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Effect of artisanal kid rennet paste on lipolysis in semi-hard goat cheese

J. Fontecha [a,*], I. Castillo [a], L. Blasco [a], L. Alonso [b], M. Juárez [a]

[a] Departamento de Productos Lácteos, Instituto del Frío (CSIC), José Antonio Novias 10, Ciudad Universitaria, 28040 Madrid, Spain
[b] Instituto de Productos Lácteos de Asturias (CSIC), Carretera de Infiesto s/n. 33300 Villaviciosa, Principado de Asturias, Spain

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Abstract

This study examined the use of hygienised kid rennet pastes in model cheese systems and also in goat milk semi-hard cheeses to promote lipolysis. The results obtained indicated that the use of rennet paste caused greater lipolysis and increased, mostly, the short-chain free fatty acid (FFA) content. The model systems made with whole goat's milk using rennet paste and commercial rennet mixture exhibited a higher FFA content than did the rennet paste-free controls (31,600 vs. 25,600 μmol/kg cheese). For the pilot cheeses made with bovine rennet and rennet paste mixture, the increase in FFA level after 45 days of ripening compared with the cheeses prepared only with commercial rennet was as much as 6600 (μmol/kg cheese) and the increase in the butyric acid content was also 1650 (μmol/kg cheese). Moreover, after 15 days of ripening, industrially prepared cheeses made with rennet paste exhibited greater levels of FFA than did the cheeses made with commercial rennet (11,500 μmol/kg at 45 days of ripening). Their flavour was stronger and the organoleptic characteristics were better accepted, which implies less ripening time for commercial cheese manufacture.

Keywords: Goat's cheese; Rennet paste; Lipolysis

1. Introduction

Goat's milk is becoming increasingly important in Spain, especially because of the popularity of its products, in particular cheese. In fact, virtually all the milk from this species is used for making semi-hard cheese with a short ripening period. Nevertheless, sometimes, commercialised products do not have the desired flavour and they are sometimes bitter in taste. This is due to the industry's use of rapid culture starters with high protease activity. There are not many technological studies on specifically improving the preparation and ripening conditions of goat's cheese. One solution is to speed up ripening by increasing lipolysis and proteolysis, since these are the changes that are essential for the development of flavour in cheeses.

Goat's cheese is not ripened by mould and exhibits low levels of lipolysis (De la Fuente, Fontecha, & Juárez, 1993), since the lipolytic activity of the lactic bacteria used in the starter culture is low (Fox & Stepaniak, 1993). Sources of lipases for improving the aroma of sheep's milk cheese, and particularly goat's, have traditionally been animal tissues, namely pancreatic glands and pregastric tissues of milk ruminants. Rennet pastes used for clotting the milk contain pregastric lipases, which cause the hydrolysis of triglycerides in the milk and release acids in position sn-3 where most of the short-chain acids (butyric, caproic and caprylic) are esterified; these short-chain acids are responsible for the typical "piquant" flavour in these cheeses (Hill, Ghannouchi, & García, 2001; Rampilli & Barzaghi, 1995).

* Corresponding author. Tel.: +34 915492300; fax: +34 915493627.
E-mail address: jfontecha@if.csic.es (J. Fontecha).

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It has also been reported that the flavour is better with rennet pastes than with purified pregastric lipases (Barzaghi, Davoli, Rampilli, & Contarini, 1997). However, rennet pastes do have some drawbacks. The major disadvantages are the complexity and variability of the rennet paste composition, its low clotting activity and poor microbiological quality – which also depends on the animal species, the way in which the pastes are obtained and the preservation system. Analytical controls (microbiological and enzymatic), hygienisation and characterisation of these rennet pastes are, therefore, essential so that they can be more widely used to produce uniform quality products.

There are several studies that have shown that lamb rennet pastes contain lipolytic activities (Fox & Guinee, 1987) and that the use of these pastes directly affects lipolysis in cheeses (Virto et al., 2003). However, there are fewer studies on the use of kid paste to increase the levels of lipolysis in cheeses in a controlled way.

Artisanal rennet kid pastes are used on the Island of Fuerteventura to obtain artisanal *Majorero* cheese. They are prepared by filling the stomach of the suckling kid with milk, which is then sun-dried and put in salt or preserved in brine. In our laboratory, artisanal kid pastes of different origin were characterised and prepared from the contents of stomachs preserved under both conditions (Calvo & Fontecha, 2004).

