ELSEVIER

Food Chemistry 70 (2000) 227–233

Food Chemistry

www.elsevier.com/locate/foodchem

"Quesucos de Liébana" cheese from cow's milk: biochemical changes during ripening

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Received 19 August 1999; received in revised form and accepted 29 November 1999

Abstract

The changes in chemical composition, main physico-chemical parameters, classical nitrogen fractions, caseins and their degradation products, and some fat characteristics were studied during the ripening process of 10 batches of Quesucos de Liébana cheese, a traditional variety made in the north of Spain. The values of the different compositional and physico-chemical parameters at the end of the ripening did not differ significantly from those found in other cow's milk cheeses elaborated with similar technology. The presence of residual lactose at the end of the ripening process is outstanding. The total soluble nitrogen increased by a factor of 2 at the end of the ripening compared to the values shown at the beginning. However, non-protein nitrogen increased very little. The final values of the different nitrogen fractions show that this cheese undergoes a proteolysis moderate in extension but not very intense and that the rennet is the main agent of the proteolysis produced during ripening. Using PAGE techniques, it was possible to show that, throughout ripening, 56% of α_s-casein and 11% of β-casein turned out to be degraded. The TBA number and acidity index of the fat values show that the processes of lipolysis and fat autooxidation during the ripening of this cheese are not very intense. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Quesucos de Liébana cheese is a traditional variety made in the north of Spain from cow's, ewe's or goat's milk or from mixes of milk from two or three of these animal species through an enzymatic coagulation. The cheese elaborated from cow's milk is the most frequent. It is cylindrical in form with a diameter of 8–12 cm and a height of 3–10 cm, varying in weight between 200 and 500 g. The mass is firm, compact and slightly yellowish in colour with a few irregularly distributed holes. The ripening period is of about 2 months. This variety has been protected by a "Designation of Origin" since 1993.

To date, no information exists on the characteristics, either chemical or microbiological, of this cheese variety which hinders the improvement of its quality and uniformity and impedes a wider distribution in markets beyond the production area. The aim of this article is to study, in a representative number of batches of this cheese variety, the changes in the gross composition, the main physico-chemical parameters, and the protein and lipid fractions during the ripening process, with special emphasis on the degradation of the proteins, the most important phenomenon during the cheese ripening.

2. Materials and methods

2.1. Cheesemaking and sampling

Ten batches of cheese were produced by 10 different industrial cheesemakers following the traditional method. Pasteurized whole cow's milk, after addition of a lyophilized lactic mesophilic starter culture (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*), was coagulated at 28–32°C by adding 25 ml of commercial calf rennet (1/10 000 strength) per 100 l of milk. One hour after the addition of the rennet, the curd was cut to the size of a pea and was transferred to the moulds where it stayed for 2 days without applying pressure. The elimination of the whey was carried out by self-oozing. The cheeses were salted by rubbing in

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PII: S0308-8146(00)00087-X

dry salt in a proportion of 2–3%; after 1 day in the moulds, the cheeses were salted on the upper side, and after 48 h in the moulds they were taken from the moulds and salted on the lower side and on the lateral sides. Then, cheeses were ripened in rooms at 8–12°C and relative humidities of 85–95% for 2 months, being turned over periodically.

From each batch, samples of cheese were taken after salting (3-day-old cheese) and after the ripening has been finished (2-month-old cheese). Each sample consisted of one whole cheese. The cheeses were transported to the laboratory under refrigeration conditions (below 4°C). In the laboratory, the rind of the cheeses was discarded and the cheeses were triturated and held in airtight containers at −40°C until they were analyzed.

2.2. Compositional and physico-chemical parameter analysis

Total solids, protein, fat, salt, ash and lactose were determined according to the FIL-IDF standards 4A (IDF, 1982), 20B (IDF, 1993), 5B (IDF, 1986), 88A (IDF, 1988), 27 (IDF, 1964), and 43 (IDF, 1967), respectively. D- and L-lactic acids were determined by the spectrophotometric method recommended by Boehringer Mannheim (1995). pH and titratable acidity were measured using AOAC methods 14.022 (AOAC, 1980a) and 16.247 (AOAC, 1980b), respectively. Water activity was measured in a DECAGON CX-1 Water Activity System apparatus (Decagon Devices Inc., Pullman, USA). All the determinations were carried out in duplicate.

