ELSEVIER

Food Chemistry 74 (2001) 463–469

Food Chemistry

www.elsevier.com/locate/foodchem

p

Study of the biochemical changes during ripening of Ahumado de Áliva cheese: a Spanish traditional variety

I. Franco[a], B. Prieto[a], R. Urdiales[b], J.M. Fresno[b], J. Carballo[a,*]

[a]*Área de Tecnología de los Alimentos, Facultad de Ciencias de Orense, Universidad de Vigo, 32004 Orense, Spain*
[b]*Departamento de Higiene y Tecnología de los Alimentos, Universidad de León, 24071 León, Spain*

Received 14 November 2000; received in revised form 12 February 2001; accepted 12 February 2001

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 of ten batches of Ahumado de Áliva 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 cows' milk cheeses elaborated by similar technology. The low pH values are outstanding. The presence of residual lactose at the end of ripening is also relevant. Total soluble nitrogen and non-protein nitrogen increased very little during ripening. The evolution of the values of the different nitrogen fractions show that this cheese undergoes very little proteolysis and that the rennet is the main proteolytic agent. Using PAGE, it was possible to show that, throughout ripening, only 22% of α_s-casein and 9% of β-casein were degraded. The TBA value indicated that the fat of Ahumado de Áliva cheese does not undergo noticeable autooxidation during ripening. The acidity index of the fat also indicated that this cheese underwent little lipolysis during ripening.

Keywords: Ahumado de Áliva cheese; Cow cheese; Ripening; Biochemical changes; Proteolysis; Lipolysis

1. Introduction

The latest Catalogue of Spanish Cheeses (MAPA, 1990) includes 81 varieties, of which a large number are artisanal or semi-industrial cheeses, the present production volume of which is very small. Spain is not yet a large producer or consumer of cheese. Although, over the last few decades, production has increased from 132,752 tonnes in 1980 to 230,000 tonnes in 1998, the production volume is much lower than those of other countries of the European Union. The cheese consumption in Spain has also increased from 2.8 kg/ caput per annum in 1974 to approximately 8.7 kg at present, but these figures are among the lowest in the European Union. There has been a marked increase in imports, from 26,447 tonnes in 1985 to 81,511 tonnes in 1996; exports also increased during this period, but the negative balance has increased from 25,887 tonnes in 1985 to 58,577 tonnes in 1996.

From the tendency observed over the last few years, and from the comparative data for the production and consumption in Spain and in neighbouring countries, it is possible to deduce that both the production and consumption of cheese in Spain will noticeably increase in the future and this increasing situation can be exploited by Spanish artisanal cheeses if they become attractive to the consumer and capable of competing with other varieties.

In order to establish the scientific basis for the large-scale production of these cheeses while retaining the properties and peculiarities of the original products, a thorough study is needed, to allow us to define, with accuracy, both the biochemical and microbiological characteristics of these cheeses.

Ahumado de Áliva cheese is included in these artisanal cheeses. It is produced in northern Spain, in the region of Cantabria, from cows', ewes' or goats' milk or from mixtures of milk from two or three of these species; cheese produced from cows' milk is the most common. It is cylindrical in shape and small in size, varying from 0.5 to 2 kg per piece. Its rind is rough and the interior is compact and white. Its taste is mild with a slight flavour of smoke.

* Corresponding author. Tel.: +34-988-387052; fax: +34-988-387001.
E-mail address: carbatec@uvigo.es (J. Carballo).

0308-8146/01/$ - see front matter
PII: S0308-8146(01)00164-9

To date, no information on the characteristics, either chemical or microbiological, of this cheese variety is available. The aim of this article is to study, in a representative number of batches of this cheese, the changes which occur in the gross composition, the main physico-chemical parameters, and the protein and lipid fractions during ripening.

