

# Review: Technological and organoleptic issues pertaining to cheeses with denomination of origin manufactured in the Iberian Peninsula from ovine and caprine milks

## Revisión: Aspectos tecnológicos y sensoriales de quesos con denominación de origen elaborados en la Península Ibérica con leche de oveja y de cabra

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A few European countries have created *Appellations d'Origine Protégée* (AOP) in order to support legal protection and hence promote enforcement of high-quality standards of selected food products. This paper comprehensively reviews and updates fundamental and applied aspects encompassing technological and organoleptic characteristics of AOP traditional cheeses manufactured in the Iberian Peninsula, from ovine and/or caprine milks. Those cheeses from Portugal and Spain can be divided into four distinctive groups, based on milk source and rennet type: (i) *Azeitão*, *Castelo Branco*, *Évora*, *Nisa*, *Serpa*, *Serra da Estrela* and *La Serena* cheeses, manufactured with raw ovine milk and coagulated via plant rennet; (ii) *Terrincho*, *Idiazábal*, *Manchego*, *Roncal* and *Zamorano* cheeses, elaborated with raw ovine milk and coagulated via animal rennet; (iii) *Cabra Transmontano* and *Majorero* cheeses, manufactured with raw caprine milk and coagulated via animal rennet; and (iv) *Amarelo da Beira Baixa*, *Picante da Beira Baixa* and *Rabaçal* cheeses, manufactured with mixtures of raw ovine and caprine milks and coagulated via animal rennet. Considerable differences between Portuguese and Spanish AOP cheeses exist; the former are, in general, characterized by softer consistencies and similarities to one another in terms of manufacturing protocols (usually encompassing coagulation of plain raw milk followed by slow draining of the curd), whereas the latter are, in general, larger and firmer.

**Keywords:** artisanal cheeses, dairy foods, sensory characteristics

Algunos países europeos han creado el sistema de "denominación de origen" para dar protección legal y promover y reforzar patrones de calidad de ciertos alimentos selectos. En este trabajo se revisan los fundamentos y los aspectos prácticos en torno a las características organolépticas de quesos tradicionales con denominación de origen elaborados en la Península Ibérica con leche de oveja, de cabra o de la mezcla de ambas. Estos quesos, procedentes de Portugal y España, se dividen en cuatro grupos diferentes en función del tipo de leche y de cuajo: (i) *Azeitão*, *Castelo Branco*, *Évora*, *Nisa*, *Serpa*, *Serra da Estrela* y *La Serena*, que se preparan con leche de oveja y cuajo de origen vegetal; (ii) *Terrincho*, *Idiazábal*, *Manchego*, *Roncal* y *Zamorano*, elaborados con leche de oveja y cuajo animal; (iii) *Cabra Transmontano* y *Majorero*, obtenidos a partir de leche de cabra y cuajo de origen animal; y (iv) *Amarelo da Beira Baixa*, *Picante da Beira Baixa* y *Rabaçal*, elaborados con leche de oveja y de cabra y cuajo de origen animal. Existen diferencias considerables entre los quesos portugueses y españoles con denominación de origen, los primeros tienen, en general, una consistencia más suave y poseen características muy similares en cuanto a su elaboración (que implican coagulación con leche cruda seguida de un drenaje lento del suero) mientras que los españoles son, en general, más grandes y más firmes.

**Palabras clave:** quesos artesanos, lácteos, características sensoriales

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## INTRODUCTION

Southern European countries account for production of a major fraction of caprine and ovine milks worldwide (Delgado, 1993); almost all such milk (usually in raw

form) is converted into cheeses, most of which are manufactured following traditional protocols. These traditional dairy products possess extremely high intrinsic value arising from their unique sensory characteristics. They are manufactured chiefly on the farm level, although an increasing fraction has been taken up by small industrial dairies; artisanal production of regional cheeses is indeed a part of the cultural heritage of many countries in Europe, viz. Portugal and Spain (Cogan and Rea, 1996).

Most artisanal cheeses obtained from ovine and caprine milks are seasonal, with a peak of production in spring and a valley in fall. Increasing logistic difficulties associated with artisanal production, e.g. exodus of farmers towards urban areas and suboptimal exploitation of complementary agricultural activities, have led to a drastic reduction in the fraction of active population dealing with some artisanal cheese varieties; however, Medina (1996a) and Canada (1998) reported that the production of artisanal cheeses in EU countries actually increased 65% (on average) from 1984 to 1996, with an extremely high increase (223%) in Spain and a far more moderate increase (19%) in Portugal.

A few European countries have invested in *Appellation d'Origine Protégée* regions (Nuñez *et al.*, 1989), in order to technically support legal protection and thus promote sustained high quality standards. They account for 13 traditional cheeses in Portugal and 12 in Spain. In Portugal, *Serra da Estrela*, *São Jorge*, *Azeitão*, *Serpa*, *Castelo Branco*, *Picante da Beira Baixa* and *Amarelo da Beira Baixa* cheeses were granted AOP status between 1985 and 1988, whereas *Nisa* cheese was granted that status 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 and/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 range between 75 000 and 95 000 t/year, whereas that of caprine milk is estimated to range between 37 000 and 42 000 t/yr (Roseiro and Barbosa, 1996), and both are almost exclusively associated with the inland area (Keating, 1997). According to the same authors, these two types of milk from small ruminants account for production of 18 000 to 20 000 t/year of cheese, which represents c. 40% of all cheese produced in Portugal; of this amount, only 10–15% is currently certified by AOP organisms (Canada, 1998).

Although Spain possesses a wider diversity of traditional cheeses (81 referenced types), most of which are well established and duly described (MAPA, 1990), only 12 such cheeses possess an AOP status, and only six of these are manufactured with ovine and/or caprine milks. From 1981 to 1991, *Cabrales*, *Cantabria*, *Idiazábal*, *Mahón* and *Roncal* cheeses were granted AOP status, whereas *La Serena*, *Tetilla* and *Zamorano* cheeses reached

that status in 1993, and *Picón Bejes-Tresviso*, *Quesucos de Liébana*, *Manchego* and *Majorero* cheeses between 1994 and 1996. Approximately 200 000 t of cheeses were produced in Spain in 1990; although the numbers for the artisanal production of cheese are again difficult to estimate, they are thought to lie in the neighborhood of 25 000 t/year (Herrero, 1993).

The aforementioned ovine and/or caprine AOP cheeses from Portugal and Spain can be divided into four distinctive groups, based on milk source and rennet type: (i) *Azeitão*, *Castelo Branco*, *Évora*, *Nisa*, *Serpa*, *Serra da Estrela* and *La Serena* cheeses, manufactured with raw ovine milk and coagulated by plant rennet (*Cynara* spp.); (ii) *Terrincho*, *Idiazábal*, *Manchego*, *Roncal* and *Zamorano* cheeses, which are manufactured with raw ovine milk and coagulated by animal rennet; (iii) *Cabra Transmontano* and *Majorero* cheeses, elaborated with raw caprine milk and coagulated by animal rennet; and (iv) *Amarelo da Beira Baixa*, *Picante da Beira Baixa* and *Rabaçal* cheeses, obtained with mixtures of raw ovine and caprine milks and coagulated by animal rennet.

As will be detailed below, there are considerable differences between Portuguese and Spanish AOP cheeses; the former are, in general, softer and usually encompass coagulation of plain raw milk followed by slow draining of the curd, whereas the latter are, in general, larger and firmer. This paper presents and discusses state-of-the-art knowledge pertaining to technological and organoleptic characteristics of AOP traditional cheeses manufactured from ovine and/or caprine milks in the Iberian Peninsula, in a more or less standard format.

The major operations associated with manufacture of ovine and/or caprine milk traditional cheeses (some of which are, in certain cases, absent) are milk handling, coagulation, cutting and syneresis, pressing, salting, and ripening. Each cheese will be described below in a condensed fashion, encompassing its region of origin, ovine and caprine breeds that produce the milk, manufacturing protocol, and textural and organoleptic characteristics. For methodological purposes, the subdivision by milk type and rennet type, tentatively introduced in this section, will be followed hereafter.