2.3. Nitrogen fraction analysis

The total nitrogen content (TN) was determined by the Kjeldahl method as described by the FIL-IDF 20B (IDF, 1993) standard. The Vakaleris and Price (1959) procedure was followed for the extraction of the total soluble nitrogen (TSN) and non-protein nitrogen (NPN), and that of Johnson (1941) was used for their determination. In the case of NPN, previous precipitation of proteins with trichloroacetic acid at 12% was necessary. The method described by Ordoñez (1974) was used for the determination of ammonia nitrogen (NH_3–N) and amino nitrogen (NH_2-N). Protein, casein, oligopeptides and proteose-peptone nitrogen were calculated as described by Prieto, Fresno, Carballo, Bernardo and Martín Sarmiento (1994). All the nitrogen fractions were analyzed in quadruplicate.

2.4. Electrophoretic analysis

The casein degradation was studied using PAGE techniques following the procedure of Andrews (1983). For the identification and quantification of the casein fractions, the software package Diversity One™ 1.0 (pdi, New York, USA) was used after having duly scanned the electrophoresis gels. All electrophoresis analyses were performed in duplicate. The optical density of each region was expressed as a percentage of the total optical density.

2.5. Fat characteristics

The TBA number was determined as described by Tarladgis, Watts, Younathan and Dugan (1960). The acidity index of the fat was determined as described by the FIL-IDF 6B (IDF, 1989) standard.

3. Results and discussion

3.1. Compositional and physico-chemical parameters

Tables 1 and 2 show the average ± standard deviation values of the main compositional and physico-chemical parameters, respectively, of the cheeses after salting (3-day-old cheeses) and of the ripened cheeses (2-month-old cheeses) in the 10 batches studied.

Total solids content increased slightly during the ripening process, reaching final values (59.33 ± 4.39 g/100 g of cheese) very close than those described in other similar cow's milk cheeses such as Bola (Marcos, Fernández-Salguero, Esteban, Léon, Alcalá & Beltrán de Heredia, 1985), Edam (Marcos et al., 1985), Gouda (Marcos, Alcalá, Léon, Fernández-Salguero & Esteban, 1981), Mahón (Alcalá, Beltrán de Heredia, Esteban & Marcos, 1982), San Simón (Marcos, Millán, Esteban,

Table 1
Changes in chemical composition during the ripening of Quesucos de Liébana cheese[a]

	Cheese after salting	Ripened cheese
Total solids (%)	50.24 ± 2.21	59.33 ± 4.39
Protein (% T.S.)	37.0 ± 4.49	40.2 ± 3.49
Fat (% T.S.)	57.65 ± 5.20	54.99 ± 3.79
Ash (% T.S.)	4.84 ± 1.26	6.33 ± 1.41
NaCl (% T.S.)	1.08 ± 0.87	2.73 ± 0.71
Lactose (% T.S.)	1.98 ± 0.52	0.27 ± 0.46
D-lactic acid (% T.S.)	0.16 ± 0.15	0.56 ± 0.45
L-lactic acid (% T.S.)	2.05 ± 0.50	1.37 ± 0.78

[a] Means of 10 batches ± standard deviations.

Table 2
Changes in physico-chemical parameters during the ripening of Quesucos de Liébana cheese[a]

	Cheese after salting	Ripened cheese
pH	5.21 ± 0.15	5.45 ± 0.12
Titratable acidity (g lactic acid/100 g T.S.)	1.85 ± 0.17	1.78 ± 0.21
a_w	0.982 ± 0.007	0.967 ± 0.009

[a] Means of 10 batches ± standard deviations.

Alcalá & Fernández-Salguero, 1983; Millán, Saavedra, Sanjuán & Castelo, 1996), and Tetilla (Marcos et al., 1985). These values, however, were lower than those reported for other cow's milk cheeses such as Afuega'l Pitu (Cuesta, Fernández-García, González de Llano, Montilla & Rodríguez, 1996), Cheddar (Marcos et al., 1981; Muir, Hunter & Watson, 1995; Sapru, Barbano, Yun, Klei, Oltenacu & Bandler, 1997) and León (Prieto et al., 1994).

Final protein and fat contents (expressed as g/100 g of total solids) were in the range of those observed for other cow's milk cheeses (Marcos et al., 1981, 1983, 1985; Muir et al., 1995; Paleari, Soncini, Beretta, Dragoni & Piantoni, 1993; Prieto et al., 1994; Sieber, Badertscher, Fuchs & Nick, 1994).

The NaCl and ash contents were higher at the end of the ripening process compared to the values observed in 3-day-old cheese. This variation is very probably due to the salting system used. In 3-day-old cheese the salt had not totally penetrated the depth of the cheese, which means that the NaCl and ash values observed are lower than those obtained in 2-month-old cheese. Final NaCl values are in the lower extreme of the range of values observed for different cow cheeses (Cuesta et al., 1996; Marcos et al., 1981, 1983, 1985; Muir et al., 1995; Prieto et al., 1994; Sieber et al., 1994).