2. Materials and methods

2.1. Cheesemaking and sampling

Ten batches of cheese were manufactured by 10 different semi-industrial cheesemakers, according to the traditional method. Pasteurized whole cows' milk, after addition of a lyophilized lactic mesophilic starter culture (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*), was coagulated at 30–32°C by adding 20 ml of commercial calf rennet (1/10,000 strength) per 100 l of milk. One hour after the addition of the rennet, the coagulum was cut to the size of a pea and transferred to the moulds where it stayed for 2 days without the application of pressure (the elimination of the whey was carried out by self-oozing). The cheeses were salted by rubbing in dry salt at a level of 2–3%; after 24 h in the moulds, the cheeses were salted on the upper side and, after 48 h in the moulds, they were removed from the moulds and salted on the lower and on the lateral sides. Then, cheeses were ripened in rooms at 8–12°C and a relative humidity of 85–95% for 28 days, being turned periodically. After ripening, cheeses were smoked for 3 h on each of 2 days, using smoke produced by burning sawdust from oak, beech and poplar wood.

From each batch, one whole cheese was taken after salting (3-day-old cheese), and after the smoking (32-day-old cheese), and transported to the laboratory under refrigeration (below 4°C). At the laboratory, the rind was removed and discarded and the cheeses were grated and held in airtight containers at −40°C until they were analyzed.

2.2. Compositional and physico-chemical parameter analysis

Total solids, protein, salt, fat, ash and lactose were determined according to the FIL-IDF standard methods 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 of Boehringer Mannheim (1995). pH and titratable acidity were measured using AOAC methods (AOAC, 1980a, 1980b). Water activity was measured in a DECAGON CX-1 Water Activity System apparatus (Decagon Devices Inc., Pullman, USA). All determinations were made in duplicate.

2.3. Analysis of nitrogen fractions

The total nitrogen content (TN) was determined by the Kjeldahl method (IDF, 1993). The procedure of Vakaleris and Price (1959) was followed to prepare 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 12% trichloroacetic acid was necessary. The method of Ordóñez (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 degradation of caseins was studied using PAGE, 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 scanned the electrophoresis gels. All electrophoretic analyses were performed in duplicate. The optical density of each region was expressed as 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 IDF (1989).

3. Results and discussion

3.1. Compositional and physico-chemical parameters

Tables 1 and 2 show the average values for the main compositional and physico-chemical parameters, respectively, of the cheeses after salting (3-day-old cheeses) and of the ripened cheeses (32-day-old cheeses) from the 10 batches studied.

Total solid content increased noticeably during the ripening process, reaching final values (60.42 ± 8.47 g/100 g of cheese) very close than those reported for other similar cows' milk cheeses, such as Bola (Marcos, Fernández-Salguero, Esteban, León, Alacalá, & Beltrán de Heredia, 1985), Edam (Marcos, et al., 1985), Gouda (Marcos, Alcalá, León, Fernández-Salguero, & Esteban, 1981), Mahón (Alcalá, Beltrán de Heredia, Esteban, & Marcos, 1982), Quesucos de Liébana (Prieto, Urdiales, Franco, Fresno, & Carballo, 2000), San Simón (Marcos, Millán, Esteban, Alcalá, & Fernández-Salguero,

1983; Millán, Saavedra, Sanjuán, & Castelo, 1996), and Tetilla (Marcos et al., 1985). However, these values were lower than those reported for other cows' 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 levels of protein and fat (expressed as g/100 g of total solids; TS) were in the range of those observed for other cows' milk cheeses (Marcos et al., 1981, 1983, 1985; Muir et al., 1995; Paleari, Soncini, Beretta, Dragoni, & Piantoni 1993; Prieto et al., 1994, 2000; Sieber, Badertscher, Fuchs, & Nick, 1994).

The levels of NaCl and ash (expressed as g/100 g of TS) varied slightly between the beginning and the end of ripening. These variations are very probably due to the salting method used. Cheeses are individually salted by the addition of solid salt during the moulding operations and each sample is made up of one entire cheese. It is then possible that all the cheeses do not receive exactly the same quantity of salt, and it is also possible that the degree of penetration of the salt into the cheese is also different for each unit. The final level of NaCl was at the lower end of the range of values reported for different cows' milk cheeses (Cuesta et al., 1996; Marcos et al., 1981, 1983, 1985; Muir et al., 1995; Prieto et al., 1994, 2000; Sieber et al., 1994).

