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Effect of a hygienized rennet paste and a defined strain starter on proteolysis, texture and sensory properties of semi-hard goat cheese

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Abstract

A hygienized rennet paste (HRP) and a defined strain starter culture, including *Lactobacillus casei* subsp. *casei* IFPL as adjunct, were considered for manufacturing Majorero cheese, a Spanish traditional variety made from goat milk. Influence of both factors on physicochemical characteristics, proteolysis, rheological and sensory properties, was evaluated throughout the ripening. Cheeses produced either industrially (IL) or in artisanal manner (AL) were compared with the experimental lot (EL), which included HRP and IFPL starter in its manufacture. Results showed a low level of primary proteolysis, expressed by a low content of non-casein nitrogen (NCN), in experimental cheeses. Despite the slightly poor texture (hard and crumbly) related to the high TS and salt contents, a good general acceptability was attained for EL, with the best scores for aroma and flavour intensities achieved at 30 ripening days. In fact, the sensory panel detected the "piquant" flavour (typical of the artisanal cheese variety) in EL after 15 days of ripening.

Keywords: Hygienized rennet paste; Goat cheese; Proteolysis; Texture

1. Introduction

Majorero Protected Designation of Origin is a traditional variety of semi-hard cheese from Fuerteventura Island (Canary Island, Spain) manufactured with majorera goats' milk (BOE, 1996). Previous characterization performed in our laboratory, evidenced noticeable differences between industrial Majorero cheese made with pasteurized milk (Martín-Hernández, 1987) and artisanal cheese manufactured with raw milk (Fontecha et al., 1990). Besides the different milk treatments employed, factors, such as the microbial flora (autochthonous or added), type of rennet, and physical/mechanical procedures employed during cheesemaking markedly affected the final product features. It has been proven that the use of pasteurized milk in cheesemaking diminishes cheese aroma and flavour development. Pasteurization eliminates autochthonous microflora of milk, whose enzymatic activities are not always replaced successfully by those of bacteria present in the starter cultures. Nevertheless, pasteurization allows the standardization of the cheese processing and final sensory characteristics, as well as the improvement of hygienic and sanitary quality. Requena, de la Fuente, Fernández de Palencia, Juárez, & Peláez (1992) developed a specific starter culture able to impart some peculiar sensory features of the artisanal Majorero cheese to the industrial variety manufactured from pasteurized milk.

On the other hand, it is known that the piquant taste development, characteristic of artisanal Majorero cheese, is directly related to the use of rennet pastes for milk curdling (Fontecha et al., 1990). These rennet pastes, produced by macerating the stomachs from suckling ruminants, have also been used in elaboration of other traditional Spanish

Abbreviations: HRP, hygienized rennet paste; IL, industrial lot; EL, experimental lot; AL, artisanal lot; TN, total nitrogen; NCN, non-casein nitrogen; NPN, non-protein nitrogen; PPN, proteose peptone fraction.

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cheeses (Bustamante et al., 2003; Irigoyen, Izco, Ibañez, & Torre, 2002; Virto et al., 2003), as well as of some Italian hard cheese varieties (Fox, Law, McSweeney, & Wallace, 1993), and are still used in Feta cheese manufacture (Moatsou et al., 2004). In our laboratory, a technological process (based on filter sterilization) has been designed to obtain a hygienized rennet paste from artisanal kid pastes (Calvo & Fontecha, 2004). In addition to the better microbiological quality achieved, this procedure overcomes the difficulty of standardizing both milk-clotting and lipase activities which represent the main disadvantage (Harboe, 1994) associated with the traditional rennet pastes. The effect of this hygienized rennet paste (HRP) and a defined strain starter (IFPL) on lipolysis and the volatile fraction of Majorero cheese has been examined by Castillo, Calvo, Alonso, Juárez, & Fontecha (2007). The results showed that both factors positively affect lipolysis, increasing the mono- and diglicerides concentrations as well as the content of short-chain FFA, particularly butanoic acid, an important flavour component of Majorero cheese.