From our data, the 86% of lactose present in 3-day-old cheese turned out to be degraded throughout the ripening process, reaching final average values of 0.27 ± 0.46 g/ 100 g T.S. This final content is higher than presented by the majority of ripe cow cheeses in which the presence of lactose is not usually detected after 30 days of ripening. The presence and evolution of lactose in cheese is dependent on the deepness of whey drainage and on the intensity of degradation of lactose held in the curd. The non-application of pressure during the moulding favours the retention of lactose in the curd, and the high contents in lactose observed in 3-day-old cheese. The non-total degradation of lactose throughout the ripening of Quesucos de Liébana cheese could be related to the high values of salt/moisture ratio observed (6.71% at the end of ripening). Thomas and Pearce (1981) and Turner and Thomas (1980), working on Cheddar cheese, observed that high salt/moisture ratios strongly inhibit the lactose degradation.

As a consequence of lactic acid bacteria activity (mainly lactococci) in the first stages of the manufacture, high levels of L-lactic acid (the isomer produced by these lactic acid bacteria from lactose) were observed in 3-day-old cheese. The L-lactic acid content slowly decreased during the ripening, reaching final values of 1.37 ± 0.78 g /100 g T.S. in 2-month-old cheese. The low degradation of lactic acid throughout ripening is usual in cheeses ripened by bacteria in contrast to those ripened by moulds which, together with yeasts, are the main lactic acid-consuming microorganisms during cheese ripening. The fall in L-lactic acid content during the ripening of Quesucos de Liébana cheese could also be due in part to its transformation into D-lactic acid via racemization by enzymes of microbial origin (Thomas & Crow, 1983). This racemization could justify the slight increase in D-lactic acid content observed during ripening. This same behaviour of the L- and D-lactic acid contents was observed in other cow's milk cheeses such as Cheddar, Colby, Gouda and Cheshire (Thomas & Crow, 1983).

Due to the the lactic acid bacteria activity in the early stages of the ripening, low pH values were observed in 3-day-old cheese. Recorded shifts in pH during ripening are hardly significant. The values slowly increased, reaching final figures (5.45 ± 0.12) which are in the range of those observed by different authors for other cow's milk cheeses (Coulon, Verdier, Pradel & Almena, 1998; Marcos et al., 1981, 1983, 1985; Muir et al., 1995; Watkinson et al., 1997). The low increase of the pH values during ripening can be explained in part by the previously mentioned low consumption of lactic acid throughout the ripening process. The moderate protein degradation undergone by Quesucos de Liébana cheese and the consequent scarce liberation of alkaline nitrogen compounds could also contribute to this phenomenon.

The water activity values decreased in a moderate way during the ripening process reaching final values (0.967 ± 0.009) very similar to those observed for other cow's milk cheeses such as Bola (Marcos et al., 1981), Cheddar (Marcos et al., 1981), Edam (Marcos et al., 1981), Gallego (Marcos et al., 1985), Gouda (Marcos et al., 1981), Mimolette (Marcos et al., 1981), Munster (Marcos et al., 1981), San Simón (Marcos et al., 1983) and Tetilla (Marcos et al., 1983). The relatively low decrease undergone by the water activity values throughout the ripening of Quesucos de Liébana cheese could be explained in part by the scarce dehydration undergone by the cheeses during the ripening process. The moderate proteolysis observed in Quesucos de Liébana cheese during ripening could also have an influence; the falling effect, which the nitrogen components of low molecular weight generated during proteolysis have on the water activity, is well known.

3.2. Proteolytic parameters

Table 3 shows the average values obtained for the different nitrogen fractions (expressed as percentage of total nitrogen) at the beginning and at the end of the ripening process.

The total soluble nitrogen increased during ripening, reaching final values of double of those observed in 3-day-old cheese. The final values of this nitrogen fraction were similar to those reported for other cow's milk cheeses such as Bola (Marcos et al., 1985), Cheddar (Lau, Barbano & Rasmussen, 1991; Sapru et al., 1997),

Table 3
Changes in nitrogen fractions during the ripening of Quesucos de Liébana cheese[a]

	Cheese after salting	Ripened cheese
Total nitrogen (%)	2.91 ± 0.38	3.74 ± 0.48
Protein nitrogen (% T.N.)	93.0 ± 1.05	90.1 ± 3.42
Non protein nitrogen (% T.N.)	7.06 ± 1.05	9.89 ± 3.42
Casein nitrogen (% T.N.)	88.5 ± 2.85	79.0 ± 7.73
Total soluble nitrogen (% T.N.)	11.5 ± 2.85	21.1 ± 7.73
Proteose-peptone nitrogen (% T.N.)	4.42 ± 2.63	11.2 ± 4.81
Oligopeptide nitrogen (% T.N.)	4.87 ± 0.85	6.04 ± 2.40
Amino nitrogen (% T.N.)	1.70 ± 0.40	2.62 ± 1.08
Ammonia nitrogen (% T.N.)	0.49 ± 0.09	1.23 ± 1.21

[a] Means of 10 batches ± standard deviations.

