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Effect of artisanal liquid rennet from kids and lambs abomasa on the characteristics of Feta cheese

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Abstract

Feta cheeses were manufactured with commercial calf rennet and artisanal liquid rennet from kids and lambs abomasa with an aim to investigate the influence of rennet type on the characteristics of this cheese variety. The two rennets had similar chymosin to pepsin ratios and they were used in quantities with the same total milk coagulating activity. The use of traditional rennet had no significant effect on the evolution of physicochemical composition (pH, total solids, ash, NaCl, fat, total protein) or on the evolution of proteolysis (water-soluble nitrogen expressed as percentage of total nitrogen, free amino acids expressed as mmol Leu, residual α_s-casein and reversed phase HPLC profiles) during the ripening. The textural characteristics of mature 60d-old cheeses were not significantly different. The microbiological quality of traditional rennet significantly affected the enterococci and coliform counts of 3d-old cheeses but this influence was eliminated in the mature 60d-old cheeses. The significantly higher flavour scores of mature Feta made with traditional artisanal liquid rennet were in accordance with the significantly higher C4:0 and C10:0 FFA contents resulting from the lipase activity of traditional rennet.

Keywords: Kids and lambs rennet; Feta cheese; Proteolysis; FFA

1. Introduction

Rennet is the enzymatic preparation that is a key-factor for cheesemaking, consisting of the acid aspartic proteinases, chymosin and pepsin. Its hydrolytic action on the 105–106 bond of κ-casein results in the formation of the cheese curd. Moreover, it is one of the main proteolytic factors involved in cheese ripening. The most common type of rennet, used in many cheese varieties worldwide, is calf rennet that is extracted from the abomasa of young calves.

In Mediterranean countries, rennets from kids or lambs abomasa are also used. The well-known rennet paste, used in the manufacture of Italian cheeses such as Provolone and Pecorino-Romano, is a paste prepared from partially dried and ground abomasa of kids and lambs. In fact, it is an enzymatic preparation that also contains, apart from the milk clotting proteinases, pregrastic esterases (PGEs). The PGEs are secreted by oral glands (Nelson, Jensen, & Pitas, 1977) and they are responsible for the strong lipolysis and the characteristic "picante" flavour of Italian cheese varieties (Battistotti & Corradini, 1993; Collins, McSweeney, & Wilkinson, 2003). Despite the particular organoleptical characteristics of cheeses made with kids or lambs rennets, only a few studies have been carried out with the aim to compare the influence of kid's and lamb's rennet with that of calf rennet, and to study the characteristics of the resulting cheese (Anifantakis, 1976; Bustamante et al., 2003; Irigoyen, Izco, Ibanez, & Torre, 2000, 2002; Santoro & Faccia, 1998; Vicente, Ibánez, Barcina, & Barron, 2001; Virto et al., 2003).

In the past, Feta cheese was almost exclusively manufactured using an artisanal liquid rennet preparation that is made from minced, dried and salted, mixed lamb's and kid's whole abomasa. The rennet

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preparation is extracted using a NaCl solution (Anifantakis & Green, 1980; Moschopoulou, 2003). In recent years, the commercial calf rennet has replaced the artisanal liquid rennet in industrially-made Feta. However, it is still used in the traditionally-made Feta, which is considered to be more tasteful by most consumers (Moatsou, Massouras, Kandarakis, & Anifantakis, 2002).

In the present work, artisanal liquid kid's and lamb's rennet used in cheese plants located in the Argos region of Peloponnese, has been adopted for the manufacture of Feta cheese according to the industrial method (packaging and ripening in tins filled with brine). A second series of Feta cheeses was manufactured with commercial calf rennet from the same cheesemilk. The aim of this work was to investigate the effect of artisanal liquid rennet on the physicochemical composition, the evolution of proteolysis, the FFA profile, the microbiological characteristics and the textural properties and flavour scores of Feta cheese.

2. Materials and methods

2.1. Rennets

Greek artisanal liquid rennet extracted from mixed dried and salted lamb's and kid's whole abomasa and commercial calf rennet (HALA, Chr. Hansen's, DK-2970, Hersholm, Denmark), were used. The milk-clotting activities of both rennets were determined according to the IDF (1997a). The chymosin to pepsin ratio was determined according to the chromatographic method of the IDF (1997b). The lipolytic activity of artisanal liquid rennet was determined by pH-Stat at pH 5.5, according to Barzaghi and Rampilli (1996).

