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Microbiology and Biochemistry of Cheeses with Appélation d'Origine Protegée and Manufactured in the Iberian Peninsula from Ovine and Caprine Milks

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ABSTRACT

To support legal protection with objective technical data and to promote enforcement of high quality standards a few European countries have created Appélation d'Origine Protegées. This paper reviews and updates fundamental and applied aspects encompassing microbiological and biochemical characteristics of traditional cheeses with Appélation d'Origine Protegée manufactured in the Iberian Peninsula from ovine, caprine, or both milks. Ovine and caprine cheeses with Appélation d'Origine Protegée from Portugal and Spain can be divided into four distinct groups based on milk source and rennet type: 1) Azeitão, Castelo Branco, Évora, Nisa, Serpa, Serra da Estrela, and La Serena cheeses are manufactured with raw ovine milk and coagulated via plant rennet; 2) Terrincho, Idiazábal, Manchego, Roncal, and Zamorano cheeses are manufactured with raw ovine milk and coagulated via animal rennet; 3) Cabra Transmontano and Majorero are manufactured with raw caprine milk and coagulated via animal rennet; and 4) Amarelo da Beira Baixa, Picante da Beira Baixa, and Rabaçal are manufactured with mixtures of raw ovine and caprine milks and coagulated via animal rennet.
(**Key words:** dairy foods, enzyme, microflora, Mediterranean)

Abbreviation key: AOP = Appélation d'Origine Protegée, **FA** = fat acidity, **LAB** = lactic acid bacteria, **TN** = total nitrogen, **WSN** = water-soluble nitrogen, **WSP** = water-soluble peptides.

INTRODUCTION

Southern European countries undoubtedly account for production of most caprine and ovine milks worldwide (26). Almost all such milk (usually in raw form) is converted into cheese. The cheesemaker often relies on adventitious lactic acid bacteria and nonstarter microflora to lower pH and bring about ripening.

Artisanal production of regional cheeses is a part of the cultural heritage of many countries in Europe (e.g., Portugal and Spain) (21). Records of said traditional cheese-making date back to the Roman occupation of the Iberian Peninsula, when cheeses were often used to pay rents, tolls, and charges because of their high market value (24). Currently, these cheeses with ancient roots are manufactured chiefly on the farm level, although an increasing fraction has been associated with small industrial dairies. Such cheeses originate from some of the poorer regions in Portugal and Spain and account for an important part of the incomes of local farmers (21). These traditional dairy products have high intrinsic value, arising from their unique organoleptic characteristics, coupled with long-recognized social and economic impacts in maintaining local employment and retaining farmer families on peripheral, otherwise desert regions (72).

Most artisanal cheeses obtained from ovine and caprine milks are seasonal, and peak production is in spring and lowest production is in fall. Increased logistic difficulties associated with artisanal production (e.g., exodus of farmers toward urban areas and suboptimal exploitation of complementary agriculture activities) have led to a drastic reduction in numbers of persons associated with some artisanal cheese varieties. However, Medina (98) and Canada (16) reported that the production of artisanal cheeses in European Community countries has increased 65% (on average) from 1984 to 1996, with an extremely high increase (223%) in Spain and a moderate increase (19%) in Portugal.

To technically support legal protection and thus promote sustained high quality standards, a few European countries have created Appélation d'Origine Protegée (**AOP**) regions (118). Those in the Iberian Peninsula are represented in Figures 1 and 2 and account for 13 traditional cheeses in Portugal and 12 in Spain (see Table 1 for details).

In Portugal from 1985 to 1988, Serra da Estrela, São Jorge, Azeitão, Serpa, Castelo Branco, Picante da Beira Baixa, and Amarelo da Beira Baixa cheeses were granted AOP status; Nisa cheese was granted that sta-

Received February 23, 1999.
Accepted August 18, 1999.
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tus in 1993; Cabra Transmontano, Évora, Rabaçal, and Terrincho cheeses in 1994; and Pico cheese in 1996. The majority of these cheeses are manufactured with raw ovine or caprine milk (except São Jorge and Pico cheeses, which are manufactured with bovine milk) without deliberate addition of any starter or nonstarter microflora. Production of ovine milk in Portugal is estimated to be between 75,000 and 95,000 tonnes/yr; that of caprine milk is estimated to range between 37,000 and 42,000 tonnes/yr (143). Both are almost exclusively associated with the inland area (66). According to the same authors, these two types of milk from small ruminants account for production of 18,000 to 20,000 tonnes of cheese/yr, which represent approximately 40% of all cheese produced in Portugal. Of this amount, only 10 to 15% is currently certified by AOP organizations (16).

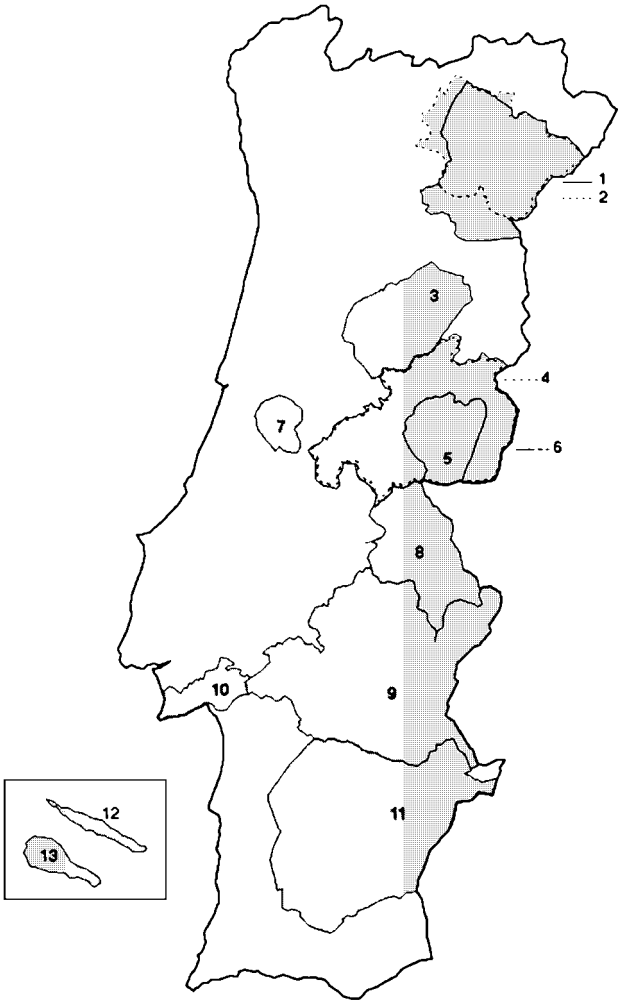

Figure 1. Appélation d'Origine Protegée regions in Portugal: 1. Terrincho cheese; 2. Cabra Transmontano cheese; 3. Serra da Estrela cheese; 4. Picante da Beira Baixa cheese; 5. Castelo Branco cheese; 6. Amarelo da Beira Baixa cheese; 7. Rabaçal cheese; 8. Nisa cheese; 9. Évora cheese; 10. Azeitão cheese; 11. Serpa cheese; 12. S. Jorge cheese; and 13. Pico cheese.

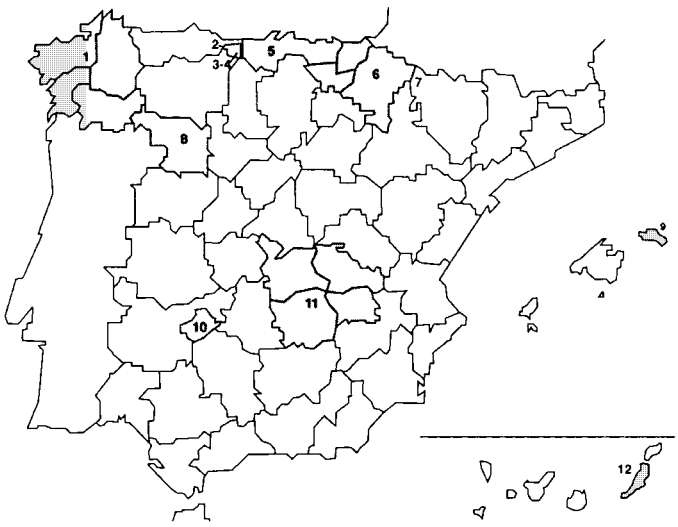

Figure 2. Appélation d'Origine Protegée regions in Spain: 1. Tetilla cheese; 2. Cabrales cheese; 3. Picón Bejes-Tresviso cheese; 4. Quesucos de Liébana cheese; 5. Cantabria cheese; 6. Idiazábal cheese; 7. Roncal cheese; 8. Zamorano cheese; 9. Mahón cheese; 10. La Serena cheese; 11. Manchego cheese; and 12. Majorero cheese.

Although Spain possesses a wider diversity of traditional cheeses (81 referenced types), most of which are well established and duly described (107), only a few of such cheeses possess an AOP status. Of these, only six are manufactured with ovine or caprine milks. From 1985 to 1991, Cabrales, Cantabria, Idiazábal, Mahón, and Roncal cheeses were granted AOP status; La Serena, Tetilla, and Zamorano cheeses were granted that status in 1993; and Picón Bejes-Tresviso, Quesucos de Liébana, Manchego, and Majorero cheeses were so considered between 1994 and 1996. Approximately 200,000 tonnes of cheese were produced in Spain in 1990. Although the numbers for the artisanal production of cheese are difficult to estimate, they are thought to be near 25,000 tonnes (58).

Ovine and caprine AOP cheeses from Portugal and Spain can be divided into four distinctive groups, based on milk source and rennet type: 1) Azeitão, Castelo Branco, Évora, Nisa, Serpa, Serra da Estrela, and La Serena cheese, which are manufactured with raw ovine milk and coagulated by plant rennet (*Cynara* spp.); 2) Terrincho, Idiazábal, Manchego, Roncal, and Zamorano cheeses, which are manufactured with raw ovine milk and coagulated by animal rennet; 3) Cabra Transmontano and Majorero cheeses, which are manufactured with raw caprine milk and coagulated by animal rennet; and 4) Amarelo da Beira Baixa, Picante da Beira Baixa, and Rabaçal cheeses, which are manufactured with

Table 1. Legal requirements of Portuguese and Spanish cheeses with Appélation d'Origine Protegée status.