Edam (Marcos et al., 1985), Gouda (Marcos et al., 1981), Mimolette (Marcos et al., 1981), and Saint-Paulin (Lenoir, 1963; Marcos et al., 1981).

The non-protein nitrogen increased very little throughout ripening. The final values of this fraction were lower than those observed in the above-mentioned cheeses; only Saint-Paulin cheese (Lenoir, 1963; Marcos et al., 1981) showed non-protein nitrogen values as low as those observed in Quesucos de Liébana cheese.

In accordance with the scarce increase in non-protein nitrogen, the components of the same (oligopeptides, amino and ammonia nitrogen fractions) underwent a very small increase throughout the ripening process. The final amino nitrogen values (2.62 ± 1.08% of T.N.) were fairly low; only León (Prieto et al., 1994), Mimolette (Marcos et al., 1981) and Saint-Paulin (Lenoir, 1963; Marcos et al., 1981) cheeses showed values as low as those determined in Quesucos de Liébana cheese. The final ammonia nitrogen values (1.23 ± 1.21% T.N.) were, however, in the range of those observed for the different cow's milk cheeses (Lenoir, 1963; Marcos et al., 1981, 1983, 1985). The values of the amino N/ammonia N ratio at the end of the ripening process were very much higher than 1 (2.13 on average), which corresponds to the cheeses in which lactic acid bacteria have a greater role in the ripening than the rest of the microbial groups.

The proteose-peptone nitrogen fraction is made up of large-sized peptides. This was the nitrogen fraction that underwent a larger increase throughout ripening, showing high values in 2-month-old cheese. The final contents in large-sized peptides show the result of the equilibrium between their production, mainly due to the chymosin of the rennet, and their degradation, carried out by the enzymes of microbial origin responsible for the formation of the fractions which make up the non-protein nitrogen (Desmazeaud, Gripon, Le Bars & Bergère, 1976; Gripon, Desmazeaud, Le Bars & Bergère, 1975; O'Keeffe, Fox & Daly, 1976, 1978; Visser, 1977; Visser & De Groot-Mostert, 1977). The final high values of the proteose-peptone nitrogen fraction and the low values of the non-protein nitrogen allow us to deduce that the rennet is the main agent of the proteolysis in Quesucos de Liébana cheese.

From the values of the different nitrogen fractions at the end of the ripening process, we can conclude that Quesucos de Liébana cheese undergoes a proteolysis which is moderate in extension and not very intense.

Fig. 1 shows the typical electrophoretogram and densitogram of the caseins and their degradation products in 3-day-old cheese and in 2-month-old cheese with values of Rf of each one of the bands detected. Table 4 shows the changes in the percentage of total optical density of the electrophoretical regions in the stained

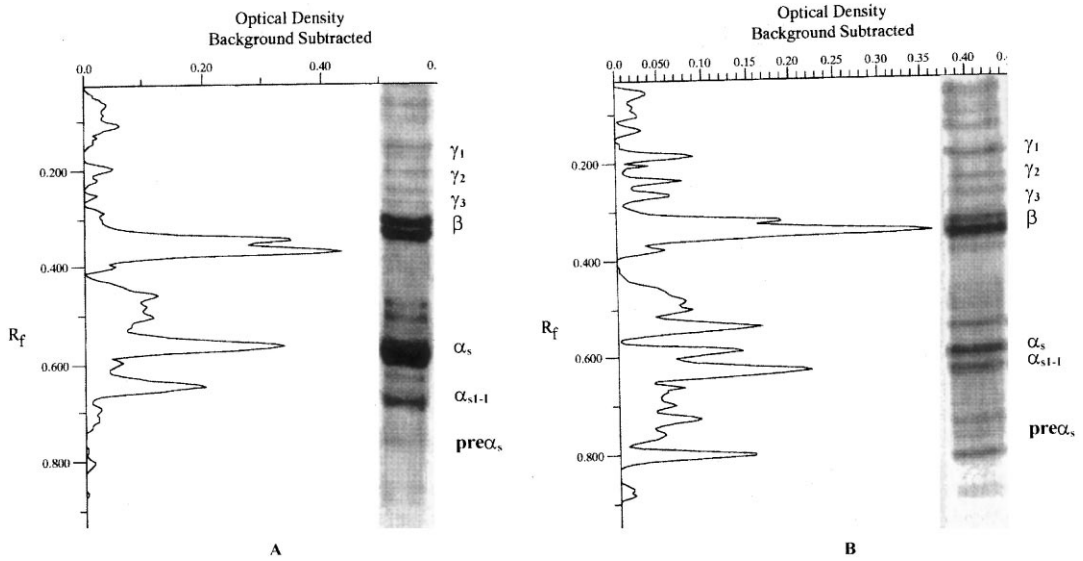

Fig. 1. Typical electrophoretogram of the casein fractions in cheese after salting (A) and in ripened cheese (B) with Rf and optical density values of each one of the bands detected.

