Food Chemistry 124 (2011) 583–588

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Influence of an artisanal lamb rennet paste on proteolysis and textural properties of Murcia al Vino cheese

E. Ferrandini [a], M.B. López [a,*], M. Castillo [b], J. Laencina [a]

[a] Departamento de Tecnología de los Alimentos, Nutrición y Bromatología, Universidad de Murcia, Campus de Espinardo, 30071 Murcia, Spain
[b] Departamento de Ciencia Animal y de los Alimentos, Universidad Autónoma de Barcelona, Edificio V, Campus de Bellaterra, 08193 Barcelona, Spain

ARTICLE INFO

Article history:
Received 7 April 2010
Received in revised form 20 May 2010
Accepted 21 June 2010

Keywords:
Rennet paste
Nitrogen fractions
Goat cheese
Texture

ABSTRACT

The aim of this study was to evaluate the influence of lamb rennet paste on the proteolysis and textural properties of Murcia al Vino cheese, compared with calf rennet. The enzyme concentration was adjusted according to its milk-clotting activity. The use of rennet paste led to higher values of all nitrogen fractions studied. Significant increases were observed in the water-soluble nitrogen fraction as a result of the lower pH of rennet paste cheeses; although the rennet paste is not characterised, three proteases are reported in the references consulted which can justify the greater proteolysis compared with calf rennet. The use of natural rennet paste produces a cheese with a more hydrolysed protein matrix, which is associated with significant changes in texture. The greater firmness determined in the rennet paste cheese was associated with higher fracture stress, lower fracture strain and lower moisture content.

1. Introduction

Proteolysis is the major and most complex biochemical process that takes place during the ripening of most cheese varieties. Degradation of the protein matrix during ripening influences the changes, which occur in the cheese texture, especially elasticity, brittleness, adhesiveness, hardness and chewiness properties. The same process is responsible for the development of basic tastes and for changes in cheese flavour, due to the ammonia, amines, sulphur compounds and volatile free fatty acids that develop during this process. The proteolytic enzymes employed in the different stages of cheese processing determine the characteristics of the casein residues. Active proteolytic agents include indigenous enzymes (plasmin), rennet (chymosin and pepsin) or the enzymes released by starter and non-starter microorganisms (Hayaloglu, Brechany, Deegan, & McSweeney, 2008).

Cheese proteolysis is characterised by the solubilisation of caseins, which translates into an increase in soluble nitrogen (Fox & Law, 1991). According to Freitas, Fresno, Prieto, Malcata, and Carballo (1997), Freitas and Malcata (2000), and Freitas, Macedo, and Malcata (2000), the effect starts with the proteolytic breakdown of the para-κ-casein, mediated by residual rennet and/or plasmin, to generate polypeptides, which then follow a process of degradation by proteases from the lactic acid bacteria and/or secondary microbial flora. The phenomenon of primary proteolysis of cheese involves the formation of high-molecular-weight peptides, which are insoluble in water, and small water-soluble peptides.

As regards primary proteolysis, the type of rennet used plays an important role. Different types of rennet are commercially available, which may differ in their origin (i.e., animal, vegetable, microbial, or recombinant rennet) or physical state (liquid, powder or paste). According to Ferrandini (2006), and Addis, Piredda, and Pirisi (2008), the most commonly used rennet is calf rennet in liquid or powder form. However, in Mediterranean countries, where goat and sheep milk is traditionally used, lamb and kid rennet paste is frequently used in certain types of cheese. The difference in the enzymatic composition of this type of rennet form is mainly due to the presence of lipolytic enzymes (pregastric and gastric) in the rennet paste (Bustamante, 2002).

In this study, a Protected Designation of Origin cheese (Murcia al Vino cheese) from Murcia (Spain) was manufactured with liquid calf rennet and lamb rennet paste to evaluate any differences in primary proteolysis and the changes in texture which take place during the ripening period.

2. Materials and methods

2.1. Experimental design

A randomised factorial design with two factors and two replications was used to study the effect of the different rennets (liquid calf rennet and experimental lamb paste rennet). The liquid rennet employed was considered as a control. Three levels of cheese

* Corresponding author. Tel.: +34 868884710; fax: +34 868884147.
E-mail address: mbelen@um.es (M.B. López).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.06.079

ripening time (2, 45 and 60 days) were investigated. The cheeses obtained were analysed, to assess their physicochemical composition, proteolysis profile (nitrogen fractions) and texture.