#### **Cheeses manufactured with ovine milk and coagulated by plant rennet**

*Cynara cardunculus* is a prickly variety of thistle with purple flowers, which is rather similar to the globe artichoke (*Cynara scolymus*). It has been used since ancient times in the traditional manufacture of several varieties of Portuguese cheeses (Barbosa *et al.*, 1981) and of Spanish ovine cheeses (Medina, 1996a). This plant grows wild and abundantly in dry, stony and uncultivated places in Mediterranean regions, as well as in the Canary and Madeira islands (Roseiro and Barbosa, 1996). *Cynara* spp. produce large heads that bloom during summer; the flowers are then picked up and dried in the

open air, in the shade, without any special treatment (Macedo *et al.*, 1993). Because no standard protocol exists for cutting and drying, the activity of the thistle flower extract is extremely variable, and depends on the variety, the stage of maturity, the portion of the flower used, the drying time and the final moisture content (Barbosa, 1983; Cordeiro *et al.*, 1991). Purification and partial characterization of the milk clotting proteases naturally present in the flowers of *C. cardunculus* (cardosins) have been performed by several authors (Campos *et al.*, 1990; Heimgartner *et al.*, 1990; Faro, 1991), and studies conducted on the specificity of said cardosins have indicated that these aspartic proteases can act both as endopeptidases (Heimgartner *et al.*, 1990; Faro, 1991) and exopeptidases (Heimgartner *et al.*, 1990). Literature pertaining to applications of thistle flower extracts in cheesemaking has been reviewed by Roseiro (1991) and a more recent body of work has been produced by Sousa and Malcata (1995, 1996, 1997a, b, c, d).

This tentative family of cheeses includes the most famous, and undoubtedly the most sought after, Portuguese cheeses. It is interesting to note that, despite the relatively similar manufacturing procedures, they acquire distinctive final characteristics; a likely rationale lies in the raw milk employed in cheesemaking, which originates from several regions and from various breeds. These cheeses are in general soft or semi-soft, a feature directly associated with the activities of enzymes that are part of the plant rennet, which are very proteolytic in terms of substrate proteins (Roseiro and Barbosa, 1996) but markedly less proteolytic in terms of medium-size peptides (Sousa and Malcata, 1997e). The preparation of plant rennet extracts differs from cheesemaker to cheesemaker, but Macedo *et al.* (1993) noted that most cheesemakers have elected one of the following three methods: (1) direct addition of dry thistle flowers to warm milk, followed by efficient mixing and filtration of the milk through a fine, clean cloth; (2) maceration of dry thistle flowers with small amounts of water and salt until a paste results, dilution of the paste in water, filtration of the extract through a fine, clean cloth, and addition of this crude extract directly to the milk; and (3) maceration of dry thistle flowers with salt and water, and recovery of this paste inside a cloth under closed ends, followed by submersion in milk, agitation and squeezing. Table 1 depicts the major manufacturing procedures of ovine milk cheeses that are coagulated via plant rennet, whereas Table 2 summarizes their organoleptic characteristics.

#### *Azeitão* cheese

The manufacture of *Azeitão* cheese is restricted to the counties of Palmela, Sesimbra and Setúbal, and its production is promoted and monitored by a regional association of sheep breeders in Serra da Arrábida. The edapho-climatic conditions prevailing in the aforemen-

tioned area provide fields with a very rich flora, which eventually affects the sensory properties of this cheese (Anonymous, 1992).

*Azeitão* cheese is a cured cheese with a well-established manufacturing protocol (Rasteiro, 1905; Burguete, 1942; Soares Franco, 1945); its organoleptic characteristics have been described by Silva (1980) and by Vieira de Sá and Barbosa (1990). Ripening of *Azeitão* cheese is carried out in two distinct stages; the first, at room temperature, takes c. 10 days and cheeses are submitted to an environment ranging from 90–95% relative humidity, which promotes surface fermentation leading to formation of a viscous smear (Vieira de Sá and Barbosa, 1990); during this initial period, the cheeses are turned upside down daily, and washed with salted or tap water. Afterwards the cheeses are subject to a drier environment (85–90% relative humidity) and kept at 10–15 °C for two to three weeks, during which they are turned upside down and washed several times (Vieira de Sá and Barbosa, 1990). This type of cheese can be manufactured in two sizes (Anonymous, 1986): (1) a small size, characterized by c. 100 g, 5–7 cm in diameter and 3–5 cm high; or (2) a regular size, characterized by c. 250 g, 8–11 cm in diameter and 3–5 cm high; in both cases, the minimum ripening period is 20 days.

The traditional technology for manufacture of this cheese is labor-intensive and allows great variations in technological parameters. Vasconcelos (1990) attempted to assess the effect of technology on cheese quality; procedures tested included use of double-wall cheese vats, horizontal and vertical knives, controlled additions of rennet and NaCl, and control of temperatures and time for each operation. All such improvements seem to have no disturbing effect that might eventually be perceived in the final cheese, but show advantages in terms of reduction of labor, increase in manufacturing capacity and improvement of hygiene throughout processing.

#### *Castelo Branco* cheese

*Castelo Branco* cheese is manufactured in central western Portugal, in a region where both ovine and caprine milks are produced, and sometimes mixed, for cheesemaking (Anonymous, 1988; Vieira de Sá and Barbosa, 1990). *Castelo Branco* cheese is, in a sense, related to *Serra da Estrela* cheese; however, owing to somewhat different weather conditions (i.e. higher average temperatures and lower average relative humidities throughout the year), it exhibits a stronger flavor and a more compact rind (Vieira de Sá and Barbosa, 1990). This cheese is an example of a soft (or semi-soft) consistency, and the major steps in its manufacture do not differ much from those initially set by Cruz (1945); however, said steps are currently performed under much more hygienic conditions, including temperature-controlled coagulation vats and mechanical presses, as well as controlled humidity and temperature ripening cham-

**Table 1.** Main technological characteristics of Portuguese and Spanish AOP cheeses manufactured with raw ovine milk and coagulated with plant rennet.**Tabla 1.** Principales características tecnológicas de los quesos portugueses y españoles con denominación de origen, elaborados con leche de oveja y cuajo de origen vegetal.

Cheese variety	Coagulation		Cutting	Pressing	Salting		Ripening			References
	Temperature (°C)	Time (min)			Milk % (w/v)	Surface	Temperature (°C)	Relative humidity (%)	Time (days)	
Portuguese <i>Azeitão</i>	30–35	variable	Crude milling of curd with a spoon	Manual	2	—	10–15	85–95	20–30	Soares Franco, 1945; Vieira de Sá and Barbosa, 1990
<i>Castelo Branco</i>	20–30	30–45	Crude milling of curd	Manual or mechanical	—	By rubbing both faces	8–14	80–90	40	Cruz, 1945; Anonymous, 1988; Vieira de Sá and Barbosa, 1990; Santos, 1996
<i>Évora</i>	30	15–120	Manual crude milling	Manual	3	By rubbing both faces	8–15	80–95	30–90	Anonymous, 1994; Pinheiro <i>et al.</i> , 1999
<i>Nisa</i>	25–28	45–60	Manual crude milling	Manual	—	By rubbing both faces	8–14	80–90	45	Anonymous, 1993
<i>Serpa</i>	28–30	60	With vertical and horizontal knives	Manual or mechanical	—	By rubbing both faces	6–11	75–100	30–40	Anonymous 1987, 1992; Di, 1998
<i>Serra da Estrela</i>	27–29	60	Stirring for 10–15 min with bare hand or knife	Manual	1–3	By rubbing both faces	6–12	85–90	40–60	Antunes and Santos, 1943; Vieira de Sá <i>et al.</i> , 1970; Saramago <i>et al.</i> , 1975; Anonymous, 1992; Macedo <i>et al.</i> , 1993
Spanish <i>La Serena</i>	25–32	50–75	Milling to size of grain of 10 to 20 mm	Manual or mechanical	—	By rubbing both faces for 16–24 h or brining for 24 h	4–15	>75	40–60	Fernández del Pozo <i>et al.</i> , 1988a; Nuñez <i>et al.</i> , 1989

**Table 2.** Main organoleptic characteristics of Portuguese and Spanish AOP cheeses manufactured with raw ovine milk and coagulated with plant rennet.