In addition to flavour changes during ripening, cheese quality is also determined by texture development. Therefore, understanding the factors controlling texture is essential to make products of the highest quality (Foegeding, Brown, Drake, & Daubert, 2003). Proteolysis contributes to cheese texture through the hydrolysis of proteins, the increase of pH and the retention of water by the amino and carboxylic groups that are formed during ripening. Both coagulant and starter are considered as determinant proteolytic agents and hence their influence on textural properties of cheese should be evaluated.

For the above reasons, and due to the renewed interest in maintaining the authenticity of traditional cheeses, the aim of this work was to discover whether the addition of a hygienized rennet paste (instead the traditional rennet paste), along with a defined strain starter culture IFPL, affects both texture and sensory characteristics of Majorero cheese. Possible implications for the dairy industry in mimicking artisanal cheeses were also considered.

2. Materials and methods

2.1. Hygienization process of rennet pastes

The hygienized rennet paste (labelled as HRP) was obtained according to Calvo & Fontecha (2004). The suckling kids stomachs (full of milk and salted in brine for at least 2 months) were washed and sectioned in order to extract their contents, which were minced and homogenized in Milli-Q water until a semi-liquid homogeneous paste was obtained. The crude homogenate was centrifuged (15,000*g*, 4 °C for 30 min). The supernatant was submitted to vacuum filtration in a Millipore unit, coupled to an Eyela pump, model A-3S (Tokyo Rikakikai Co.) and Whatman No. 1 filters, employed to eliminate particles in suspension. Subsequently, the solution was filtered through 0.22 μm pore-size filters (Millipore) in order to avoid bacterial contamination of the samples. HRP displayed values of 2.66 and 0.79 ± 0.01 U/mg for clotting and lipase activities, respectively.

2.2. Starter culture preparation

The defined strain starter culture (IFPL) employed in this study had been developed in our laboratory (Requena et al., 1992). It included microorganisms previously isolated from Majorera goat raw milk. This IFPL starter contained the isolate *Lactococcus lactis* subsp. *lactis* IFPL359, and the adjunct *Lactobacillus casei* subsp. *casei* IFPL731, *Lactobacillus plantarum* IFPL935, *Leuconostoc mesenteroides* subsp. *dextranicum* IFPL709 and *Leuconostoc paramesenteroides* IFPL705. For starter preparation, strains were grown separately in milk and were then combined and inoculated, together into sterile skimmed milk, at a final concentration of 1%.

2.3. Cheese manufacture

Three different cheesemaking trials were done in duplicate. All lots of cheese were manufactured using Majorera goat's milk from flocks of Fuerteventura island. The industrial lot (IL) was manufactured using 6500 l of pasteurized milk (72–76 °C, 20 s) and a commercial homofermentative starter culture (EZAL®MA011, including *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) from Larbus S.A. Madrid, (Spain). Commercial lamb rennet (strength 1:150,000) from Epasa (Spain) was employed for milk curdling. The artisanal lot (AL) was produced from 300 l of raw goat's milk in the traditional manner. As coagulant, HRP ($1\ ml\ l^{-1}$ milk, rennet strength 1:10,000), obtained according to the procedure described by Calvo and Fontecha (2004), was employed. The native microflora of the milk were used for acidification. Experimental lot (EL) was manufactured from 300 l of pasteurized goat's milk (72–76 °C, 20 s). The defined strain starter (IFPL) for Majorero cheese, as mentioned above, and the HRP ($1\ ml\ l^{-1}$ milk) were also employed.