2.2. Artisanal lamb rennet paste

Only whitish abomasums from lambs sacrificed with a full stomach were selected to manufacture the lamb rennet paste, according to the procedure described by Ferrandini (2006). To characterise this rennet, the following parameters were determined: chymosin (71.1%), total milk-clotting activity (177 IMCU/ml) and lipase activity (4.6 U/g), as described by Ferrandini et al. (2008). Before rennet addition to the milk, 60 g of rennet paste were mixed with 100 ml of distilled water and filtered using a thin cloth to obtain an aqueous extract. The lamb rennet paste extract was added to milk at a concentration of 0.20 ml/kg milk.

2.3. Commercial liquid rennet

The enzymatic composition and milk-clotting activity of liquid animal rennet were provided by Caglio Star S.A. (Cieza, Murcia, Spain). The sample had 80% chymosin and a total milk-clotting activity of 180 IMCU/ml. The calf rennet was added at a concentration of 0.14 ml/kg milk.

2.4. Murciano-Granadina goat milk

The physicochemical characterisation of the milk samples used in this study was carried out as follows: pH, dry matter, fat and protein (Ferrandini, 2006). Every assay was made in triplicate and the resulting mean values were pH (6.60 ± 0.00), dry matter (13.30% ± 0.02), fat (4.98% ± 0.03) and protein (3.43% ± 0.04).

2.5. Experimental Murcia al Vino cheese

The cheeses were produced on 2 days at the University of Murcia Food Technology Pilot Plant with 1 day in between. A total of four cheeses were made, with each type of rennet (lamb and calf) being produced on both days. A 50-L double-O, stainless steel cheese vat (AISI-310 Cameselle SL, Vigo, Spain) was used to manufacture the cheese.

In this paper, the total milk-clotting activity of each rennet is known, which enables us to calculate the exact dose based on this activity. Preliminary tests were used to select enzyme concentrations, the concentrations of both rennets were selected to obtain a curd cutting time of 40 ± 5 min. All cheese manufacturing processes were performed with the collaboration of an accredited expert cheesemaker and under the supervision of a representative member of the Board of P. D. O. Murcia al Vino cheese to ensure the manufacturing process was according with the standard procedure for Murcia al Vino cheese. Before sampling, the cheese rind was discarded and the cheeses were grated and kept in airtight plastic containers at −80 °C until the analyses were performed.

The physicochemical characterisation of cheese samples was carried out in triplicate as follows: pH, total nitrogen content by the Kjeldahl method, NaCl, protein and fat as described by Ferrandini (2006). The means and standard deviations of the measured properties of the cheeses can be seen in Table 1.

2.6. Nitrogen fractions

From the total nitrogen content in the samples of cheese (FIL-DF 1964), the following nitrogen fractions were calculated as described by Bütikofer, Rüegg, and Ardö (1993): water-soluble nitrogen (WSN), soluble nitrogen at pH 4.4 ($SNpH_{4.4}$); 12% trichloroacetic acid-soluble nitrogen (TCASN); 5% phosphotungstic acid-soluble nitrogen (PTASN) and 28.5% ethanol-soluble nitrogen (ETSN). All samples were analysed in duplicate.

2.7. Texture analysis

Uniaxial compression and stress relaxation tests were performed using a texture analyser TA-TX2 (Stable Micro Systems Ltd, Godalming, UK). From each ripening day (45 and 60) cube-shaped samples (1 cm^3) were cut from a rindless cheese, wrapped in aluminium foil and equilibrated at 20 °C ± 0.5 °C for 3 h before testing. Samples were lubricated with Vaseline® oil on both sides of the contact surface, to minimise the effect of friction (Buffa, Trujillo, Pavia, & Guamis, 2001). The texture analysis was carried out six times for each sample. Cheese cubes were compressed to $\varepsilon = 0.02$ ($\varepsilon = \Delta L/L_o$, where L_o is the initial length) using a 245-N load cell and a crosshead speed of 80 mm/min.

Fracture stress (σ_f, in kPa), fracture strain (ε_f, dimensionless), and fracture work (W_f, in kJ/m^3) were obtained from the stress–strain curves.

Cheese cubes were analysed for stress relaxation to $\varepsilon = 0.001$ for 3 min, using a 245-N load cell and a crosshead speed of 200 mm/min test. This produces a characteristic experimental curve for viscoelastic materials, which enables the relaxation parameters e (dimensionless) and r (s^{-1}) to be calculated.

Table 1
Physicochemical parameters in experimental cheeses.