The lactose retained in the curd was hardly degraded during ripening, eight of the 10 cheeses analyzed showing residual lactose after 32 days of ripening with an average value of 1.95 g/100 g TS. This level was higher than for other ripened cows' milk cheeses in which the presence of lactose is not usually detected after 30 days of ripening. The presence of lactose in cheese is dependent on the extent of whey drainage and on the degree of degradation of lactose held in the curd. The non-application of pressure during moulding favours the retention of lactose in the curd, as a result, high levels of lactose were observed in 3-day-old cheese. The persistence of the lactose in the cheese during ripening could be due to a moderate glycolytic activity of the starter culture used.

Table 1
Changes in the chemical composition of Ahumado de Áliva cheese during ripening[a]

	Cheese after salting	Ripened cheese
Total solids (TS; %)	45.76±5.65	60.42±8.47
Protein (% TS)	35.1±3.35	36.8±1.90
Fat (% TS)	54.98±1.53	54.71±4.98
Ash (% TS)	5.29±1.17	4.49±2.13
NaCl (% TS)	1.50±0.99	2.03±0.82
Lactose (% TS)	2.49±0.52	1.95±0.99
D-lactic acid (% TS)	0.12±0.10	0.79±0.29
L-lactic acid (% TS)	2.19±0.46	1.62±1.04

[a] Means of 10 batches±standard deviations.

As a consequence of the activity of the starter culture during the early stages of the manufacture, high levels of L-lactic acid (the isomer produced by the lactococci from lactose) were observed in 3-day-old-cheese. The L-lactic acid content decreased slowly during ripening, reaching final values of 1.62±1.04 g /100 g TS in 32-day-old cheese. The low degradation of lactic acid during ripening is a common phenomenon 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 slight decrease in L-lactic acid content during the ripening of Ahumado de Áliva cheese could also be due in part to its transformation into D-lactic acid, due to the racemizing activity of some microorganisms (mainly pediococci and lactobacilli) which grow in cheese under the conditions of low pH and high L-lactate concentrations (Thomas & Crow, 1983). This racemizing activity could explain in part the increase in D-lactic acid content observed in Ahumado de Áliva cheese during ripening, although the main source of D-lactic acid is the activity of the non-starter lactic acid bacteria which utilizes the residual lactose (Turner & Thomas, 1980). This same behaviour of the L- and D-lactic acid contents was observed in other bacterial-ripened cow's milk cheeses such as Cheddar, Colby, Gouda and Cheshire (Thomas & Crow, 1983) and Quesucos de Liébana (Prieto et al., 2000) cheeses.

Due to the activity of lactic acid bacteria during the clotting, wheying and moulding operations, low pH values were observed in 3-day-old cheese. The pH continued to fall during ripening, reaching a final value (4.69±0.51) lower than those reported for other cows' milk cheeses (Coulon, Verdier, Pradel, & Almena, 1998; Marcos et al., 1981, 1983, 1985; Muir et al., 1995; Prieto et al., 2000; Watkinson et al., 1997). Only for Afuega'l Pitu (Margolles, Rodríguez, & Reyes Gavilán, 1996) and León (Prieto et al., 1994) cheeses were such low pH values reported. Normally, the pH of cheese increases during ripening as a consequence of the consumption of lactic acid on the one hand and, on the other, of the alkalizing effect of the compounds (mainly amines and ammonia) generated during protein degradation during ripening. In Ahumado de Áliva cheese, the D + L-lactic

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

	Cheese after salting	Ripened cheese
pH	5.07±0.54	4.69±0.51
Titratable acidity (g lactic acid/100 g total solids)	1.74±0.22	1.68±0.45
a_w	0.980±0.003	0.972±0.007

[a] Means of 10 batches±standard deviations.

acid content, instead of decreasing throughout ripening, increase slightly as a consequence of increase in the content of the D-isomer. Very little protein degradation occurred during the ripening of this cheese, as will be discussed in the next section. These phenomena explain the changes in pH during the ripening of Ahumado de Áliva cheese.