Table 4
Changes in the percentage of total optical density of the electrophoretical regions in the stained gels of caseins during the ripening of Quesucos de Liébana cheese[a]

	Cheese after salting	Ripened cheese
#[b]	3.77 ± 1.22	6.44 ± 2.62
γ_1	2.46 ± 0.18	3.29 ± 1.10
γ_2	1.34 ± 0.40	1.65 ± 0.83
γ_3	1.52 ± 0.28	1.93 ± 0.70
β	27.88 ± 2.33	24.71 ± 2.85
#[b]	0.79 ± 0.12	1.97 ± 1.02
α_s	39.57 ± 2.08	17.26 ± 4.08
#[b]	3.06 ± 0.33	6.22 ± 3.60
$\alpha_{s1\text{-}I}$	12.16 ± 1.34	16.96 ± 4.41
Pre-α_s	7.47 ± 1.72	19.22 ± 6.35
α/β	1.43 ± 0.15	0.69 ± 0.13

[a] Means of 10 batches ± standard deviations.
[b] #, Unknown degradation products.

gels of caseins during the ripening of Quesucos de Liébana cheese.

Very little degradation (around 11%) of the β-casein occurs during ripening, this fraction representing, at the end of the ripening, on average, 24.7 ± 2.85% of the O.D. The α_s-fraction turned out to be more degraded (56%) during ripening, final average values being 17.3 ± 4.08% of the O.D. The fall in the α_s-casein fraction was accompanied by an increase in the fractions which corresponded to its degradation products; the α_{s1}-I-casein increased to almost 40% and the pre-α_s-casein to 157%, with these fractions representing, on average 17.0 ± 4.41% and 19.2 ± 6.35% of the O.D., respectively, at the end of ripening. These results again show the importance of the rennet in the protein degradation in Quesucos de Liébana cheese, since the degrading action of rennet is mainly carried out on α_s-caseín (McSweeney, Olson, Fox, Healy & Hojrup, 1993; Mulvihill & Fox, 1977) and to a much lesser extent on β-casein which is very resistant to its action.

The quantification results of the caseins and their degradation products again show the limited proteolysis undergone by this cheese variety throughout ripening. The high values of the salt/moisture ratio in the last stages of the ripening process (6.71% on average) could be in part responsible for this scarce protein degradation. Noomen (1978) proved that the most favourable conditions for the action of rennet on α_{s1}-casein in cheese are at pH values of about 5 and salt/moisture ratio values of 4%. The effect of the salt/moisture ratio values on the degradation of α_{s1}-casein has also been widely demonstrated by Thomas and Pearce (1981). These authors, working on Cheddar cheese, showed that, after a month's ripening at 10°C, at a salt/moisture ratio value of 4%, α_{s1}-casein was degraded almost totally, while at a salt/moisture ratio value of 8% only was degraded 40% of this casein. Mulvihill and Fox (1978) and Thomas and Pearce (1981), at the same time, showed that the rennet action on β-casein is also influenced by the NaCl concentration.

With regard to the degrading action of the microorganisms, different authors have pointed out the slight activity of lactic acid bacteria on the intact caseins (Farkye, Fox, Fitzgerald & Daly, 1990; Law, Fitzgerald, Uniacke-Lowe, Daly & Fox, 1993; Oberg, Davis, Richardson & Ernstrom, 1986; Visser & De Groot-Mostert, 1977).

3.3. Fat parameters

The average values of T.B.A. number and acidity index of the fat at the beginning and at the end of the ripening are shown in Table 5.

The values of T.B.A. number, indicators of the autooxidation grade of fat, increased very slightly during ripening. The existing information on the evolution of T.B.A. number during cheese ripening is very limited with only a few studies carried out on some homemade cheese varieties such as Armada (Fresno, Tornadijo, Carballo, Bernardo & González-Prieto, 1997), Babia-Laciana (Argumosa, Carballo, Bernardo & Martín, 1992), León (Prieto et al., 1994) and Valdeteja (Carballo, Fresno, Tuero, Prieto, Bernardo & Martín Sarmiento, 1994). The values of T.B.A. number found in Quesucos de Liébana cheese at the end of ripening turned out to be similar to those found in the cited varieties of cheese for a similar ripening period and seem to indicate that the fat does not undergo a noticeable autooxidation during ripening.