2.2. Cheese manufacture

Three cheesemaking trials were carried out in a cheese pilot plant. Each day, 120 kg of standardized (casein to fat ratio: 0.68–0.72) pasteurized (72 °C/20 s) ewes' milk were equally divided in two cheesevats. A mixture of mesophilic and thermophilic strains was used as starter culture (FRC-60, Chr. Hansen's, DK-2970, Hersholm, Denmark).

After 30 min, 2.1 g calf rennet, were added to one cheesevat. In the other cheesevat, artisanal kid's and lamb's rennet were added in quantities equivalent to calf rennet in IMCU. Cheese curd was cut into 20 mm cubes, 45 min after the rennet addition. After 10 min, the fresh cheese curds, hereafter abbreviated to C (made with commercial calf rennet) and T (made with traditional artisanal liquid rennet), were put into Feta moulds and they were left to drain at 18 °C. Dry salt was added and, after 4 h, the blocks of the fresh cheese curd were turned and dry-salted again. After 20 h of draining they were cut into pieces, dry-salted and left in containers until pH reached the value 4.80–4.60 (3 days). Then, the cheese pieces were washed and kept with dry salt in sealed tins for a further 10 days at 18 °C. Finally, the cheeses were washed and packaged into tins filled with 7.5% brine, sealed and stored at 4 °C.

2.3. Biochemical analyses

Total solids (TS) were determined in triplicate according to the IDF (1982). Ash was determined in triplicate, at 550 °C, to constant weight. Total nitrogen (TN), was determined in triplicate by the Kjeldahl method. Water-soluble nitrogen (WSN) was prepared by homogenizing of 1 g cheese with 5 ml H_2O according to 'Method I' for WSN extraction, cited in 'AIR-FLO-RA Laboratory Manual' (1997) and nitrogen content was determined, in duplicate, by the Kjeldahl method. Free amino acids ($N\text{-}NH_2$) content was determined in 100 μl WSN in triplicate, by the Cd-ninhydrin method (Folkertsma & Fox, 1992).

Cheese samples were analyzed in duplicate by urea–PAGE, using the method of Andrews (1983) with direct staining using Coomassie Brilliant Blue G-250 (Blakesley & Boezi, 1977). Electrophoresis was carried out on a vertical slab unit (LKB vertical electrophoresis unit 2001, Bromma, Sweden) in slabs 140×160 mm with thickness 1.5 mm. After destaining with water, gel slabs were digitized by a scanner (Hewlett Packard, ScanJet 4c/T) linked to an image processing system (GelCompar v. 4.0, Applied Maths, Belgium). A suspension of 240 mg cheese in 10 ml stacking gel buffer containing 6 M urea, 0.1 M β-mercaptoethanol and 0.4 ml tracking dye solution, was kept at 40 °C for 15 min, and then centrifuged at 3000 g for 15 min at 4 °C. The solidified fat layer was discarded and 10 μl of the supernatant were used for electrophoresis.

Biochemical analyses were carried out in Feta samples at 3, 16, 30, 60 and 180 days of ripening.

2.4. Reversed-phase HPLC (RP-HPLC)

Analyses of 50 μl water-soluble extracts were performed using an automated HPLC system of WATERS (WATERS, 34 Marple Street, Milford, MA 01757, USA) consisting of WATERS 600 pump, a diode array UV/Vis detector (WATERS 996), a helium degasser and a Rheodyne injector (model 7125, Rheodyne Inc., Cotati, California, USA). The data acquisition and processing were performed by the MILLENIUM[32] software v. 3.05.01 (1999, WATERS Corporation). A RP C_{18} Nucleosil wide pore column (5 μm, 30 nm, 250×4.0 mm, Macherey–Nagel, Duren, Germany) with a guard column (30×4.0 mm) was used. Chromatographic conditions were: solvent A, 1 ml/l trifluroacetic

acid (TFA) in water; solvent B, a mixture of 600 ml/l acetonitrile, 399 ml/l water and 1 ml/l TFA; flow rate 0.75 ml min^{-1}. The method used for sample elution was as follows: first, 100% A for 10 min, then a gradient of 0–80% B over 80 min and finally 100% B for 10 min. The absorbance of the eluate was monitored at 220 nm. The solvents and the samples were filtered through 0.45 μm filters (Millipore Corporation, Bedford, MA, USA).

Water-soluble extracts (WSN) of Feta at 3, 16, 30, 60 and 180 days of ripening were analysed.