The water activity hardly decreased during the ripening, reaching a final value (0.972±0.007) very similar to those observed for other cows' 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), Quesucos de Liébana (Prieto et al., 2000), San Simón (Marcos et al., 1983) and Tetilla (Marcos et al., 1983). The very slight decrease in the water activity of Ahumado de Áliva cheese throughout the ripening could be explained in part by the slight dehydration undergone by the cheeses during the ripening process. The slight protein degradation in Ahumado de Áliva cheese during ripening could also influence this phenomenon because low molecular weight nitrogen components generated during proteolysis reduce the water activity of cheese.

3.2. Proteolytic parameters

Table 3 shows the average values for the different nitrogen fractions at the beginning and end of ripening.

The total soluble nitrogen increased slightly during ripening, reaching a final average value (16.6±6.56 g/100 g TN) lower than those reported by other authors in other cows' milk cheeses, such as Bola (Marcos et al., 1985), Cheddar (Fritsch, Martens, & Belitz, 1992; Marcos et al., 1981; Thakur, Kirk, & Hedrick, 1975), De Nata (Marcos et al., 1981), Edam (Marcos et al., 1981; Poznanski & Rymazewski, 1965), Gallego (Marcos et al., 1985), Mahón (Marcos et al., 1985; Ramos, Barneto, Suárez, & Íñigo, 1982), Munster (Marcos et al., 1981), Quartirolo (Paleari et al., 1993), Quesucos de Liébana (Prieto et al., 2000), San Simón (Marcos et al., 1983, 1985), Taleggio (Marcos et al., 1981) and Tetilla (Marcos et al., 1981, 1985). Of the cows' milk cheeses studied to date, only Afuega'l Pitu (González de Llano, Polo, & Ramos, 1995), León (Prieto et al., 1994) and Saint-Paulin (Lenoir, 1963; Marcos et al., 1981) show similar or even lower values.

Table 3
Changes in the nitrogen fractions of Ahumado de Áliva cheese during ripening[a]

	Cheese after salting	Ripened cheese
Total nitrogen (TN; %)	2.52±0.41	3.47±0.40
Protein nitrogen (% TN)	92.1±1.51	91.8±3.06
Non protein nitrogen (% TN)	7.90±1.51	8.22±3.06
Casein nitrogen (% TN)	87.5±2.35	83.4±6.56
Total soluble nitrogen (% TN)	12.5±2.35	16.6±6.56
Proteose-peptone nitrogen (% TN)	4.59±2.29	8.39±5.67
Oligopeptide nitrogen (% TN)	5.34±0.96	5.41±2.38
Amino nitrogen (% TN)	1.65±0.61	2.42±0.92
Ammonia nitrogen (% TN)	0.90±0.33	0.55±0.35

[a] Means of 10 batches±standard deviations.

The level of NPN also increased very little throughout ripening. The final average values of this fraction were also lower than those observed in the above-mentioned cheeses; only León (Prieto et al., 1994), Saint-Paulin (Lenoir, 1963; Marcos et al., 1981) and Tetilla (Marcos et al., 1981, 1985) cheeses showed NPN values as low as those observed for Ahumado de Áliva cheese.

In accordance with the small increase in NPN, the components of this nitrogen fraction (oligopeptides, amino and ammonia nitrogen) changed very little during ripening. The final value for amino nitrogen (2.42±0.92% of TN) was fairly low; only León (Prieto et al., 1994), Mimolette (Marcos et al., 1981), Quesucos de Liébana (Prieto et al., 2000) and Saint-Paulin (Lenoir, 1963; Marcos et al., 1981) cheeses showed values as low as those determined in Ahumado de Áliva cheese. The final ammonia nitrogen values (0.55±0.35% of TN) were also very low and were at the lower end of the range of values reported in literature for the different cows' milk cheeses. The values of the amino N/ammonia N ratio, at the end of the ripening process, were very much higher than 1 (4.4 on average), which corresponds to the cheeses in which lactic acid bacteria play the main role in ripening.