Cheese	pH (pH units)			Total solids (%)			NaCl (%)			Protein (%)			Fat (%)		
	2 d	45 d	60 d	2 d	45 d	60 d	2 d	45 d	60 d	2 d	45 d	60 d	2 d	45 d	60 d
A	5.57	5.36	5.41	54.18	65.50	66.30	1.65	1.95	2.14	19.85	23.28	23.84	29.68	39.40	43.54
B	6.14	5.45	5.46	54.11	62.92	64.54	1.50	1.77	1.94	18.24	21.11	22.38	29.86	36.33	40.83
C	5.40	5.28	5.36	52.34	65.56	68.15	1.42	1.84	2.05	19.05	23.34	24.50	28.65	42.07	42.04
D	5.25	5.36	5.36	54.56	69.64	70.27	1.42	1.79	2.03	19.40	23.72	24.64	29.97	44.80	43.08
SEM	0.01	0.00	0.00	0.06	0.06	0.07	0.01	0.02	0.03	0.08	0.18	0.10	0.38	0.28	0.40
S. level	***	***	***	***	***	***	***	**	**	***	***	***	NS	***	**
Contrasts SEC															
A,B *vs* C,D	0.02***	0.01***	0.00***	0.13***	0.12***	0.14***	0.03***	0.05 NS	0.06 NS	0.17 NS	0.36***	0.20***	0.77 NS	0.56***	0.81 NS

D, days; SEM, standard error of means; S. level, significance level; NS, not significant ($p > 0.05$); SEC, standard error of contrast; A and B cheeses made with commercial rennet; C and D cheeses made with paste rennet.
** $p < 0.01$.
*** $p < 0.001$.

2.8. Statistical methods

The data were analysed using the general linear modelling (GLM) procedure in Statistica for Windows (Version 8.0, Statsoft, Tulsa, OK).

3. Results and discussion

3.1. Nitrogen fractions

The results of the different nitrogen fractions obtained during the ripening of the cheeses made with different rennets are detailed in Table 2.

As expected, the values of the different nitrogen fractions increased as a function of time during ripening, indicating that the cheeses were developing a clear process of protein fractionation involving the release of lower molecular weight substances that were more soluble in water than those of the initial matrix.

Both at the initial (2 days) and final stage (60 days) of ripening, the value of WSN, TCASN and ETSN showed significant differences ($p < 0.001$) between the cheeses made with the commercial rennet (A and B) and lamb rennet paste (C and D), the higher values, being reached in the latter. At 45 days, the WSN did not show significant differences.

Regarding the $SNpH_{4.4}$ fraction, in a manner similar to that observed for PTASN, we identified significant differences ($p < 0.01$) between all the cheeses at 2 days of ripening. However, when the cheeses were compared according to the type of rennet used, this difference showed a higher level of confidence (99.9%), as seen in the last line of Table 2. At 45 days of ripening the values of all the nitrogen fractions considered, except WSN, showed significant differences ($p < 0.001$) between cheeses made from commercial rennet (A and B) and those made from lamb rennet paste (C and D).

However, at 60 days the PTASN fraction only showed a significant difference at $p < 0.01$ (between all the cheeses and between the two types of cheese).

Several authors (Bustamante et al., 2003; Irigoyen, Izco, Ibáñez, & Torre, 2001; Moatsou et al., 2004) have compared the effect of lamb rennet paste or bovine rennet on proteolysis in different cheeses. However, in these studies there is no information about the total milk-clotting activity of the enzymes added to the cheese vat, so that the results may be confusing. In general, significant differences were observed between the values recorded with both types of rennet, those derived from the rennet paste cheeses being higher, which agrees with the previous studies made by Bustamante (2002).

Several authors identified similar values for WSN in different cheeses: Manchego (Pavia, Trujillo, Guamis, & Ferragut, 2000), Murciano-Granadina goat cheeses (Buffa et al., 2001), and Cheddar at 90 days (Fox & Law, 1991). However, higher values were determined by Martín-Hernández and Juárez (1992) in goat cheeses. Lower WSN values are mentioned by Freitas et al. (1997) in Picante goat cheese; Lane, Fox, Johnston, and McSweeney (1997) in Cheddar cheese; Bütikofer et al. (1993) in Fontina (17.95%); and in Feta cheese by Moatsou et al. (2004).

Higher WSN values observed in the cheeses produced with rennet paste may be related with higher chymosin retention in curd but also with the lower pH values found in cheese produced with this rennet paste (Table 1). WSN had an inverse relationship with pH. Lower pH values corresponded to higher WSN values, in agreement with Martín-Hernández and Juárez (1992). The amount of chymosin retained in the curd is strongly influenced by the pH at the moment the whey is eliminated, being higher at lower pH values (Prieto et al., 2004). According to Bustamante et al. (2003) the increase in WSN during the early stages of ripening is due to the initial hydrolysis of the caseins by the residual coagulant and endogenous milk proteases.