2.5. Determination of free fatty acids (FFA)

Free fatty acids (FFA) were extracted from 60d-old cheese and determined in triplicate by gas chromatography, according to the method described by Nieuwenhof and Hup (1971). The FFA (C2:0–C12:0) were separated with a 1.50 m × 3.175 mm outer diameter glass column packed with 5% Carbowax 20 M-terephthalic acid on Chromosorb W-AW-DMCS (60–80 mesh), using a Hewlett–Packard (model 5700A) gas chromatograph. The gas chromatograph was equipped with a flame ionization detector (FID) and was connected to a Varian recorder (model 4270). The GC conditions were: 250 °C injector and detector temperatures and a helium gas flow rate of 30 ml/min, initial column temperature 65 °C, temperature programme rate 4 °C/min, final temperature 240 °C.

2.6. Microbiological analyses

The different microbial groups in cheese samples and artisanal rennet were enumerated as follows: total mesophilic flora on plate count agar at 30 °C for 72 h, mesophilic lactococci on M-17 agar (CM785, Oxoid, Hampshire, RG24 0PW) at 30 °C, for 48 h, thermophilic lactococci on M-17 agar at 37 °C for 48 h, thermophilic lactobacilli on MRS agar (CM361, Oxoid), pH 5.4 at 42 °C for 48 h anaerobically, non-starter lactic acid bacteria (NSLAB) on Rogosa agar (CM627, Oxoid) at 30 °C for 5 d anaerobically, lipolytic micrrorganisms on tributyrin agar (Sigma–Aldrich Chemie, 89552 Steinheim, Germany) at 30 °C for 72 h, enterococci on kanamycin aesculin azide agar (KAA) at 37 °C for 48 h, according to Oxoid manual instructions, coliforms on violet red bile agar (CM107, Oxoid) at 30 °C for 24 h according to the IDF (1985a), micrococci on mannitol salt agar (CM85, Oxoid) supplemented with 100 μg/ml cycloheximide (C7698, Sigma–Aldrich Chemie), *Staphylococcus aureus* on Baird Parker agar (CM 272, Oxoid) at 35 °C for 24 h, confirmed by a positive coagulase test (IDF, 1990b) and *Salmonella* sp. according to the IDF (1985b).

Also, in the cheese samples, the following were determined: *Listeria* sp. according to the IDF (1990a) and *E. coli* in lauryl sulfate broth (10266, Merck KgaA, Darmstadt, Germany) and in EC broth (10765, Merck) according to IDF (1994).

Microbiological analyses were performed in Feta samples at 3 and 60 days of ripening.

2.7. Textural properties

A Shimadzu testing Instrument, model AGS-500NG (Shimadzu Corp., Japan) equipped with a 50 kg load cell was used for determining the textural properties of cheese. A plunger (diameter 50 mm) was attached to the moving crosshead. The speed of the crosshead was at 30 mm/min in a downward direction. Feta cheese samples (25 × 25 × 25 mm) were compressed at 20 °C to 80% of their original height. From the compression curves, hardness (the force at 80% compression), fracturability (the force at the point of fracture) and % compression at fracturability point were calculated. The textural characteristics of the two series of cheeses were evaluated simultaneously. Five replicate measurements were made on each 60d-old cheese sample.

2.8. Sensory analyses

Cheeses were tested with regard to flavour at 60 and at 180 days of ripening in a random order, by a panel of 8 persons familiar with cheese grading, who also recorded flavour and textural defects.

2.9. Statistical analysis

Multifactor analysis of variance was used to test the influence the rennet type, the days of ripening and the interaction between them. The software STATGRAPHICS Plus for Windows v. 5.2 (1995, Manugistics, Inc., Rockville, Maryland 20852, USA) was used.

3. Results and discussion

3.1. Rennet characteristics

Total milk coagulating activity of artisanal liquid rennet was 31 IMCU/ml and its lipase activity was 1.67 U/ml. The total milk coagulating activity of calf rennet was 1000 IMCU/g. The chymosin to pepsin ratios were 70:30 and 67:33 for artisanal and calf rennets, respectively. The microbial populations of artisanal rennet were: total mesophilic count 3×10^5, thermophilic lactococci 7.8×10^5, proteolytic microorganisms 1.7×10^4, lipolytic microorganisms 3.0×10^6, enterococci 2.2×10^3, coliforms 2.7×10^3, micrococci 7.6×10^3, yeasts and moulds 30 and anaerobic spore-forming microorganisms 70. No *Salmonella* sp. and *Staphylococcus aureus* had been detected. According to the manufacturer, the calf rennet was free of microorganisms.

3.2. Cheesemaking

The respective mean clotting times of C and T cheese curds were 9.7 ± 1.0 min and 9.7 ± 1.2 min. The respective mean compositions of C and T wheys just after the removal of cheese curds, determined by Milkoscan apparatus, were: fat, 0.63% and 0.62%, protein, 1.98% and 1.96%, lactose 3.84% and 3.77% and total solids 7.14% and 7.03%, respectively. After 20 h of draining, the respective pH values were 4.99 and 4.97. The use of traditional rennet (T) had no significant influence ($P > 0.05$) on the cheesemaking characteristics of Feta cheese.