**Tabla 2.** Principales características organolépticas de los quesos portugueses y españoles con denominación de origen, elaborados con leche de oveja y cuajo de origen vegetal.

Cheese variety	External color	Shape	Size		Weight (g)	Rind	Texture	Flavor	References
			diameter (cm)	height (cm)					
Portuguese <i>Azeitão</i>	Yellow	Flat cylinder	5–11	3–5	100–250	Smooth and extremely thin	Semi-hard or soft with few or no holes	Delicate, slightly acidic and fresh	Silva, 1980; Vieira de Sá and Barbosa, 1990; Anonymous, 1992
<i>Castelo Branco</i>	Yellow	Flat cylinder	2–16	5–8	800–1300	Dense and smooth	Semi-hard or semi-soft with few small holes	Strong and slightly piquant	Anonymous, 1988
<i>Évora</i>	Yellow	Flat cylinder	6–15	1.5–4	60–300	Smooth and slightly wrinkled	Closed, semi-hard or hard with small holes	Strong, acidic and slightly piquant	Anonymous, 1994
<i>Nisa</i>	Straw to dark yellow	Flat cylinder	10–16	3–8	200–1300	Hard and smooth or slightly wrinkled	Closed, semi-hard or hard with few eyes	Intense and slightly acidic	Anonymous, 1993
<i>Serpa</i>	Yellow	Flat cylinder	10–30	3–8	200–2500	Smooth and slightly wrinkled	Closed, soft, buttery with few eyes	Strong, slightly hot and spicy	Anonymous, 1987; Amaral, 1996; Di, 1998
<i>Serra da Estrela</i>	Yellow	Flat cylinder	15–20	4–6	1000–1500	Smooth with regular surface	Soft butter-like texture with few eyes	Clean and slightly acidic	Anonymous, 1992; Macedo <i>et al.</i> , 1993
Spanish <i>La Serena</i>	Yellow	Flat cylinder	18–24	4–8	750–2000	Smooth with regular surface	Creamy to semi-hard with few small eyes	Strong and slightly bitter	Fernández del Pozo <i>et al.</i> , 1988a

bers. *Castelo Branco* cheese is obtained by slowly draining the curd after coagulation of pure raw ovine milk via plant rennet (Anonymous, 1988). Depending on the cheesemaker, coagulation can take 30–45 min at 20–30 °C (Cruz, 1945); Mata (1989) found average values of 42 min at 26.5 °C and Santos (1996) reported average values of 50 min at 29 °C.

According to a survey by Santos (1996), an average of 25.6 cheeses are manufactured per farmer and per day, and each cheese requires an average of 5 L of ovine milk to yield a ripened cheese within 40–50 days and weighing, on average, 1057 g.

The only work (Dias, 1992) that has addressed technological studies pertaining to *Castelo Branco* cheese is rather basic; however, it indicates that this cheese should be technologically improved in order to standardize its microbiological quality. The effects of the salting pattern (salting by direct addition to plain milk, by cheese brining or by cheese surface rubbing), as well as the effects of using cheese vats, horizontal and vertical knives, mechanical presses, controlled levels of addition of salt, and temperature- and moisture-controlled chambers were all duly tested. Unfortunately, the conclusions of this work lack statistical significance, even though they have shown that the aforementioned cheese may advantageously be manufactured according to more rational and hygienic protocols.

#### Évora cheese

Production of *Évora* cheese is restricted to a few counties of Alentejo, where sheep are raised under a distinct Mediterranean climate, characterized by hot and dry summers, as well as cold and wet winters, with pastures appearing spontaneously. *Évora* cheese is a ripened cheese, which is manufactured in three sizes: (1) 60–90 g, 6–8 cm in diameter and 2–4 cm high; (2) 120–200 g, 12–14 cm in diameter and 2–4 cm high; or (3) 200–300 g, 13–15 cm in diameter and 1.5–3 cm high (Anonymous, 1994). Coagulation of ovine milk is performed at *c.* 30 °C, with temperature being, in general, empirically monitored in a manual fashion by the cheesemaker; the duration of the coagulation step varies widely from 15 up to 120 min, depending on the rennet activity (Pinheiro *et al.*, 1999). The duration of the ripening period depends on the desired final consistency; for semi-hard cheeses, the ripening period should be nearly 30 days, whereas hard cheeses require a minimum of 90 days, at 8–15 °C and 80–95% relative humidity (Anonymous, 1994).

Pinheiro *et al.* (1999) performed sensory analyses with the goal of establishing a pattern for organoleptic characteristics of cheeses manufactured from two different ovine milk breeds, *Merina* and *Saloia*; cheeses with 30 and 60 days of ripening were assessed, and the main conclusions were that the former were softer, and possessed a thinner paste and a stronger flavor than the latter.

#### Nisa cheese

*Nisa* cheese is produced in the middle region of Alentejo. The cheese has a few distinctive characteristics that are attributable to the unique method of rearing sheep, with natural pastures full of oak groves and a rich Mediterranean flora. *Nisa* cheese is a ripened cheese, usually manufactured in two sizes: (1) 200–400 g, 10–12 cm in diameter and 3–5 cm high; or (2) 800–1300 g, 13–16 cm in diameter and 5–8 cm high (Anonymous, 1993). To date virtually no studies have focussed on this variety of cheese.

#### Serpa cheese

*Serpa* cheese is produced in the village and county of *Serpa*, and its artisanal production dates back to immemorial times. It is manufactured on the farmhouse scale only by farmers that own large flocks of *Merino* sheep (1 000 to 2 000 animals), the milk quality of which depends strongly on the edapho-climatic characteristics of this region in Alentejo (Anonymous, 1992). Most dairies have stayed in the same family for a long time and the traditional manufacturing processes have been passed, as empirical knowledge, from generation to generation (Di, 1998). During manufacture of this cheese, the cheesemaker receives help to fill up the mold, although some steps of cheesemaking have already been mechanized. *Serpa* cheese is somewhat similar to *Serra da Estrela* cheese, but possesses a stronger flavor, and the aged cheese is actually rather hot and spicy. Modern equipment has recently been purchased by some dairies, thus clearly improving processing hygiene (Di, 1998).

*Serpa* cheese is cylindrical in shape and is produced in four different sizes: (1) 200–250 g, 10–12 cm diameter and 3–4 cm high; (2) 800–900 g, 15–18 cm in diameter and 4–5 cm high; (3) 1000–1500 g, 18–20 cm in diameter and 4–6 cm high; or (4) 2000–2500 g, 25–30 cm in diameter and 6–8 cm high (Anonymous, 1987). The ripening period is generally divided into stages with different environmental conditions (temperature and humidity). According to Di (1998), cheeses ripened in controlled atmosphere chambers are submitted, in the first stage of ripening, to temperatures of 6–10 °C and relative humidities of 95–100%, whereas the second stage is characterized by temperature ranges of 7–11 °C and relative humidities of 75–90%. Traditional dairies create those environmental conditions in the two sequential stages by the use of appropriate wall and floor building materials, and ensuring frequent washes with tap water. The first stage of ripening aims at improving microbial growth and retarding drying of the surface, whereas the second stage is related to formation of the rind and set so as to permit development of several microbiological groups.

Amaral (1996) coordinated sensory evaluation panels for 40 and 55 day old ripened *Serpa* cheeses manu-

factured with milk obtained from three distinct ovine breeds (*Merina*, *Serra da Estrela* and *Lacaune*); ovine breed was not a statistically significant factor for sensorial quality of cheese, but time of cheese production within the lactation period and ripening period were noted to be statistically significant: extension of ripening from 40 to 55 days affected negatively the final quality of cheese.

Di (1998) performed textural analyses of *Serpa* cheeses using time of ripening, milk state (refrigerated versus non-refrigerated), lactation period and position within the cheeses as manipulated parameters. Texture quantification in the two types of cheese has indicated that: hardness in general decreased throughout ripening and was higher in cheese manufactured from refrigerated milk; adhesiveness displayed an identical evolution, but random oscillations were observed along ripening for the two types of cheese; and 28 day old cheeses manufactured with refrigerated milk presented the highest values.