This work was designed to study the possible positive effect of characterized kid rennet pastes on goat milk semi-hard cheese to improve the organoleptic characteristics of the final product. Moreover, with the use of rennet paste, the ripening period could be shortened, which would be a clear advantage for commercial manufacturers. For this purpose, three cheesemaking types were performed: model, pilot and industrial.

2. Materials and methods

2.1. Preparation and enzymatic assays of rennet pastes

Rennet pastes from a selected artisanal cheesemaker in Fuerteventura (Canary Islands, Spain), prepared from suckling kids' stomachs, according to the local traditional procedures, were used in this study. The kids' stomachs were salted in brine for at least 2 months before preparing the rennet paste. In order to discover the effect of the extraction system on the enzymatic activity of the paste, two different methods were compared. In the first preparation (A), the kids' stomachs were washed and sectioned in order to extract their contents, which were minced and homogenised in Milli-Q water using a Stomacher (model 400, Seward) until a semi-liquid homogeneous paste was obtained, as in Calvo and Fontecha (2004). In the second preparation (B), all the washed stomach was cut into pieces and minced in an Omni-mixer held in an ice bath for 2 min with 100–150 ml of Milli-Q water until a homogeneous paste was obtained. Water dilutions (1:4 v/v) of both rennet pastes were employed as starting material to perform the hygienisation process, and the mixtures were magnetically stirred for 16 h at 4 °C. The hygienisation process and microbiological analysis, as well as the clotting time, coagulum characteristics, proteolytic, esterase and lipase activity were performed as suggested by Calvo and Fontecha (2004).

2.2. Preparation of the cheese model systems

Four cheese model systems were prepared with 10 l of milk each, two with whole goat's milk (4.2% fat) (one using commercial rennet – WCR, and another using a rennet paste and commercial rennet mixture – WRP) and another two with semi-skimmed milk (2.5% fat) (using the same types of rennet – SCR and SRP). The whole or semi-skimmed milk, which had previously been pasteurised (70 °C 15 min), was tempered at 30 °C in the 15-l capacity vat by stirring. The pH fell to 5.7 with a dissolution of δ-gluco-lactone (l g/ml). Ten millilitre of $CaCl_2$ (20%) and 10 ml of sodium azide (0.1 g/ml) were added. Sodium azide was added to prevent the proliferation of microorganisms. Commercial rennet of animal origin (Chr. Hansen Laboratorium, Copenhagen, Denmark) (15 mg/l) was added for the control system. For the rennet paste model systems (preparation B was selected), a mixture solution prepared from 7.7 mg/l of commercial rennet and hygienised kid rennet paste ($F = 1{:}10{,}000$) was added so that the clotting occurred in ≈40 min, which was similar to the clotting time obtained from commercial rennet. The curd was then cut to the size of 1 cm and stirred at 37 °C for 30 min. The moulded cheeses (weighing ≈500 g) were salted in 20% brine for 4 h and ripened at 12 °C and 85% relative humidity. Cheese samples for analysis were taken at 2, 15 and 30 days.

2.3. Preparation of pilot cheeses

Two batches of three lots of semi-hard cheese were prepared, varying the kind of rennet used (commercial rennet – control, rennet paste and mixture of both rennets). Each cheese lot was made from 30 l of pasteurised goat's milk (76 °C for 45 s) with whole milk (fat content of 4.2%). The commercial liquid rennet was lamb Bio-Star, consisting of 93.7% chymosin and 6.3% pepsin, with a rennet strength of $F = 1{:}150{,}000$. The milk was pre-heated to 31 °C to which 12 ml of $CaCl_2$ (54%) were added. The starter (300 ml "IFPL starter culture," including the microorganisms *Lactococcus lactis* ssp. *lactis* IFPL 359, *Lactobacillus casei* ssp. *casei* IFPL 731, *Lactobacillus plantarum* IFPL 935, *Leuconostoc mesenteroides* ssp. *dextranicum* IFPL 709 and *Leuconos-*