3.3. Biochemical characteristics of the cheeses

The evolution of biochemical characteristics during the ripening of the two series of cheeses is shown in Table 1 and the ANOVA results, expressed as *P*-values, are shown in Table 2. According to the *P*-values of Table 2, only ripening significantly affected the physicochemical composition and the nitrogenous fractions, with the exception of fat on total solids. The use of artisanal liquid rennet had no significant effect ($P > 0.05$) on these values.

Total solids and NaCl contents remained steady from 60 d, which is the minimum ripening period for Feta, and thereafter. The main proteolytic agent responsible for the primary proteolysis in Feta is residual rennet because of the low pH, which is not favourable for plasmin action. Primary proteolysis was estimated from the residual α_s-casein percentage and the percentage of WSN on total nitrogen (WSN/TN) during the first days of ripening (Table 1). It is notable that ovine α_{s1}- and α_{s2}-caseins cannot be completely distinguished by urea–PAGE; therefore, they were considered together as α_s-caseins. The changes of α_s-caseins are due to rennet action on α_{s1}-casein, which is the preferable substrate for chymosin action during cheese ripening (Sousa, Ardö, & McSweeney, 2001).

Although no significant differences were detected by ANOVA, the accumulation of WSN in cheeses T, made with artisanal rennet within the first 16 d of ripening, was faster. The same was also true for the changes of residual α_s-casein. Since the pH changes of the two series of cheeses during the draining were similar, the retentions of whey proteins in the cheese curds were also similar. Therefore, this trend of WSN during the first stage of Feta ripening, could be attributed mostly to rennet action on caseins.

Other researchers who have carried out comparative studies on the use of rennets of lamb's and kid's abomasa and the use of bovine rennets in different cheese varieties, report contradictory results about proteolysis. Anifantakis (1976) found no significant differences in the soluble nitrogen of Kefalotyri cheeses made with calf or lamb rennet. Santoro and Faccia (1998) found only slight differences between the values of SN/TN of

Table 1
Biochemical characteristics during the ripening of Feta cheese made with calf rennet (C) or traditional artisanal liquid rennet from lamb's and kids's abomasa (T)

	Rennet	3 d[a]	16 d	30 d	60 d	180 d
pH	C	4.72	4.50	4.43	4.38	4.37
	T	4.74	4.44	4.43	4.58	4.26
Total solids (TS), % of cheese	C	45.0	46.8	46.7	48.8	48.1
	T	44.6	46.7	47.6	48.5	48.5
Ash, % of cheese	C	2.43	2.61	3.57	3.63	3.83
	T	2.47	2.75	3.50	3.73	3.82
NaCl, % of cheese	C	1.16	1.75	2.74	3.40	3.26
	T	1.26	1.80	2.61	3.73	3.22
Fat/TS, %[b]	C	51.4	55.6	54.3	54.8	54.8
	T	52.5	54.9	53.9	55.1	54.1
Protein/TS, %[c]	C	40.8	35.4	37.2	35.5	34.8
	T	39.1	36.3	37.2	36.5	35.9
WSN/TN, %[d]	C	10.7	13.7	13.6	12.2	11.6
	T	9.6	14.1	14.2	12.9	10.8
$N\text{-}NH_2$ (mmol Leu)[e]	C	0.08	0.20	0.30	0.54	0.36
	T	0.07	0.25	0.30	0.67	0.31
Residual α_s-CN, %[f]	C		70.1	69.1	60.0	48.4
	T		63.4	64.1	57.7	46.5

Values are means of three cheesemaking trials.
[a] Days of ripening.
[b] Fat, expressed as percentage of total solids.
[c] Total protein, expressed as percentage of total solids.
[d] Water-soluble nitrogen, expressed as percentage of total nitrogen.
[e] Free amino acid contents, expressed as mmol of leucine.
[f] Residual α_s-caseins, expressed as percentage of the area of α_s-caseins at 3 d.