Cheese variety	Milk type	Small ruminant breed	Rennet type	Cheese type	Region of origin	Overall production (tonnes/yr)	Reference
Portuguese cheeses							
Amarelo da Beira Baixa	Ovine and caprine	Charnequeira	Animal	Semi hard	Castelo Branco	40	NP[1]
Azeitão	Ovine	Saloia or Bordaleira or Friserra or Merina	Plant	Semi hard	Palmela and Sesimbra and Setúbal	29	NP
Cabra Transmontano	Caprine	Serrana	Animal	Extra hard	Bragança and Vila Real	. . .	. . .
Castelo Branco	Ovine	Merina	Plant	Semi hard	Castelo Branco	30	NP
Évora	Ovine	Merina	Plant	Semi hard or hard	Évora	4	NP
Nisa	Ovine	Merina	Plant	Semi hard or hard	Nisa and Portalegre	23	NP
Picante da Beira Baixa	Ovine and caprine	Merina or Charnequeira	Animal	Semi hard or hard	Castelo Branco	16	NP
Pico	Bovine	. . .	Animal	Soft	Pico	151	NP
Rabaçal	Ovine and caprine	. . .	Animal	Semi hard or hard	Coimbra	. . .	. . .
São Jorge	Bovine	. . .	Animal	Semi hard or hard	São Jorge	. . .	. . .
Serpa	Ovine	Merina	Plant	Soft	Serpa and Beja	19	NP
Serra da Estrela	Ovine	Bordaleira	Plant	Soft	Serra da Estrela	22	NP
Terrincho	Ovine	Churra	Animal	Semi hard	Bragança and Viseu and Guarda	. . .	. . .
Spanish cheeses							
Cabrales	Bovine and ovine and caprine	. . .	Lactic acid	Blue	Cabrales and Peflamellera Alta	200	(63)
Cantabria	Bovine	. . .	Animal	Soft	Gallicia	319	(63)
Idiazábal	Ovine	Lacha or Carranzana	Animal	Semi hard or hard	Navarra and Pais Basco	896	(63)
La Serena	Ovine	Merina	Plant	Soft or semi hard	Badajoz	150	(60)
Mahón	Bovine	. . .	Animal	Semi hard or hard	Minorca	1471	(63)
Manchego	Ovine	Manchego	Animal	Semi hard or hard	Albacete and Ciudad Real and Cuenca and Toledo	2557	(63)
Majorero	Caprine	Majorera	Animal	Semi hard or hard	Fuerteventura	. . .	. . .
Picón Bejes-Tresviso	Bovine and ovine and caprine	. . .	Animal	Semi hard	Liébana	. . .	. . .
Quesucos de Liébana	Bovine and ovine and caprine	. . .	Animal	Soft	Liébana	92	(63)
Roncal	Ovine	Lacha or Rasa	Animal	Semi hard or hard	Roncal Valley and Navarra	359	(63)
Tetilla	Bovine	. . .	Animal	Semi hard	Galicia	. . .	. . .
Zamorano	Ovine	Churra or Castilian	Animal	Semi hard or hard	Zamora	600	(43)

[1]Not published.

mixtures of raw ovine and caprine milks and coagulated by animal rennet.

This review focuses on microbiological and biochemical characteristics of AOP traditional cheeses manufactured from ovine and caprine milks in the Iberian Peninsula.

MICROBIOLOGICAL STUDIES

As shown previously, all AOP cheeses manufactured at the farm level from raw milk typically are not inoculated with starter cultures and may pose health hazards because the indigenous microflora are qualitatively and quantitatively unknown. Microbiological aspects of bovine cheeses manufactured worldwide, especially on an industrial scale and almost exclusively from pasteurized milk (e.g., Cheddar cheese), have been studied for decades. Not until recently have in-depth studies focused on artisanal cheeses manufactured in Mediterranean countries from ovine and caprine milks. Such studies have especially focused on the characterization of those microbial groups related to sanitary issues and ripening aspects [viz., enterobacteria, staphylococci, lactic acid bacteria (**LAB**) and yeasts]. Because of the wide acceptance that *Enterobacteriaceae* and coliforms are indicator microorganisms for the microbiological and sanitary quality of foods, large populations of these microbial groups in ripened cheese may represent serious handicaps for extended trade of said products (100). Promotion of artisanal raw milk cheeses requires a set of standard manufacturing practices and final product characteristics, which can be achieved only after comprehensive (and integrated) characterization of chemical, microbiological, and sensorial profiles. According to Keating (66), the major problems yet to be solved in the Iberian Peninsula concerning use of raw milk in cheese-making are the strict sanitary control of dairy herds coupled with strict manufacturing practices.

Cheeses Manufactured with Ovine Milk and Coagulated by Plant Rennet

Azeitão cheese. According to Mimoso et al. (105, 106), the total mesophilic microflora in Azeitão cheese increased in the first week of ripening, attaining values in the range of 10^9 to 10^{10} cfu/g of cheese. Their concentration decreased slightly thereafter until 20 d, when typical values were about 10^9 cfu/g. The LAB were the major constituents of the microflora in this type of cheese, and *Lactobacillus* was the predominant genus. *Leuconostoc* was also an important genus, which increased in concentrations from 10^6 to 10^7 cfu/g of cheese at 0 d to approximately 10^8 cfu/g by 20 d of ripening. The species of LAB most frequently identified were *Lactococcus lactis*, *Lactobacillus casei* spp. *casei*, *Lactobacillus casei* spp. *pseudoplantarum*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Leuconostoc dextranicum*, *Leuconostoc mesenteroides*, and *Leuconostoc lactis*. The concentration of coliforms decreased throughout ripening and ranged from 10^7 to 10^8 cfu/g of cheese at 0 d to 10^6 to 10^7 cfu/g by 20 d. The concentration of yeasts and fungi increased more than 1-log cycle during the first week and then decreased slightly to 10^6 cfu/g.

Castelo Branco cheese. Very few reports can be found on the microbiological characteristics of Castelo Branco cheese. In attempts to study and characterize this cheese, Mata (96) and Marques (85) reported total viable microflora of 10^8 cfu/g of cheese for 40-d ripened cheeses. The concentration of coliforms ranged from 10^{-5} to 10^{-2} cfu/g of cheese. Both authors reported that *Staphylococcus aureus* and *Salmonella* spp. could not be detected in 0.1 or in 25 g of cheese, respectively. More recently, the incidence of *Listeria monocytogenes* (46.0%), *Listeria innocua* (36.5%), and *Listeria seeligeri* (3.2%) was assessed in several cheeses that were produced in dairy farms and small plants scattered throughout the subregion of Castelo Branco (134). Fungus and yeast viable concentrations were in the range of 10^2 to 10^3 cfu/g of cheese (96). Viable counts of surface yeasts by Batalha (12) were 1.2×10^4 and 2.1×10^7 cfu/g of cheese by 0 and 46 d, respectively. Carreira (18) and Batalha (12) have developed techniques to determine which microflora were responsible for the browning pigmentation of some Castelo Branco cheeses throughout ripening. This surface pigmentation creates problems for the producers because it is a negative factor in assessment of cheese quality by the consumers. According to both authors, the microflora that might be responsible for pigmentation are yeasts (e.g., *Yarrowia lipolytica*, which is able to produce pigments in the presence of Tyr). However, Batalha (12) claimed that inoculation of cheese samples with *Y. lipolytica* did not lead to full development of pigmentation.

Évora cheese. According to work by Matos (97), LAB were the dominant microbiological group throughout 45 d of ripening with maximal counts (10^5 to 10^6 cfu/g of cheese) at 5 to 7 d. Saraiva (147) reported concentrations of LAB of approximately 10^7 to 10^8 cfu/g for Évora cheeses by 60 d of ripening. Cardoso (17) and Reis (137) identified *Lactobacillus plantarum*, *Lb. paracasei* spp. *paracasei*, *Lb. delbrueckii* spp. *delbrueckii*, *Lb. delbrueckii* spp. *lactis*, *Lb. acidophilus*, *Lb. brevis*, *Lb. curvatus*, *Lactococcus lactis* spp. *lactis*, *Leuconostoc mesenteroides* spp. *mesenteroides*, and *Pediococcus* spp. as the LAB present in Évora cheese by 3 to 7 d of ripening. Enterococci and staphylococci were more constantly present between 3 and 45 d; their counts ranged be-

tween 10^4 and 10^5 cfu/g of cheese (97). Pereira (130) identified five major groups of lipolytic bacteria in Évora cheese by 30 to 60 d of ripening, viz., the families *Enterobacteriaceae* and *Micrococcaceae* and the genera *Staphylococcus*, *Streptococcus*, and *Bacillus*. The presence of yeasts in the bulk of Évora cheese was quantified by Dias (28); samples of fresh cheese and cheese by 30, 45, and 60 d of ripening displayed concentrations ranging from 10^2 to 10^7 cfu/g of cheese with maximum values at approximately 30 d of ripening. This author claimed that the dominant yeast identified was *Debaryomyces hansenii*, although *Candida zeylanoides* and *Rhodotorulla* spp. could also be identified.

Potes and Marinho (135) monitored changes in the microbial profile of Évora cheese throughout the ripening period (between 3 and 60 d) and during the lactation season (from mid-January until mid-May) for total viable bacteria, LAB, coliforms, enterobacteria, enterococci, and yeasts. The results, in general, showed clear differences between winter and spring, in agreement with Cardoso (17). Counts of LAB were higher by the end of the ripening period for cheeses produced in winter; the highest counts were recorded at 3 to 7 d of ripening for those produced in spring. The counts of enterobacteria and coliforms decreased toward the end of the ripening period, and the lowest values were obtained for cheeses manufactured in spring. These data show an apparent relationship between microbial viable numbers and degree of environmental contamination (135).

