	442 ± 24	429 ± 9	NS	885 ± 31	914 ± 32	NS	56 ± 3	52 ± 1	NS	505 ± 36	518 ± 16	NS	ND	ND	NS

Area values are mean ± standard deviation of duplicate analyses. ND, not detected. Significance: *$P \leq 0.05$; NS, not significant.
[a] Linear retention index.

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Alcohols

Primary and secondary straight-chain alcohols and primary branched-chain alcohols were mainly identified in all cheeses. Alcohols are common constituents of the volatile profile of goat's milk cheeses, but, owing to their high perception threshold values, some authors have suggested that they play a minor role in cheese flavour.[63]

Among primary straight-chain alcohols, ethanol was found at higher levels in cheeses made with *L. plantarum* and *E. faecium* than in their corresponding control cheeses. Irigoyen *et al*.[35] reported high concentrations of ethanol in ewe's milk cheeses made with the addition of *L. plantarum* as adjunct culture. This compound is formed from lactose and citrate metabolism or alanine catabolism by Strecker degradation.[8,72] Ethanol is recognised as a major alcohol present in the volatile profile of goat's and ewe's milk cheeses.[35,60,63,67,73] Although it is not considered a key compound in cheese aroma, its importance resides in the fact that it participates in the biosynthesis of ethyl esters. For other compounds belonging to this category, such as 1-butanol, 1-pentanol and 1-hexanol, no adjunct effect on their production in cheeses was observed. These primary alcohols are produced by reduction of the corresponding aldehydes originating from fatty acid or amino acid catabolism.[74]

Among secondary alcohols, 2-propanol and 2-butanol presented the same behaviour in all trials. As can be seen in Table 4, no differences in their area values were found between cheeses with adjunct cultures and control cheeses. On the other hand, 2-pentanol and 2-heptanol showed higher area values in cheeses made with *E. faecium* and *L. rhamnosus* respectively than in their control cheeses. These compounds have been recognised as major contributors to goat's cheese aroma.[58,59,62]

With respect to branched-chain alcohols, 3-methyl-1-butanol showed an increased area value only in one repetition in cheeses made with *E. faecium* and *L. rhamnosus*. This alcohol is a typical compound found in goat's milk cheeses[58,67,75] and provides a pleasant aroma of fresh cheese.[68] Its presence in cheeses is related to leucine catabolism.[72]

Esters

Three ethyl esters (ethyl acetate, ethyl butanoate and ethyl hexanoate) and one isoamyl ester (isoamyl butanoate) were detected in cheese samples. Esters are common constituents of the aroma array of cheeses.[76] According to Di Cagno *et al*.,[61] esters represented the second most abundant group of compounds in semi-hard goat's milk cheeses. Among this group, ethyl esters are considered potent flavour compounds in cheeses made from goats' milk.[53,60,62,75] In particular, those containing few carbon atoms have low perception thresholds and thus can contribute to cheese aroma by providing fruity and floral notes.[57,68]

The results of this study showed that the production of ethyl hexanoate and isoamyl butanoate in cheeses did not seem to be affected by the addition of any adjunct culture. However, ethyl acetate and ethyl butanoate were present at higher levels in cheeses made with *L. plantarum* and *E. faecium* than in control cheeses.

Several LAB have demonstrated their ability to produce ethyl esters by either esterification or alcoholysis mechanisms, but the yield of biosynthesis is highly strain-dependent.[76] Abeijón Mukdsi *et al*.[77] studied the ester-synthesising activity of *L. plantarum* ETC17 and *L. rhamnosus* ETC14, two of the strains assayed in the present study, in cell-free extracts (CFEs). They reported that the *L. plantarum* strain showed high ethyl acetate and butanoate production when CFEs were incubated in the presence of tricaproin + ethanol and tributyrin + ethanol respectively, suggesting an alcoholysis mechanism.

Bioavailability of ethanol has been considered the most important factor limiting the extent of ester biosynthesis.[76] As can be seen in Table 4, area values of ethyl butanoate, the main ester identified, showed a correlation with those of ethanol.

Aldehydes

Only acetaldehyde, 2-methylbutanal and 3-methylbutanal were identified in both control and experimental cheeses. Aldehydes are transitory compounds that do not accumulate in cheeses, since they are rapidly converted to alcohols or acids.[8] However, some of them have low perception thresholds and may play a significant role in goat's cheese aroma.[59,60,63] Branched-chain aldehydes are mainly derived from amino acid catabolism. In particular, 2-methylbutanal and 3-methylbutanal are formed from isoleucine and leucine respectively.[77] 3-Methylbutanal, with a green malty odour that becomes fruity and pleasant at low concentrations,[68] has been reported as the major aldehyde in Majorero cheese.[60] Acetaldehyde is formed from lactose and citrate metabolism or by the breakdown of threonine,[78] being the most important aldehyde present in Teleme cheese.[67]

Overall, no positive influence of adjunct cultures on aldehyde production could be observed. Only 3-methylbutanal presented higher area values in cheeses with *E. faecium* than in control cheeses.

