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A Ripening and Storage Study of Soft Goat Cheese with *Penicillium candidum* on the Surface*

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ABSTRACT

The present study analyzed physico-chemical characteristics, proteolysis, and lipolysis over a 14-day ripening period and a 14-day storage period in two batches of industrially produced goat cheese with Penicillium candidum *on the surface.*

After the 28 days the pH inside the cheese was considerably lower than that on the surface, due to the activity of the moulds. At the end of the study the water-soluble nitrogen was 16% of the total nitrogen, and 84% of the water-soluble nitrogen was non-protein nitrogen. Degradation of α_s *and* β*-caseins was low, but at the end of the storage period a series of bands, chiefly degradation products of* β*-casein, was observed in the* γ*-casein region; weak bands with higher electrophoretic mobility than that of* α_s*-casein were also found. Electrophoretic analysis of the water-soluble protein fraction showed no variation. No change in the triglyceride fatty acid composition was observed during the study period. The total free fatty acid content increased from 9881 to 12 137 ppm.*

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INTRODUCTION

Although goat's milk production represents only a modest share of world milk production, it is, nevertheless, important in certain parts of the world, particularly in Mediterranean countries and the Middle East, and it has increased gradually in recent years. About 50% of goat's milk is produced in Asia, 20% in Africa and nearly 30% in Europe, where the increase represents 8%. In Spain, the fourth largest goat's milk producer in Europe with a production of 353 million liters, following the USSR, Greece and France, the tendency is similar, with an increase of 7%.

The only goat's milk product which has been extensively researched is cheese, while data on low fat, enriched and aromatized liquid products, as well as fermented products, ices, butter and powdered milk are very scarce.

The leading countries in cheese production are Greece (40 277 tons), France (36 000 tons), and Spain (16 022 tons). In Spain 33·5% is produced by the goat farmers themselves and 26·9% by small local factories. Only 39·6% of the total is sent to the central dairies.

The majority of Spanish goat cheeses can be classified into three types: soft cheeses, either unfermented or with lactic fermentation, which are consumed fresh or within a 15–20 day period; semi-hard, lightly pressed pastes, which have ripened for 1–1·5 months; and hand-made hard cheeses. A large part of goat's milk is sent to be processed into cheeses which also contain cow's milk.

Data available on Spanish goat cheese are scarce. Some studies on composition and nutritive values of some local cheeses have been made and are cited by Martín-Hernández *et al.* (1984). Aside from these, only data on average composition are given in other studies.

The present study analyzes physico-chemical characteristics, proteolysis and lipolysis of industrially produced goat cheese with *Penicillium candidum* on the surface. We have studied this type of goat cheese, which has surface flora, because of its interest as a potential use for goat's milk.

MATERIAL AND METHODS

Samples

Two batches of goat cheese were prepared at industrial level in a small dairy in the province of Cáceres using pasteurized milk (72–74°C, 15 s) $CaCl_2$ (0·1–0·2 g/liter) was incorporated with aromatizers and acidifiers, mesophilic starter and animal rennet (6–7 ml per 100 l milk; 1/10 000). The coagulation time was 18–20 h at 20–22°C at which point it turns, and the curd separates from the whey until content of total solids in the curd is approximately 45%.

Cylindrically shaped cheeses of 150 g (15 cm high, 5 cm diameter) were salted in brine for 1–2 h, and were then sprayed with a solution containing *Penicillium candidum* spores, and were ripened for 14 days at 10–12°C with a relative humidity of 85–87%. Later they were stored under refrigeration at 0–4°C for an additional 14 days, which is the estimated commercialization period. The following samples were taken from the inside of the cheese for analytical purposes: milk, curd and cheeses after 2, 7, 14, 21, and 28 days of ripening and storage. In the same way, a rind 5 mm thick separated, in which the pH, ash and total solids were analyzed.

Analytical methods

Physico-chemical determinations

The pH was determined on a slurry obtained by homogenizing 50 ml of water and 10 g of cheese. Fat, total solids, ash and salt were analyzed according to the standards of the International Dairy Federation (IDF).