The general cheesemaking procedure (applicable to all three lots) was as follows: milk was heated to 32 °C and then $CaCl_2$ ($0.2\ g\ l^{-1}$) was added. For EL, starter culture was also inoculated (10^7 cfu ml^{-1}) at this point and was left to act until the milk pH decreased to 6.5. Once the corresponding rennet was added, the cutting point for the curd was reached in 35 min for IL and ~75 min for AL and EL. The curd was cut to a grain size of 5–15 mm and then stirred while the vat temperature was gradually increased to 36 °C at a rate of 0.3 °C min^{-1}. The curd was placed in plastic moulds where it was dripped and pressed until the pH was 5.3–5.2. Cheeses corresponding IL and EL were salted in brine (24.6% w/v) at 10 °C for 12 h. Cheeses from AL were salted dry, applying salt directly on their surface for 6 h on each side, according to the traditional procedure. Once removed from the moulds, the cheeses were transferred to a ripening room where they remained at

15–17 °C and ~85% HR for 60 days. Cheese samples for the analysis were taken at 7, 15, 30 and 60 days of ripening. In each replicate lot, 12 cheeses, with a mean weight of about 2.6 kg, were analysed. Although all cheeses showed a diameter ~18 cm, the height in AL and EL cheeses was lower (~1 cm) than in industrial cheeses.

2.4. Microbiological analysis of cheeses

Sampling and dilutions were performed in accordance with International Dairy Federation standards (IDF, 1985). Cheese samples (10 g) were homogenized in 90 ml 2% (w/v) of sterilized sodium citrate at 45 °C for 1 min in a Colworth Stomacher 400 (Seward, London, UK). Tenfold dilutions were prepared in sterile Ringer solution and plated on specific medium in duplicate. In all cases, the plates with counts between 30 and 300 colonies were counted after the adequate incubation period. Counts of total viable microorganisms were determined in plate count agar (PCA) (Oxoid), after incubation at 30 °C for 72 h, following the IDF, standard (IDF, 1987a). *Lactococci* were determined on M-17 Agar (Scharlau) and the plates were anaerobically incubated at 30 °C for 72 h. Rogosa agar (Oxoid) was employed to enumerate *Lactobacilli*, using the pour-plate and overlay technique. Only white lenticular-shape colonies, with size diameter $\geqslant 0.5$ mm, were counted after incubation at 30 °C, for 72 h. *Leuconostoc* species were estimated by surface inoculation on MSE agar (Scharlau), previously solidified, and colonies were counted after incubation at 30 °C for 48 h.

2.5. Physicochemical composition

Analytical determinations of total solids (IDF, 1982), fat (IDF, 1986) and salt (IDF, 1972) content, as well as pH (Ministère de l'Agriculture method, 1974), were carried out. All determinations were performed in triplicate for each lot and for each ripening time.

2.6. Assessment of proteolysis

The total nitrogen (TN) content was determined according to IDF (1993). Total protein content was obtained multiplying the TN value by 6.38, the factor corresponding to milk protein (IDF, 1964). The method of Kuchroo & Fox (1982) was followed to fractionate cheese nitrogen into the non-casein nitrogen (NCN) and non-protein nitrogen (NPN) fractions. The nitrogen content in each fraction was determined according to the Kjeldahl method (IDF, 1993). The NCN minus NPN gave the polypeptides previously called proteose peptone fraction (PPN) which is formed by fragments of β-casein PP8 fast (β-CN f1-28), PP8 slow (β-CN f29-105 and f29-107) and PP5 (β-CN f1-105 and 1-107), as has been pointed by Farrell et al. (2004), McSweeney (2004), Sousa, Ardö, & McSweeney (2001).

2.7. Rheological analysis

Cylindrical samples (20 mm height and 18 mm diameter), equilibrated for 6 h at room temperature, were analysed by means of an Instron Texture Analyser Model 4501 (Instron Ltd.High Wycombe, Bucks, UK) fitted with a 5 kN load cell. Compression to fracture tests performed for texture analysis at several ripening times, were carried out according to a published procedure (Fontecha, Kalàb, Medina, Peláez, & Juárez, 1996). Cheese samples were compressed between two parallel plates (58 mm of diameter) at a vertical displacement speed of 100 mm min^{-1}. The deformation was set at 75%. The following parameters were determined at fracture point: hardness (maximum strength in N), deformation percentage (% D) and firmness (F) in N mm^{-1}. Texture values were the means of at least five replicates at each sampling time.