The proteose-peptone nitrogen fraction is made up of large peptides. This fraction underwent a large increase throughout ripening, showing a value in 32-day-old cheese approximately double that for the 3-day-old-cheese. The final level of large peptides is the result of the equilibrium between their production, mainly due to chymosin, and their degradation by enzymes of microbial origin to NPN (Desmazeaud et al., 1976; Gripon et al., 1975; O'Keeffe et al., 1976, 1978; Visser, 1977; Visser & de Groot-Mostert, 1977). The final high values of the proteose-peptone nitrogen and the low values of the NPN allow us to conclude that the rennet is the main agent of the proteolysis in Ahumado de Áliva cheese and that the microbial enzymes are not very important in protein degradation.

From the values of the different nitrogen fractions at the end of ripening we can conclude that Ahumado de Áliva cheese undergoes very limited proteolysis.

Fig. 1 shows the typical electrophoretogram and densitogram of the caseins and their degradation products in 3-day-old cheese and in 32-day-old cheese with the Rf values of each band detected. Table 4 shows the changes in the percentage of total optical density of the regions of the stained PAGE gels.

Very little degradation (around 9%) of the β-casein occurs during ripening, this fraction representing, at the

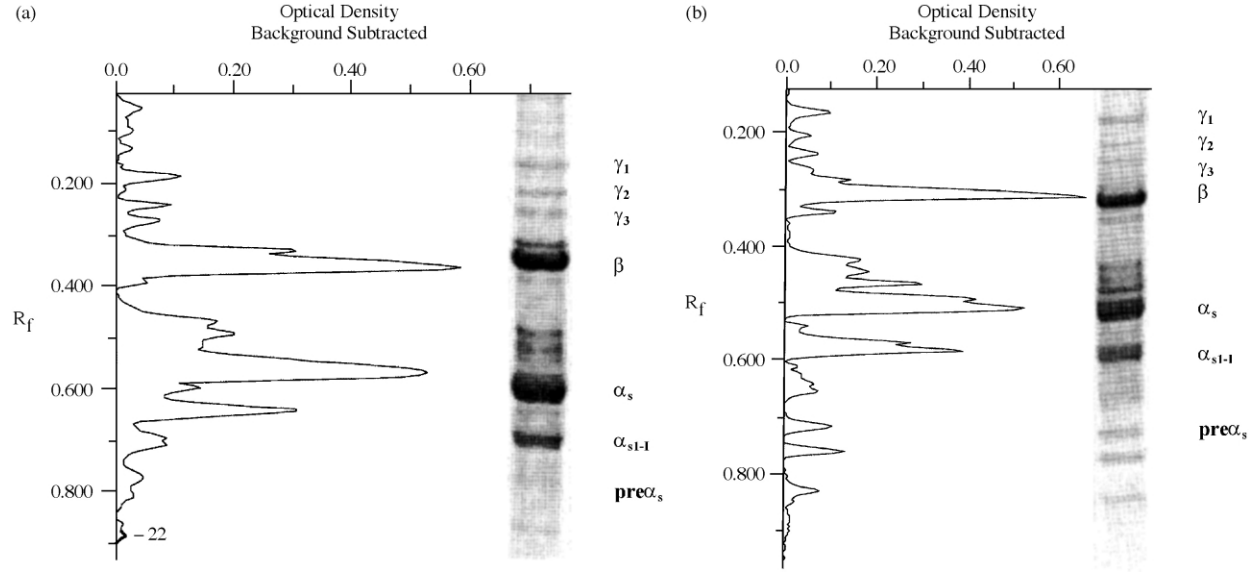

Fig. 1. Typical electrophoretogram of the casein fractions in cheese after salting (A) and in ripened cheese (B), with Rf and optical density values of each one of the bands detected.