Acids

Straight-chain acids with an even number of carbon atoms ($C_{2:0}-C_{10:0}$) and branched-chain acids such as 2-methylpropanoic acid and 3-methylbutanoic acid (or isovaleric acid) were mainly identified.

In the case of ethanoic acid (or acetic acid), higher levels were detected in cheeses with *L. plantarum* than in control cheeses. Ethanoic acid is formed via various pathways, including lactose and citrate metabolism and amino acid catabolism.[8,72] Several studies have demonstrated that *L. plantarum* and *L. rhamnosus strains* can produce it from citrate metabolism.[39]

Straight-chain acids with an even number of carbon atoms from $C_{4:0}$ are formed primarily by lipolysis. Butanoic and hexanoic acids presented higher area values in all experimental cheeses than in their respective control cheeses. This would confirm the esterase activities observed in the adjunct cultures assayed.[15] Among branched-chain acids, 3-methylbutanoic acid had higher area values in cheeses made with *L. plantarum*, being formed from leucine catabolism.[72]

The typical flavour of goat's cheese has been attributed to the fatty acid fraction.[75,79] Even at low levels, SCFA, MCFA and branched-chain fatty acids can play a key role in the overall flavour,[59,60,62,63,67] giving the characteristic 'goaty' note.

Other compounds

Two hydrocarbons (*p*-xylene and *m*-xylene) and two terpenes (D-limonene and β-myrcene) were identified. These compounds are commonly reported to be present in goat's cheeses.[53,58,59] However, they likely do not contribute to cheese flavour.[59]

No differences between control and experimental cheeses were observed. This is not surprising, since the presence of terpenes in cheeses appear to be related more to the fodder given to ruminants[71] than to biosynthesis by micro-organisms.

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Sensory analysis

No significant differences in global sensory perception ($P < 0.05$) were found for experimental cheeses made with *L. plantarum* and *L. rhamnosus* compared with control cheeses. In the case of cheeses with *E. faecium*, significant differences were detected in comparison with corresponding controls ($P < 0.05$). The sensory analysis reflected the results observed in peptide analysis, lipolysis and volatile compound profiles, which demonstrated that cheeses made with *E. faecium* ETC3 had a different global perception in comparison with control cheeses. These observations do not imply a preference by the sensory panel for cheeses made with adjunct cultures, which should be evaluated in future research.

CONCLUSION

Semi-hard goat's milk cheeses made with and without the addition of autochthonous adjunct cultures were mainly characterised regarding chemical composition, lipolysis, proteolysis and volatile compound profiles in order to determine the effect of these adjunct cultures on ripening parameters.

The presence of adjunct cultures did not significantly affect the gross composition of cheeses. In relation to microbiological analysis, among the more relevant results can be mentioned that mesophilic lactobacilli showed a higher development in all cheeses made with adjunct cultures than in control cheeses at the end of ripening, whereas enterococci reached high counts in those cheeses made with *E. faecium* ETC3, irrespective of ripening time. None of the strains assayed seemed to affect the primary proteolysis. PCA of the peptide profiles of water-soluble extracts of cheeses evidenced that variability among cheese makings was not negligible. Nevertheless, in spite of some differences observed between the two trials, the *E. faecium* strain clearly had a higher effect on peptide profiles than the tested lactobacilli. Likewise, slight differences in lipolysis profiles were found for cheeses made with the addition of *E. faecium*. In particular, SCFA levels were higher in this type of cheese, which could be related to the esterase activity of this strain. Analysis of the volatile compound profiles showed a particular enzymatic action of each adjunct culture in the production of volatile compounds. However, the levels of several volatiles that have been mentioned as key compounds in the overall flavour of goat's cheeses were affected by the presence of *E. faecium*. These results were reflected in the sensory analysis, which demonstrated that cheeses made with *E. faecium* had a different global perception in comparison with control cheeses.

The present work represents a first contribution to the characterisation of the ripening process of Argentinean goat's cheeses. *Enterococcus faecium* ETC3 looks very promising for use as an adjunct culture in such cheeses. In further studies the preference of the sensory panel for cheeses with and without adjunct strains and the effect of both primary and secondary autochthonous cultures will be evaluated. Thus the development of a product with higher regional characteristics is expected.

ACKNOWLEDGEMENTS

The authors wish to thank to Ing. Víctor Herrera from INTA EEA Santa Cruz, Catamarca for providing goat's milk. They also acknowledge CONICET, Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán and Project PICT 0582 for financial support of this work.

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