2.8. Sensory analysis

A sensory test was carried out, following the IDF recommendations (IDF, 1987b), by trained panellists (10 tasters minimum) selected from the Instituto del Frío staff.

Cheeses were sampled at four ages (7, 15, 30, 60 days). A wedged-slice (1 cm thickness) was removed from the block of cheese and the rind was discarded. The attributes evaluated were appearance, aroma intensity, taste intensity and overall acceptance, as well as the flavour descriptors (characteristic taste, acid, bitter, salty, piquant, rancid), and the texture parameters (hardness, creaminess, plastic, adherence, brittleness and grittiness). Intensity of each attribute was rated on a 10-point intensity scale.

2.9. Statistical analysis

The SPSS package (SPSS 11.5 for Windows, SPSS Inc. Chicago, IL, USA) was used for statistical analysis of the results. Analysis of variance (ANOVA) was undertaken and the meaningful level was established for $P \leqslant 0.05$. Mean comparisons were performed with the Tukey's honest significant differences (HDS) test. Thus, the a, b, c superscripts were employed to state significant differences between lots for the same ripening time.

3. Results and discussion

3.1. Physicochemical composition

The evolution of the physicochemical characteristics during the ripening of the three cheese batches is shown in Table 1. IL cheeses showed significantly higher pH values ($P < 0.05$) than and EL and AL cheeses. All pH values were found to be lower than those described for goats' cheeses showing similar characteristics (Fontecha et al., 1990; Martín-Hernández, Juárez, & Ramos, 1992; Requena et al., 1992), and remained low at the end of the ripening,

Table 1
Mean values of physicochemical parameters

Ripening (days)	Lot	pH	%TS	Protein (%TS)	Fat (%TS)	NaCl (%TS)
7	IL	4.97[a]	59.16[a]	38.2[b]	55.01[a]	2.01[b]
	EL	4.83[b]	59.23[a]	41.4[a]	56.12[a]	2.29[a]
	AL	4.79[b]	56.66[b]	38.3[b]	52.97[b]	2.24[a]
15	IL	5.00[a]	58.55[b]	39.9[a]	55.72[a]	2.63[b]
	EL	4.72[b]	68.28[a]	38.4[b]	55.81[a]	3.92[a]
	AL	4.74[b]	60.19[b]	37.8[b]	53.06[b]	3.58[ab]
30	IL	4.97[a]	60.51[b]	38.6[b]	56.01[a]	2.71[c]
	EL	4.73[b]	71.97[a]	42.7[a]	55.95[a]	4.36[a]
	AL	4.68[b]	62.81[b]	36.4[c]	53.62[b]	3.77[b]
60	IL	5.03[a]	65.48[c]	41.2[a]	55.41[a]	2.99[b]
	EL	4.75[b]	79.63 [a]	40.5[a]	56.23[a]	4.34[a]
	AL	4.78[b]	71.42[b]	37.7 [b]	53.95[b]	4.19[a]

Each value corresponds to an average of three cheeses analysed in triplicate ($n = 9$).
[a,b,c] Different letters in the same column indicate significant differences ($p < 0.05$) between lots for the same ripening time.

which could be interpreted as a consequence of the moderate proteolysis detected in these cheeses.

Initially the TS content ranged from 57% to 59% in the three cheese lots. Although the percentage of TS increased progressively in all lots during the maturation, this trend was more evident in artisanal and experimental lots ($P < 0.05$). It is well known that the intense dehydration occurring throughout the ripening is the main drawback for artisanal goats' cheese commercialization. The low moisture content, at least in initial stages, has been partly attributed to the great ability of goat's milk to undergo acidification (Yubero, 1985). Although the three lots were aged under similar conditions, the lower size of EL, which could favour salt diffusion, was likely related to the decrease in moisture detected. Thus, the high content of dry matter shown by EL cheeses (reaching TS values of nearly 80% after 60 ripening days) was to some extent expected. Similar results have been described in artisanal Majorero cheese (Fontecha et al., 1990) and in other varieties of goat milk cheese, such as Armada (Fresno, Tornadijo, Carballo, González Prieto, & Bernardo, 1996) and Babia-Laciana (Franco, Prieto, Bernardo, González Prieto, & Carballo, 2003).