toc paramesenteroides IFPL 705), was then added (Requena, de la Fuente, Fernández de Palencia, Juárez, & Peláez, 1992), and left to pre-ripen for ≈30–40 min. When the pH dropped to ~6.5 from 6.9, which was the initial milk's pH, the rennet was added. The control lots were prepared by adding 3.3 ml of commercial rennet ($F = 1{:}150{,}000$). A solution of rennet prepared from 50% commercial rennet used in the control cheeses and 50% hygienised and lyophilised kid paste ($F = 1{:}10.000$) was added to the lots with a commercial rennet and rennet paste mixture to maintain the clotting time. Two gram of rennet paste was used in the lots with just this paste. For all the cheese lots, the cutting point of the curd (the size of a hazelnut) was reached between 30 and 35 min. The curd was placed in plastic moulds where it was dripped and pressed until the pH was ≈5.5. The salting was done in brine (18°Bme) for 12 h. The cheeses prepared for this study weighed,average, 1 kg and were ripened at 12 °C and 85% relative humidity. Cheese samples for the analyses were taken at 2, 15, 30 and 45 days of ripening.

2.4. Preparation of commercial cheeses

Two lots of cheeses were made from whole goat's milk (4.2% fat). The first lot was made from 4000 l milk, using a commercial starter culture consisting of 5% mesophilic culture and 0.5% thermophilic culture and a 400 ml commercial rennet coagulant (consisting of l00% of chymosin). About 1500 l milk and the same starter were used in the second lot, but this time a commercial rennet (80 ml) and kid rennet paste (17 g) mixture was added.

2.5. Cheese analysis

The pH, total solids (TS) and total nitrogen were analysed according to Alonso, Juárez, Ramos, and Martin-Alvárez (1987). Non-casein nitrogen (NCN) was analysed according to Kuchroo and Fox (1982); non-protein nitrogen (NPN) was analysed using the method described by Ordóñez, Barneto, and Ramos (1978).

2.6. Cheese enzyme extract

To obtain the cheese enzyme extract, 15 g of the model system and cheeses were taken and mixed with 30 ml of buffer Pipes 50 mM, pH 6.7, which contained 0.86 M of NaCl and 0.1 M of $CaCl_2$. The mixture was homogenised in an Omni-mixer for 5 min. Then, it was stirred at 4 °C for 4 h and centrifuged at 12,000*g* for 15 min at 4 °C. The supernatants were filtered first using Whatman 40 filters, followed by Millipore 0.45-μm pore-size filters and the filtrate was kept at −20 °C until analysed. The protein content was determined using the Bio-Rad protein assay. In the model system, and for cheese enzyme extracts, the determination of proteolytic activity was measured using azocasein, and it was expressed as an increase of 0.1 absorbance units at 440 nm per min and per mg of protein (arbitrary units, AU) according to the method described by Fontecha, Requena, and Swaisgood (1996). The esterase activity was expressed as μmol of β-naphthol released from the substrate β-naphthyl caprylate per min and per mg of protein and the lipase activity with tributyrin was expressed as milli-equivalents of butyric acid per min and per mg of protein, according to the procedures described by Calvo and Fontecha (2004). For the determination of proteolytic activity we began with 0.5 ml of cheese enzyme extract whereas, for the esterase activity and lipase activity, 100 and 250 μl filtrates were used, respectively.

2.7. Free fatty acid analysis

Lipid extraction was carried out on an acidified cheese (10 g) slurry, using ethyl ether, followed by methylation with 20% of tetramethylammonium hydroxide in methanol (Martín-Hernández, Alonso, Juárez, & Fontecha, 1988). FFA were analysed on a fused silica column (60 m × 0.22 mm × 0.22 μm) coated with BPX 70, using a Perkin–Elmer 8420 apparatus (Perkin–Elmer, Beaconsfield, UK) equipped with a PTV injector, with helium as the carrier gas (split ratio 1:20). Oven temperature was held at 70 ° C for 3 min, then raised to 190 °C at a rate of 13 °C/min and held at this temperature for 25 min. Other details are as described by Juárez, de la Fuente, and Fontecha (1992). The FFA determinations were done in duplicate for each sample.

2.8. Sensory analysis

For the sensory analysis of cheeses, the International Dairy Federation (IDF, 1987) recommendations were followed and a group of 10 tasters (minimum) was selected from the Instituto del Frío staff.