Total nitrogen (TN) was determined according to the FIL–IDF standard (25: 1964); soluble nitrogen (SN) by the method of Sirk (Juárez *et al.*, 1983), non-protein nitrogen (NPN) as in Juárez *et al.* (1983) and free amino acids (NH_2N) in the sulphosalicylic acid-soluble fraction were determined by the ninhydrin method and expressed as leucine.

Milk and cheese fats for the index analysis and the triglycerides fatty acid composition were extracted following the IDF standard (32: 1962) using ethyl ether as the extraction solvent and taking an aliquot of the ethyl ether fat solution, without evaporating the solvent, to determine the free fatty acid (FFA) index. The soluble and insoluble volatile fatty acids, Reichert-Meissl, Polenske, Kirschner and FFA indices of the fat were determined according to IDF standards 37:1966 and 6A:1969, respectively.

Fat extraction for the determination of individual free fatty acids and total and triglyceride composition in curds and cheeses was done by the Woo & Lindsay (1982) and Needs *et al.* (1983) methods adapted in our laboratory (Martín-Hernández *et al.*, 1988). 10 g of the sample were mixed with 7 g of distilled water and acidified with 11 N H_2SO_4 to bring the pH down to 1·5 ($\sim$0·4 ml). The acidified homogenate was mixed with 15 ml ice-cold diethyl ether and shaken in an electric homogenizer for 3 min. Following centrifugation at 3000 rpm for 5 min at 2°C, the upper organic phase was removed to a bottle containing 1 g of anhydrous Na_2SO_4. After 5 min the diethyl ether layer was transferred to a screw-capped glass for further study by GLC analysis.

Electrophoretic analysis

The casein breakdown was studied by vertical polyacrylamide urea gel

electrophoresis in a 0·7 mm slab or tube according to the procedure reported by Akroyd (1968) ($T = 7·7\%$, $C = 2·6\%$, pH = 8·6).

The non-caseinic fraction was also analyzed by electrophoresis by the Hillier method ($T = 9·4\%$, $C = 4·25\%$, pH = 8·9). The slab for caseins and whey proteins were stained with Commassie Blue without decolouration.

Quantitative measurement of gels was done by densitometry. The nitrogen distribution was calculated as described by Marcos *et al.* (1983).

Chromatographic analysis

Esterification of the different fractions was carried out by the Metcalfe & Wang procedure (1981) modified in our laboratory (Martínez Castro *et al.*, 1986). 2 ml of the diethyl ether solution containing the milk fat were taken. 1 ml more of diethyl ether and 0·2 ml 20% tetramethyl ammonium hydroxide (TMAH) in methanol were added, and the mixture was shaken for 1–2 min and allowed to settle. The upper layer then contained the fatty acid methyl esters (FAME) from the glycerides, while the TMAH FFA soaps were in the lower layer, which was neutralized before injection. The total composition can also be obtained by adding enough methanol to make a single phase and injecting an aliquot thereof.

The methyl esters were analyzed by programmed GLC as previously described (Martínez-Castro *et al.*, 1979), using a WCOT fused silica column (25 m × 0·25 mm, CP-Sill 88).

To avoid any discrimination in the pyrolytic methylation analysis of the FFA mixture, the programmed GLC analysis of this fraction was done with packed columns (2 m × 3·2 mm, with 4% DEGA on Chromosorb G, AW-DMCS 80–100 mesh) in Perkin–Elmer F-990 equipment. Quantitative determination of individual free fatty acid content in the cheese was made using *n*-nonanoic acid, added to the cheese at the beginning of the analysis, as internal standard.

Statistical treatment

Analysis of variance was applied using the BMDP V Program (BMDP series, 1981) with a CDC 180/185 computer, to study the differences throughout the ripening in all studied parameters.

RESULTS AND DISCUSSION

Global composition

Table 1 shows the mean values of total solids (TS), NaCl and ash content of the milk, curd and cheese during the different ripening and storage stages.