The increase in salt content detected in all lots, throughout the maturation (Table 1), is probably related to the decrease in moisture. In addition, the diffusion of salt from the surface into the centre of the cheese might also affect NaCl levels (Martín-Hernández et al., 1992). At the end of maturation, the salt contents of AL and EL were significantly higher ($P < 0.05$) than that of IL. These differences are probably related to the lower size of AL and EL cheeses but also to the salting process employed (Park, 2001). Moreover, since the stomachs from which HRP were derived had been preserved in brine, the use of HRP may represent an additional reason for the higher salt contents of AL and EL cheeses.

The total protein (~40%) and the fat (~56%) contents in experimental cheeses were similar to those found in IL cheeses, but slightly higher than the values corresponding to the artisanal lot. These results were comparable to those previously reported for Majorero cheese (Fontecha et al., 1990; Martín-Hernández et al., 1992).

3.2. Microbiological composition

Table 2 summarizes the evolution of the microbial populations during the ripening in the three cheese lots. Initially, the highest total viable counts were found in industrial cheeses. These total counts decreased in a slow manner throughout the maturation in all lots, except in EL ($P < 0.05$), where levels lower than normal for semi-hard cheeses were reached after one month of ripening. It is known that an excess of salt negatively affects both the growth and the activity of the lactic bacteria. The high NaCl content found in EL, in addition to the great dehydratation occurring in this lot, could explain this decrease in microorganism counts.

These data were in accordance with those previously published for artisanal (Fontecha et al., 1990) and industrial (Gómez, Peláez, & de la Torre, 1989) Majorero cheese. *Lactococci* constituted the predominant microbiota, both in cheeses made from raw milk and those where a starter culture was added. Counts decreased throughout the ripening by one logarithmic unit in IL and AL cheeses while EL cheeses showed a reduction, by more than two logarithmic units, in the last 30 days, due to the low humidity, as discussed above. Although initial counts of *lactobacilli* were higher in AL than in IL, comparable levels in both lots of cheese were reached after the first ripening month. Both EL and AL cheeses displayed equivalent initial counts of *leuconostocs*, but these microorganisms declined sharply

Table 2
Mean levels of total viable microorganisms, *Lactococci*, *Lactobacilli* and *Leuconostoc* in AL, EL and IL cheeses during ripening

Ripening (days)	Lot	Counts (log cfu g^{-1})			
		Totals	*Lactococci*	*Lactobacilli*	*Leuconostoc*
7	IL	9.2[a]	9.2[a]	6.3[c]	n.d.
	EL	8.9[b]	8.9[b]	8.8[a]	7.2[a]
	AL	8.5[c]	8.6[c]	7.4[b]	7.0[a]
15	IL	8.5[a]	8.4[a]	7.4[b]	n.d.
	EL	8.4[a]	8.4[a]	8.2[a]	5.8[a]
	AL	8.3[a]	8.3[a]	7.8[b]	5.7[a]
30	IL	8.2[a]	8.3[a]	7.9[ab]	n.d.
	EL	8.2[a]	8.2[b]	8.1[a]	3.4[b]
	AL	8.3[a]	8.3[a]	7.8[b]	5.6[a]
60	IL	8.3[a]	8.2[a]	7.7[a]	n.d.
	EL	5.9[c]	6.1[b]	6.0[b]	n.d.
	AL	8.1[b]	7.9[a]	7.2[a]	4.1

n.d., not detected.
[a,b,c] Different letters in the same column indicate significant differences ($p < 0.05$) between lots for the same ripening time.

in EL being not detectable at the end of ripening (Table 2). *Leuconostocs* were not detected in IL cheeses as they were not included in the starter culture employed. As previously indicated, IFPL starter was designed from isolated strains of predominant species present in artisanal Majorero cheese (Requena et al., 1992). This would explain that, at the beginning, EL cheeses showed bacterial flora levels comparable to those found in AL cheeses made from raw milk.