#### *Serra da Estrela* cheese

*Serra da Estrela* cheese is the most famous of all traditional Portuguese cheeses and, owing to its unique organoleptic characteristics, it has retained the craft hallmark for hundreds of years, making it a crucial element of the rich cultural heritage of the Beira plateau in the center of Portugal (EEC, 1992). It is produced mostly in the neighborhood of Estrela, the highest mountains in Portugal (c. 2 000 m high), in a region characterized by long, cold, rainy and snowy winters, but hot and dry summers. Natural pastures comprise spontaneous perennial grasses, whereas cultivated ones are covered in white clover and subterranean clover, which help give the ovine milk (and cheese obtained therefrom) its unique flavor (Anonymous, 1992). The manufacturing protocols of *Serra da Estrela* cheese have been comprehensively reviewed by Macedo *et al.* (1993). The studies available pertaining to this type of cheese have indicated that the best coagulation temperature is 27–29 °C, which requires an average coagulation time of 60 min. The ripening conditions are 6–12 °C and 85–90% relative humidity, with maturation being essentially surface driven; the low temperatures prevailing during winter help in controlling microbial contamination, which occurs as a consequence of the poor sanitary conditions prevailing during cheese manufacture (Macedo *et al.*, 1993).

Several technological studies were performed with *Serra da Estrela* cheese in the past, and encompassed several easily manipulated parameters such as temperature and time of milk coagulation (Antunes and Santos, 1943; Saramago *et al.*, 1975; Barbosa, 1986), pressing (Lopes *et al.*, 1963), and temperature and humidity in the ripening room (Antunes and Santos, 1943; Vieira de Sá *et al.*, 1970; Saramago *et al.*, 1975; Barbosa, 1986); these parameters were assessed with respect to cheese yield

and final organoleptic characteristics. Macedo *et al.* (1998) reviewed the individual and interactive effects of four manufacturing parameters (amount of rennet, temperature of coagulation, pressing and salting of fresh cheese) and two maturation factors (temperature and relative humidity prevailing during ripening) on microbiological, physicochemical, biochemical, textural and sensorial characteristics of *Serra da Estrela* cheese, using a factorial design methodology. Salting and ripening relative humidity had significant effects upon the characteristics of the final cheese; addition of salt to the surface of the fresh cheese affected microbial growth negatively, as well as  $a_w$ , moisture content, lactic acid content, proteolysis, lipolysis, aroma and softness of cheese; conversely, an increase in the relative humidity during ripening affected all these characteristics positively (Macedo *et al.*, 1998). In order to optimize cheesemaking, a few experimental cheeses were then manufactured according to a second-order composite factorial design using temperature of coagulation, amount of rennet added and amount of salt added as deliberately manipulated technological variables (Macedo and Malcata, 1997); the amount of salt added to the fresh cheese affected significantly the sensorial, microbiological and biochemical characteristics of 60 days old *Serra da Estrela* cheese, whereas the amount of rennet added and the coagulation temperature affected significantly only the sensorial and microbiological attributes. None of the three technological parameters (at least at the levels tested) played a statistically significant role ( $p > 0.05$ ) on the chemical composition of cheese. According to Macedo and Malcata (1997), 0.3 g of rennet added per L of milk, coagulation at 28 °C and use of 0.05 g of salt per cm<sup>2</sup> of fresh cheese surface provided the best quality cheese in terms of sensory scores, as well as the lowest numbers of viable enterococci and coliforms.

#### *La Serena* cheese

*La Serena* cheese is manufactured from ovine raw milk in western Spain around the Badajoz area. Because of the seasonal production of ovine milk, *La Serena* cheese is only manufactured from January to May; cheese is made twice daily, after the morning and evening milkings, in farms and small dairies (Fernández del Pozo *et al.*, 1988a). According to Nuñez *et al.* (1989), manufacturing procedures are essentially similar to those of Portuguese *Serra da Estrela* cheese. The use of *Merino* sheep, which produce ovine milk possessing a high fat content (8–10% w/v), and plant rennet, with a strong proteolytic activity (Vieira de Sá and Barbosa, 1972), results in a cheese with a pronounced flavor and a creamy texture (Fernández del Pozo *et al.*, 1988a).

The technological studies performed on *La Serena* cheese encompassed the effects of manufacture and ripening temperature (González *et al.*, 1990), the influence

of rennet type (animal versus plant) (Nuñez *et al.*, 1991a), and the influence of ovine breed and dairy farm (González *et al.*, 1991) on the final cheese characteristics. The effect of ripening temperature is more notorious on cheeses produced according to the traditional technology: cheeses ripened at 15 °C received a lower sensory score than those ripened at 10 °C; and the best cheeses were obtained when the traditional technology was followed and ripening was at 10 °C. According to Nuñez *et al.* (1991a), cheeses produced with animal rennet have a higher moisture content and display a lower pH than those produced with plant rennet; proteolysis is more rapid in cheese manufactured with plant than animal rennet, although lipolysis follows an inverse relationship. Softening of cheese is considerably more pronounced in plant rennet cheese, which also exhibits significantly better flavor quality and higher flavor intensity. Medina *et al.* (1991) assessed the influence of a starter on the microbiological quality of *La Serena* cheese: more acidic conditions in the bulk of the cheese were found when a selected *Lactococcus lactis* strain was employed, which also drove a reduction of Enterobacteriaceae, coliform and *Staphylococcus* viable counts. Driven by concerns about homogeneous quality of AOP cheeses, Roa *et al.* (1997) analyzed the effects of milk composition of the *Merino* breed with respect to aptitude for coagulation with *Cynara cardunculus* rennet, as well as upon changes in physicochemical and microbiological characteristics throughout ripening using large scale manufacturing technology in order to assess their impact upon the final product. The clotting properties were monitored with a Formagraph, and the estimates obtained for the time to reach the gelification point and for the hardness of the curd by 30 min after the gelification point were  $46.3 \pm 5.2$  min and  $59.4 \pm 2.7$  mm, respectively; those figures led to an estimated normal cutting time of 79 min. Cheese yield and other physicochemical parameters were assessed throughout ripening, and were similar to those associated with traditional cheese counterparts, e.g. pH was 5.42 by 60 days, whereas González *et al.* (1991), Fernández del Pozo *et al.* (1988b) and Marsilla de Pascual (1979) reported pH values of 5.32, 5.34 and 5.42, respectively, by the same time; WSN and NPN values were 36.2 and 14.1% (w/w<sub>TN</sub>) by 60 days, which are very similar to the values reported by Fernández del Pozo *et al.* (1988b), viz. 38.8 and 14.6% (w/w<sub>TN</sub>), respectively.

Rheological studies carried out by Fernández del Pozo *et al.* (1988b) have indicated that, during the first month of ripening, the cheese texture softens owing to hydrolysis mainly of  $\alpha_s$ -casein, thus leading to development of a more consistent body as ripening time elapses. With respect to sensory assessment, flavor quality and flavor intensity were analyzed by the same author; TCA-soluble nitrogen was the only chemical characteristic, and viable numbers of Enterobacteriaceae and coliforms were the only microbial characteristics that

were significantly implicated with flavor intensity. Traditional technology, using old vats, esparto grass molds and wooden tables, combined with ripening at 10 °C, produced cheeses possessing significantly better sensorial characteristics than those manufactured commercially (González *et al.*, 1990).

#### Cheeses manufactured with ovine milk and coagulated by animal rennet

Pepsins and chymosins, referred to in general as gastric proteinases, are secreted by the stomach walls of a large number of vertebrates, including the adult cow and calf, adult goat and kid and adult sheep and lamb, as well as pig and chicken, as inactive pro-enzymes or zymogens (Guinee and Wilkinson, 1992). These are converted into the active enzyme form by acidification to pH values in the range 2–5, both *in vivo* and *in vitro*; in the latter case, the active enzymes reside in the supernatant, which is recovered after centrifugation of its saline homogenate following maceration of stomach tissues (Foltman, 1971, 1987; Green, 1977). Stomach secretions generally contain a number of different zymogens, each of which yields an acid proteinase which may differ slightly from one another in molecular weight and substrate specificity (Guinee and Wilkinson, 1992).