The acidity index of the fat values increased during ripening by a factor of about 3, reaching final average values of 3.99 mmol of KOH/100 g of fat. These final values are quite lower than those determined by other authors in different varieties of cow cheeses (Marcos et al., 1985; Vanbelle, Vervack & Foulon, 1978) and only similar to those obtained by Prieto et al. (1994) in León cow cheese. The low acidity index of the fat values indicates that Quesucos de Liébana cheese undergoes a very scarce lipolysis during ripening.

The native lipase of the milk and the microbial lipases are responsible for the degradation of the lipids in cheese. The pasteurization treatment of the milk could

Table 5
Changes in acidity index of the fat and T.B.A. number during the ripening of Quesucos de Liébana cheese[a]

	Cheese after salting	Ripened cheese
Acidity index[b]	1.39 ± 0.31	3.99 ± 2.82
T.B.A. number[c]	0.35 ± 0.16	0.48 ± 0.26

[a] Means of 10 batches ± standard deviations.
[b] Expressed as mmol of KOH/100 g of fat.
[c] Expressed as mg of malonaldehyde/kg of cheese.

partially inactivate the native milk lipase. Moreover, the pH and NaCl values in Quesucos de Liébana cheese are far from the range of optimum values of the action of this enzyme (Driesen, 1989). Vlaemynck (1992), working in Gouda-type cheese, showed that the native milk lipase activity is reduced by between 80 and 85% at pH values of 5.3 and NaCl concentrations of 1 M, with regard to the activity which presents in cheeses with a pH value of 7.8 and an NaCl concentration of 0.1 M. In agreement with this, under the conditions which Quesucos de Liébana cheese experiences during ripening (pH ranging from 5.21 to 5.43 and NaCl concentrations ranging from 0.37 to 1.14 M), it is to be expected that its activity should be very inhibited.

The lipolytic activity of the lactic acid bacteria is very limited and it is mainly aimed at the degradation of mono- and diglycerides previously formed by the native milk lipase (Stadhouders & Veringa, 1973). Moreover, the majority of the lipolytic enzymes of the lactic acid bacteria show their maximum activity at pH values close to neutral. According to Downey (1980), pH values of 4.75 (not far from those observed in Quesucos de Liébana cheese during ripening) act as inhibitors of the bacterial lipases.

References

Alcalá, M., Beltrán de Heredia, F. H., Esteban, M. A., & Marcos, A. (1982). Composición química y contenido energético del queso de Mahón. *Archivos de Zootecnia, 31*, 131–139.

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *Journal of Dairy Research, 50*, 45–55.

AOAC (Association of Official Analytical Chemists) (1980a). Hydrogen-Ion activity (pH). 14.022 potentiometric method. In W. Horwitz, *Official methods of analysis* (13th ed.) (p. 213). Washington: AOAC.

AOAC (Association of Official Analytical Chemists) (1980b). Acidity. 16.247 method. In W. Horwitz, *Official methods of analysis* (13th ed.) (p. 266). Washington: AOAC.

Argumosa, O. G., Carballo, J., Bernardo, A., & Martín, R. (1992). Chemical characterization of a Spanish artisanal goat cheese (Babia-Laciana variety). *Microbiologie-Aliments-Nutrition, 10*, 69–76.

Boehringer Mannheim (1995). *Methods of enzymatic bioanalysis and food analysis*. Mannheim, Germany: Boehringer Mannheim GmbH Biochemicals.

Carballo, J., Fresno, J. M., Tuero, J. R., Prieto, J. G., Bernardo, A., & Martín Sarmiento, R. (1994). Characterization and biochemical changes during the ripening of a Spanish hard goat cheese. *Food Chemistry, 49*, 77–82.

Coulon, J. B., Verdier, I., Pradel, P., & Almena, M. (1998). Effect of lactation stage on the cheesemaking properties of milk and the quality of Saint-Nectaire-type cheese. *Journal of Dairy Research, 65*, 295–305.

Cuesta, P., Fernández-García, E., González de Llano, D., Montilla, A., & Rodríguez, A. (1996). Evolution of the microbiological and biochemical characteristics of Afuega'l Pitu cheese during ripening. *Journal of Dairy Science, 79*, 1693–1698.

Desmazeaud, M. J., Gripon, J. C., Le Bars, D., & Bergère, J. L. (1976). Étude du rôle des micro-organismes et des enzymes au cours de la maturation des fromages. III. Influence des micro-organismes. *Lait, 56*, 379–396.