TABLE 1
Mean Values for Total Solids (TS), Salt and Ash Contents of Soft Goat's Cheese with Surface Flora during Ripening (2 to 14 days) and Storage (21 and 28 days)

Variable	*Milk*	*Curd*	*Cheeses (days)*				
			2	*7*	*14*	*21*	*28*
TS (%)							
center	14·12	46·44	50·91	54·47	59·16	58·37	58·24
surface			52·11	54·11	51·06	49·99	50·81
NaCl (%TS)							
center	—	—	3·53	3·71	3·77	3·92	4·29
Ash (%TS)							
center	0·78	0·59	2·94	3·34	3·88	3·13	2·94
surface			3·62	4·34	6·05	6·20	6·26

Total solids content of the inside significantly ($p \leq 0{\cdot}01$) increased with up to 14 days of ripening from 50·91% to 59·16%; on the surface it increased up to the end of the first week, and later descended from 54·11% to 50·81%. A similar evolution was found in Camembert cheese by Le Graet *et al.* (1983), who attributed the migration of water from the center to the surface to a transfer of hydrated ions, an increase in the hydration of protein as a result of the increase in pH, and to the absorption of water by *Penicillium*. NaCl (%TS) in the center increased until the end of the study period as a consequence of salt diffusion. Ash content (%TS) also increased until the fourteenth day, after which the level on the surface continued to increase, while the inside level fell, due to the movement of minerals towards the surface, as a result of changes in pH level, just as the alkalization of the surface took place due to the action of mould (Le Graet *et al.*, 1983).

Fat and protein contents (%TS) did not change during ripening and storage and represented, in the cheese, values of $60{\cdot}76 \pm 0{\cdot}63$ and $33{\cdot}6 \pm 1{\cdot}09$%, respectively.

pH decreased during the coagulation period down to values of 4·45, as a result of the lactic fermentation throughout the coagulation period; it then increased noticeably, especially on the surface (Fig. 1). At the end of the study period, the pH inside the cheese was 5·06, while on the surface it was 7·14, as a consequence of the activity of *Penicillium*, which metabolizes lactic acid and lactate (Trieu-Cuot & Gripon, 1982*a*) and causes the breakdown of curd components, which, in turn, causes the increase in pH. Similar changes

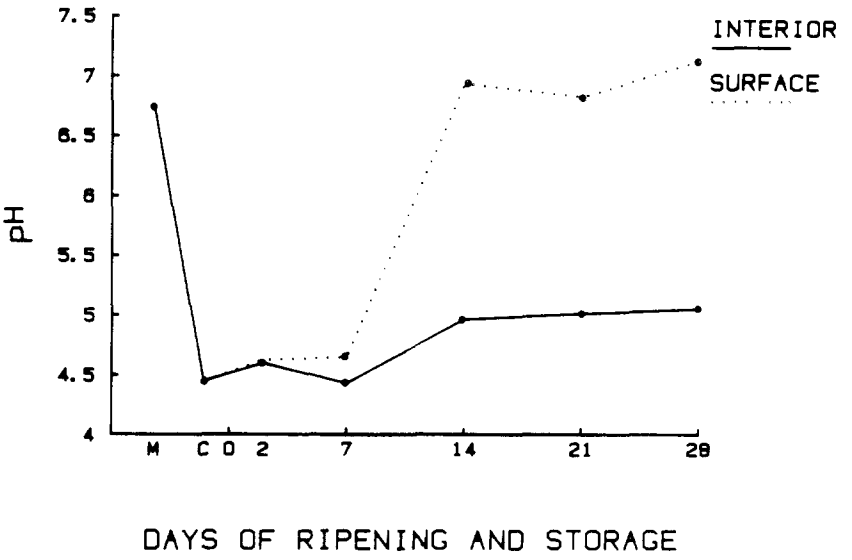


Fig. 1. Mean changes in pH of goat's cheese with surface flora during ripening and storage: M, milk; C, curd.

in pH were found during the ripening of Camembert cheeses (Trieu-Cuot & Gripon, 1982*a*).