Calf rennet and adult bovine rennet contain both chymosin and pepsin, but at different levels. Factors responsible for this variability include source breed, physiological state and age, method of enzyme extraction and environmental conditions (including pH values) at which the milk clotting activity is measured (Garnot *et al.*, 1972; Rothe *et al.*, 1976, 1977; Collin *et al.*, 1981; Andren and Collin, 1986, 1988). Gastric proteinases from young calves, kids and lambs were traditionally used for the manufacture of most cheese varieties, with calf chymosin being the most common. Lamb and kid chymosins are still used in production of hard cheeses in some parts of the Mediterranean countries. These were traditionally obtained as rennet pastes (i.e. macerated stomachs and contents thereof), which also contain pregastric esterases; the latter enzymes are essential for development of the unique spicy taste of such Italian cheese as *Romano* (Nelson *et al.*, 1977). Rennet and pregastric esterase preparations (i.e. liquid extract of calf, lamb and/or kid oral tissues) have currently replaced most rennet paste in the manufacture of major traditional cheeses.

Tables 3 and 4 present the main procedures of manufacture, as well as a summary of the corresponding organoleptical characteristics of ovine milk cheeses coagulated via animal rennet.

#### Terrincho cheese

*Terrincho* is a ripened cheese established ever since the *Foral de Mirandela* was granted by king Manuel the first

**Table 3.** Main technological characteristics of Portuguese and Spanish AOP cheeses manufactured with raw ovine milk and coagulated with animal rennet.**Tabla 3.** Principales características tecnológicas de los quesos portugueses y españoles con denominación de origen, elaborados con leche de oveja y cuajo de origen animal.

Cheese variety	Coagulation		Cutting	Pressing	Salting		Ripening			References
	Temperature (°C)	Time (min)			Milk % (w/v)	Surface	Temperature (°C)	Relative humidity (%)	Time (days)	
Portuguese <i>Terrincho</i>	35	—	Milling to size of rice grain with spoon or knife	Manual	—	By rubbing 15–20 % (w/w) on both faces	5–12	80–85	30	Anonymous, 1992
Spanish <i>Idiazábal</i>	28–32	20–45	Milling to 5–10 mm grain, stirring and scalding at <38 °C	Mechanical for 5–6 h (2 kg/cm <sup>2</sup> )	—	By rubbing or brining for <48 h	8–15	80–95	60–90	Núñez <i>et al.</i> , 1989; Anonymous, 1992; Ibáñez <i>et al.</i> , 1999
<i>Manchego</i>	28–32	45–60	Milling to 5–10 mm grain, and scalding at 36–40 °C for 30–40 min	Mechanical for 6–18 h	—	By brining for 24–48 h	12–15	75–85	60–90	Medina <i>et al.</i> , 1989
<i>Roncal</i>	32–37	60	Crude milling but intense cutting to obtain paste	Manual or mechanical	—	By rubbing both faces or brining for 24 h	12	85	120	Núñez <i>et al.</i> , 1989
<i>Zamorano</i>	28–32	30–45	Milling to 5–10 mm grain, and scalding at 40 °C	Mechanical	—	By rubbing or brining for <36 h	<15	75–95	100–240	Anonymous, 1992

**Table 4.** Main organoleptic characteristics of Portuguese and Spanish AOP cheeses manufactured with raw ovine milk and coagulated with animal rennet.

**Tabla 4.** Principales características organolépticas de los quesos portugueses y españoles con denominación de origen, elaborados con leche de oveja y cuajo de origen animal.

Cheese variety	External color	Shape	Size		Weight (g)	Rind	Texture	Flavor	References
			diameter (cm)	height (cm)					
Portuguese <i>Terrincho</i>	Straw yellow	Flat cylinder	13–20	3–6	800–1200	Elastic, uniform, smooth and well formed	Semi-hard, slightly oily with small eyes	Delicate and clean	Anonymous, 1992
Spanish <i>Idiazábal</i>	Yellow to grey	Flat cylinder	10–30	8–12	1000–3000	Hard and smooth	Compact, semi-hard or hard	Strong, slightly piquant and buttery	Anonymous, 1992
<i>Manchego</i>	Pale yellow	Flat cylinder	9–22	7–12	1000–3500	Flat with impressions made by woven esparto grass	Semi-hard or hard with few small eyes	Pronounced but not strong, slightly, buttery and oily	Fontecha <i>et al.</i> , 1994a; Medina <i>et al.</i> , 1999
<i>Roncal</i>	Straw yellow to brown	Flat cylinder	Variable	8–12	1000–2000	Hard and thick	Compact, semi-hard or hard	Slightly piquant and buttery	Nuñez <i>et al.</i> , 1989
<i>Zamorano</i>	Pale yellow to dark grey	Flat cylinder	<24	<14	<4000	Hard and flat	Firm, compact, semi-hard with no eyes	Intense but not strong, slightly piquant, buttery and oily	Anonymous, 1992

of Portugal, back in 1512, with very few references to it until the present century (Anonymous, 1992). The manufacturing protocol of this cheese is similar to that of Portuguese cheeses manufactured with ovine milk, except that it is coagulated with animal rennet; it is obtained by slowly draining the curds following coagulation. The orographical layout of the land of production creates unique weather characteristics, which range from a sub-Atlantic to a semi-arid Mediterranean climate, thus providing this area with specific ecological conditions which, together with traditional methods of animal rearing, result in production of milk with unique characteristics. Studies of this cheese are extremely scarce.

#### *Idiazábal* cheese

*Idiazábal* is a full-fat, ripened cheese manufactured in the Autonomous Community of the Basque Country and part of the Autonomous Community of Navarre. As a result of the climatic conditions prevailing in this area, an exceptional breed was allowed to thrive. *Lacha* sheep graze on high pastureland in spring and summer, and in humid and cold valleys in autumn and winter; the combination of the milk of *Lacha* and *Carranzana* ewes produces one of the world's most original and characteristic cheeses (Anonymous, 1992). Gradual increases in the number of sheep have been recorded, and in 1995 they accounted for 4.9 million L of ovine milk, which were transformed into 979 t of cheese, 75.3% of which was officially certified (Ibáñez *et al.*, 1999).

*Idiazábal* is manufactured, at both artisanal and industrial levels, using raw milk. Use of acidifying starters, viz. *Lactococcus lactis* spp. *lactis* and *Lc. lactis* spp. *cremoris*, is acceptable. After two to three months of ripening, the cheeses may receive a smoke treatment by burning beech tree (Nuñez *et al.*, 1989). The cheese is submitted to smoking for 24 h at 6–15 °C and 85–95% relative humidity, or alternatively, smoking for 4 h at 23–25 °C and 80% relative humidity (Ibáñez *et al.*, 1999), following traditional practices.

Pérez-Elortondo *et al.* (1993b) have expanded the technological knowledge of *Idiazábal* cheese through testing the effect of brining and smoking times on microbiological attributes, whereas Ibáñez *et al.* (1993) studied the physicochemical changes induced by brining time; finally, Nájera *et al.* (1994) ascertained the influence of brining and smoking times on the free fatty acid profile of cheese throughout ripening. Brining time has a considerable effect on microbial growth, especially in cheeses brined for 12 and 24 h; the differences between unsmoked and smoked cheeses during ripening are significant for all microbial groups, except lactobacilli and enterococci (Pérez-Elortondo *et al.*, 1993b). In terms of physicochemical changes during ripening induced by brining time, decreases in water activity and pH were noted, coupled with rises in the degree of proteolysis

when the sodium chloride content was lowered (Ibáñez *et al.*, 1993).

Analysis of variance of the results produced for unsmoked cheeses at distinct brining times indicated that the concentration of free fatty acids, individually and lumped together, as well as of NaCl were significantly different throughout ripening (Nájera *et al.*, 1994); data pertaining to the effect of brining time on free fatty acid production indicated that the latter was greater in cheeses brined both for 12 and 24 h than for more than 24 h. Student's *t*-tests applied to data generated with unsmoked and smoked cheeses brined for 24 h indicated that the concentrations of all individual free fatty acids were significantly different over the ripening period after smoking; by 90 days in the post-smoking period, all free fatty acids (except butyric acid) were more concentrated in smoked than in unsmoked cheeses, although smoking increased lipolytic activity from 15 days on with regard to medium- and long chain fatty acid residues (Nájera *et al.*, 1994).