3.3. Nitrogen fractions

Fig. 1 shows the evolution of the nitrogen fractions in the three batches of cheese throughout the ripening.

The NCN increased slightly during ripening of the EL and AL cheeses, reaching similar levels in both lots (~11% of TN). This low level of primary proteolysis would be partly attributed to the type of rennet used. Both lots, made using a HRP, showed NCN levels lower than those found in artisanal Majorero cheese made with crude kid rennet paste (Fontecha et al., 1990). Probably this fact was a consequence of the hygienization process, as HRP showed a lower proteolytic activity (0.025 ± 0.002 U mg^{-1}) than did the original rennet paste (0.033 ± 0.003 U mg^{-1}) from which it derives (Calvo & Fontecha, 2004). On the other hand, it is known that salt content and the method of salting markedly affect the rate and/or extent of proteolysis, with high salt levels being related to delay in proteolysis (Park, 2001). Moisture level also has a major effect on the rate of proteolysis. Thus, both the high NaCl content and the low cheese moisture could also explain the lower proteolytic activity found in EL and AL.

The liberation of large peptides that form the proteose peptone fraction (PPN), which is included in the NCN (Fig. 1), is due to the action of plasmin on the β-casein. Because of the greater proteolytic activity of the commercial rennet, IL cheeses showed higher levels of PPN than did the other lots and these increase throughout the ripening. On the other hand, a noticeable decrease of PPN level occurred in EL during maturation (although its initial value (~5%) was similar to that in IL), whilst this index hardly changed in AL. These data confirm that the role of the HRP in the proteolysis during ripening is limited.

At the end of the ripening, the NPN level in IL (made using a commercial rennet) was also significantly higher (16.8% TN) than in those lots manufactured using HRP (~9.8% TN). The formation of NPN (made up of small peptides, free amino acids, and ammonia nitrogen) is due, fundamentally, to the enzymes of microbial origin that act on the large peptides released by the rennet as it acts on the α-caseins. The higher quantity of large peptides (PPN) detected in IL could favour the transformation of these peptides into nitrogen substances of lower molecular size. The action of the microbial enzymes included in the IFPL starter was obvious, as the NPN value for EL was comparable to that found in AL (made from raw milk) after 60 days of ripening.

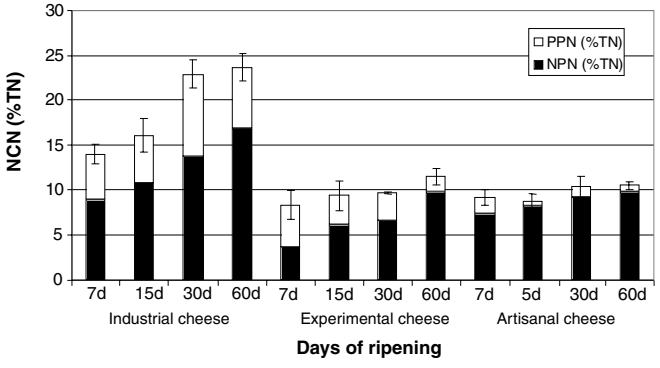

Fig. 1. Evolution of nitrogen fractions throughout the ripening of cheese from the three studied systems. NCN = non-casein nitrogen, NPN = non-protein nitrogen and PPN = proteose peptone fraction. Data were expressed as percentage of the total nitrogen (%).