Serpa cheese. According to Roseiro and Barbosa (143), the microbial flora of Serpa cheese is qualitatively identical to that of the milk from which it is produced; lactobacilli predominate, and coliforms and streptococci, which are common in fresh cheese (10^7 cfu/g of cheese), decrease during ripening. However, other authors (8) have claimed that mesophilic LAB (10^8 cfu/g of cheese) and enterococci (10^7 cfu/g of cheese) are predominant groups, and *Leuconostoc* and *Lactococcus* are the most abundant genera. Proteolytic bacteria are found in the fresh cheese and their concentration increased slightly during ripening (143) to 10^4 to 10^5 cfu/g (19). Total viable bacteria ranged from 10^8 to 10^9 cfu/g of cheese for ripened cheeses and coliforms and enterococci have been detected at levels of 10^{-3} to 10^{-5} and 10^{-6} to 10^{-8} cfu/g, respectively. *Staphylococcus aureus* was not detected in 1 g of cheese. Fungi and yeasts existed in the range 10^3 to 10^5 cfu/g of cheese. More recently, Amaral (1) studied several microbiological characteristics of Serpa cheese manufactured from three different ovine breeds (Merina, Serra da Estrela, and Lacaune) and ripened for 40 to 55 d. No significant differences were found between breeds in terms of total viable microflora, which ranged from 10^8 to 10^9 cfu/g of cheese. In terms of *Staphylococcus aureus*, 34% of the cheeses analyzed contained concentrations above 2×10^3 cfu/g of cheese; for coliforms and *Escherichia coli*, values were from 2.5×10^6 to 2.5×10^7 and from 1.4×10^3 to 9.0×10^4 cfu/g of cheese, respectively. Microbial enumeration during ripening of standard Serpa cheese, and of cheese made from refrigerated milk, was performed in two distinct seasons (April to May and June to July) and in three cheese axial positions (smear, surface, and bulk) (31). The main microbial groups isolated were LAB (10^3 to 10^9 cfu/g of cheese), coliforms (10^1 to 10^8 cfu/g of cheese), and yeasts (10^2 to 10^8 cfu/g of cheese), without significant differences between the two cheese types and the two seasons. The incidence of yeasts on the surface was about 100-fold that in the cheese interior; 51 yeast strains isolated from April to May were identified, and *Debaryomyces hansenii* was the most frequent species, followed by *Yarrowia lipolytica*, both on the cheese surface and in the interior.

Serra da Estrela cheese. Several microbiological studies have been performed on Serra da Estrela cheese (the most traditional Portuguese cheese), which encompass viable counts and identification of microflora, namely LAB (2, 23, 60, 80, 146). More recently, the microflora of Serra da Estrela cheese were monitored, in two sequential years during a 35-d ripening period throughout the cheese-making season (with sampling between November and May) (69, 79) and throughout the AOP region (156), with cheeses produced according to traditional protocols in several different dairy farms. The concentration of LAB enumerated on Rogosa and on M17 agars increased during the first week of ripening by 4- and 3-log cycles, respectively, with respect to their initial numbers in the curd (approximately 10^2 and 10^4 cfu/g of cheese). Thereafter, viable LAB tended to increase at a much slower rate from 7 to 21 d of ripening and eventually stabilized by 35 d at 10^7 cfu/g of cheese (71). In these groups, *Lc. lactis*, *Enterococcus faecium*, and *Leuc. mesenteroides* were the dominant species in curd, with frequencies of 43, 27, and 18%, respectively, of all LAB identified positively; *Leuc. lactis*, *Lb. paracasei* spp. *paracasei* and *Lb. plantarum* were also found at lower frequencies, approximately 9, 2, and 1%, respectively. Throughout the whole ripening period, *Leuc. lactis* was the most abundant (up to 72%) and resistant LAB found in Serra da Estrela cheese; *Enter. faecium* and *Lc. lactis* spp. *lactis* exhibited the highest decrease in percentage frequency, followed by *Leuc. mesenteroides*. The initial numbers of coliforms in the curd (approximately 10^6 cfu/g of cheese) increased 2-log cycles during the first week of ripening and decreased thereafter at a very slow rate, so the numbers of coliforms in 35-d-old cheese could still be considered as high (approximately 10^7 cfu/g of cheese). The most

abundant and proliferating coliform found in curd and 35-d-old cheese was *Hafnia alvei*, which accounted for 63 and 84%, respectively, of all coliform isolates. Significant concentrations of *Escherichia coli* and *Citrobacter freundii* were detected in 35-d-old cheese (approximately 9 and 7%, respectively, of all *Enterobacteriaceae* isolates); however, these bacteria could not be detected in all samples, probably a result of very low initial contamination of the milk with members of this microbial group. The maximum numbers of viable staphylococci were obtained by 7 d of ripening (approximately 10^3 cfu/g of cheese); by 35 d the cheeses contained a concentration of staphylococci similar to that in the curd (approximately 10^2 cfu/g of cheese). The predominant *Staphylococcus* spp. found in curd were *Staphylococcus xylosus*, *Staph. aureus*, and *Staph. epidermidis* (with percentages of 40, 30, and 21%, respectively, of all staphylococcal isolates). *Staphylococcus simulans* and *Staph. hominis* were also found in several samples of the curd but at much lower levels; failure to detect these bacteria may be attributed to very low levels of these microorganisms when compared with those of other staphylococci. The staphylococcal flora by the end of ripening is mainly composed of *Staphylococcus xylosus* (83%), which has been revealed to be the most resistant and proliferating *Staphylococcus* species throughout ripening. *Staph. simulans* (14%) and *Staph. aureus* (3%) were also identified in 35-d-old cheese; *Staph. epidermidis* was not isolated from 35-d-old cheeses. *Pseudomonas fluorescens* was the only pseudomonad found in the curd and was at approximately 10^3 cfu/g of cheese; after 7 d of ripening, its concentration was negligible. The viable yeasts in the curd (approximately 10^2 cfu/g of cheese) increased up to 21 d of ripening to approximately 10^3 cfu/g of cheese and tended to stabilize thereafter. A large spectrum of yeasts was found in both curd and 35-d-old Serra da Estrela cheese, and *Sporobolomyces roseus* was dominant (at 43 and 54%, respectively, of all yeast isolates) followed by *Leucosporidium scottii-Debaryomyces hansenii* (33 and 22%, respectively), *Rhodotorula aurantiaca* (6 and 7%, respectively), and *Yarrowia lipolytica* (4 and 6%, respectively); *Kluyveromyces marxianus*, *Pichia membranaefaciens*, and *Trichosporum beigelii* were also found in both the curd and several 35-d-old cheeses.

Macedo et al. (69, 79) claimed that the microbiological profile of Serra da Estrela cheese is significantly affected by the period within the cheese-making season and the axial location within the cheese, although to a much lower extent than by ripening time. The 35-d-old cheeses manufactured in spring exhibited the lowest numbers of LAB and yeasts, whereas those manufactured in winter showed the lowest numbers of coliforms and staphylococci. This observation might be explained by the higher moisture contents and the higher LAB growth rates in cheeses ripened in winter than in spring, which lead to faster decreases of viable numbers in cheese during winter than during spring. This environment also indirectly favors growth of microorganisms less sensitive to acidic environments (e.g., LAB and yeasts), while selectively reducing growth of bacteria more sensitive to acidic environments (e.g., coliforms and staphylococci). No statistically significant differences were detected between the surface and interior of the cheese in terms of viable numbers of LAB and coliforms; however, the numbers of staphylococci and yeasts were significantly higher on the rind (by approximately 1- and 2-log cycles, respectively) than in the innermost part of the cheese.

Dominant strains within the most important genera isolated from 35-d-old traditional Serra da Estrela cheese were further screened, in pure form, for their ability to release FAA and FFA with ovine milk coagulated by thistle flower as substrate (74). Significant protease and peptidase activities were displayed by pure cultures of *Leuc. mesenteroides* spp. *mesenteroides-dextranicum*, *Lc. lactis* spp. *lactis* and *Leuc. scottii-Debaryomyces hansenii*; *Leuc. lactis* and *Enter. faecium* exhibited only peptidase activity. The *Leuc. mesenteroides* spp. *mesenteroides-dextranicum*, *Lc. lactis* spp. *lactis*, and *Enter. facecium* could break down tributyrin but only after a long period of incubation. Although short- and medium-chain fatty acid residues were released preferentially by microbial lipases, *Leuc. mesenteroides* spp. *mesenteroides-dextranicum* could also hydrolyze long-chain fatty acids. Because of hydrolytic activities, greater consistency in the quality levels of Serra da Estrela cheese (considered in both sensory and safety aspects) is anticipated for cheeses manufactured from milk inoculated with a mixed-strain mesophilic starter of *Lc. lactis* spp. *lactis* and *Leuconostoc* spp., possibly combined with *Enter. faecium* or *Debaryomyces hansenii*, or both.