In relation with $SNpH_{4.4}$, similar values were found by Franco, Prieto, Bernardo, González, and Carballo (2003) in the initial stage of ripening in Babia Laciana cheese, although higher values were reported by López et al. (1990) in fresh goat cheese. At the end of ripening, Tejada et al. (2008) reported slightly higher values at 45 days using calf rennet, while values close to ours at 60 days of ripening were recorded by Irigoyen et al. (2001) in Roncal cheese.

Murcia al Vino cheese made with lamb rennet paste (C and D) exhibited significantly higher proteolytic activity ($p < 0.001$) than those obtained from commercial rennet (A and B) as seen in the values obtained for $SNpH_{4.4}$, which agrees with López et al. (1990). This reflects the higher proteolytic effect of lamb rennet paste compared with calf rennet; although the aqueous extract of the lamb rennet paste is not completely characterised, three proteases have been reported (Foltman, 1993), which can justify the greater proteolytic activity compared to calf rennet. The increase in the percentage of soluble nitrogen at pH 4.6 in the first stages of ripening is caused by the activity of endogenous proteases in milk, the coagulant and to a lesser extent the residual activity of starter proteases (Fox & Law, 1991). The $SNpH_{4.4}$ levels observed in this study indicate that the degradation of casein and peptides in cheeses made with lamb rennet paste is more intense than in

Table 2
Soluble nitrogen fractions from cheese during ripening (% of total nitrogen).

Cheese	Soluble nitrogen fractions														
	WSN			$SNpH_{4.4}$			TCASN			PTASN			ETSN		
	2 d	45 d	60 d	2 d	45 d	60 d	2 d	45 d	60 d	2 d	45 d	60 d	2 d	45 d	60 d
A	6.76	15.45	16.51	7.27	16.51	17.54	2.28	6.69	7.86	0.61	1.27	1.63	3.84	9.48	10.61
B	5.58	15.58	17.34	7.12	15.09	17.44	2.09	6.45	7.53	0.56	1.21	1.68	3.60	9.17	10.37
C	7.99	16.55	21.91	9.28	19.42	21.24	2.83	9.09	11.12	0.75	1.71	2.18	4.08	12.70	15.25
D	7.55	15.80	19.39	9.32	17.05	20.08	2.93	8.50	10.24	0.73	1.56	1.91	5.25	11.58	14.85
SEM	0.12	0.71	0.31	0.50	0.28	0.32	0.09	0.07	0.13	0.03	0.03	0.10	0.06	0.05	0.14
S. Level	***	NS	***	**	***	***	***	***	***	**	***	**	***	***	***
Contrast															
SEC	0.25	1.42	0.62	1.00	0.56	0.65	0.18	0.15	0.27	0.06	0.06	0.21	0.13	0.10	0.29
A,B *vs* C,D	***	NS	***	***	***	***	***	***	***	***	***	**	***	***	***

WSN, water-soluble nitrogen; $SNpH_{4.4}$, pH 4.4 soluble nitrogen; TCNSN, 12% trichloroacetic acid-soluble nitrogen; PTASN, 5% phosphotungstic acid-soluble nitrogen; ETSN, 28.5% ethanol-soluble nitrogen. A and B, cheese made with commercial rennet; C and D, cheese made with rennet paste; 2, 45 and 60, days of ripening; SEM, standard error of means; S. level, significance level; NS, not significant ($p > 0.05$); SEC, standard error of contrast.

** $p < 0.01$.

*** $p < 0.001$.

those obtained from bovine rennet, which reflects the findings of Bustamante (2002) in Idiazabal cheese, when lamb rennet paste and calf rennet were compared.

López, Luna, Laencina, and Falagán (1999) determined values of TCASN almost three times higher in fresh goat cheese using commercial rennet, than the values we observed in cheeses A and B. However, our results coincide with those of Tejada et al. (2008) in Murcia al Vino cheese.

Higher levels of TCASN were determined in Roncal cheese (Irigoyen et al., 2001), and Fiore Sardo cheese (Addis, Pirisi, Di Salvo, Podda, & Piredda, 2005), using lamb rennet paste. Murcia al Vino cheese is a washed curd cheese. During cheesemaking, 40% of the whey is removed and replaced with 40% of water at 40 °C, lowering the lactose content and also lactic acid bacteria activity. For the abovementioned reason it was difficult to compare with cheeses having the same technological characteristics.