Changes in the microflora of *Idiazábal* cheese after addition of a commercial lactic starter, composed of *Lactococcus lactis* spp. *lactis* and *Lc. lactis* spp. *cremoris*, were deliberately introduced by Pérez-Elortondo *et al.* (1993a); viable counts of aerobic mesophiles and psychrotrophs in these cheeses were approximately one log unit lower, and viable counts of enterococci were about two log units lower than those recorded in similar cheeses without starter addition. Enterobacteriaceae and coliforms were no longer detected in the cheeses after ripening for 30 days, whereas in their starterless counterparts these bacteria were still detected by 60 days at levels of  $c. 10^2$  cfu/g<sub>cheese</sub>; in terms of LAB, negligible differences were observed throughout ripening; finally, the pH was lower when starter was present, especially by the end of pressing.

Molina *et al.* (1997) studied the evolution of physicochemical and microbiological parameters of *Idiazábal* cheese preserved in the traditional fashion or vacuum packaged, and concluded that higher moisture contents (and consequently higher microbiological viable counts) were attained in the latter.

#### *Manchego* cheese

*Manchego* is a semi-hard or hard (depending on the extension of the ripening period) variety of cheese produced in central Spain, namely in the region of La Mancha, which is characterized by an area of 43 000 km<sup>2</sup> and encompasses the provinces of Ciudad Real, Albacete, Cuenca and Toledo. This arid region possesses an extreme climate, with sparse rainfall, variable winds and clear skies. The landscape is flat, with few trees, and the soil is rich in limestone and marl, which makes it highly suitable for sheep grazing and which will eventually lead to high quality milk.

The manufacture of this cheese is possible by both traditional and industrial practices starting from raw

milk, as well as pasteurized milk. The former is generally manufactured without addition of a starter, whereas starters are typically employed in the manufacture of the latter. These starters are mesophilic cultures composed of *Lactococcus lactis* spp. *lactis*, *Lc. lactis* spp. *cremoris*, *Lc. lactis* spp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* spp. *cremoris*; *Streptococcus thermophilus* is occasionally included in the starter (Nuñez *et al.*, 1989; Medina, 1996b). *Manchego* cheese is sometimes cured by immersion in olive oil (Nuñez *et al.*, 1989).

The production and trade of *Manchego* cheese has increased continuously ever since establishment of its AOP, and the number of registered cheesemaker facilities in 1995 was 76 (37 artisanal and 39 industrial); this number was globally responsible for 4111 tons of cheese per year, a figure which represents 54% of all AOP cheeses produced in Spain (Medina *et al.*, 1999).

*Manchego* cheese is probably one of the most thoroughly studied AOP cheeses in the Iberian Peninsula in terms of attempts to improve manufacture and ripening procedures; a comprehensive description can be found in Medina *et al.* (1999). Particular emphasis was given to acceleration of ripening via use of elevated temperatures (Nuñez *et al.*, 1986b; Gaya *et al.*, 1990). Higher temperatures of ripening were associated with larger values for the nitrogen soluble fractions coupled with more extensive lipolysis, but overall flavor quality was not improved. Addition of such free enzymes as neutral proteinases (Nuñez *et al.*, 1991b) and recombinant chymosin (Nuñez *et al.*, 1992), or lipolytic and proteolytic enzymes in soluble form (Fernández-García *et al.*, 1994), and, more recently, liposome-encapsulated enzymes (Picón *et al.*, 1994, 1995, 1996) generally brought about increases in the levels of proteolysis without impairing flavor.

Another area that has received attention is freezing and storage of fresh cheese aiming at decreasing the seasonality of production of *Manchego* cheese (Fontecha *et al.*, 1994b); despite the observed differences in texture (formation of ice crystals during freezing clearly affected this parameter), in chemical properties (higher rates of proteolysis were observed in frozen cheeses) and in microbiological properties (lower viable counts were observed in frozen cheeses), sensory analyses led to essentially similar results by 90 days of ripening.

The changes in microbiological, chemical, rheological and sensory characteristics of vacuum-packaged *Manchego* cheese were also monitored throughout a six month ripening period (Nuñez *et al.*, 1986a); while packaging had no significant effect upon viable numbers, as well as upon extent of proteolysis, lipolysis and flavor, moisture content and rheological characteristics were conversely different to a significant degree, between packaged and unpackaged cheeses, by one to two weeks after salting. Packaged cheese displayed a lower flavor intensity than unpackaged cheese, but no significant differences in flavor quality were found; weight losses by

six months were negligible in packaged cheese, but were c. 15.2% in its unpackaged counterpart (Nuñez *et al.*, 1986a).

The use of starters has improved the manufacture of *Manchego* cheese (Barneto and Ordoñez, 1979; Nuñez *et al.*, 1981, 1982; Ramos *et al.*, 1981). These works consisted of testing strains of different lactic acid bacteria isolated from raw sheep's milk as starters for the manufacture of *Manchego*-type cheese. According to Nuñez *et al.* (1981), *Streptococcus lactis* was the most suitable candidate for starter. Ramos *et al.* (1981) compared the microbiological, biochemical and organoleptical performance of several starters, composed of as many combinations of lactic acid bacteria, on pasteurized milk cheeses; analysis of variance yielded no significant differences between soluble nitrogen (and non-protein nitrogen) in cheeses manufactured with the experimental starters, and the control cheese manufactured with raw milk but without starter. Significant differences were substantiated for amino acid nitrogen and for organoleptic scores: cheeses manufactured with a starter culture combining *Streptococcus lactis*, *Lactobacillus casei* and *Leuconostoc lactis* received the highest scores for flavor, texture and overall acceptance, and these results have backed up successful scale up to semi-industrial levels (Barneto and Ordoñez, 1979).

Studies by Fontecha *et al.* (1994a) on the texture of pasteurized *Manchego* cheese have indicated that shear strength decreased clearly until 30 days, followed by a slight increase between 30 and 45 days of ripening; firmness values decreased slightly until 30 days, and this decrease was then followed by an increase between 30 and 45 days. Mean elasticity values exhibited a decrease after 15 days of ripening, probably arising from proteolytic processes (Fontecha *et al.*, 1994a).

Appearance, aroma, taste, flavor and texture are sensory parameters that have been assessed by Fontecha *et al.* (1994a) in pasteurized *Manchego* cheese, between two and 90 days of ripening; appearance was rated as quite acceptable by the taste panel for two day old cheeses, which were also judged as compact in appearance; taste, flavor, texture and overall acceptability scores by the taste panel were also found reasonable for 90 day old cheeses; the taste panelists judged aroma via scores that consistently rose as ripening time elapsed.

#### Roncal cheese

*Roncal* is a hard cheese manufactured in Northern Spain following a technology that resembles that of *Manchego*, except for the higher renneting temperature (32–37 °C) and the smaller size (1.8–2.0 kg) (Nuñez *et al.*, 1989). The AOP area comprises the seven counties in the Roncal Valley of Navarre. This area has a great cheesemaking tradition, which was established back in the 18th century; census data show the importance of livestock in that valley, with a large population of shep-

herd families who insist on preserving their cheesemaking tradition. This area is characterized by high rainfall, and the *Lacha* and *Rasa* breeds have evolved towards a state of remarkable adaptation to the outer environment. These conditions lead to a cheese possessing a strong flavor, slightly spicy and buttery to the palate. In 1996, 2 483 million L of milk were produced, which gave rise to 401 t of *Roncal* cheese (Ibáñez *et al.*, 1999).

#### Zamorano cheese

*Zamorano* is a full-fat cheese, and its production region comprises the Zamora province, centered at Toro. In this province, the primary producer of ovine milk in Spain (Anonymous, 1992), the flocks graze on the bare plateau covered with pastures, in a region where the continental climate favors growth of trees. The manufacture of this cheese is similar to that of *Castellano* cheese, yet *Zamorano* is manufactured only with ovine milk, coagulation is performed at lower temperature and ripening lasts for longer periods. In remote times, this cheese was ripened in winery facilities, or in the manor house, where the environmental conditions are normally fresh and characterized by high humidities (above 85%), both of which promote development of grey fungi that will eventually be responsible for pigmentation of the external surface of the cheese (Canut, 1988). Studies of this cheese are scarce.