The cheese samples were prepared without the rind and in ≈1-cm thick portions. All the analyses were rated on a hedonic scale from 1 to 10. The attributes analysed were: aroma strength, flavour strength, basic flavours, presence of other flavours, texture and general acceptability. The presence of certain characteristics for each attribute was also rated as were any negative aspects of the taste, aroma or appearance of the cheeses (e.g., rancid taste).

2.9. Analysis of volatile compounds

Internal standard solution (80 μl of 1.14 mg/ml of propionic acid ethyl ether) and 10 g of anhydrous sodium sulphate were mixed with 10 g of the cheese sample using a spatula in a 20-ml headspace vial that was sealed hermetically with a polytetrafluoroethylene coated rubber septum and an aluminium cap. The mixture was

stored at −20 °C until further analysis, as described by Alonso, Fontecha, and Juárez (1999).

2.10. Statistical analyses

Two-way variance analysis (ANOVA) was performed, with time and sample type as factors, to determine significant differences ($P < 0.05$). The software used was SPSS 11.5 (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Rennet pastes

The proteolytic activity in the rennet paste obtained from inside the stomach (A) and characterised in the earlier work (Calvo & Fontecha, 2004) was higher than the proteolytic activity in the complete rennet paste obtained from mincing all the stomach (B) (0.025 against 0.013 activity units). The esterase activity of this latter paste was slightly higher but not statistically significant (0.33 against 0.28 μmol of β-naphthol/min/mg protein), since the enzyme content also present in the stomach tissues was included.

The lipase activity values were similar in the two kinds of preparations, 0.3 μmol fatty acid released/min/mg of protein.

Regarding the clotting activity, the clotting time was less with B rennet paste (0.5 min against 2.5 min), although the theoretical cheese yields were similar for the two pastes.

Given that the aim of these pastes was to increase FFA in cheese, the complete rennet paste (B) obtained from mincing all the stomach was used in this study because of its slightly higher esterase activity and shorter clotting time.

3.2. Cheese model systems

The average fat content of the whole milk cheese model systems (WCR and WRP) at the start of the period was 30.5% and that of the semi-skimmed milk cheese model systems (SCR and SRP) was 21.5%; the TS levels were 57.2% and 46.9%, respectively. The total protein contents in the four systems were similar, with an average value of 17.8%. Although proteolysis (measured as soluble nitrogen content) increased slightly throughout the study, it exhibited low levels as corresponds to a system similar to a fresh cheese prepared without a starter culture (Martín-Hernández, Juárez, & Ramos, 1992). Average soluble nitrogen contents (referred to as total nitrogen) in the four systems at the end of the period were around 12%. The pH in the different systems remained around 6.0 and dropped very slightly (~5.8) until 30 days. Therefore, the levels of the composition and proteolysis characteristics were similar to those in fresh cheese (Martín-Hernández et al., 1992; Rodríguez et al., 1997).

Proteolytic and esterase activities were higher in the systems made with rennet pastes (WRP and SRP) at the beginning of ripening, although there were wide intervals of variation, especially for the proteolytic activity (Fig. 1). This result is in agreement with the similar proteolysis indices observed. Throughout the period under study, the decline in proteolytic and esterase activity that was observed can be justified (Fig. 1). This decline was due to the protein present in the soluble extract, which increased throughout ripening.

As for lipolytic activity, especially at the beginning of ripening, higher levels were observed in the systems prepared with the rennet paste (Fig. 1), and thus greater levels of lipolysis were expected. FFA contents were