La Serena cheese. According to Fernández del Pozo et al. (31), high concentrations of microorganisms (10^7 cfu/ml) are found in the raw milk used for manufacture of La Serena cheese. The microflora of La Serena cheese have been studied by Fernández del Pozo et al. (31) and Martínez-Manso and Fernández-Salguero (90) and, more recently, by Sanchez-Rey et al. (145), with a focus mainly on microbiological quality and incidence of pathogenic species throughout ripening. Most microbial groups increased by 1.5 to 2.0 log during milk coagulation and whey drainage, and a further increase of approximately 1 log for total viable counts, lactobacilli, leuconostoc, and enterococci was observed within the first 2 d of ripening. These bacteria tend to attain maxi-

mum numbers in 15-d-old cheese. *Lactococcus lactis*, *Lb. casei*, *Lb. plantarum*, *Leuc. mesenteroides*, and *Enter. faecium* predominate in the bulk of cheese. Their concentrations decrease gradually from 15 d toward the end of ripening. The LAB, yeasts, and molds predominate on the cheese surface. Viable counts of microorganisms were enumerated in 60-d-old La Serena cheese, including lactococci (10^8 cfu/g of cheese), lactobacilli (10^8 cfu/g of cheese), micrococci and staphylococci (10^6 cfu/g of cheese), coliforms (10^3 cfu/g of cheese), and yeasts and molds (10^4 cfu/g of cheese) (31, 90). In addition, coagulase-positive staphylococci had not been detected by 45 d, and fecal coliforms had not been found by 60 d in the interior of La Serena cheeses. Contrasting results were reported by Sanchez-Rey et al (145), who indicated that the microbiological quality of the samples was very poor and contained 10^4 to 10^7 cfu/g of cheese of coliforms (including fecal coliforms) by 60 d of ripening. *Salmonella arizonae* was the only species of *Salmonella* detected; *Listeria monocytogenes* could be isolated in a few samples at very low concentrations (145). High viable numbers of yeasts (10^7 to 10^8 cfu/g of cheese) and molds (10^6 to 10^7 cfu/g of cheese) on the cheese surface were found from 30 d of ripening onwards. These were mainly lactic acid-utilizing species, which suggested that they make a significant contribution to cheese ripening through utilization of lactic acid (which leads to higher pH values) and production of proteinases or lipases (which increase hydrolysis of proteins and triglycerides, respectively) (31).

Cheeses Manufactured with Ovine Milk and Coagulated by Animal Rennet

Idiazábal cheese. Few studies of the microbial flora of Idiazábal cheese have been carried out and have encompassed either the raw ovine milk used for cheesemaking or the cheese itself throughout ripening (12, 131, 132, 144).

Mesophiles, psychrotrophs, lactobacilli, lactococci, *Leuconostoc*, *Micrococcaceae*, and yeasts undergo continuous declines and eventually level off at constant values toward the end of ripening; such asymptotic value is attained by leuconostocs by 30 d of ripening (approximately 10^6 cfu/g of cheese) and by psychrotrophs (10^7 to 10^8 cfu/g of cheese), lactobacilli (approximately 10^6 cfu/g of cheese), and yeasts (10^2 cfu/g of cheese) by 60 d of ripening. The counts of *Clostridium* spp. and enterococci rose initially but tended eventually toward a constant value (approximately 10^1 and 10^6 cfu/g of cheese, respectively) (132). Rua et al. (144) reported that 53% of 150 strains of LAB isolated from milk, curd, and cheese belong to the *Lactococcus* genus, 37% to *Lactobacillus*, and the remaining 10% to *Leuconostoc*. Microbiological identification showed that the main species was *Lc. lactis* (48%), followed by *Lb. casei* (20.4%), *Lb. plantarum* (13.3%), and *Leuc. lactis* (10%). *Enterobacteriaceae* were no longer present after 60 d of ripening; total coliforms and fecal coliforms were no longer present after 90 d (132). *Salmonella* spp. (10, 132) and *Staphylococcus aureus* (10) were negative in all determinations. In a report on the fungal microflora present in cheeses throughout ripening, Arizcun et al. (3) obtained counts below 10^3 cfu/g of cheese in all samples; nine different genera of molds were identified (viz., *Penicillium*, *Cephalosporium*, *Aspergillus*, *Geotrichum*, *Pullularia*, *Mucor*, *Paecilomyces*, *Candida*, and *Acremonium*).

Manchego cheese. The microbial flora of Manchego cheese manufactured from raw milk is well documented (91, 92, 93, 111, 112, 113, 117, 120, 121, 124). The total viable microbiological counts in raw milk Manchego cheese throughout ripening are characterized by high increases in the first days, attaining maximum values by 7 d of ripening (1 to 5×10^9 cfu/g of cheese), up to values of approximately 10^8 cfu/g of cheese by 90 d of ripening (102). Lactococci, mainly *Lc. lactis* spp. *lactis* (91), predominate during the first month of ripening; thereafter, they are outnumbered by mesophilic homofermentative lactobacilli, mainly *Lb. plantarum* and *Lb. casei* (111). Enterococci (mainly *Enter. durans*), leuconostocs (mainly *Leuc. mesenteroids* spp. *dextranicum* and *Leuc. paramesenteroides*) and pediococci (mainly *Pediococcus pentosaceus*) are also found in significant numbers (91, 112, 113). Data from Ordónez et al. (120) indicated *Enter. faecalis*, *Lb. casei*, and *Lc. lactis* as the most abundant species of all LAB isolated.

Considerable differences are observed in the concentrations of coliforms by 90 d of ripening with viable counts ranging from 10^1 to 10^6 cfu/g of cheese (117). In fresh curd, micrococci and coagulase-positive staphylococci are commonly found at levels of 10^5 to 10^6 cfu/g and 10^4 to 10^5 cfu/g; both groups decrease gradually as ripening time elapses (92). *Micrococcus lactis*, *M. saprophyticus*, and *M. roseus* were the major *Micrococcus* species identified by Ortiz de Apodaca and Burgos (124). Psychrotrophic counts in cheese increased from 24 to 96 h in milk stored at 4°C (119).

Serrano et al. (151) claimed that the yeasts most frequently isolated in both artisanal and industrial Manchego cheese belong to the *Debaryomyces* and *Candida* genera, followed by *Yarrowia*, *Pichia*, *Saccharomyces*, and *Torulaspora*.

The microbiology of Manchego cheese made from pasteurized milk has also been investigated (48, 114, 142). Lactococci from the starter predominate, but numbers of lactobacilli (*Lb. casei* and *Lb. plantarum*) may reach 10^8 cfu/g of cheese by 30 d following manufacture, which

are concentrations similar to those of raw milk cheese. Low concentrations of leuconostocs, micrococci, and yeasts are usually found (142). Data obtained by García et al. (48) from pasteurized milk Manchego cheese, collected at random from retail shops, exhibited a wide range of mean log microbiological counts per gram of cheese for coliforms (10^2), enterococci (10^3), LAB (10^7), mesophiles (10^8), psychrotrophs (10^3), yeasts and molds (10^4), and staphylococci (10^4).

According to Medina et al. (102), microbiologically acceptable upper limits are 1×10^3 cfu/g of cheese for *E. coli*, 1×10^2 cfu/g of cheese for *Staph. aureus* and absence of *Salmonella*, *Shigella* spp., and *List. monocytogenes* in 25 g of cheese. Several works were also performed with a focus on the survival of several pathogenic microorganisms throughout manufacture and ripening of Manchego cheese and included *Staph. aureus* (49, 115, 125), *Salmonella* (101), *Enterobacteriaceae* (50, 116), and *List. monocytogenes* (29, 140); the more relevant conclusions of these studies were reviewed by Medina et al. (102).

Roncal cheese. A study of the microbial flora of Roncal cheese was carried out by Ordoñez et al. (123): LAB increase strongly during the first days of ripening, eventually stabilize, and comprise mainly *Strept. lactis*, *Lb. casei*, *Lb. plantarum*, *Leuc. dextranicum*, and *Leuc. lactis*. According to the same authors, micrococci and staphylococci decrease by 10 to 30 d of ripening, and three species of micrococci have been identified (viz., *Micrococcus saprophyticus*, *M. lactis* and *M. roseus*). Yeasts are characterized by a stable concentration throughout ripening up to 3 mo (approximately 10^3 cfu/g of cheese). Arizcun et al. (3) reported that counts below 10^3 cfu/g of cheese for fungi undergo the highest decrease by 30 d of ripening, although their presence is still detected by 150 d. Nine different genera of molds were identified: *Penicillium*, *Cephalosporium*, *Aspergillus*, *Geotrichum*, *Pullularia*, *Mucor*, *Paecilomyces*, *Candida*, and *Acremonium*.

Cheeses Manufactured with Caprine Milk and Coagulated by Animal Rennet

Majorero cheese. According to Gómez and Casla (52), lactococci, leuconostoc, and enterococci predominate in Majorero cheese manufactured artisanally during the first month of ripening, and lactococci predominate in its industrial counterpart. Fontecha et al. (36) reported lactic streptococci (*Lc. lactis* spp. *lactis* and *Lc. lactis* spp. *cremoris*) as the major contributors to the microbial flora during ripening of artisanal cheese. Lactobacilli species present were mainly *Lb. plantarum* and *Lb. casei*, although *Lb. brevis* and *Lb. fermentum* were also detected in smaller amounts. High concentrations of *Leuconostoc* were recorded (10^5 cfu/g of cheese) and suggested that they might play an important role in ripening. Species identified included *Leuc. paramesenteroides*, *Leuc. mesenteroides* spp. *dextranicum*, and *Leuc. mesenteroides* spp. *mesenteroides*. Growth of enterococci followed the same pattern as growth of the lactic streptococci and attained very high values (approximately 10^8 cfu/g of cheese) early in ripening, but growth fell off sharply at later stages. The species found for this group of microorganisms were *Enter. faecalis* spp. *liquefaciens* and *E. faecalis* spp. *faecalis*. Several strains of *Lc. lactis* spp. *lactis*, *Lb. casei*, and *Lb. plantarum* isolated from traditional Majorero cheese have been studied by Requena et al. (138); *Lc. lactis* spp. *lactis* displayed proteolytic activity in skim milk greater than that of *Lb. casei*, but such activity in *Lb. plantarum* was very low.

The development of microflora was reported by Gomes et al. (54) for semi-hard Majorero cheese industrially produced from pasteurized caprine milk inoculated with a starter composed of *Streptococcus lactis* and *S. cremoris*. These authors observed that the total counts increased initially, primarily as a result of the growth of mesophilic lactococci and, subsequently, both these counts stabilized or even decreased. The concentration of the *Lactobacillus* genus increased, and by the end of ripening (90 d), they were the predominant microorganisms with dominant species being *Lb. casei* spp. *casei*, *Lb. casei* spp. *rhamnosus*, *Lb. casei* spp. *plantarum*, and *Lb. cellobiosus*. Counts of enterococci never exceeded 10^3 cfu/g of cheese, and this family vanished by the end of the second month of ripening; leuconostocs were not found in any cheese.