#### Cheeses manufactured with caprine milk and coagulated by animal rennet

Tables 5 and 6 include the main procedures of manufacture, as well as a summary of the corresponding organoleptic characteristics of caprine milk cheeses coagulated via animal rennet.

#### *Cabra Transmontano* cheese

*Cabra Transmontano* cheese is an extra hard ripened cheese, which owes its existence mainly to the activity of the National Association of Serrana Goat Breeders. The animals are raised in a very traditional way, with natural feeding only, following a diet in which indigenous, spontaneous plants predominate and which creates a few distinctive characteristics in the milk. Scientific knowledge pertaining to this cheese is virtually nil.

#### *Majorero* cheese

*Majorero* cheese is one of the most important Spanish semi-hard cheeses, and is manufactured in one of the Canary Islands—Fuerteventura island. Because of the island's low altitude, absence of trees and frequent droughts, the livestock can only subsist on the higher, humid zones of the Jandía Peninsula and the Betancuria mountain massif.

The manufacture of this AOP cheese takes place both at the farm level from raw ovine milk and at the industrial level from pasteurized ovine milk (Gómez and Casla, 1996); the former is manufactured without addition of a starter, unlike the latter which uses mesophilic cultures (consisting of *Lactococcus lactis* spp. *cremoris* and, to a lesser extent, *Lc. lactis* spp. *lactis*). These cultures are prepared by growth in reconstituted skim milk at 22 °C for c. 16 h (pH 4.6–5.0) (Gómez and Casla, 1996).

Rennet pastes are used in the production of artisanal *Majorero* cheese; these are obtained by macerating kid stomachs that had previously been filled with milk and sun-dried for at least two months (the pastes contain both rennet and pregastric esterase, and probably also contaminating microorganisms); calf rennet (at the ratio of 6 g per 100 L of milk, diluted to 1:10 000) is used in the manufacture of industrial *Majorero* cheese (Gómez and Casla, 1996).

Requena *et al.* (1992) evaluated a specific starter for production of semi-hard *Majorero* cheese using several wild strains belonging to the *Lactococcus*, *Lactobacillus* and *Leuconostoc* genera as starter cultures; screening of these strains for cheesemaking was done *in vitro*, and those strains that had received the best scores were further assayed in pilot scale cheesemaking. The starter containing *Lactococcus lactis* spp. *lactis*, *Lactobacillus casei* spp. *casei*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides* spp. *dextranicum* and *Leuconostoc paramesenteroides* yielded cheeses with the best organoleptic characteristics by 60 days of ripening.

The mean hardness scores at both the beginning and the end of ripening of industrial *Majorero* cheese were reported by Martín-Hernández *et al.* (1992); this parameter increased throughout ripening from 0.71 to 1.07 kg /  $\bar{S}_{\text{cheese}}$  and this increase was associated with a concomitant increase of total solids.

#### Cheeses manufactured with mixtures of ovine and caprine milks and coagulated by animal rennet

##### *Amarelo da Beira Baixa* cheese

*Amarelo da Beira Baixa* cheese is, together with *Castelo Branco* and *Picante da Beira Baixa* cheeses, one of the three AOP cheeses from the *Castelo Branco* region. *Amarelo da Beira Baixa* and *Picante da Beira Baixa* (which will be discussed in the next subsection) have similar manufacturing procedures, usually termed *à cabreira* (Cruz, 1945; Cruz and Borrego, 1948), and can be obtained from either ovine milk or caprine milk or, more usually, from a mixture of both; the different ripening procedures are responsible for distinct characteristics. *Amarelo da Beira Baixa* cheese is ripened for a minimum of 40 days in ripening chambers under natural or controlled environmental conditions. Santos (1996) reported that an average 58.2 cheeses are manufactured per day by each producer; this type of cheese dominates (c. 50%) within the

**Table 5.** Main technological characteristics of Portuguese and Spanish AOP cheeses manufactured with raw caprine milk or mixtures of ovine and caprine milks and coagulated with animal rennet.

**Tabla 5.** Principales características tecnológicas de los quesos portugueses y españoles con denominación de origen, elaborados con leche de cabra o mezcla de oveja y de cabra y cuajo de origen animal.

Cheese variety	Coagulation		Cutting	Pressing	Salting		Ripening			References
	Temperature (°C)	Time (min)			Milk % (w/v)	Surface	Temperature (°C)	Relative humidity (%)	Time (days)	
<b>Caprine cheeses</b>										
Portuguese										
<i>Cabra Transmontano</i>	35	—	Crude manual cutting in irregular pieces	Manual	—	By rubbing both faces	5–18	70–85	60	—
Spanish										
<i>Majorero</i>	28–32	60	Sequential cuts to 5–15 mm grains	Mechanical	—	By rubbing or brining for 24 h	12–18	>75	60	Gómez and Casla, 1996
<b>Mixed-milk cheeses</b>										
Portuguese										
<i>Amarelo da Beira Baixa</i>	28–32	40–50	Crude manual cutting or with 1–1.5 cm spaced wires	Manual	—	By rubbing both faces	10–18	50–70	40–50	Cruz, 1945; Cruz and Borrego, 1948
<i>Picante da Beira Baixa</i>	28–30	40–50	Crude manual cutting or with 1–1.5 cm spaced wires	Manual	—	By rubbing both faces	10–18	70–80	120–180	Cruz, 1945; Anonymous, 1988; Santos, 1996
<i>Rabaçal</i>	30	45–60	By both hands to irregular grains	Manual	—	By rubbing both faces	10–15	70–85	20	Anonymous, 1992

**Table 6.** Main organoleptic characteristics of Portuguese and Spanish AOP cheeses manufactured with raw caprine milk or with mixtures of ovine and caprine milks and coagulated with animal rennet.**Tabla 6.** Principales características organolépticas de los quesos portugueses y españoles con denominación de origen, elaborados con leche de cabra o mezcla de oveja y de cabra y cuajo de origen animal.

Cheese variety	External color	Shape	Size		Rind	Texture	Flavor	References	
			diameter (cm)	height (cm)					
<b>Caprine cheese</b>									
Portuguese <i>Cabra Transmontano</i>	White	Flat cylinder	12–19	3–6	600–900	Semi-hard, compact and smooth	Closed, extra hard with few small eyes	Intense, clean and slightly piquant	—
Spanish <i>Majorero</i>	White to yellow or orange	Flat cylinder	15–35	6–9	1000–6000	Flat with imprints: edge-palm-leaves braid; surface-parallel grooves	Compact, creamy, with or without few small eyes	Pronounced but not strong, fatty, slightly sour and salty	Gómez and Casla, 1996
<b>Mixed-milk cheeses</b>									
Portuguese <i>Amarelo da Beira Baixa</i>	Straw to dark yellow	Flat cylinder	12–16	3–5	600–1300	Semi-hard, well formed and smooth	Closed, creamy semi-hard with few eyes	Intense and slightly acidic	Cruz, 1945; Cruz and Borrego, 1948; Santos, 1996
<i>Picante da Beira baixa</i>	White to grey	Flat cylinder	10–15	3–5	400–1000	No rind	Closed, brittle, semi-hard or hard with few eyes	Strong, active, salty and piquant	Cruz, 1945; Anonymous, 1988
<i>Rabaçal</i>	Straw yellow	Flat cylinder	10–12	3–4	300–500	Well formed, flat and slightly greasy	Firm, closed, semi-hard or hard with few eyes	Fresh, milky and slightly acidic	Anonymous, 1992

aforementioned set of three cheeses. According to Santos (1996), each *Amarelo da Beira Baixa* cheese requires an average of 4 L of milk (coagulated at 30 °C and for 43 min), which in turn yields a ripened cheese with c. 1000 g weight by 45 days.