Table 2
ANOVA results (*P*-values) for the biochemical characteristics during the ripening of Feta cheese made with calf rennet (C) or traditional artisanal liquid rennet from lamb's and kid's abomasa (T)

Factors	*P*-values								
	pH	TS[a]	Ash	NaCl	Fat/TS[b]	P/TS[c]	WSN/TN[d]	N-NH_2[e]	α_s-CN[f]
Rennet type (R)	0.304	0.819	0.746	0.801	0.856	0.586	0.951	0.255	0.140
Days of ripening (D)	0.000	0.001	0.000	0.000	0.095	0.000	0.000	0.0000	0.000
Interaction (R×D)	0.617	0.928	0.985	0.976	0.599	0.259	0.653	0.122	0.904

[a] Total solids.
[b] Fat, expressed as percentage of total solids.
[c] Total protein, expressed as percentage of total solids.
[d] Water-soluble nitrogen, expressed as percentage of total nitrogen.
[e] Free amino acid contents, expressed as mmol of leucine.
[f] Residual α_s-caseins, expressed as percentage of the area of α_s-caseins at 4 d.

Canestrato Pugliese cheeses made with lamb or bovine rennet. It has to be mentioned that they used bovine rennet with a chymosin:pepsin ratio of 40:60 and lamb rennet with a chymosin:pepsin ratio of 70:30. Vicente et al. (2001) report that the changes of SN/TN, were faster during the ripening of Idiazabal cheese made with artisanal lamb rennet (chymosin:pepsin ratio 78:22) than in cheese made with commercial calf rennet (chymosin:pepsin ratio 40:60). Also, Irigoyen et al. (2002) found that SN/TN and NPN/TN of Roncal cheese made with lamb rennet (78% chymosin coagulation activity) were significantly higher than those of cheese made with commercial calf rennet (>70% chymosin coagulation activity). However, Bustamante et al. (2003) found that the type of rennet (bovine or lamb with chymosin:pepsin ratios 90:10 and 80:20, respectively), at comparable amounts of total milk coagulating activity, did not significantly affect the percentages of the nitrogen fractions in Idiazabal cheese.

Free amino acid (FAA) contents of Feta cheeses expressed as mmol Leu (N-NH_2) were not influenced by the rennet type but only by the ripening time (Tables 1 and 2); although they accumulated more rapidly in cheeses made with artisanal rennet. In 60d-old cheeses, the N-NH_2 contents were similar to those reported for Feta cheese of the same age (Kandarakis, Moatsou, Georgala, Kaminarides, & Anifantakis, 2001). At 180 days, they decreased as a result of the diffusion of low molecular weight substances into the brine and chemical modification that leads to the formation of volatile flavour compounds (McSweeney & Sousa, 2000).

According to Santoro and Faccia (1998), the use of lamb rennet increased the low mol weight nitrogen, expressed as PTA-soluble N, of Canestrato Pugliese cheese compared to calf rennet. However, the type of rennet (lamb or calf) does not have a major influence on the concentration of total and individual FAA in ovine Idiazabal cheese (Bustamante et al., 2003). It is noteworthy that Santoro and Faccia (1998) compared a lamb rennet that contained almost 75% more chymosin than the bovine, while Bustamante et al. (2003) used lamb rennet with 11% less chymosin (Virto et al., 2003).

Changes in residual α_s-caseins of Feta made with traditional artisanal rennet were faster but they were not significantly different ($P > 0.05$) from the respective values of cheeses made with calf rennet. Santoro and Faccia (1998) report that degradation of α_{s1}-casein was faster in the lamb rennet cheese, while hydrolysis of β-caseins was not influenced. According to Irigoyen et al. (2000), lamb or calf industrial rennet has no influence on hydrolysis of α-caseins in ovine Roncal cheese, while hydrolysis of β-caseins in cheese made with lamb rennet is higher. Vicente et al. (2001) reported that after 60 d of ripening, α_{s1}-casein of Idiazabal cheese made with artisanal lamb rennet was lower than that of made with calf rennet, which has much lower chymosin to pepsin ratio, as mentioned above. In contrast to theses results, Bustamante et al. (2003), who used lamb and bovine rennets with almost similar chymosin to pepsin ratios, found that the type of rennet did not affect the hydrolysis of α_{s1}-caseins, and that the hydrolysis of β-casein was higher in cheeses made with bovine rennet.

Therefore, a critical parameter for the effect of lambs or kids rennet on the proteolytic characteristics of cheese, seems to be the chymosin to pepsin ratio, providing that rennet quantities with equal total milk coagulating activities are used.

3.4. Reversed-phase HPLC (RP-HPLC) profiles of WSN

Fig. 1 shows the chromatograms of the WSN of the two series of cheeses at 60 d and 180 d of ripening. Before 30 min, amino acids and low molecular mass nitrogenous compounds of Feta WSN (<600 Da) were eluted (Moatsou et al., 2002). After 70 min, hydrophobic peptides and peptides of high molecular mass, insoluble in 12% trichloroacetic acid, were eluted (Lee & Warthesen, 1996; Moatsou et al., 2002). The ovine whey proteins were eluted after 89 min.