Although coliforms exist in high concentration until salting, they drop sharply and virtually disappear by the third month of ripening in artisanal cheeses (36) and by the second month of ripening in industrial cheeses (54). Micrococci, staphylococci, yeasts, and molds have also been found in artisanal and industrial cheeses; in particular, *Staph. aureus* is found in artisanal cheeses until the third month of ripening (36, 54).

Cheeses Manufactured with Mixtures of Ovine and Caprine Milks and Coagulated by Animal Rennet

Amarelo da Beira Baixa cheese. Studies pertaining to the microbiological characteristics of Amarelo da Beira Baixa cheese are virtually nonexistent. Martins (95) reported that in 50-d ripened cheeses the presence of coliforms is positive at 10^{-3} to 10^0 g of cheese, whereas *Staph. aureus* is not detected in 10 g of cheese, enterococci are detected in the range 10^{-3} to 10^0 g of cheese, and yeasts are found at approximately 10^4 cfu/g of cheese.

Picante da Beira Baixa cheese. The evolutions of LAB, enterobacteria, staphylococci, and yeasts in Picante da Beira Baixa cheese were studied throughout a 6-mo ripening period (44, 47). The LAB were the dominant constituents of the microbial population during the entire ripening period and were also the microorganisms most resistant to the increasingly adverse environmental conditions prevailing in cheese (e.g., low water activity, high salt content, and low pH). They decreased by 2-log cycles during the whole ripening period, although the death rate was significantly higher after 140 d of ripening (they decreased by 1-log cycle between 140 and 180 d) than before that time. The high fraction of enterococci ascribes a possible role to these microorganisms in terms of the ripening process; the microbial population of cheeses ripened for 6 mo was in fact limited to enterococci, of which *Enter. faecium* was the most abundant (with a frequency of occurrence of 57% of all LAB isolates) followed by *Enter. durans* (29%) and *Enter. faecalis* (14%). The dominant LAB in 9-d-old cheeses were *Leuc. mesenteroides* (19%) and *Lc. lactis* (19%), followed by *Lb. plantarum* (15%), and *Lb. paracasei* (15%) and at lower concentrations by *Enter. faecalis* (8%), *Enter. faecium* (4%), *Lb. curvatus* (4%), and *Leuc. lactis* (4%). However, *Lc. lactis* and *Leuconostoc* spp. were not detected by 40 d of ripening; this observation was somewhat expected because these bacteria are rather salt-sensitive. *Lactobacillus plantarum* and *Lb. paracasei* survive as ripening progresses until 140 d; *Lb. brevis* and *Lb. fermentum* can also be detected in those cheeses by this time.

The concentration of enterobacteria did actually decrease significantly after 9 d of ripening. These bacteria showed the highest death rate of all microorganisms investigated, and they virtually had vanished by 83 d of ripening. The most commonly identified species of *Enterobacteriaceae* in fresh cheese was *Serratia liquefaciens* (frequency of occurrence was 55% of all *Enterobacteriaceae* isolates) followed by *Enterobacter cloacae* (18%), *Serratia rubiadae* (18%), and *Citrobacter freundii* (9%). *Escherichia coli* and *Hafnia alvei* were not detected in the curd; however, *E. coli* (67%) and *Serratia liquefaciens* (33%) were the only species detected in 83-d-old cheese. *Hafnia alvei* was the dominant *Enterobacteriaceae* in 25- and 40-d-old cheeses (50 and 89%, respectively) but could not be detected in cheeses before 9 d and after 55 d; *E. coli* was the only bacterium detectable in almost all cheeses by 83 d of ripening.

During the first week of ripening, staphylococci increased by 1-log cycle with respect to their initial numbers in curd (approximately 10^6 cfu/g of cheese); thereafter, these bacteria tended to decrease (almost by 1-log cycle) up to 140 d and to decrease at a much higher rate (by 2-log cycles) between 140 and 180 d. As a consequence, the 6-mo-old ripened cheeses still showed high concentrations of staphylococci (approximately 10^5 cfu/g of cheese). Only approximately 40% of the isolates from Baird-Parker medium could be safely considered as staphylococci, and the most abundant species present throughout ripening were *Staphylococcus hominis* and *Staphylococcus xylosus*, followed by *Staph. aureus* (which accounted for only 6% of the isolates); *Staphylococcus saprophyticus* was only detected in fresh cheeses. The relatively high numbers of coagulase-positive staphylococci in ripened cheeses potentiates health hazards, and hence, extensive improvement in hygiene during cheese-making is in order.

High concentrations of yeasts were found in curd (approximately 10^6 cfu/g of cheese), which tended to increase by 1-log cycle during the first month; thereafter, the numbers of yeasts stabilized at 83 d of ripening and then decreased rapidly until disappearance by 110 d. Most yeasts detected in Picante da Beira Baixa cheese are nonfermenting and are able to utilize lactic acid. *Debaryomyces hansenii* was the most abundant and frequently identified species throughout the remainder of the ripening period; *Debaryomyces polymorphus* and *Rhodotorula* spp. tend to disappear as ripening time elapses, and they could not be detected after 40 d. *Cryptococcus laurentii* and *Y. lipolytica* could be identified only at some sampling times. The predominance of *Y. lipolytica* (50%) by 110 d of ripening could be responsible for some sensorial characteristics of this cheese; *Deb. hansenii* (38%) and *Crypt. laurentii* (12%) could also be detected by this time.

Four species of bacteria (*Enter. faecium*, *Enter. faecalis*, *Lb. plantarum*, and *Lb. paracasei*) and three species of yeasts (*Debar. hansenii*, *Y. lipolytica*, and *Crypt. laurentii*) isolated from Picante da Beira Baixa cheese were assayed by Freitas et al. (45, 46) for such biochemical events as glycolysis, proteolysis, and lipolysis. The milk type (caprine or ovine), the ripening time (0 to 65 d), and the concentration of NaCl [0 to 14% (wt/vol)] have been studied in terms of their effects on in vitro curdled milk. Production of lactic acid was correlated with lactose degradation and was highest for *Lb. paracasei* followed by *Enter. faecium*. Citrate metabolism was clearly apparent for *Enter. faecalis* and, to a lesser extent, for *Enter. faecium*, *Lb. plantarum*, and *Lb. paracasei*. Evidence of proteolytic and peptidolytic activities was provided for *Y. lipolytica* and at much lower levels for the other strains. Milk type, ripening time, and content of NaCl appeared to be statistically significant processing factors for proteolysis. Clear lipolytic activity was detected for *Y. lipolytica*, but release of FFA to lesser extents was observed for the other strains under study. Ripening time was statistically significant for lipolysis, but milk type was not. Lipolytic activities were

strongly affected by NaCl content, and the extent of fat hydrolysis was much more affected by the increase of NaCl from 0 to 7% than from 7 to 14%. In view of the experimental evidence, a mixed-strain starter is of potential interest for Picante da Beira Baixa cheese provided that it includes *Lb. plantarum*, *Enter. faecium* (or *Enter. faecalis*), and *Debar. hansenii* (or *Y. lipolytica*) (45).

Rabaçal cheese. Information on Rabaçal cheese with regard to its microbiology can be obtained from Delgado (25), Rodrigues (139), and Martins (94). Total viable mesophilic microorganisms increase in the first 16 d of ripening and attain values of 10^8 to 10^9 cfu/g of cheese; this tendency is reversed afterward, attaining values of 10^6 cfu/g of cheese by 32 d of ripening. A similar result was observed for total coliforms, which presented the highest values between 8 and 16 d of ripening; high numbers of *E. coli* (10^4 to 10^5 cfu/g of cheese) were found by Pereira et al. (129) in this period of time.

PHYSICOCHEMICAL AND BIOCHEMICAL STUDIES

Cheese ripening is a set of relatively slow biochemical processes that involve the concerted action of several viable microorganisms and cell-free enzymes. The primary biochemical phenomena of ripening are glycolysis, proteolysis, and lipolysis, although the relative importance of each depends on the cheese variety in question (38).

One of the first events in the manufacture of most, if not all, cheese varieties is fermentation of lactose to lactic acid (and other end metabolites) by selected LAB or, in traditional cheese-making made from raw milk, by the indigenous microflora (37). Acidification (and consequent lowering of pH) affects almost all aspects of cheese manufacture and, hence, final composition of cheese and its associated quality.

Proteolysis plays a crucial role in development of typical cheese flavor and texture. Initial proteolysis of caseins during ripening is caused chiefly by residual rennet and produces large- and medium-sized peptides that can subsequently be degraded. Degradation is via exocellular or endocellular enzymes from lysed microorganisms that eventually yield small peptides and FAA (161). For development of acceptable flavors, a balanced hydrolysis of curd protein (i.e., casein) into small peptides and free amino acids is necessary (161). These products can contribute directly to flavor (4, 5, 20) or, alternatively, serve as precursors for synthesis of small organic compounds associated with strong and sharp flavor intensities.

Lipolysis is the third biochemical phenomenon of great importance in ripening. Analysis of the profile of short- and medium-chain FFA is often used for chemical characterization of the extent of cheese ripening (30, 68, 162) because such FAA make significant contributions to the flavor of different types of cheese. Furthermore, FFA act also as precursors for chemical formation of such other important aroma components as esters, aldehydes, and ketones (67, 149).

Cheeses Manufactured with Ovine Milk and Coagulated by Plant Rennet

Azeitão cheese. The first known physicochemical studies about Azeitão cheese date back to the beginning of the century (127), and no further studies were published until Burguete (14), Soares Franco (155), and Mendes and Almeida (103) revisited that subject. Starting in the 1980s, several studies (153, 157, 158, 159) attempted to develop a more systematic and scientific approach to the physicochemical and biochemical profile of Azeitão cheese. Table 2 tabulates the major physicochemical and biochemical characteristics of this variety of cheese.