Nitrogen fractions

Figure 2 shows the mean values of the soluble and non-protein nitrogen during the various ripening and storage stages. A slight but significant ($p \leq 0{\cdot}01$) increase in the nitrogen fractions was observed. By the end, the water-soluble nitrogen had risen up to a value of 16% TN, of which about 84% was NPN. The slight proteolysis found in this cheese could be partly justified by the fact that initial pH is very low, a special factor that affects the activity of residual rennet and the level of proteases produced by the *P. candidum*, which is at its optimum point of activity when pH is 5·5 to 6·5 (Holmes *et al.*, 1977; Stepaniak *et al.*, 1980; Lenoir, 1984). In fact, differences in the proteolysis level in the two batches were found depending on the pH level; on the 28th day, Batch A, with a pH of 4·67, showed a 12·9% SN, while Batch B, with a 5·54 pH, represented 19·1% SN. Moreover, it has been shown that proteases of *P. candidum* diffuse only to a very limited extent into the cheese (Karahadian & Lindsay, 1987).

On the other hand, in spite of the relatively low content of SN, the value of the NPN (%SN) is even higher than for other types of cheese ripened with other species of *Penicillium*, such as Cabrales cheese ripened with *P. roqueforti* for the same length of time (Juárez *et al.*, 1983).

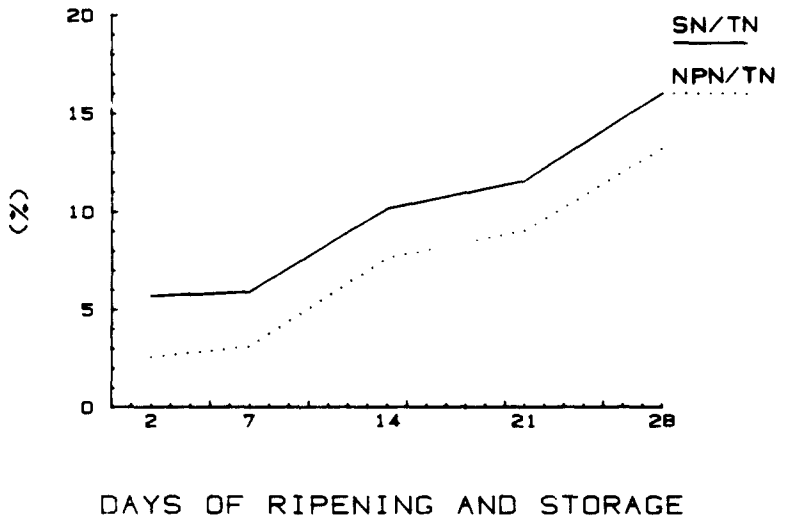


Fig. 2. Mean values in soluble nitrogen (SN) and non-protein nitrogen (NPN) (% total nitrogen, TN) of goat's cheese with surface flora during ripening (2, 7 and 14 days) and after 14 days of storage (21 and 28 days).

The free amino acid content increased from 0·121 to 1·49 (% total solids). The values found for these ripening indices are similar to those obtained in Camembert type cheese and goat cheese with surface flora for the same ripening period (Polychroniadou & Manolkidis, 1984; Jarmul *et al.*, 1985).