Table 4
Changes in the percentage of total optical density of the electrophoretic regions in the stained gels of Ahumado de Áliva cheese during ripening[a]

	Cheese after salting	Ripened cheese
#[b]	3.60±0.94	2.52±1.68
γ_1	3.26±0.44	2.76±1.04
γ_2	1.60±0.42	1.11±0.69
γ_3	1.75±0.47	1.72±1.01
β	29.2±3.68	26.5±5.37
#[b]	1.83±1.63	2.47±1.53
α_s	40.1±3.02	31.2±14.79
#[b]	2.34±0.94	1.96±1.02
α_{s1-I}	12.1±1.95	15.9±8.25
Pre-α_s	4.13±0.57	14.0±11.00
α/β	1.40±0.25	1.17±0.54

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

end of the ripening, on average 26.5±5.37% of the O.D. The α_s-fraction was degraded more (22%) during ripening, its final value being 31.2±14.79% of the total O.D. The decrease in the α_s-casein fraction was accompanied by an increase in the fractions which corresponded to its degradation products; the pre-α_s-casein increased by a factor of 3.4 at the end of the ripening compared to the values for 3-day-old-cheese. These results again show the importance of the chymosin of the rennet in the protein degradation in Ahumado de Áliva cheese, since the degrading action of rennet is mainly on α_s-casein (McSweeney et al., 1993b; Mulvihill & Fox, 1977) and to a much lesser extent on β-casein which is very resistant to its action. The dominant role of the rennet in the proteolysis has also been shown in other cows' milk cheeses such as Cheddar (McSweeney, Fox, Lucey, Jordan, & Cogan, 1993a), Gouda (Visser, 1977; van den Berg, & Exterkate, 1993; Exterkate, Lagerwerf, Haverkamp, & Van Schalkwijk, 1997), and Quesucos de Liébana (Prieto et al., 2000).

The results of the quantification of the caseins and their degradation products show, again, the limited proteolysis undergone by this cheese variety during ripening. Even though the short ripening period could be partly responsible for this limited proteolysis, it would seem that the low pH value of this cheese from the beginning of ripening and which is maintained, even decreasing, during this period, is responsible for this phenomenon. Various authors (Jong & de Groot-Mostert, 1977; Mulvihill & Fox, 1977; Mulvihill & Fox, 1978; Visser & Slangen, 1977) showed, in model systems, that the casein degradation by the rennet was reduced at pH values lower than 5.8. On the other hand, the lactic acid bacteria, the main microbial group in Ahumado de Áliva cheese, have slight activity on intact caseins (Farkye, Fox, Fitzgerald, & Daly, 1990; Law, Fitzgerald, Uniacke-Lowe, Daly, & Fox, 1993; Oberg, Davis, Richardson, & Ernstrom, 1986; Visser & de Groot-Mostert, 1977) which could also contribute to the low level of proteolysis in this cheese during ripening.

3.3. Fat parameters

Table 5 shows the average values of the TBA number and the acidity index of the fat at the beginning and at the end of the ripening. The TBA number, which is an index of autooxidation of fat, remained constant during ripening.

The average acidity index of the fat values increased very little during ripening, reaching a final average value of 3.10 mmol of KOH/100 g of fat which is lower than

Table 5
Changes in acidity index of the fat and TBA number of Ahumado de Áliva cheese during ripening[a]

	Cheese after salting	Ripened cheese
Acidity index[b]	2.33±1.06	3.10±2.88
TBA number[c]	0.37±0.13	0.30±0.13

[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.

those reported by other authors for different cows' milk cheese varieties (Marcos et al., 1985; Vanbelle, Vervack, & Foulon, 1978) and similar only to those found for León (Prieto et al., 1994) and Quesucos de Liébana (Prieto et al., 2000) cheeses. The low acidity index of the fat indicates that Ahumado de Áliva cheese undergoes very little lipolysis during ripening.

The degradation of lipids in cheese during ripening is catalyzed by the indigenous lipase of the milk (LPL) and by microbial lipases. Pasteurization of the milk could partially inactivate milk lipase. On the other hand, this enzyme is optimally active at pH value of 8.0–9.0 and temperature of 35 to 40°C, and is inhibited by NaCl (Vlaemynck, 1992). The values of these parameters in Ahumado de Áliva cheese (pH 5.07 to 4.69, temperature 8–12°C, NaCl 0.68 to 1.22%) are far from the optimum requirements of LPL.