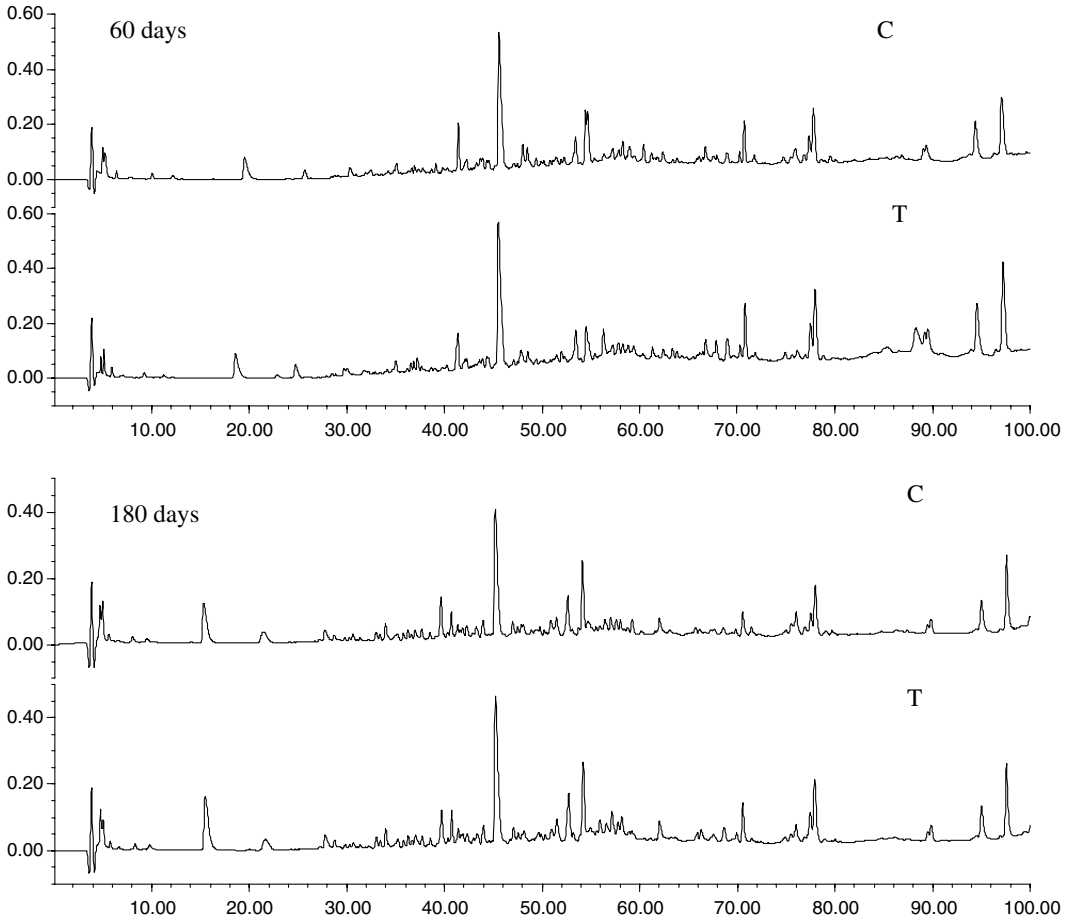

Fig. 1. Reversed phase HPLC profiles of water-soluble extracts (WSN) of Feta cheeses made with calf rennet (C) and traditional artisanal liquid rennet from kids and lambs abomasa (T) at 60 and at 180 days of ripening.

The use of artisanal liquid rennet caused no major qualitative differences in the WSN profiles. Moreover, the areas of the partial regions of the profiles, expressed as percentages of the total chromatographic area, were significantly affected only by the ripening time but not by the type of rennet (Table 3). The evolution of the ratios of the areas of the different chromatographic regions is presented in Fig. 2. Santoro and Faccia (1998) found that the ratio of polar and non-polar compounds estimated in the RP-HPLC profiles of WSN of 240d-old Canestrato-Pugliese cheese was about 20% higher in cheeses made with lamb rennet than in those made with calf rennet. However, as mentioned above, they used lamb rennet, with a higher chymosin content than calf rennet.