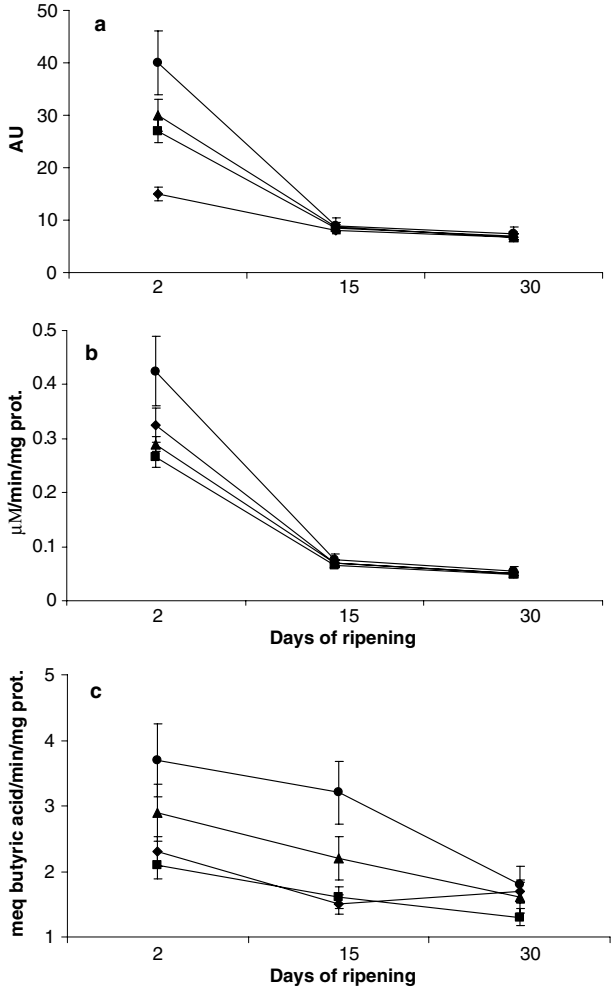

Fig. 1. Enzymatic activities in the model cheese systems prepared with: whole goat's milk and commercial rennet (–◆– WCRX), semi-skimmed milk and commercial rennet (–■– SCR), whole goat's milk and a rennet paste and commercial rennet mixture (–●– WRP), semi-skimmed milk and a rennet paste and commercial rennet mixture (–▲– SRP), throughout ripening. (a) Proteolytic activity; (b) Esterase activity; (c) Lipolytic activity. AU (Arbitrary units): increase of 0.1 absorbance units at 440 nm per min and per mg of protein.

Table 1
Average free fatty acid content(μmoles/kg cheese) in the model systems at 30 days of ripening

	WCR	SCR	WRP	SRP
C4	2140	2282	3526	2315
C6	1454	1350	1270	1250
C8	1248	1265	1542	870
C10	3121	3390	4310	2428
C12	1428	1398	1901	1229
C14	2359	1909	2979	2223
C16	5812	4602	6606	5681
C18	2461	1304	2655	2216
C18:1	5234	3012	6188	4448
C18:2	318	382	620	570
Total	25,575	20,894	31,597	23,230

WCR, model system prepared with whole goat's milk and commercial rennet; SCR, model system prepared with semi-skimmed goat's milk and commercial rennet; RP, model system prepared with whole goat's milk and a rennet paste and commercial rennet mixture; SRP, model system prepared with semi-skimmed goat's milk and a rennet paste and commercial rennet mixture.

greater (31,600 against 25,600 μmol/kg) in the systems with all the fat levels (Table 1). Of the total FFA, the short- and medium-chain acids (C4–C10) of WRP were about 2700 μmol/kg higher than WCR. The systems with a lower fat content also exhibited higher total FFA values when rennet paste was included in the preparation (23,200 against 20,900 μmol/kg) (Table 1). Nevertheless, in these systems, the short- and medium-chain acids of SCR were higher than SRP. From the results obtained, we can conclude that the addition of rennet paste would increase the level of lipolysis, especially in the cheeses produced with whole milk.

3.3. Pilot cheeses

Changes in pH were similar to those found in other goat milk semi-hard cheeses, with figures close to 5.5 at 45 days of ripening (Fontecha et al., 1990; Requena et al., 1992). The TS mean content of the cheeses, at two days, exhibited values of 54.4%. The mean fat content of the cheeses studied at the beginning of ripening was 28.0%. The total mean protein content in the cheeses, at two days, was 21.7%, which increased as a result of dehydration in the reduced humidity environment during ripening.

The two proteolysis indices studied, NCN(%TN) and NPN(%TN), increased throughout ripening, exhibiting, at 45 days, average values of 17.0% and 12.5%, respectively. From the beginning of ripening, higher values were observed in the lot prepared with whole milk and commercial rennet, due to greater proteolytic activity in this rennet, and the differences in NCN and NPN values remained unchanged until 30 days of ripening. However, after 30 days of ripening, similar levels of proteolysis were observed in the cheeses made with rennet paste.