According to Vasconcelos et al. (159) and Vasconcelos (158), pH decreases from approximately 6.6 in the curd to 5.4 to 5.8 by 7 to 8 d of ripening; afterward, the pH increases and attains values of approximately 6.0 to 6.2 by 18 to 21 d. The concentration of total solids increases linearly during ripening until 18 to 21 d, when a mean moisture loss of approximately 17% is observed.

Proteolysis in Azeitão cheese is characterized by a gradual degradation of soluble nitrogen fractions; variations from 15 to 20% to 30 to 35% of pH 4.4-soluble nitrogen and from 0 to 4% to 8 to 12% of nitrogen soluble in 12% TCA were observed between 0 and 18 to 21 d of ripening (158, 159).

Castelo Branco cheese. Scientific and technological knowledge of the physicochemical and biochemical characteristics of Castelo Branco cheese is restricted to the studies by Mata (96) and Marques (85), which report data that are summarized in Table 2.

Évora cheese. Data produced in a consistent fashion regarding the physicochemical and biochemical characteristics of Évora cheese were reported by Mendes and Almeida (103), Silva (153), and Câmara Municipal de Évora (15). Pinheiro et al. (133) have compiled all such data, and the corresponding average and standard error for each analytical parameter considered are displayed in Table 2.

In studies conducted by Silva (154) and Pinheiro et al. (133), the variation of a few physicochemical and biochemical parameters throughout the ripening period and throughout the lactation season (extending from January to June) were discussed. Total solids content of Évora cheese increased significantly with both time

Table 2. Physicochemical and biochemical characteristics throughout ripening of Portuguese and Spanish cheeses with Appélation d'Origine Protegée status, manufactured with raw ovine milk and coagulated with plant rennet.

	Azeitão	Castelo Branco	Évora		Nisa	Serpa		Serra da Estrela				La Serena		
						40–55 d		0 d		35 d				
Parameters	18–21 d	40 d	$\bar{X}$	SE	ca. 45 d	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	2 d	60 d	60 d
pH	5.86	5.10	6.29		5.2	5.67	0.17	6.45	0.06	5.24	0.14	5.29	5.34	5.17
Water activity	. . .	. . .	. . .		. . .	. . .		. . .		. . .		. . .	. . .	. . .
Moisture (% wt/wt)	46.95	39.35	26.97	8.92	40.6	44.99	3.1	55.61	2.71	49.65	1.83	52.90	42.63	40.71
NaCl (% wt/wt of TS)	4.04	6.17	4.93	1.09	4.2	5.04	0.89	2.32	0.34	4.68	0.50	2.45	3.54	3.93
Ash (% wt/wt of TS)	7.32	. . .	. . .		. . .	. . .		6.06	0.04	7.90	0.40	. . .	8.7	. . .
Fat (% wt/wt of TS)	46.92	54.00	46.38	10.82	53.5	49.66	3.06	43.66	1.61	49.07	2.45	. . .	43.0	53.30
Total protein (% wt/wt of TS)	41.13	36.54	32.95	4.09	41.8	50.03	3.93	38.53	0.82	37.51	2.48	. . .	46.2	41.50
Soluble N (% wt/wt of TN)	33.71	35.73	29.90	8.53	42.4	25.9	4.35	9.53	2.20	36.41	4.49	25.57	38.80	36.66
NPN N (% wt/wt of TN)	10.41	. . .	. . .		. . .	. . .		1.75	0.28	5.77	0.38	4.72	14.55	12.70
PTA N (% wt/wt of TN)	. . .	. . .	. . .		. . .	. . .		0.93	0.04	1.24	0.08	2.58	10.59	. . .
Reference	(158)	(87)	(133)		(26)	(1, 18)		(80)				(36, 60, 88)		

% wt/wt of TS—as mass percentage of total solids.

% wt/wt of TN—as mass percentage of total nitrogen.

variables [e.g., 45-d-old cheeses manufactured in June contained higher levels (82.85 ± 0.73% wt/wt) than did similar cheeses manufactured in January (63.50 ± 0.39% wt/wt)]. The variation of pH was also significant, and from 0 to 45 d of ripening it ranged from 6.30 ± 0.00 to 5.75 ± 0.08 for cheeses manufactured in January. Those cheeses manufactured in June exhibited pH variations from 6.77 ± 0.06 to 5.29 ± 0.05 within the same ripening period. The minimum values were, in general, observed between 10 and 15 d of ripening, which, according to Pinheiro et al. (133), correlates well with counts of LAB and enterococci as reported by Fonseca (34). Both time variables considered were statistically significant ($P < 0.001$) for both proteolysis indices measured, viz., water-soluble nitrogen (**WSN**), as a percentage of total nitrogen (**TN**) (% wt/wt of TN), and NPN (% wt/wt of TN). Forty-five-day-old cheeses manufactured in June contained higher concentrations (38.55 ± 0.85% wt/wt of TN and 13.97 ± 0.05% wt/wt of TN for WSN and NPN, respectively) than did similar cheeses manufactured in January (24.35 ± 0.36% wt/wt of TN and 12.92 ± 0.69% wt/wt of TN for WSN and NPN, respectively). This result is apparently related to the total solids content and textural consistency of the cheese paste.

Nisa cheese. The only available data on the physicochemical and biochemical characteristics of Nisa cheese were produced by Condessa (22), and the most relevant characteristics thereof are depicted in Table 2.

Serpa cheese. Work concerning the physicochemical and biochemical characteristics of Serpa cheese was developed by a variety of researchers (1, 13, 19, 24, 27, 160). The most relevant data, which encompass mainly ripened cheeses, are compiled in Table 2. Concerns about the effect of the ovine breed on the quality of Serpa cheese have been raised (1, 13) because, despite the tradition of manufacturing Serpa cheese with milk from the local Merino ovine breed, most milk producers have gradually been replacing it by dairy breeds improved elsewhere (e.g., Serra da Estrela and Lacaune). According to Bettencourt et al. (13), statistical differences of the physicochemical properties of milk exist regarding breed and period of lactation. The average total protein is 7.2 ± 0.1, 6.5 ± 0.1, and 5.8 ± 0.1 g/100 g of milk for Merino, Serra da Estrela, and Lacaune, respectively ($P < 0.01$); the variation of this parameter between December and March is 5.8 ± 0.2 and 7.3 ± 0.2 g/100 g of milk ($P < 0.01$). Fat content is affected only by period of lactation. Acidity, as well as chloride and solids contents, are all statistically affected by both breed and lactation period ($P < 0.05$). Finally, cheese quality is, in general, not influenced by the ovine breed used but is significantly affected by the period of lactation ($P < 0.05$), so the hypothesis by Bettencourt et al. (13) and Amaral (1) could not be proven.

Di (27) studied the effects of ripening, milk state (refrigerated versus nonrefrigerated), lactation period, and cheese location on several physicochemical parameters. The main effects in terms of pH, acidity, and moisture content were consubstantiated in ripening time and cheese location; milk type and lactation period did not cause significant differences. Values of pH in the bulk and on the rind of Serpa cheese were 6.42 ± 0.12 and 6.43 ± 0.10, respectively, at 0 d and 5.03 ± 0.15 and 7.10 ± 0.34, respectively, by 28 d. Acidity values were 0.29 ± 0.11 and 0.21 ± 0.09 (percentage lactic acid) and 1.57 ± 0.18 and 0.37 ± 0.15 (percentage lactic acid), respectively. Moisture content values were 55.25 ± 2.87 and 51.00 ± 2.45 (percentage wt/wt) and 51.00 ± 3.56 and 37.00 ± 3.83 (percentage wt/wt), respectively.

Serra da Estrela cheese. The physicochemical and biochemical characteristics of Serra da Estrela cheese have been studied extensively by many authors (2, 6, 7, 61, 103, 146, 148, 153); the most important data generated thereby were duly compiled and discussed by Macedo et al. (80). Macedo et al. (69, 70, 77) and Macedo and Malcata (73, 74, 75, 76, 77, 78) have also extensively researched this cheese. Table 2 includes means and standard deviations of selected physicochemical and biochemical characteristics of Serra da Estrela cheese manufactured from November to May.

Changes in the contents of lactose, lactic acid, and acetic acid in Serra da Estrela cheese were monitored over a 35-d ripening period at several times within the cheese-making season (77). Lactose content in cheese decreased consistently from 6.17 to 0.21% (wt/wt of TS) as ripening time elapsed. Lactic acid content increased from 0.01 to 2.10% (wt/wt of TS), and acetic acid content increased from 0 to 0.24% (wt/wt of TS) during the same period. Lactose content and pH were statistically correlated with lactic acid content but not with acetic acid content.

The degrees of proteolysis and lipolysis in Serra da Estrela cheese were measured in cheeses manufactured from experiments laid out as a three-way factorial design replicated twice (70). Independent variables studied were location within the cheese, time within the lactation season (from October to June), and ripening time (from 0 to 35 d). All three variables had significant effects ($P < 0.05$) on the concentration of water-soluble peptides (**WSP**), but only time within the lactation season affected fat acidity (**FA**) in a significant fashion. The extents (after the given ripening period) and the rates (averaged over the ripening period) of generation of WSP and FA were highest for spring and lowest for autumn. The lowest values for both extent of proteolysis and rate of proteolysis were obtained for the rind. The rates of proteolysis and lipolysis tended to decrease with ripening time; most lipolysis took place during the first week, but proteolysis was still in progress by 35 d after manufacture.

According to Macedo and Malcata (76), hydrolysis of the major caseins in Serra da Estrela cheese is characterized by α_s-CN and β-CN being degraded up to 82 and 76%, respectively, by 35 d of ripening. The α_s-CN displayed two variants (α_{s2}-CN and α_{s3}-CN) with similar degradation patterns, and β-CN also appeared as two variants (β_1-CN and β_2-CN). The time during the cheese-making season significantly affected hydrolysis of only β_2-CN and α_{s3}-CN. Degradation of α_{s3}-CN was slower in February than in November or May for 21-d-old cheese; cheeses ripened for 7 or 21 d showed more intact β_2-CN when manufactured in May than in November or February. Proteolysis in 35-d-old cheese was quantitatively high, with average values of 34.6 and 11.9% (wt/wt of TN) for WSN and 2% (wt/vol) TCA-soluble N, respectively, but qualitatively low; average values were 5.8 and 1.2% (wt/wt of TN) for 12% (wt/vol) TCA-soluble N and 5% (wt/vol) phosphotungstic acid-soluble N (PTA-soluble N), respectively. Parameters WSN and 2% (wt/vol) TCA-soluble N were lowest for cheeses ripened in February; the ratio 12% (wt/vol) TCA-soluble N was highest for cheeses ripened in November (75).