#### *Picante da Beira Baixa* cheese

*Picante da Beira Baixa* cheese is produced in a region that borders *Serra da Estrela*, and is manufactured manually once a day, from December to April, in small quantities, departing from a mixture of c. 60% (v/v) raw ovine milk (either from *Merino da Beira Baixa*, *Churra do Campo* or *Frízia* breeds) and 40% (v/v) raw caprine milk (either from *Serrana* or *Charnequeira* breeds), using liquid rennet extracted from calf's stomach and without deliberate addition of any starter (Cruz, 1945). It is also possible to manufacture this AOP cheese with plain ovine or with plain caprine milk (Anonymous, 1988). Ripening of *Picante da Beira Baixa* is different from that of most cheeses because the curd is rubbed with dry salt immediately upon manufacture and maintained in the maturation room for two to six days in order to allow the salt content reach the value of c. 3.5% (w/w); the cheeses are thereafter rubbed once again with dry salt, grouped in small horizontal sets of two or three cheeses and left in that way for two to three weeks. The purpose of such cheese stacking is to allow a greater degree of whey expression in order for the cheese to attain a high salt content, which is determined by the cheesemaker via empirical assessment of the cheese consistency. These sets are later brought together in vertical stacks of several cheeses supported by intercalated layers of clean sand and wheat straw, and kept in that way for a minimum of four months. The cheeses are periodically washed with tap water or salted water, and the ripening process is allowed to proceed in the absence of any control of temperature or relative humidity. The prevailing weather conditions usually range from 10–18 °C and from 70–80% relative humidity. By six months of ripening, *Picante da Beira Baixa* cheese typically acquires a rindless, flat cylinder shape with well-defined sharp edges.

The manufacture of *Picante da Beira Baixa* cheese is intimately related with that of *Amarelo da Beira Baixa* cheese, but less than 10% of all producers currently manufacture the former variety of cheese within the AOP region (Santos, 1996). Typically, an average of 4.5 L of a mixture of ovine and caprine milks is coagulated at 29 °C for 43 min; the ripening period varies between 150 and 190 days, which produces ripened cheeses of c. 950 g weight (Santos, 1996).

In order to gain information on the influence of several manufacturing conditions, viz. source of rennet (animal rennet versus plant rennet), proportion of ovine and caprine milks, amount of NaCl applied to the cheese surface and duration of the ripening period, on the fi-

nal characteristics of *Picante da Beira Baixa* cheese, several experimental cheeses were produced following a factorial design (Freitas and Malcata, 1996). The main conclusions were that milk type is a statistically significant technological parameter in terms of viable microorganisms of various genera and extent of proteolysis, probably as a consequence of higher initial contamination of caprine milk; the type of coagulant had a major effect on proteolysis, and values of WSN for cheese coagulated with animal rennet were in general lower than those for cheeses coagulated with plant rennet, even though much smaller differences were detected between values of NPN; and breakdown of  $\alpha_s$ - and  $\beta$ -caseins was more extensive in cheeses manufactured with plant rennet (Freitas and Malcata, 1996). According to Freitas *et al.* (1998a), experimental cheeses manufactured with 20% (v/v) caprine milk, coagulated with commercial animal rennet in liquid form and salted only once exhibited the highest content of total free amino acids by 120 days of ripening, whereas all four manufacturing parameters were found to be statistically significant, at the 0.5% level of significance, in terms of total concentration of free amino acids. The level of NaCl was a statistically significant parameter for all microbiological, physicochemical and chemical characteristics, whereas ripening time did not play a significant role in the numbers of viable staphylococci (Freitas and Malcata, 1996).

In order to optimize selected technological parameters of cheesemaking of *Picante* cheese, several cheeses were produced according to a second-order composite factorial design using volumetric percentage of caprine milk, ripening time and percentage of salt added to fresh cheese as the technological variables manipulated (Freitas and Malcata, 1999); microbiological, physicochemical, biochemical and textural analyses were carried out for all experimental cheeses. Of the manufacturing variables studied with respect to their effect upon microbiological growth, the most important in magnitude was ripening time, whereas milk composition and amount of salt added were also significant factors, especially in terms of total viable mesophilic microorganisms, Enterobacteriaceae and staphylococci. In terms of proteolysis and lipolysis, ripening time was also the dominant factor, but milk composition and NaCl content were also found to be significant; a higher content of caprine milk was associated with a higher extension of both phenomena, whereas a higher content of NaCl led to the opposite behavior. It was found that: (i) the range 50–80% (v/v) caprine milk for cheesemilk composition seems to be adequate, since the differences in terms of microbiological safety, proteolysis and lipolysis extents are minor; (ii) 195 days is suitable for ripening time, which confirmed once more that this type of cheese should be ripened for no less than 120 days; and (iii) NaCl content should be 15% (w/w<sub>TS</sub>), which corresponds to salting fresh *Picante* cheese (c. 66%, w/w, moisture) with c. 5% (w/w) NaCl (Freitas *et al.*, 1998b).

The traditional ripening procedures of *Picante da Beira Baixa* cheese are excessively labor-intensive, so attempts to manufacture and mature this cheese under more standard conditions have been scrutinized by Freitas and Malcata (1998). Two alternative ripening protocols were considered, the traditional one and another with controlled environmental conditions that uses maturation chambers set at different temperatures; characterization of the experimental cheeses was done in microbiological, physicochemical, biochemical, sensorial and textural terms. Ripening time and temperature were statistically significant parameters for the whole microflora: cheese ripened isothermally at temperatures ranging from 8 to 15 °C exhibited significant differences in terms of several microbiological, physicochemical, biochemical, textural and sensorial parameters when compared with that ripened following traditional practices. The magnitude of these differences decreased for cheeses ripened at higher temperatures, thus indicating that low temperatures are not suitable for ripening of this type of cheese, although an increase in the ripening temperature from 11.5 to 15 °C was in general not statistically significant for most parameters studied. These results have led to the conclusion that the standard ripening method was close to the traditional one in terms of final cheese characteristics, provided that the ripening temperature was above 11.5 °C. This conclusion is consistent with optima obtained via response surface analysis, which indicated that the best ripening temperatures are between 11 and 13 °C.

Cheeses manufactured with several volumetric ratios of ovine and caprine milks and ripened for 180 days were organoleptically assessed by a group of experienced panelists (Freitas *et al.*, 1997); parameters considered were form, surface, texture and flavor. Appearance of cheeses, as well as texture and flavor, were rated as satisfactory; the effect of composition of the milk mixture was not statistically significant in terms of the organoleptic characteristics assayed, but cheeses manufactured with either 75% or 100% caprine milk obtained the highest scores in terms of texture and flavor (Freitas *et al.*, 1997).

#### Rabaçal cheese

The existence of *Rabaçal* cheese cannot be understood without remembering its intimate relationship with the population of the *Rabaçal* region (Carvalho and Fróis, 1991); however, there are not many references to this cheese, and often they are not coincident (Hansens, 1938; Arnaut, 1961; Silva, 1980; Gonçalves and Silva, 1983; Bernardo, 1986; Torres, 1991).

The flocks are raised extensively in the farms, and the smell and flavor of this cheese reveal the unique regional flora that serves as pasture for said sheep, which is in turn a natural consequence of the weather conditions prevailing in this area (Anonymous, 1992).

This type of cheese is manufactured with variable proportions of ovine and caprine milks; however, according to Delgado (1993), the volumetric proportion 2:1 of ovine to caprine milk is ideal.

Several sensory studies were performed on *Rabaçal* cheese sequentially during four years. One such study attempted to establish which sensorial characteristics could distinguish *Rabaçal* from other cheeses produced in the same region (Alves, 1992); this type of cheese was found to exhibit peculiar characteristics in terms of aroma, flavor and texture. Aroma and flavor were described as not too intense, milky, floral and acid, and texture ranged from semi-hard and not elastic to very elastic (which correlated well with acidity). Another study tried to assess the sensorial quality of *Rabaçal* cheese when different proportions of ovine and caprine milks, as well as of bovine milk were employed (Carvalho *et al.*, 1996); the main conclusions were that the elastic texture correlates with incorporation of bovine milk, and extremely acid cheeses can be avoided via control of ripening conditions.

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