In agreement with López et al. (1990) and Prieto et al. (2004), we deduce that cheeses made with lamb rennet paste (C and D) show throughout the maturation period an exopeptidase activity of microbial origin that is significantly greater than that observed in cheeses made with animal rennet (A and B), as reflected in the values obtained for TCASN (Table 2).

This TCASN fraction is traditionally considered as an index of "ripening depth" and is composed mainly of ammonia nitrogen, small peptides of 2 and 20 residues and free amino acids (Prieto et al., 2004; Tejada et al., 2008). The components of this nitrogen fraction (small peptides, free amino acids and ammonia nitrogen) are mainly due to the enzymes of microbial origin acting on the large peptides released by the chymosin and on α-casein. The higher quantity of large peptides that are released in the cheese made with commercial rennet and also the greater availability of substrate could facilitate the action of the agents that convert these peptides into nitrogen compounds of lower molecular size (Prieto et al., 2004).

The 5% phosphotungstic acid-soluble nitrogen fraction provides an index of the production of amino acids during cheese ripening (Fox & Law, 1991) and is related to ammonia nitrogen, which reflects the capacity of the deamination exercised on the free amino acids by the microbial flora (Prieto et al., 2004). The values found in our cheeses made with lamb rennet paste are higher than in those made from commercial rennet during the ripening period studied. At 60 days, higher values were found in La Serena and Fiore Sardo cheeses by Freitas et al. (1997) and Addis et al. (2005), respectively. However lower values were found in León cow milk cheese (Prieto et al., 2004).

The literature consulted contains very little on ETSN in goat cheese because it is usually only used for the electrophoretic study of cheese proteins. In Idiazabal cheese at 180 days Bustamante (2002) found ETSN values that were 9.7% lower than those of the cheeses in this study at 60 days. This author also pointed out that in Idiazabal cheese the soluble nitrogen fraction in ethanol increases with maturation and is significantly higher in cheeses made with lamb rennet paste compared with those obtained from commercial rennet, which agrees with our results as well as those of Irigoyen et al. (2001).

3.2. Texture analysis

3.2.1. Uniaxial compression test

The parameters determined through uniaxial compression were fracture stress, fracture strain and work or energy of fracture (Table 3).

Significant differences ($p < 0.001$) for fracture stress at 45 days were determined between cheeses A, C and D, which showed similar values, and cheese B, which had a lower value (22.35 kPa). At 60 days, cheeses made with commercial rennet (A and B) were significantly more fragile than those made with lamb rennet paste (C and D), the highest value corresponding to cheese D. Only in cheese D did the value of this parameter increase from 45 to 60 days of ripening.

At 60 days, higher fracture stress values were found in Manchego cheese (Pavia et al., 2000), goat cheese (Buffa et al., 2001) and in Caciocavallo Pugliese cheese made with calf rennet paste (Gobbetti et al., 2002). As can be seen in Table 1, dry matter was the highest in the cheese made with rennet paste. The decrease in cheese water content during ripening, which leads to dehydration of the protein, results in less freedom of movement for the protein molecules and therefore in greater firmness of the cheese matrix (Buffa et al., 2001). This may explain the higher values found in lamb rennet cheeses compared to those made with commercial liquid bovine rennet.

With regard to fracture strain, significant differences ($p < 0.001$) existed between both cheese types at 45 and 60 days. At 45 days fracture strain in the cheeses obtained from commercial rennet (average 46%) was significantly higher than in those produced with lamb rennet paste (40%). The highest value was determined in cheese B (47%) and the lowest in cheeses C and D.

The same behaviour was determined at 60 days, when cheeses made with commercial rennet (A and B) were more deformable (39% in both samples) than those obtained with lamb rennet paste (C and D), which had significantly lower values of 32% and 35%, respectively. Lower values were determined in goat cheese by Buffa et al. (2001).

The values for fracture strain were higher in commercial rennet cheeses, which reflects the fact that cheeses made with lamb rennet paste were less deformable and matured faster than those obtained with commercial rennet. These results agree with those obtained by Calvo, Castillo, Díaz-Barcos, Requena, and Fontecha

Table 3
Uniaxial compression test values.