The concentrations of FFA were monitored throughout ripening and throughout the cheese-making season (73), and both these time variables were statistically significant ($P < 0.05$). The major FFA released throughout ripening were butyric (short chain, saturated), capric (medium chain, saturated), palmitic and stearic (long chain, saturated), and oleic (long chain, unsaturated). Saturated and unsaturated long-chain FFA were present at the highest concentrations at all stages of ripening. According to Macedo and Malcata (73), lipolysis in Serra da Estrela cheese proceeds slowly to a final overall FFA concentration of 21.7 g/kg of fat by 35 d.

La Serena cheese. Physicochemical and biochemical characteristics of ripened La Serena cheese have been made available by Fernández-Salguero et al. (33), Marsilla de Pascual (86), and Fernández del Pozo et al. (31, 32). According to Fernández del Pozo (31), pH decreases from 6.65 in milk to 6.38 in molded curd as a consequence of acid production by adventitious LAB. The pH variation from 2-d-old cheese to 60-d-old cheese is depicted in Table 2. A high moisture content (65% wt/wt) is found in the curd because the cheese is not cooked; moisture decreases to 43% (wt/wt) by the end of ripening.

Considerable proteolysis takes place during ripening of La Serena cheese. Degradation of α_s-CN is faster than that of β-CN; by 15 d of ripening, 61% of α_s-CN and 29% of β-CN have already been hydrolyzed (32). As a consequence of the activity of the plant rennet upon β-CN, two (or three) bands with electrophoretic mobility higher than that of α_s-CN can be detected in fresh cheese, and they increase considerably in density during ripening (152). Several bands are also detected in the γ-CN region and result from degradation of β-CN by plasmin, plant rennet, or both. Differences in the extent of hydrolysis of α_s-CN and β-CN persist until the end of ripening. The levels of different fractions of soluble N in La Serena cheese are depicted in Table 2.

According to Fernández del Pozo (32), the level of FFA increases gradually during ripening to reach 127 mmol/kg of fat by 60 d.

More recently, further information has been obtained for a few physicochemical components and milk clotting

Table 3. Physicochemical and biochemical characteristics throughout ripening of Portuguese and Spanish cheeses with Appélation d'Origine Protegée status, manufactured with raw ovine milk and coagulated with animal rennet.

	Idiazábal			Manchego		Roncal		Terrincho	Zamorano
Parameters	1 d	60 d	180 d	Artisanal	Industrial	2 d	180 d	30 d	100 d
pH	4.94	4.78	5.04	5.66	5.45	5.69	5.67	. . .	5.1 to 0.8
Water activity	0.957	0.926	0.898	. . .	0.97	. . .	. . .	. . .	. . .
Moisture (% wt/wt of TS)	42.55	35.12	29.80	37.0	36.0	43.27	37.73	55–65[1]	<45
NaCl (% wt/wt of TS)	1.90	2.85	3.16	. . .	3.36	4.11	4.25	. . .	. . .
Ash (% wt/wt of TS)	7.32	7.98	8.53	. . .	. . .	. . .	. . .	. . .	. . .
Fat (% wt/wt of TS)	55.60	58.97	59.83	55.6	54.69	46.24	46.87	45–60	>45
Total protein (% wt/wt of TS)	36.11	35.41	34.77	. . .	36.91	45.17	44.47	. . .	>25[2]
Soluble N (% wt/wt of TN)	3.83	13.96	16.84	23.55	21.05	5.55	21.52	. . .	. . .
NPN N (% wt/wt of TN)	0.81	8.34	11.88	16.46	14.63	3.62	13.5	. . .	. . .
PTA N (% wt/wt of TN)	. . .	. . .	. . .	9.89	6.74	. . .	. . .	. . .	. . .
Reference		(66)		(39, 52, 55, 150)		(106, 123)		(5)	(3)

[1]Moisture in nonfat basis.

[2]Cheese basis.

% wt/wt of TS—as mass percentage of total solids.

% wt/wt of TN—as mass percentage of total nitrogen.

parameters for La Serena certified cheeses ripened for 60 d (56). According to these authors, a notable variability in the composition of milk exists, which likely accounts for the observed heterogeneity of cheeses. Higher homogeneity in terms of cheese characteristics obviously requires improvement of milk quality and further standardization of cheese-making technology.

Cheeses Manufactured with Ovine Milk and Coagulated by Animal Rennet

Idiazábal cheese. Several workers have focused on the physicochemical and biochemical properties of Idiazábal cheese (9, 62, 63, 141). Table 3 depicts the typical composition of this type of cheese during ripening (62). Some of these values differ from those reported by Rodríguez et al. (141), especially in terms of pH and dry matter content by 75 d of ripening. The results pertaining to the various nitrogen fractions during ripening indicate a low level of proteolysis (62), possibly because the relatively low pH values do not promote enzyme activity (32). The SDS-PAGE revealed the presence of seven bands, tentatively identified as α_{s1}-CN, α_{s2}-CN, β-CN, α_{s1}-I-CN, γ_1-CN, β-I-CN, and para-κ-CN. In relative units (optical density/mm^2 per mg of casein), these bands were characterized by 2.48, 12.31, 0.74, 0.97, 0.39, and 2.03 for cheeses by 1 d of ripening and 0.42, 8.34, 1.27, 1.27, 1.67, and 1.89 for cheeses by 180 d of ripening, respectively (62). The band corresponding to α_{s1}-CN disappeared more rapidly than that corresponding to β-CN. Percentages of these bands present at 180 d of ripening were 16.9 and 67.8%, respectively. Concentrations of FAA were monitored over a ripening period of 1 yr by Barcina et al. (9); total FAA varied between 140 by 1 d and 15 mg/g of dry matter by the end of this period. The major FAA were Glu, Leu, Val, Phe, Lys, and Ala, which accounted for approximately 50% of the total FAA.

According to Nájera et al. (110), the FFA content of Idiazábal cheese undergoes considerable increase during the first stage of ripening (from approximately 0.9 to 2.5 g/kg of cheese between 1 and 90 d) and eventually levels off toward the end of the ripening period. In cheeses brined for 24 h, the total FFA evolution was similar to that of butyric acid, which increased at a faster rate before 90 d, followed by caproic, capric, and caprylic acids. According to the same authors, lipolysis during ripening leads to production mostly of short- and medium-chain FFA (e.g., butyric acid).

Manchego cheese. Physicochemical characteristics of Manchego cheese have been reported extensively (35, 48, 50, 93, 117, 150), and these works were reviewed by Pardo et al. (126). Table 3 reports data typical of ripened Manchego cheese manufactured with raw and pasteurized milk, which is characterized by a pH of 4.8 to 5.8, a minimum of 55% (wt/wt) total solids, a minimum of 50% (wt/wt) fat in dry basis, a minimum of 30% (wt/wt) of total protein in dry basis, and a maximum of 2.3% (wt/wt) NaCl.

Proteolysis in Manchego cheese has been assessed via casein hydrolysis and several soluble nitrogen fractions (81, 82, 83, 109, 136), as well as in terms of free amino acid profile (83, 84, 122). Table 3 depicts data on the different nitrogen fractions obtained for 4-mo-old Manchego cheese manufactured with raw and pasteurized milk and ripened at 12°C (51).

According to Medina (101), extensive proteolysis of α_s-CN and β-CN takes place during the first 2 h following manufacture. Residual α_s-CN by 24 h in raw and pasteurized milk cheese is 45.1 and 43.2% (of total milk

casein) and the corresponding values for β-CN are 29.9 and 27.2% (51). Pasteurization significantly enhances degradation of α_s-CN and β-CN within the first 24 h, probably because of retention of higher amounts of rennet in pasteurized milk cheese (99). Gaya et al. (51) reported that greater degradation of α_s-CN than β-CN goes on as ripening time elapses, and by 4 mo at 12°C residual α_s-CN in raw and pasteurized milk cheeses is 23.9 and 18.4%, respectively, whereas β-CN counterparts are 30.5 and 20.0%.

Ordoñez and Burgos (1980) observed that all FAA increased in concentration during ripening, although Arg was maximum by 6 mo and decreased afterward and Glu was maximum by 4 mo and remained unchanged thereafter. The predominant FAA in the last stages were Lys, Leu, Val, Phe, and Ile. In general, these data agree with those of Marcos and Mora (84), except that Arg was found as one of the predominant FAA and that aromatic amino acids (viz., Tyr, Trp, and Phe) were negligible. These authors concluded that the liberation of FAA was faster in Manchego cheese manufactured with pasteurized milk than with raw milk.

Lipolysis occurs only to a limited extent in Manchego cheese (136). Gaya et al. (51) found lower levels of FFA in pasteurized milk cheese than in raw milk cheese, probably because of inactivation of the native milk lipase by pasteurization. Concentrations of FFA by 24 h after manufacture were 128.6 and 58.9 mmol/kg of fat in raw and pasteurized milk cheeses, respectively, and these values increased slightly during ripening at 12°C to attain 140.0 and 88.5 mmol/kg of fat, respectively, by 4 mo. Martínez-Castro et al. (89) analyzed volatile components in Manchego cheese. Four homologous series of compounds were found: FFA (from butyric to myristic), methyl ketones (from 2-pentanone to 2-undecanone, at approximately 30 μg/g of cheese), ethyl esters (within the range 5 to 18 μg/g of cheese), and methyl esters (at approximately 2 μg/g of cheese) of fatty acids with an even number of carbon atoms.