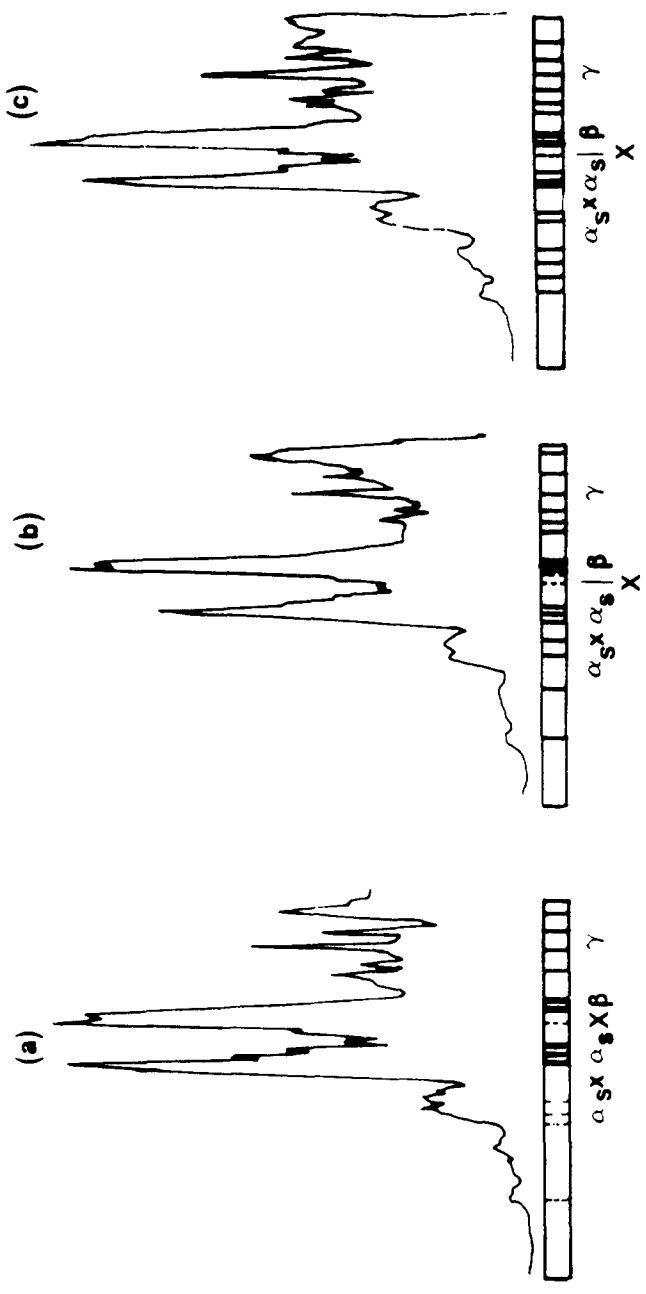
Nevertheless, there is a 50% increase of the SN from the second to the third week in Camembert cheese (Jarmul *et al.*, 1985). This did not occur in the cheese used in this study because, for the last two weeks (14th to 28th days) it was kept under refrigeration and not ripening, and at 0°C the proteolytic activity of the mould decreases considerably.

Casein breakdown

Figure 3 shows the electrophoretic diagrams and densitometric curves of caseins of cheeses at the different ripening and storage stages (2 to 28 days) from one of the batches studied.

The electrophoretic diagram is typical of goat's milk. In the diagram of cheese 2 days into ripening, besides the γ, β (β_1 and β_2) and α_s (α_{s1} and α_{s2}) caseins, there can be seen 2–3 bands of higher electrophoretic mobility than that of α_s-caseins, which are degradation products from α_s-casein by the rennet action (α_s,χ). Another product appears between the β and the α_s-caseins (χ fraction).

Table 2 shows the mean values for the nitrogen distribution in the cheeses analyzed during ripening and storage. Contents are calculated on the basis