Cheese	Fracture stress (kPa)		Fracture strain (dimensionless)		Work of fracture (kJ/m^3)	
	45 days	60 days	45 days	60 days	45 days	60 days
A	$34.80^{a} \pm 8.21$	$28.45^{c} \pm 2.77$	$0.45^{a} \pm 0.02$	$0.39^{a} \pm 0.01$	$0.91^{a} \pm 0.20$	$0.67^{b} \pm 0.13$
B	$22.35^{b} \pm 3.28$	$22.20^{c} \pm 3.10$	$0.47^{a} \pm 0.03$	$0.39^{a} \pm 0.01$	$0.61^{b} \pm 0.07$	$0.56^{b} \pm 0.05$
C	$40.99^{a} \pm 7.71$	$36.75^{b} \pm 5.31$	$0.40^{b} \pm 0.01$	$0.32^{b} \pm 0.03$	$0.96^{a} \pm 0.24$	$0.78^{b} \pm 0.17$
D	$38.58^{a} \pm 4.22$	$50.08^{a} \pm 5.66$	$0.40^{b} \pm 0.02$	$0.35^{b} \pm 0.02$	$0.88^{ab} \pm 0.09$	$1.14^{a} \pm 0.17$
SEM	2.55	1.80	0.00	0.00	0.06	0.05
S. level	***	***	***	***	**	***
Contrasts SEC						
A,B *vs* C,D	5.09***	3.59***	0.01***	0.01***	0.13*	0.11***

A and B, Cheese made with commercial rennet; C and D, cheese made with rennet paste; SEM, standard error of means significance level; SEC, standard error of contrast.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

(2007), who found that the hardness and firmness significantly increase as a consequence of the low moisture and pH found in Majorero cheese (Table 1). Also, low cheese pH values are responsible for brittle textures with lower fracturability values (Creamer, Gilles, & Lawrence, 1988).

Creamer et al. (1988) consider fracture strain as an indicator of the rheological behaviour of the structures of the gel network formed by the action of rennet, which could be related to the content of α_{s1}-casein. Lamb rennet has a greater proteolytic activity against α_{s1}-casein than calf rennet, and consequently the α_{s1}-casein degradation occurs more quickly in cheese made using lamb rennet (Addis et al. 2008; Irigoyen et al., 2001), which can partly explain the lower values found in our lamb rennet paste cheese.

As regards energy of fracture, statistical significant differences were found between cheeses made with rennet paste and calf rennet, with different levels of significance at day 45 ($p < 0.05$) and 60 days ($p < 0.001$). After 45 days, cheese B had the lowest value (0.61 kJ/m^3), while the highest value was observed in D at 60 days.

Cheeses made with rennet paste need more energy than cheese made with commercial rennet, which is associated with the low moisture content resulting from faster maturity. The presence of small cracks and mechanical eyes, observed within some cheeses, generated heterogeneous areas, which is why the absence of significant differences between the values found did not always reflect the sensory evaluation, these results coinciding with those Farkye and Fox (1990). There are no references related to this parameter in goat cheeses.

3.2.2. Relaxation parameters

The experimental results for the different cheese types are shown in Table 4. The parameter r is related to the behaviour of an elastic material, with values between 0 and 1. Values close to 0 indicate an ideal elastic behaviour, while values close to 1 indicate the contrary. There were no significant differences between the cheeses for this parameter at 45 days, all of them showing the same elastic behaviour, while at 60 days there were significant differences ($p < 0.05$) but not between types. At this time, cheese B was the most elastic, followed by the cheeses made with rennet paste (C and D). The least degree of elasticity was seen in cheeses C and D.

The parameter e also has values between 0 and 1. Values close to 0 indicate that the material behaves like a solid block and a level near 1 indicates the typical behaviour of viscous liquids. This parameter showed no significant differences at the end of the time studied as can be seen in Table 4, although at 45 days significant differences ($p < 0.05$) were determined between all the cheeses and between the types of cheese. Cheese D shows the lowest value, which indicated that it is the hardest.

Table 4
Relaxation parameters r and e.

Cheese	r (s^{-1})		e (dimensionless)	
	45 days	60 days	45 days	60 days
A	0.184	0.193	0.885	0.891
B	0.180	0.157	0.884	0.896
C	0.175	0.188	0.883	0.927
D	0.186	0.176	0.872	0.872
SEM	0.005	0.008	0.003	0.017
S. Level	NS	*	*	NS
Contrasts SEC				
A,B *vs* C,D	0.009 NS	0.015 NS	0.006*	0.035 NS

A and B, cheese made with commercial rennet; C and D, cheese made with rennet paste; SEM, standard error of means significance level; NS, not significant ($p > 0.05$); SEC, standard error of contrast.
* $p < 0.05$.

As can be seen, cheese deformation decreases with increasing time of ripening, a phenomenon that is related to the elasticity produced by the chemical structures of the substances inside the cheese and the degree of hydration of the protein (Buffa et al., 2001).