From the beginning of ripening, slightly significantly higher FFA contents were obtained in the lots that used just rennet paste or the rennet paste and commercial rennet mixture, 44,900–48,000 μmol/kg cheese at 45 days, against 41,500 in the lot prepared with only commercial rennet (Table 2). For FFA profile by chain length, a higher level of short-chain acids (especially butyric acid, C4, with 1650 μmol/kg more than in the lot with commercial rennet) was observed, mainly responsible for the piquant flavour in the cheese lots that used rennet paste. The lipolytic enzymes present in rennet pastes have been shown to be responsible for releasing short-chain acids (Barzaghi & Rampilli, 1996). However, the lot clotted just with rennet paste showed the highest value of butyric acid at 30 days of ripening while, in the other two lots, the amount of this acid increased until the end of the period studied, but did not reach a value as high as the WRP value. The decrease of butyric acid in the WRP lot at 45 days of ripening

Table 2
Major free fatty acid content (μmol/kg) in pilot cheeses

Acid	2 Days			15 Days			30 Days			45 Days		
	WCR	WRP/CR	WRP	WCR	WRP/CR	WRP	WCR	WRP/CR	WRP	WCR	WRP/CR	WRP
C4	2879	4362	4021	2920	4265	4301	3057	4158	5579	3642	5287	4060
C6	2567	2214	2191	2882	3336	4197	2945	2352	2667	3513	3405	2621
C8	2213	1473	1505	1815	1511	1533	1903	1815	1632	1745	1935	1613
C10	5415	3916	3932	4593	3980	4024	4405	5033	4174	4356	4679	4,168
C12	1429	1247	1219	1471	1401	1318	1401	1671	1382	1513	1597	1503
C14	2283	2432	2320	2865	2878	2322	2696	3414	2634	3113	3233	3084
C16	6542	7748	7308	7500	9446	7567	8007	11,023	8595	9624	10,797	10,579
C18	3128	3735	3574	3983	4553	3751	3782	5310	4016	4640	5310	5260
C18:1	5090	6811	6619	7098	8766	7183	7396	9755	7770	8597	11,054	10,906
C18:2	265	510	785	659	805	793	391	646	765	714	744	1067
Total	31,811	34,448	33,473	35,786	40,941	36,989	35,983	45,177	39,214	41,456	48,041	44,861

WCR, cheese prepared with whole goat's milk and commercial rennet; RP/CR, cheese prepared with whole goat's milk and a rennet paste and commercial rennet mixture; WRP, cheese prepared with whole goat's milk and rennet paste.

was probably because this acid was converted into other volatile compounds (Collins, McSweeney, & Wilkinson, 2003). In the other lots, probably more time would be necessary for this to happen.

The volatile compounds present were the same in all the lots, and the main differences were quantitative. After 30 days of ripening, the amount of volatile compounds in the cheeses made just with rennet paste was slightly higher than in the other cheeses. Nevertheless, given the fact that the starter culture was the same, the differences were observed particularly in the volatile compounds resulting from lipolysis, represented by short-chain volatile fatty acids, especially butyric and hexanoic that were higher in the cheese lots that used rennet paste in their preparation (120 against slightly less than 50 μg/100 g). This result is the same as for the free fatty acids. After a few days of ripening, the rennet paste causes changes in the short-chain volatile fatty acids and free fatty acids that are responsible for the flavour in these cheeses.

On average, higher sensory scores were observed for the aroma and flavour strength in the cheeses made just with rennet paste. The scores for general acceptability at 15 days of ripening were higher for the lots made just with rennet paste and the rennet paste and commercial rennet mixture (4 vs. 5.5–6). It has been reported that the development of the typical aroma caused by rennet pastes in cheeses is more related to the relative proportions of free fatty acids that are released than to their content (Rampilli & Barzaghi, 1995). Thus, high levels of short-chain free fatty acids have been found in the volatile fractions of cheeses made with rennet paste. This confirmed that, although the differences in the total levels of free fatty acids between the lots made with rennet paste and those made with commercial paste were not high, there were differences in the sensory characteristics of the cheeses.

3.4. Industrial cheeses

Fat and protein contents in two lots of the commercially made cheeses were similar, around 50% and 40%, respectively (referred to as total solids), a figure similar to that for the three lots of pilot cheeses described earlier. The percentage of total solids in both lots was between 58% and 60%. Both primary and secondary proteolysis was higher in the cheeses that used commercial rennet in their preparation, unlike the cheeses that used the commercial rennet and rennet paste mixture. This is because there is greater proteolysis activity in the commercial powdered rennet, which includes a high percentage of chymosin in its composition (Fig. 2).