(a)
(b)
(c)
γ
X

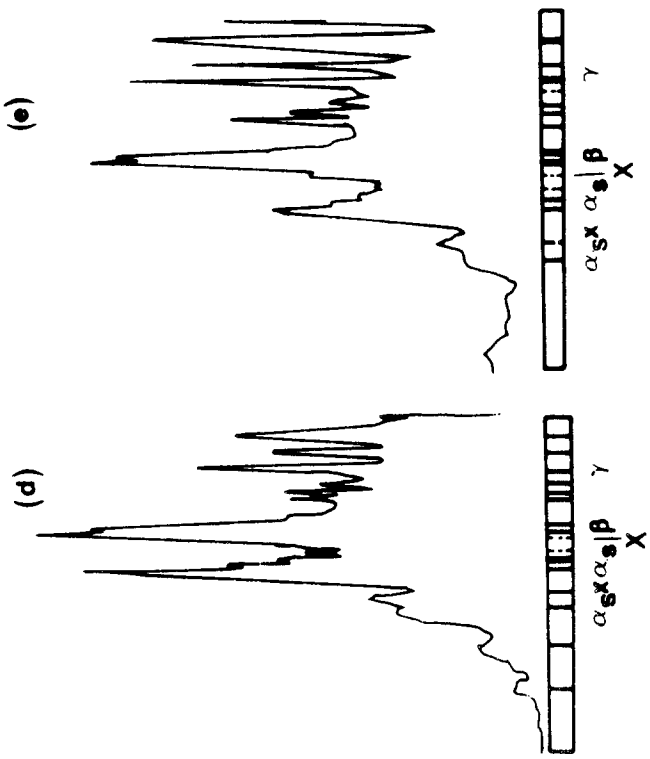


Fig. 3. Schematic diagrams and densitometric curves of electrophoretic patterns of goat's cheese caseins during 14 days of ripening and 14 days of storage: (a) 2 days; (b) 7 days; (c) 14 days; (d) 21 days and (e) 28 days.

TABLE 2
Mean Values of the Nitrogen Fractions (g/100 g TN) in Goat's Cheeses with Surface Flora at Various Stages of Ripening and Storage (2 to 28 days)

Cheeses (days)	*γ-Caseins + polypeptides*	*β-Caseins*	*X*	*α_s-caseins*	*α_s-x*	*Soluble polypeptides*	*Oligopeptides*	*NH_2-N*
2	32·5	29·9	2·7	21·1	8·2	3·1	2·3	0·3
7	32·1	28·8	2·7	21·5	9·1	2·8	2·7	0·5
14	31·0	24·2	2·9	19·9	11·9	2·5	6·1	1·6
21	36·1	23·7	2·8	16·2	9·6	2·5	7·4	1·6
28	36·2	21·7	2·6	13·1	10·3	2·8	10·3	2·9

of different nitrogen fractions (%TN) and the relative percentages of the different casein fractions and their degradation products. The electrophoresis products, which are slower than β-caseins, were recorded together with the identification γ-casein plus polypeptides. The soluble polypeptide fraction corresponds to (SN-NPN) and oligopeptides to (NPN—NH_2N).

The degradation of the α_s and β-caseins, calculated with the initial α_s and β-casein content in the milk, is not high, but at the end of the studied period a series of bands, chiefly degradation products of β-caseins, was observed in the γ-casein region. Similar results have been reported in Camembert cheese and are attributed to the action of aspartyl proteinase and metalloproteinase on the β-caseins (Trieu-Cuot & Gripon, 1982*b*). The degradation of β-caseins is 31%; this figure is higher than that found in Manchego cheese (Ordoñez *et al.*, 1978). The degradation of α_s-caseins was higher (46%). The γ-caseins plus polypeptide fraction increased from 33% to 36%, although the increase in CN was greater (34% to 43%).

The oligopeptides and NH_2N fractions increased during the ripening and are comparable to those found in Camembert cheese. (Jarmul *et al.*, 1985). The soluble polypeptide fraction did not change during the study period. This result was also tested by analysis of the non-caseinic fraction by electrophoresis. The electrophoretic diagram of this fraction did not change during the ripening and storage stages. This result of minimal change in the proteins of the whey has also been found in other types of cheese (Polo *et al.*, 1985; Ramos *et al.*, 1987), including some which are produced using ultrafiltered milk, with a high proportion of such proteins (Koning *et al.*, 1981).

Fat characteristics

The soluble and insoluble volatile fatty acid indices did not change during the study period, showing the following mean values: $24·3 \pm 0·46$ for

Reichert-Meissl; $5{\cdot}4 \pm 0{\cdot}22$ for Polenske and $20{\cdot}8 \pm 1{\cdot}84$ for Kirschner indices. These results accord with the ranges reported previously, and with the mean values published by Singh & Gupta (1982) for goat's milk.

The FFA index increased from 0·94 to 2·08 mg KOH/g fat, indicative of a slight lipolysis. The FFA contents found were similar to those reported in Camembert cheese (Jarmul *et al.*, 1985).