3.4. Rheological properties

Table 3 summarizes the mean values for the parameters analysed by the compression to fracture test. Salt concentration is one of the major factors in the ripening process, to such as extent that it determines the final quality and affects cheese functionality (Pastorino, Hansen, & McMahon, 2003). Thus, the greater hardness exhibited by AL and EL cheeses may be due to the high salt content, to the low moisture but also to the low pH (close to the isoelectric point of casein) found in these lots. Hardness and firmness both significantly increased in all cheeses during the first 15 days as a consequence of the humidity loss that took place during ripening.

Between the 15 and 30th day of ripening, firmness, which is inversely correlated with proteolysis (Park, 2001), remained constant or even decreased. However, during the same period, hardness sharply decreased in IL cheeses ($P < 0.05$) while remaining almost unchanged in AL and EL cheeses. This softening, detected in IL cheeses, may be partially explained by the proteolysis of caseins,

Table 3
Mean values obtained for rheological parameters during the compression to fracture test

Ripening (days)	Lot	H (N)	F (N mm^{-1})	D (%)
7	IL	35.57^{c}	5.73^{c}	31.13^{a}
	EL	58.58^{a}	10.63^{a}	27.81^{ab}
	AL	47.60^{b}	10.22^{b}	23.28^{b}
15	IL	57.53^{c}	12.15^{c}	23.84^{a}
	EL	142.63^{a}	31.03^{a}	23.01^{ab}
	AL	81.73^{b}	20.18^{b}	20.33^{b}
30	IL	46.57^{c}	10.30^{b}	22.60^{ab}
	EL	141.57^{a}	27.05^{a}	26.64^{a}
	AL	94.90^{b}	25.20^{a}	19.05^{b}

H = Hardness; D = % Deformation; F = Firmness.
[a,b,c] Different letters in the same column indicate significant differences ($p < 0.05$) between lots for the same ripening time.

mainly by residual coagulant (Katsiari, Voutsinas, & Kondyli, 2002) with precipitation of calcium phosphate.

These age-related changes in rheological characteristics are hence the consequence of the compromise between the hardening caused by the cheese dehydration and the softening due to the proteolysis.

Given the lower proteolytic activity in HRP, it seems more advantageous from the industrial point of view to use a HRP and a commercial rennet mixture, as suggested (Fontecha, Castillo, Blasco, Alonso, & Juárez, 2006).

3.5. Sensory analysis

IL was better scored than the other lots for cheese appearance (Table 4) throughout the ripening. The scores slightly declined with the maturation time, except for EL where a sharp decrease (from 6.5 to 4.4) was obtained. The progressive dehydratation suffered by EL cheeses results in a dry and brittle external aspect, negatively valued by the tasters. These results were in agreement with data obtained for TS content and rheological analysis.

From the beginning of the maturation, EL and AL cheeses obtained better scores for aroma and taste intensities ($P < 0.05$) than did industrial cheeses. These data confirm the changes in lipolysis and volatile fraction reported by Castillo et al. (2007) for the same cheeses. These authors found that the use of HRP and IFPL starter culture caused an increase in the mono- and diglyceride concentrations and a greater content of short-chain FFA, particularly butanoic acid, which imparts a desirable sharp, "piquant" taste to Majorero cheese. In spite of the slightly poor textures of AL and EL, the stronger flavour was considered to be positive compared with IL cheeses and to determine its better acceptability by the tasting panel.

Table 4
Mean values of scores for some sensory attributes in industrial (IL), experimental (EL) and artisanal (AL) lots of cheese after 7, 15, 30 and 60 ripening days

Ripening (days)	Lot	Appearance	Aroma intensity	Taste intensity	Overall Acceptability
7	IL	7.8[a]	3.5[b]	4.5[b]	5.3[b]
	EL	6.5[b]	6.1[a]	7.0[a]	6.1[a]
	AL	6.6[ab]	7.0[a]	6.4[a]	6.5[a]
15	IL	8.0[a]	3.6[b]	4.9[b]	5.2[b]
	EL	5.3[c]	6.9[a]	6.6[a]	6.4[a]
	AL	6.6[b]	6.5[a]	6.8[a]	5.8[a]
30	IL	7.4[a]	4.1[b]	5.1[b]	5.0[b]
	EL	4.9[b]	7.1[a]	7.3[a]	6.6[a]
	AL	5.6[ab]	6.6[a]	7.3[a]	5.4[a]
60	IL	6.8[a]	3.3[b]	5.2[b]	5.2[b]
	EL	4.4[b]	7.1[a]	7.5[a]	6.1[a]
	AL	6.2[ab]	6.8[a]	7.9[a]	6.1[a]

[a,b,c] Different letters in the same column indicate significant differences ($p < 0.05$) between lots for the same ripening time.