Relaxation of effort could be described as the ability to reduce or cope with an imposed pressure for a given time, while maintaining a constant strain. In this test, where there are large deformations of the sample, the result of stress or pressure relaxation achieved on the gel network of the cheese can be followed as a function of time. In Manchego cheese made from bovine rennet Pavia et al. (2000) found an r value of 0.068 s^{-1} (2.6 times lower) at 60 days and therefore a greater elasticity compared with our results. In goat cheese, Buffa et al. (2001) determined a lower average value (0.032 s^{-1}), which means that cheese was much more elastic (5.5 times) than those studied in this research. The authors also argue that changes in r values may be associated with greater or lesser degree of weakness of the cheese matrix, as a result of the intensity of the proteolytic process (Foegeding, Brown, Drake, & Daubert, 2003), which, in turn, would be reflected in changes in WSN values throughout ripening, and moisture loss experienced by the cheeses. For example, in Cheddar cheese, hardness and elasticity are directly related to proteolysis, while in Camembert and Brie cheese it is partly due to large changes in pH, although some degree of proteolysis is required to achieve desired texture.

4. Conclusions

The higher values of WSN and SNpH$_{4.4}$ determined in cheeses made with lamb rennet paste demonstrated a higher proteolytic activity of rennet paste, probably due to higher chymosin retention in curd, produced by lower pH values. The pH of Murcia al Vino cheese at 60 days is lower in the paste cheeses than in those made with commercial liquid rennet, due to the increased activity of lactic acid bacteria added as starter, which produce a higher concentration of amino acids, as deduced from the levels of PTASN.

The type of rennet used influences the fracture stress, fracture strain and work of fracture at 45 and 60 days of maturation, those made with lamb rennet paste being harder, less deformable and therefore less elastic and needing more energy to fracture than those made with commercial liquid rennet.

Acknowledgments

The authors acknowledge the financial support of the Ministry of Science and Technology of Spain and the collaboration of Cuajos Caporal (Cistérniga, Valladolid, Spain) and research group "Quality and Food safety of fermented foods" of the Vasque Country.

References

Addis, M., Piredda, G., & Pirisi, A. (2008). The use of lamb rennet paste in traditional sheep milk cheese production. *Small Ruminant Research, 79*(1), 2–10.

Addis, M., Pirisi, A., Di Salvo, R., Podda, F., & Piredda, G. (2005). The influence of the enzymatic composition of lamb rennet paste on some properties of experimentally produced PDO Fiore Sardo cheese. *International Dairy Journal, 15*(6–9), 1271–1278.

Buffa, M. N., Trujillo, A. J., Pavia, M., & Guamis, B. (2001). Changes in textural, microstructural, and colour characteristics during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk. *International Dairy Journal, 11*(11–12), 927–934.

Bustamante, M. A. (2002). El cuajo de cordero en pasta: Preparación y efecto en los procesos proteolíticos y lipolíticos de la maduración del Queso Idiazabal. Tesis Doctoral. Universidad del País Vasco. Vitoria-Gasteiz (Spain).

Bustamante, M. A., Virto, M., Aramburu, M., Barrón, L. J. R., Pérez-Elortondo, F. J., Albisu, M., et al. (2003). Lamb rennet paste in ovine cheese (Idiazabal) manufacture. Proteolysis and relationship between analytical and sensory parameters. *International Dairy Journal, 13*(7), 547–557.

Bütikofer, U., Rüegg, M., & Ardö, Y. (1993). Determination of nitrogen fractions in cheese: evaluation of a collaborative study. *Lebensmittel Wissenschaft und Technology, 26*(3), 271–275.

Calvo, M. V., Castillo, I., Díaz-Barcos, V., Requena, T., & Fontecha, J. (2007). Effect of a hygienized rennet paste and a defined strain starter on proteolysis, texture and sensory properties of semi-hard goat cheese. *Food Chemistry, 102*(3), 917–924.

Creamer, L. K., Gilles, J., & Lawrence, R. C. (1988). Effect of pH on the texture of Cheddar and Colby cheese. *New Zealand Journal of Dairy Science and Technology, 23*, 23–55.

IDF (1964). *Determination of the protein content of processed cheese products. [IDF Standard 25]*. Brussels, Belgium: International Dairy Federation.

Farkye, N. Y., & Fox, P. F. (1990). Objective indices of cheese ripening. *Trends in Food Science & Technology, 1*, 37–40.

Ferrandini, E. (2006). Elaboración de Queso de Murcia al Vino con cuajo natural en pasta. Tesis Doctoral, Universidad de Murcia, Murcia. (Spain).

Ferrandini, E., Castillo, M., López, M. B., de Renobales, M., Virto, M., Hernández, I., et al. (2008). Technological characterization of experimental natural rennet pastes. *Food Science and Technology International, 14*, 63–71.