No changes were observed during the study period for the triglyceride fatty acid composition, which was comparable at the beginning and end of the period (2 to 28 days). Similar results were found in other types of goat cheese (Martín-Hernández, 1987) with scarce lipolysis, in contrast to blue cheeses such as Cabrales cheese, which presented strong lipolysis and important changes in the lipid fraction (Juárez *et al.*, 1983). The main fatty acid is similar to that reported by García-Olmedo *et al.* (1979) and Gonç *et al.* (1979) for goat's milk.

Individual free fatty acids

Table 3 shows the average free fatty acid composition for the two batches of goat cheeses at 2 and 28 days of ripening and storage. Total FFA content increased from 9881 to 12 137 ppm. This value and the individual FFA were higher than the results found by other authors for Camembert and Brie cheeses (Woo *et al.*, 1984) and are indicative of a moderate lipolysis.

The results found for the FFA content, very much lower than in blue cheeses such as Cabrales cheese (Juárez *et al.*, 1983) can be justified by the low pH inside the cheese, since this is an important factor which affects the production of lipases. Moreover, the optimum pH is at alkaline values. On the other hand, the lipolytic activity in cheese is observed essentially close to the mycelium (Cerning *et al.*, 1987).

The differences found in relation to other cheese with surface flora could be justified by taking into account that the same varieties sometimes show great variability in concentration of individual major free fatty acids (Woo *et al.*, 1984).

TABLE 3
Average Free Fatty Acid Content (ppm) in Soft Goat's Cheese with Surface Flora during Ripening and Storage (2 and 28 days)

Sample days	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18:1}$	*Total*
2	298	319	400	1 228	477	1 236	2 286	1 369	2 268	9 881
28	430	466	524	1 568	682	1 146	2 954	1 572	2 795	12 137

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REFERENCES

Akroyd, P. (1968). In *Chromatographic and Electrophorietic Techniques*. ed. I. Smith. William Heinemann Medical Books Ltd., London.

Cerning, J., Gripon, J. C., Lamberet, G. & Lenoir, J. (1987). Les activités biochimiques des *Penicillium* utilisés en fromagérie. *Lait*, **67**, 3–39.

García-Olmedo, R., Carballido, A. & Arnaez, M. (1979). Contribución al estudio de la grasa de leche de cabra. II. Acidos grasos mayores y sus relaciones. *Anal. Bromatol.*, **31**, 227–70.

Gonç, S., Schmidt, R. & Renner, E. (1979). Studies on the fatty acid pattern of buffalo and goat milk. *Milchwiss.*, **34**, 684–86.

Holmes, D. G., Duersch, J. W. & Enustrom, C. A. (1977). Distribution of milk clotting enzymes between curd and whey and their survival during Cheddar cheese making *J. Dairy Sci.*, **60**, 862.

Jarmul, I., Reps, A., Wisniewska, K. & Jedrychowksi, J. (1985). Stabilisation du fromage de Camembert par congélation. *Lait*, **65**, 213–20.

Juárez, M., Alonso, L. & Ramos, M. (1983). Lipolisis y proteolisis del queso de Cabrales durante la maduración. *Rev. Agroquim. Tecnol. Aliment.*, **23**, 541–51.

Karahadian, C. & Lindsay, R. C. (1987). Integrated roles of lactate, ammonia and calcium in texture development of mould surface ripened cheese. *J. Dairy Sci.*, **70**, 909–18.

Koning, P. J., Boer, R., Both, P. & Nooy, P. C. (1981). Comparison of proteolysis in a low fat semi-hard type of cheese manufactured by standard and ultrafiltration technique. *Neth. Milk. Dairy J.*, **35**, 35–46.

Le Graet, Y., Lepienne, A., Brûlé, G. & Ducruet, P. (1983). Migration du calcium et des phosphates inorganiques dans les fromages à pâte molle de type Camembert au cours de l'affinage. *Lait*, **63**, 317–32.