3.5. Free fatty acids (FFA) of mature cheeses

The low- and medium-chain FFA of the two series of Feta are presented in Table 4. The use of artisanal rennet caused a significant increase in the butyric acid (C4:0) and capric acid (C10:0) contents. This increase also significantly affected the total C4:0 to C12:0 and could be attributed to the lipase activity of traditional

Table 3
ANOVA results (*P*-values) for the partial regions[a] (based on retention times) of the RP-HPLC profiles of WSN during the ripening of Feta cheese made with calf rennet (C) or traditional artisanal liquid rennet from lamb's and kid's abomasa (T)

Factors	*P*-values					
	0–40 min	40–70 min	70–100 min	0–70 min	40–70/0–40 min	70–100/0–70 min
Rennet type (R)	0.782	0.572	0.589	0.561	0.816	0.753
Days of ripening (D)	0.000	0.000	0.000	0.000	0.000	0.000
Interaction (R × D)	0.878	0.693	0.767	0.755	0.923	0.876

[a] The area of each partial region was expressed as percentage of the total chromatographic area.

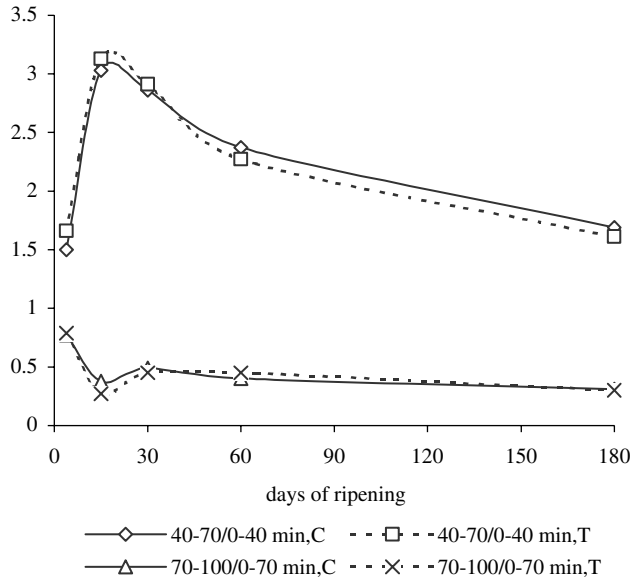

Fig. 2. Ratios of the areas of the regions of the RP-HPLC profiles of WSN of Feta cheese made with calf rennet (C) or traditional artisanal liquid rennet from lamb's and kid's abomasa (T). Values are means of three cheesemaking trials.

rennet. According to Nelson et al. (1977), the "picante" flavour of cheese is due to the release of short chain FFAs (C4:0–C10:0).

Anifantakis (1976) found that the level of low-chain FFA was higher in Kefalotyri cheese made with traditional lamb rennet than in that made with bovine rennet. Virto et al. (2003) found that short chain FFAs predominated during the ripening of cheeses made with lamb rennet paste (lipase activity from 4.0 to 6.5 U/g), whereas long chain FFAs predominated in cheeses made with bovine rennet.

It is also known that, in some hard Italian cheese varieties made with kid's or lamb's rennet paste, there is a high content of long chain FFA (Collins et al., 2003). The pregastic esterase (PGE), that is secreted by glands at the base of the tongue and is contained in the traditional kid's and lamb's rennets, is considered to be the reason for the particular FFA profile and the "picante" taste of these cheeses (Battistotti & Corradini, 1993; McSweeney & Sousa, 2000). PGE is highly specific for short chain acids esterified at the sn-3 position and butyric and other short and medium chain acids are located mainly at the sn-3 position of triglycerides (Collins et al., 2003).

Especially, kid's and lamb's PGE liberate higher totals of C4:0 to C10:0 FFA than calf PGE and kid PGE liberates the highest (Nelson et al., 1977). According to the data presented by Kim Ha and Lindsay (1993), the greatest part (>66%) of the total C4:0 to C10:0 FFA released by the lamb, kid and calf PGEs consisted of butanoic (C4:0) and decanoic (C10:0) acids. This was also true for the FFA profile of Feta made with traditional rennet (Table 4).

According to Barzaghi, Davoli, Rampilli, and Contarini (1997) and Calandrelli et al. (1997), the amount of FFA, and especially their relative compositions in Provolone cheese and caprine Semicoto cheese, are influenced by the differences in the preparation of rennet pastes. Fontecha, Peláez, Juárez, Requena, and Gómez (1990) report that FFA of goat's Majoreto cheese made with kid rennet paste has considerably higher FFA than that of Majoreto cheese manufactured with commercial rennet. Furthermore, Larráyoz, Martínez, Barrón, Torre, and Barcina (1999) found that the use of rennet prepared from lambs abomasa increases the FFA in Idiazabal cheese.