From the beginning of ripening, FFA levels were higher in the lot prepared with a rennet paste mixture, in accordance with the data for the model and pilot systems (Fig. 3). At 45 days, the cheeses made with the rennet paste and commercial rennet mixture exhibited 25% more free fatty acid content than the cheeses made with commercial rennet (58,300 against 46,800 μmol/kg), with short-chain acid levels (C4–C10) also higher (4300 μmol/kg) in the cheeses prepared with the rennet paste and commercial rennet mixture.

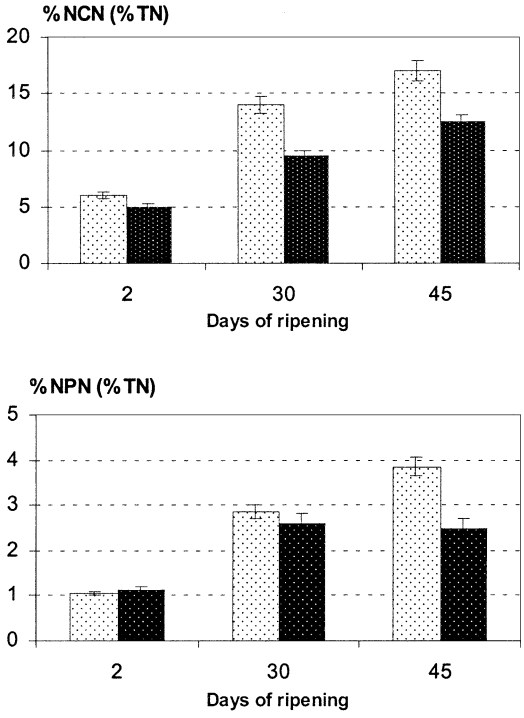

Fig. 2. Non-casein nitrogen (%NCN) and non-protein nitrogen (NPN) average percentage values referred to as a percentage of total nitrogen (TN) in the industrial cheese lots made with commercial rennet (–□– CR) and with the commercial rennet/rennet paste mixture lot (–■– CR/RP).

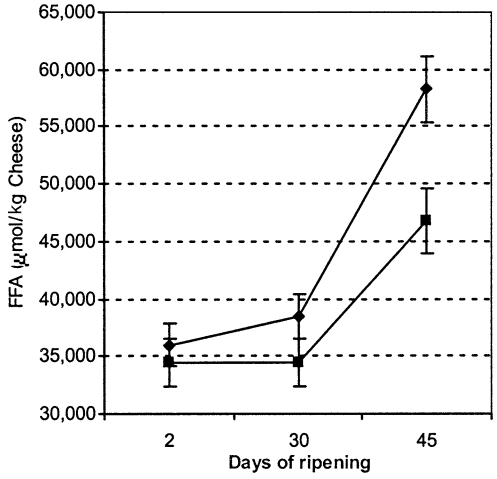

Fig. 3. Changes in total FFA concentration (μmol/kg cheese) in the industrial lot made with commercial rennet (–■– CR) and a rennet paste and commercial rennet (–◆– CR/RP) mixture at 2, 30 and 45 days of ripening.

3.5. Sensory analysis

A slightly harder, crumbly and gritty texture was observed in the rennet paste cheese lots, due to the lower proteolysis observed and this was reflected in less softening of the cheese mass. The sensory panel detected a piquant taste after 15 days of ripening in these cheeses. In spite of the slightly poor texture of the rennet paste cheese lots, the stronger flavour was considered to be positive compared with the commercial rennet lot.

The presence of a commercial starter in these cheeses, instead of a specific IFPL culture used for the pilot cheeses, meant that the acceptability of the cheeses made with rennet paste was slightly lower than that for the pilot cheese lots. The IFPL starter culture is the ideal complement for increasing the levels of proteolysis and developing the distinctive organoleptic characteristics of these cheeses.

From the results obtained, we can conclude that the use of hygienised rennet paste and a specific starter culture for making goat milk semi-hard cheese would lead to greater, controlled lipolysis. This was evident from the presence of mostly short-chain free fatty acids, which substantially enhanced the aroma, and which, in practice, would result in a shorter ripening time. Nevertheless, given that the proteolytic activity is lower in rennet paste, it seems more advantageous to use the rennet paste and commercial rennet mixture.

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