Lenoir, J. (1984). The surface flora and its role in the ripening of cheese. *IDF Bull.*, 171.

Marcos, A., Millán, R., Esteban, M. A., Alcalá, M. & Fernández-Salguero, J. (1983). Chemical composition and water activity of Spanish cheeses. *J. Dairy Sci.*, **66**, 2488–93.

Martínez-Castro, I., Juárez, M. & Martin-Alvarez, P. J. (1979). The composition of fatty acids of milk fat in Spain. *Milchwiss.*, **34**, 207–10.

Martínez-Castro, I., Alonso, L. & Juárez, M. (1986). Gas chromatographic analysis of free fatty acids and glycerides of milk fat using tetramethylammonium hydroxide as catalyst. *Chromatographia*, **21**, 37–40.

Martín-Hernández, M. C. (1987). Estudio de las características fisico-químicas de los quesos de cabra fresco y semicurado. Influencia de la congelación. Doctoral Thesis, Universidad Complutense, Madrid.

Martín-Hernández, M. C., Juárez, M. & Ramos, M. (1984). Producción y características de composición de leche y queso de cabra. *Alimentación, Equipos y Tecnología*, **3**, 61–71.

Martín-Hernández, M. C., Alonso, L., Juárez, M. & Fontecha, J. (1988). Gas chromatographic method for estimating free fatty acids in cheese. *Chromatographia*, **25**, 87–90.

Metcalfe, L. D. & Wang, C. N. (1981). Rapid preparation of fatty acid methyl esters using organic base-catalyzed transesterification. *J. Chromatogr. Sci.*, **19**, 530–5.

Needs, E. C., Ford, G. D., Owen, A. J., Tuckley, B. & Anderson, M. (1983). A method for the quantitative determination of individual free fatty acids in milk by ion exchange resin adsorption and gas–liquid chromatography. *J. Dairy Res.*, **50**, 321–9.

Ordoñez, J. A., Barneto, R., Ramos, M. (1978). Studies on Manchego cheese ripened in olive oil. *Milchwiss.*, **33**, 609–13.

Polo, M. C., Ramos, M. & Sánchez, R. (1985). Free amino acids by high performance liquid chromatography and peptides by gel electrophoresis in Mahon cheese during ripening. *Food Chemistry*, **16**, 85–96.

Polychroniadou, A. & Manolkidis, K. S. (1984). Etude biochimique d'un fromage de chèvre á croûte fleurie fabriqué à l'aide d'extraits coagulants microbiens. *Lait*, **64**, 469–83.

Ramos, M., Cáceres, I. & Polo, C. (1987). Effect of freezing on soluble nitrogen fraction of Cabrales cheese. *Food Chemistry*, **24**, 271–278.

Singh, S. & Gupta, M. P. (1982). Physico-chemical characteristics of ghee prepared from goat milk. *Asian J. Dairy Res.*, **1**, 201–5.

Stepaniak, L., Kormacki, K., Grabska, J. & Wotecki, E. (1980). Influence de certains conditions de la culture superficielle sur la production des lipases et des protéases par des souches de *Penicillium roqueforti* et *Penicillium candidum*. *Lait*, **60**, 45–55.

Trieu-Cuot, P. & Gripon, J. C. (1982*a*). A study of proteolysis during Camembert cheese ripening using isoelectric focusing and two dimensional electrophoresis. *J. Dairy Res.*, **49**, 501–10.

Trieu-Cuot, P. & Gripon, J. C. (1982*b*). *Int. Symposium Use of Enzymes in Food Technology. Versailles*. ed. P. Dupuy. Tech. Doc. Lavoisier, Paris, 293–7.

Woo, A. H. & Lindsay, M. C. (1982). Rapid method for quantitative analysis of individual free fatty acids in Cheddar cheese. *J. Dairy Sci.*, **65**, 1102–9.

Woo, A. H., Kollodge, S. & Lindsay, R. C. (1984). Quantification of free fatty acids in several cheese varieties. *J. Dairy Sci.*, **67**, 874–8.