3.6. Microbiological characteristics

The microbial populations of fresh (3 days) and mature cheeses (60 days) are shown in Table 5. All the microbial groups of Table 5, with the exception of lipolytic microorganisms, were significantly influenced ($P < 0.05$) by the days of maturation. The type of rennet significantly affected only the coliform and the enterococci groups of 3d-old cheese. This difference could be attributed to the microbiological quality of the traditional rennet used (Section 3.1), since the pH and NaCl conditions of the two series of cheese were the same. Nevertheless, they were almost eliminated at 60 d of ripening, which is the minimum ripening period for Feta according to the Codex Alimentarius (1988). It seems that the artisanal rennet did not contaminate the cheese and this observation was in accordance with the findings of Calandrelli et al. (1997). The counts of these two groups were lower than that reported for traditional Feta cheeses, while the other microbial groups were at the same level (Manolopoulou et al., 2003). No *E. coli*,

Table 4
Free fatty acids (FFA) of 60d-old Feta cheese made with calf rennet (C) or traditional artisanal liquid rennet from lamb's and kid's abomasa (T)

Rennet	FFA (ppm)					
	C4:0 Butyric acid	C6:0 Caproic acid	C8:0 Caprylic acid	C10:0 Capric acid	C12:0 Lauric acid	Total of C4:0–C12:0
C	192	101	336	471	1067	2167
T	508*	111	376	1076*	1362	3433*

Values are means of three cheesemaking trials.
* Significantly different means in the same column ($P < 0.05$).

Table 5
Microbiological characteristics of Feta cheese made with calf rennet (C) or traditional artisanal liquid rennet from lamb's and kid's abomasa (T)

Microbial groups (cfu/g of cheese)	3 days		60 days	
	C	T	C	T
Total mesophilic flora	3.4×10^9	3.1×10^9	1.9×10^8	3.4×10^8
Mesophilic lactococci	3.6×10^9	3.7×10^9	2.5×10^8	2.3×10^8
Thermophilic lactococci	2.8×10^9	2.7×10^9	1.8×10^8	2.7×10^8
Thermophilic lactobacilli	4.5×10^6	4.9×10^6	19×10^4	8×10^4
NSLAB[a]	3.9×10^3	5.6×10^3	11.5×10^7	9.1×10^7
Lipolytic microorganisms	3.8×10^3	3.4×10^3	0.6×10^3	0.7×10^3
Micrococci	1.80×10^3	1.68×10^3	0.56×10^3	0.75×10^3
Enterococci	17.2×10^3	$42\times10^{3*}$	5.8×10^3	8.9×10^3
Coliforms	375	675*	7.5	17.5

Values are means of three cheesemaking trials.
[a] Non-starter lactic acid bacteria.
* Significantly different means in the same cheese age ($P < 0.05$).

Staphylococcus aureus, *Salmonella* sp. or *Listeria* sp. were detected.

3.7. Textural properties and flavour scores

The textural characteristics of 60d-old C and T cheeses were: hardness 9.04 ± 0.73 and 10.75 ± 3.14 kg, fracturability 3.03 ± 0.63 and 4.11 ± 0.56 kg and % compression at the fracturability point 22.51 ± 2.44 and 20.14 ± 1.63, respectively. No significant differences ($P > 0.05$) were observed between the calf and artisanal rennet cheeses, since their physicochemical characteristics were not significantly different (Creamer & Olson, 1982).

At both stages of ripening, both series of cheeses received high flavour scores. The scale was from 0 to 50 points. At 60 d, the scores were 44 and 47.4 and, at 180 d, 44.7 and 49.4 points for C and T cheeses, respectively. The scores of cheeses made with artisanal rennet were significantly higher (t test, $P < 0.05$) and this could be attributed to their FFA profiles according to the discussion of Section 3.5, since all the other biochemical characteristics were not significantly different.

4. Conclusions

From the results of the present work, and on condition that the quantities of calf rennet and traditional artisanal rennet from kids and lambs abomasa have equivalent total milk coagulating activities and similar chymosin to pepsin ratios, it could be concluded that the use of traditional rennet in Feta manufacture resulted in:

- no significant effect on the evolution of physicochemical composition and on the evolution of proteolysis during the ripening. It seemed therefore, that the chymosin to pepsin ratio was the critical parameter for the physicochemical and proteolystic characteristics of Feta and not the rennet origin (calf or kid's and lamb's);
- no significant differences in the microbiological characteristics and the textural properties of mature cheeses;
- significantly higher ($P < 0.05$) C4:0 and C10:0 FFA contents of mature cheeses made with artisanal liquid rennet in accordance with their significantly higher ($P < 0.05$) flavour scores.

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