Downey, W. K. (1980). Risks from pre- and post-manufacture lipolysis. *Bulletin of the International Dairy Federation, Brussels, 118*, 4–18.

Driesen, F. M. (1989). Inactivation of lipases and proteinases (indigenous and bacterial). *Bulletin of the International Dairy Federation, Brussels, 238*, 71–93.

Farkye, N. Y., Fox, P. F., Fitzgerald, G. F., & Daly, C. (1990). Proteolysis and flavor development in Cheddar cheese made exclusively with single strain proteinase-positive and proteinase-negative starters. *Journal of Dairy Science, 73*, 874–880.

Fresno, J. M., Tornadijo, M. E., Carballo, J., Bernardo, A., & González-Prieto, J. (1997). Proteolytic and lipolytic changes during the ripening of a Spanish craft goat cheese (Armada variety). *Journal of the Science of Food and Agriculture, 75*, 148–154.

Gripon, J. C., Desmazeaud, M. J., Le Bars, D., & Bergère, J. L. (1975). Étude du rôle des micro-organismes et des enzymes au cours de la maturation des fromages. II. Influence de la présure commerciale. *Lait, 55*, 502–516.

IDF (International Dairy Federation) (1964). *Determination of the ash content of processed cheese products*. Brussels: IDF (IDF Standard 27).

IDF (International Dairy Federation) (1967). *Determination of the lactose content of cheese & processed cheese products*. Brussels: IDF (IDF Standard 43).

IDF (International Dairy Federation) (1982). *Cheese & processed cheese. Determination of the total solids content*. Brussels: IDF (IDF Standard 4A).

IDF (International Dairy Federation) (1986). *Cheese & processed cheese products. Determination of fat content*. Brussels: IDF (IDF Standard 5B).

IDF (International Dairy Federation) (1988). *Cheese & processed cheese products. Determination of chloride content*. Brussels: IDF (IDF Standard 88A).

IDF (International Dairy Federation) (1989). *Milkfat products and butter. Determination of fat acidity*. Brussels: IDF (IDF Standard 6B).

IDF (International Dairy Federation) (1993). *Milk. Determination of nitrogen content. Kjeldahl method*. Brussels: IDF (IDF Standard 20B).

Johnson, M. J. (1941). Isolation and properties of pure yeast polypeptidase. *Journal of Biological Chemistry, 137*, 575–586.

Lau, K. Y., Barbano, D. M., & Rasmussen, R. R. (1991). Influence of pasteurization of milk on protein breakdown in Cheddar cheese during aging. *Journal of Dairy Science, 74*, 727–740.

Law, J., Fitzgerald, G. F., Uniacke-Lowe, T., Daly, C., & Fox, P. F. (1993). The contribution of Lactococcal starter proteinases to proteolysis in Cheddar cheese. *Journal of Dairy Science, 76*, 2455–2467.

Lenoir, J. (1963). Note sur la composition en matières azotées des fromages affinés de Camembert, Saint-Paulin et Gruyère de Comté. *Annales de Technologie Agricole, 12*, 51–57.

Marcos, A., Alcalá, M., León, F., Fernández-Salguero, J., & Esteban, M. A. (1981). Water activity and chemical composition of cheese. *Journal of Dairy Science, 64*, 622–626.

Marcos, A., Fernández-Salguero, J., Esteban, M. A., León, F., Alcalá, M., & Beltrán de Heredia, F. H. (1985). *Quesos españoles. Tablas de composición, valor nutritivo y estabilidad*. Córdoba, Spain: Servicio de Publicaciones de la Universidad de Córdoba.

Marcos, A., Millán, R., Esteban, M. A., Alcalá, M., & Fernández-Salguero, J. (1983). Chemical composition and water activity of Spanish cheeses. *Journal of Dairy Science, 66*, 2488–2493.

McSweeney, P. L. H., Olson, N. F., Fox, P. F., Healy, A., & Hojrup, P. (1993). Proteolytic specificity of chymosin on bovine α_{s1}-casein. *Journal of Dairy Research, 60*, 401–412.

Millán, R., Saavedra, P., Sanjuán, E., & Castelo, M. (1996). Application of discriminant analysis to physicochemical variables for characterizing Spanish cheeses. *Food Chemistry, 55*, 189–191.

Muir, D. D., Hunter, E. A., & Watson, M. (1995). Aroma of cheese. 1. Sensory characterisation. *Milchwissenschaft, 50*, 499–503.

Mulvihill, D. M., & Fox, P. F. (1977). Proteolysis of α_{s1}-casein by chymosin: influence of pH and urea. *Journal of Dairy Research, 44*, 533–540.