Roncal cheese. Ordoñez et al. (123) reported physicochemical and biochemical properties of Roncal cheese, most of which are depicted in Table 3. Further data regarding proteolysis and lipolysis have been made available by Millan et al. (104), Gómez et al. (53), and La Fuente et al. (68).

Figures by Millan et al. (104), in terms of nitrogen fractions of ripened Roncal cheese, are similar to those by Ordoñez et al. (121); values of soluble nitrogen vary between 22 and 32% wt/wt of TN, whereas NPN was present as 15.8% wt/wt of TN. According to Millan et al. (104), this type of cheese exhibits an electrophoretic fingerprint with α_s-CN displaying a lower proportion (consubstantiated in 20% of optical density) when compared with β-CN (approximately 35% of optical density).

According to La Fuente et al. (68), Roncal cheese can be classified as exhibiting a moderate level of lipolysis (8.2 g/kg of cheese), which is caused by lipolytic enzymes released by some bacteria of the starter as they lyse during ripening; the FFA profiles indicated higher contents of short-chain fatty acids.

Cheeses Manufactured with Caprine Milk and Coagulated by Animal Rennet

Majorero cheese. Physicochemical and biochemical characteristics of traditional Majorero cheese have been reported by Fontecha et al. (36); those of its industrial counterpart were addressed by Barreto (11), Fernández Salguero et al. (148), Martín-Hernández and Juarez (87), and Martin-Hernández et al. (88). Gómez et al. (55) also reported results on the enzymatic activity in this cheese. Table 4 summarizes the main characteristics of both types of Majorero cheese, ripened for 2 and 60 d.

Moderate proteolysis occurs in Majorero cheese, with maximum NPN and WSN values in artisanal cheese obtained by 15 d, which accounted for 20.6 and 63.2% wt/wt of TN, respectively. These values remain essentially constant up to 90 d (36). These values are comparable to those found in industrial Majorero cheeses ripened for 2 mo (88). The high initial values of WSN have been attributed to the high initial moisture content of this cheese and the type of rennet used (52). In addition, the higher fraction of WSN in 3-mo-old industrial Majorero cheese reflects a higher level of protease activity (55). Conversely, aminopeptidase activity in the soluble extracts of Majorero cheese remains at low levels throughout ripening (55).

Degradation of α_s-CN and β-CN took place throughout ripening and was more intense in artisanal than industrial Majorero cheese by 60 d. Hydrolysis of α_s-CN was 73 and 54%, respectively; β-CN was degraded only up to 24 and 19%, respectively (36, 87).

There is considerable lipolysis in artisanal Majorero cheese as a consequence of using crude rennet pastes (52). The FFA levels are 32 g/kg of cheese by 3 mo of ripening, of which 28% are short to medium-chain fatty acids (C_4 to C_{12}). These levels of FFA indicate extensive lipolysis, which is well perceived in sensory terms as sharpness of aroma and flavor (40). In contrast, the FFA levels of industrial Majorero cheese increase only slightly during ripening, from 5 to 6 g/kg of cheese between 2 and 90 d, with no significant differences between the average fatty acid residue compositions of the triglycerides in milk, curd, and cheese (89).

Table 4. Physicochemical and biochemical characteristics throughout ripening of Portuguese and Spanish cheeses with Appélation d'Origine Protegée status, manufactured with raw caprine milk or mixtures of ovine and caprine milks and coagulated with animal rennet.

		Majorero								
	Cabra Transmontano	Artisanal		Industrial		Amarelo da Beira Baixa	Picante da Beira Baixa		Rabaçal	
Parameters	60 d	2 d	60 d	2 d	60 d	50 d	0 d	180 d	2 d	28 d
pH	. . .	5.73	5.51	5.80	5.42	. . .	5.58	6.05	6.00	4.80
Water activity	. . .	0.965	0.820	. . .	. . .	. . .	1.000	0.780	0.980	0.925
Moisture (% wt/wt of TS)	25–30[1]	55.05	17.77	45.00	39.77	47.54	57.74	40.28	62.25	35.78
NaCl (% wt/wt of TS)	. . .	1.31	3.44	0.54	4.48	6.23	8.85	19.79	. . .	. . .
Ash (% wt/wt of TS)	. . .	5.07	4.61	3.97	7.56	. . .	11.71	21.18	8.82	5.47
Fat (% wt/wt of TS)	35–50	52.29	54.12	52.73	53.54	53.94	47.92	50.65	53.35	52.48
Total protein (% wt/wt of TS)	. . .	40.80	37.52	38.00	35.50	35.63	30.50	30.50	. . .	39.90
Soluble N (% wt/wt of TN)	. . .	36.68	32.09	ca. 17	32.13	. . .	13.40	27.20	. . .	7.99
NPN N (% wt/wt of TN)	. . .	17.07	19.09	ca. 4	16.57	. . .	4.37	23.4	. . .	. . .
PTA N (% wt/wt of TN)	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
References	(6)	(40)		(90)		(97)	(44, 51)		(29, 30)	

[1]Moisture in nonfat basis.

% wt/wt of TS—as mass percentage of total solids.

% wt/wt of TN—as mass percentage of total nitrogen.

Cheeses Manufactured with Mixtures of Ovine and Caprine Milks and Coagulated by Animal Rennet

Amarelo da Beira Baixa cheese. The only study that provides useful information on physicochemical and biochemical characteristics of Amarelo da Beira Baixa cheese was that by Martins (85), the essence of which is depicted in Table 4.

Picante da Beira Baixa cheese. Until recently, only scant data existed on physicochemical and biochemical aspects of Picante da Beira Baixa cheese. Changes in physicochemical and biochemical properties throughout ripening, as well as the effect of using different proportions of ovine and caprine milks, were ascertained by Freitas et al. (40, 42, 47) and Freitas and Malcata (43). Data relating to Picante da Beira Baixa cheese manufactured with 50% ovine milk and 50% caprine milk are tabulated in Table 4.

Freitas et al. (40) claimed that both nitrogen fractions (WSN and NPN) undergo significant increases during ripening; levels of WSN reach 25 and 29% (wt/wt of TN) by 180 d of ripening in plain ovine and plain caprine milk cheeses, respectively; these values suggest a moderate ripening extension index in Picante da Beira Baixa cheese. Similar patterns were followed by NPN with respect to WSN evolution (i.e., a slight increase in the beginning and a more intense increase by the end of ripening). The maximum NPN values (viz., 87 and 92% of WSN in plain ovine and plain caprine milk cheeses, respectively) were attained by 180 d of ripening. Based on these data, one could conclude that this cheese is characterized by a high ripening depth index (40).

High molecular weight peptides (with electrophoretic mobilities similar to that of γ-CN) were detected in all types of cheese. γ-Caseins, which are mainly formed upon hydrolysis of β-CN by indigenous plasmin (57), increased in concentration during ripening, and up to four bands could be detected in their typical electrophoretic region. Higher concentrations were observed in caprine milk (40). The extent of hydrolysis of α_2-CN and β-CN, expressed as relative percentage of peak areas at 0 d, varied with the composition of the batch of milk, thus providing evidence for a higher resistance of β-CN than α_s-CN to enzymatic hydrolysis. The extents of degradation of β-CN by 180 d were 18.8 and 36.5% for ovine and caprine milk cheeses, respectively. The extents of degradation of α_s-CN were 35.9 and 68.7% for plain ovine and plain caprine milk cheeses, respectively (40).

The evolution of concentration of FAA in Picante da Beira Baixa cheese throughout ripening was ascertained for several volumetric ratios of ovine to caprine milks (42). The concentrations of all FAA, except Asn, γ-aminobutyric acid, and Cys increased as ripening time elapsed. The ANOVA indicated that ripening time and, to a lesser extent, milk composition played significant roles on the overall concentration of FAA. The major FAA present throughout the ripening period were Val, Leu, and Phe, which together account for 50, 49, 57, 46, and 42% of the total pool of FAA at 0 d and 42, 42, 43, 39, and 36% by 180 d for cheeses manufactured with 0, 25, 50, 75, and 100% caprine milk, respectively. Significant differences were detected in terms of FAA profile when the proportions of ovine and caprine milks were changed (e.g., Val changed from 251.79 ± 0.99 to

352.20 ± 16.49 mg/100 g of dry matter in 140-d-ripened cheese when ovine milk was replaced by caprine milk).

Concentrations of all FFA undergo continuous increases throughout ripening; levels of total FFA by 180 d of ripening range from 50.3 to 62.3 g/kg of fat, depending on milk composition. The C_{10}, C_{16}, C_{18}, and $C_{18:1}$ FFA attain highest concentrations throughout the whole ripening period (43). Values for the short-chain FFA (i.e., C_4, C_6, and C_8) range from 2.8 to 4.5 g/kg of fat; such concentrations, which are essentially the same irrespective of the proportions of ovine and caprine milks, contribute considerably to the characteristic piquant flavor of Picante da Beira Baixa cheese.

Rabaçal cheese. Relatively scarce physicochemical and biochemical information can be assessed in the case of Rabaçal cheese; most of it was produced recently by Delgado (25, 26), Rodrigues (139), and Martins (94) and was later revised by Pereira and Alves (128); data on cheeses ripened for 2 and 28 d are depicted in Table 4.

Proteolytic reactions in Rabaçal cheese occur to a moderate extent, as variation of WSN is slight (approximately 2.2% wt/wt of TN) from 7 to 28 d of ripening (25); identical values have been reported by Martins (96) for cheeses ripened for 32 d.

ACKNOWLEDGMENTS

Partial financial support for the bibliographic survey was provided by project PROTOLACTIS: PROdução, por Tecnologias Optimizadas, de LACticínios TradicionaIS, administered by PAMAF (INIA, Lisboa, Portugal), and by project IMPACTO: Investigação dirigida ao Melhoramento do queijo serra Por incorporação de Abordagens Científicas e TecnOlógicas, administered by PRAXIS (FCT, Lisboa, Portugal), both coordinated by author F.X.M.

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