Foegeding, E. A., Brown, J., Drake, M. A., & Daubert, C. R. (2003). Sensory and mechanical aspects of cheese texture. *International Dairy Journal, 13*(8), 585–591.

Foltman, B. (1993). General and molecular aspects of rennets (2nd ed.. In P. F. Fox (Ed.). *Cheese: Chemistry, physics and microbiology* (Vol. 1, pp. 37–68). London: Chapman & Hall.

Fox, P. F., & Law, J. (1991). Enzymology of cheese ripening. *Food Biotechnology, 5*, 239–262.

Franco, I., Prieto, B., Bernardo, A., González, J., & Carballo, J. (2003). Biochemical changes throughout the ripening of a tradicional Spanish goat cheese variety (Babia-Laciana). *International Dairy Journal, 13*(2–3), 221–230.

Freitas, A. C., Fresno, J. M., Prieto, B., Malcata, F. X., & Carballo, J. (1997). Effects of ripening time and combination of ovine and caprine milks on proteolysis of Picante cheese. *Food Chemistry, 60*(2), 219–229.

Freitas, A. C., Macedo, A. C., & Malcata, F. X. (2000). Review: Technological and organoleptic issues pertaining to cheeses with denomination of origin manufactured in the Iberian Peninsula from ovine and caprine milks. *Food Science and Technology International, 6*(5), 351–370.

Freitas, A. C., & Malcata, F. X. (2000). Microbiology and biochemistry of cheeses with Appélation d'Origine Protégée and manufactured in the Iberian Peninsula from ovine and caprine milks. *Journal of Dairy Science, 83*(3), 584–602.

Gobbetti, M., Morea, M., Baruzzi, F., Corbo, M. R., Matarante, A., Considine, T., et al. (2002). Microbiological, compositional, biochemical and textural characterisation of Caciocavallo Pugliese cheese during ripening. *International Dairy Journal, 12*(6), 511–523.

Hayaloglu, A. A., Brechany, E. Y., Deegan, K. C., & McSweeney, P. L. H. (2008). Characterization of the chemistry, biochemistry and volatile profile of Kuflu cheese, a mould-ripened variety. *Lebensmittel Wissenschaft und Technology, 41*(7), 1323–1334.

Irigoyen, A., Izco, J. M., Ibáñez, F. C., & Torre, P. (2001). Influence of rennet milk-clotting activity on the proteolytic and sensory characteristics of an ovine cheese. *Food Chemistry, 72*(2), 137–144.

Lane, C. N., Fox, P. F., Johnston, D. E., & McSweeney, P. L. H. (1997). Contribution of coagulant to proteolysis and textural changes in Cheddar Cheese during ripening. *International Dairy Journal, 7*(6–7), 453–464.

López, M. B., Luna, A., Laencina, J., & Falagán, A. (1999). Cheese-making capacity of goats milk during lactation: Influence of stage and number of lactations. *Journal of the Science of Food and Agriculture, 79*, 1105–1111.

López, M. B., Vidal, M. C., Marín, F., Garrido, M. D., Laencina, J., & Luna, A. (1990). Nuevas posibilidades en la elaboración de queso de cabra Murciano-Granadina. *I Congreso Internacional de Tecnología y Desarrollo Alimentarios Actas, 3*, 1017–1022.

Martín-Hernández, M. C., & Juárez, M. (1992). Biochemical characteristics of three types of goat cheese. *Journal of Dairy Science, 75*, 1747–1752.

Moatsou, G., Moschopoulou, E., Georgala, A., Zoidou, E., Kandarakis, I., Kaminarides, S., et al. (2004). Effect of artisanal liquid rennet from kids and lambs abomasa on the characteristics of Feta cheese. *Food Chemistry, 88*(4), 517–525.

Pavia, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2000). Proteolysis in Manchego-type cheese salted by brine vacuum impregnation. *Journal of Dairy Science, 83*(7), 1441–1447.

Prieto, B., Franco, I., Fresno, J. M., Prieto, J. G., Bernardo, A., & Carballo, J. (2004). Effect of ripening time and type of rennet (farmhouse rennet from kid or commercial calf) on proteolysis during the ripening of León cow milk cheese. *Food Chemistry, 85*(3), 389–398.

Tejada, L., Abellán, A., Cayuela, J. M., Martínez-Cacha, A., & Fernández-Salguero, J. (2008). Proteolysis in goats' milk cheese made with calf rennet and plant coagulant. *International Dairy Journal, 18*(2), 139–146.