The sensory profiles of the cheeses, throughout the maturation, were constructed using the scores awarded for some flavour descriptors and the texture parameters (see Section 2) by the sensory panel. Fig. 2 shows the sensory profile for each lot of cheese after one month of ripening. As can be seem, no significant differences between AL and EL were found with respect to the flavour attributes evaluated, thus showing overlapped plots. Both lots were awarded the best scores for all sensory features analyzed, except for bitterness. Bitter peptides are formed mainly by the action of coagulant and starter proteases. The greater proteolytic activity of the commercial rennet employed in IL may contribute to the development of bitter notes. On the other hand, salt affects the retention and activity of coagulant in the curd, and thereby may influence the development of bitterness. Salt may promote hydrophobic interactions between susceptible regions of β-casein, thus reducing chymosin action on this protein and hence the production of bitter peptides. It has also been reported that salt may decrease bitterness by inhibiting *lactoccocal* CEP (Sousa et al., 2001). Perhaps the presence of a greater salt contents in AL and EL (Table 2) could be related to the differences of bitterness.

According to the PDO specifications for this cheese variety, the higher scores for acid and piquant taste obtained in AL and EL, over the whole ripening period, may contribute to desirable flavour of mature cheese. Specifically, the rapid development of "piquant flavour" was likely related with the increase in short-chain FFA levels that result from the rennet paste action (Castillo et al., 2007). This sharp taste was detected by the sensory panel after 15 ripening days, and likely determined the good acceptability achieved in both lots in spite of their poor texture (Table 4). Experimental and artisanal cheeses were also slightly more rancid, which could be explained by the greater lipolysis level in both lots.

Scores for texture parameters given by the sensory panel were in good agreement with data from the rheological analysis. A slightly hard, crumbly and gritty texture was

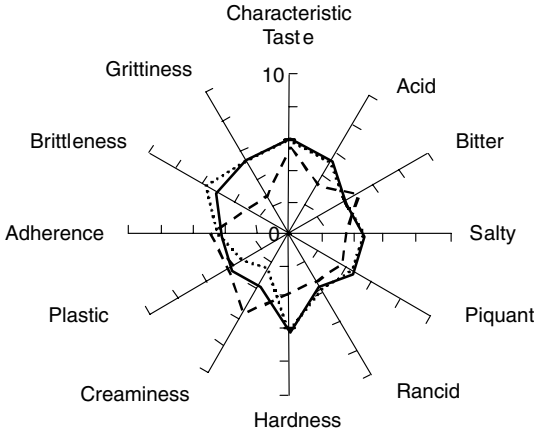

Fig. 2. Sensory profile of cheeses from AL (—), IL (- -) and EL (···) after 30 ripening days. Standard errors of the mean for all attributes were between 0.1 and 0.2.

observed in the HRP cheese lots, due to the lower proteolysis observed, and this was reflected in less softening of the cheese mass.

4. Conclusions

The use of a hygienized rennet paste and a defined strain starter for making Majorero cheese would lead to a rapid development of piquant flavour (typical of this cheese variety). However, both rheological and sensory analysis revealed a hard and crumbly texture for this experimental cheese. Perhaps, from the industrial point of view, to use a HRP and a commercial rennet mixture may be a good approach to overcome this drawback. Despite the poor texture, this could be an interesting way to produce good quality cheeses under controlled conditions, since the flavours of the traditional raw milk cheese may be mimicked.

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