The lipolytic activity of the lactic acid bacteria (the main microbial group in Ahumado de Áliva cheese), is very limited and is mainly on mono- and diglycerides formed by the action of milk LPL (Stadhouders & Veringa, 1973). Moreover, most of the lipolytic enzymes of the lactic acid bacteria show their maximum activity at pH values close to neutral. According to Downey (1980), the combination of low pH (4.75) and high salt content (2%), conditions not far from those observed in Ahumado de Áliva cheese during ripening, is inhibitory to lipolysis related to microbial growth.

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

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

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

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). Review of the progress of Dairy Science: flavour impairment from pre- and post-manufacture lipolysis in milk and dairy products. *Journal of Dairy Research*, *47*, 237–252.

Exterkate, F. A., Lagerwerf, F., Haverkamp, J., & Van Schalkwijk, S. (1997). The selectivity of chymosin action on α_{s1}- and β-caseins in solution is modulated in cheese. *International Dairy Journal*, *7*, 47–54.

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.

Fritsch, R. J., Martens, F., & Belitz, H. D. (1992). Monitoring Cheddar cheese ripening by chemical indices of proteolysis. 1. Determination of the glutamic acid, soluble nitrogen, and liberated amino groups. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, *194*, 330–336.

González de Llano, D., Polo, M. C., & Ramos, M. (1995). Study of proteolysis in artisanal cheeses: high performance liquid chromatography of peptides. *Journal of Dairy Science*, *78*, 1018–1024.

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

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

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

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

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

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

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

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

Jong, L., & de Groot-Mostert, A. E. A. (1977). The proteolytic action of rennet on different casein substrates under various conditions. *Netherlands Milk and Dairy Journal*, *31*, 296–313.

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 azotés des fromages affinés de Camembert, Saint-Paulin et Gruyère de Comté. *Annales de Technologie Agricole*, *12*, 51–57.

MAPA (Ministerio de Agricultura, Pesca y Alimentación). (1990). *Catálogo de quesos de España*. Madrid: Secretaría General Técnica del Ministerio de Agricultura, Pesca y Alimentación.

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., 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.

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.

Margolles, A., Rodríguez, A., & Reyes Gavilán, C. G. (1996). Some chemical and bacteriological characteristics of regional cheeses from Asturias, Spain. *Journal of Food Protection*, *59*, 509–515.

McSweeney, P. L. H., Fox, P. F., Lucey, J. A., Jordan, K. N., & Cogan, T. M. (1993a). Contribution of the indigenous microflora to the maturation of Cheddar cheese. *International Dairy Journal*, *3*, 613–634.

McSweeney, P. L. H., Olson, N. F., Fox, P. F., Healy, A., & Hojrup, P. (1993b). 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.

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

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.

Ordóñez, 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.

Poznanski, S., & Rymazewski, J. (1965). Proteolysis during the ripening of Edam cheese with the participation of some bacteria strains. Part I. Changes in particular nitrogen fractions. *Milchwissenschaft*, *20*, 14–20.

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.

Prieto, B., Urdiales, R., Franco, I., Fresno, J. M., & Carballo, J. (2000). "Quesucos de Liébana" cheese from cow's milk: biochemical changes during ripening. *Food Chemistry*, *70*, 227–233.

Ramos, M., Barneto, R., Suárez, J. A., & Íñigo, B. (1982). Contribution to study of Mahón cheese. I. Microbiological and biochemical aspects. *Chemie Mikrobiologie Technologie der Lebensmittel*, *7*, 167–172.

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.

Thakur, M. K., Kirk, J. R., & Hedrick, T. I. (1975). Changes during ripening of unsalted Cheddar cheese. *Journal of Dairy Science*, *58*, 175–180.

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.

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.

Van den Berg, G., & Exterkate, F. A. (1993). Technological parameters involved in cheese ripening. *International Dairy Journal*, *3*, 485–507.

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.

Visser, S., & Slangen, K. J. (1977). On the specificity of chymosin (rennin) in its action on bovine β-casein. *Netherlands Milk and Dairy Journal*, *31*, 16–30.

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.