Mulvihill, D. M., & Fox, P. F. (1978). Proteolysis of β-casein by chymosin: influence of pH, urea and NaCl. *Irish Journal of Food Science and Technology, 2*, 135–143.

Noomen, A. (1978). Activity of proteolytic enzymes in simulated soft cheeses (Meschanger type). 2. Activity of calf rennet. *Netherlands Milk and Dairy Journal, 32*, 49–68.

Oberg, C. J., Davis, L. H., Richardson, G. H., & Ernstrom, C. A. (1986). Manufacturing of Cheddar cheese using proteinase-negative mutants of *Streptococcus cremoris*. *Journal of Dairy Science, 69*, 2975–2981.

O'Keeffe, A. M., Fox, P. F., & Daly, C. (1978). Proteolysis in Cheddar cheese: role of coagulant and starter bacteria. *Journal of Dairy Research, 45*, 465–477.

O'Keeffe, R. B., Fox, P. F., & Daly, C. (1976). Contribution of rennet and starter proteases to porteolysis in Cheddar cheese. *Journal of Dairy Research, 43*, 97–107.

Ordoñez, J.A. (1974). *Microbiología y bioquímica del queso tipo "Ulloa" y preparación de un fermento para su elaboración a partir de leche pasterizada*. PhD thesis, Facultad de Veterinaria de León, Universidad de Oviedo, Spain.

Paleari, M. A., Soncini, G., Beretta, G., Dragoni, I., & Piantoni, L. (1993). A study on a typical mountain raw milk cheese. *Sciences des Aliments, 13*, 723–735.

Prieto, B., Fresno, J. M., Carballo, J., Bernardo, A., & Martín Sarmiento, R. (1994). Biochemical characteristics of León raw cow milk cheese, a Spanish craft variety. *Sciences des Aliments, 14*, 203–215.

Sapru, A., Barbano, D. M., Yun, J. J., Klei, L. R., Oltenacu, P. A., & Bandler, D. K. (1997). Cheddar cheese: influence of milking frequency and stage of lactation on composition and yield. *Journal of Dairy Science, 80*, 437–446.

Sieber, R., Badertscher, R., Fuchs, D., & Nick, B. (1994). Beitrag zur Kenntnis der Zusammensetzung schweizerischer konsumreifer Weich-und Halbhartkäse. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene, 85*, 366–381.

Stadhouders, J., & Veringa, H. A. (1973). Fat hydrolysis by lactic acid bacteria in cheese. *Netherlands Milk and Dairy Journal, 27*, 77–91.

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L. R. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of American Oil Chemists' Society, 37*, 44–48.

Thomas, T. D., & Crow, V. L. (1983). Mechanism of D(−)-lactic acid formation in Cheddar cheese. *New Zealand Journal of Dairy Science and Technology, 18*, 131–141.

Thomas, T. D., & Pearce, K. N. (1981). Influence of salt on lactose fermentation and proteolysis in Cheddar cheese. *New Zealand Journal of Dairy Science and Technology, 16*, 253–259.

Turner, K. W., & Thomas, T. D. (1980). Lactose fermentation in Cheddar cheese and the effect of salt. *New Zealand Journal of Dairy Science and Technology, 15*, 265–276.

Vakaleris, D. G., & Price, W. V. (1959). A rapid spectrophotometric method for measuring cheese ripening. *Journal of Dairy Science, 42*, 264–276.

Vanbelle, M., Vervack, W., & Foulon, M. (1978). Composition en acides gras supérieurs de quelques types de fromages consommés en Belgique. *Lait, 58*, 246–260.

Visser, F. M. W. (1977). Contribution of enzymes from rennet, starter bacteria and milk to proteolysis and flavour development in Gouda cheese. 3. Protein breakdown: analysis of the soluble nitrogen and amino acid nitrogen fractions. *Netherlands Milk and Dairy Journal, 31*, 210–239.

Visser, F. M. W., & de Groot-Mostert, A. E. A. (1977). Contribution of enzymes from rennet, starter bacteria and milk to proteolysis and flavour development in Gouda cheese. 4. Protein breakdown: a gel electrophoretical study. *Netherlands Milk and Dairy Journal, 31*, 247–264.

Vlaemynck, G. (1992). Study of lipolytic activity of the lipoprotein lipase in lunch cheese of the Gouda type. *Milchwissenschaft, 47*, 164–166.

Watkinson, P., Boston, G., Campanella, O., Coker, C., Johnston, K., Luckman, M., & White, N. (1997). Rheological properties and maturation of New Zealand Cheddar cheese. *Lait, 77*, 109–120.