The Microfloras and Sensory Profiles of Selected Protected Designation of Origin Italian Cheeses

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ABSTRACT

Approximately 39 Italian cheeses carry protected designation of origin (PDO) status. These cheeses differ in their manufacturing technology and the microbial flora which comprise the finished products. The evolution of lactic microflora in cheeses with PDO status is of particular interest because the biochemical activities of these organisms participate in cheesemaking and may play an acknowledged role in the development of organoleptic characteristics during ripening. Nonstarter lactic acid bacteria (NSLAB) constitute complex microbial associations that are characterized by the occurrence of various species and many biotypes as a result of a number of selective conditions persisting during the manufacturing process and different ecological niches. The evolution of different species during ripening of Fiore Sardo showed that, when present, Lactobacillus paracasei persists and dominates the microflora of the cheese in the last period of ripening, suggesting that this species, more resistant to the constraints of the mature cheese, could be involved in proteolysis and in other enzymatic processes occurring during cheese ripening. In contrast, the stretching step typical of pasta filata cheese, such as Ragusano, induced a simplification of the raw milk profiles, allowing the persistence only of some predominant species, such as Streptococcus thermophilus, Lactobacillus delbrueckii subsp. lactis, Lactococcus lactis, and Streptococcus macedonicus, after the stretching step. Lactobacillus plantarum and L. paracasei were isolated from ripened Castelmagno PDO cheese samples with the highest frequencies. These species, generally absent in the milk, occur in dairy ecosystems and dominate the bacterial flora of many ripened semihard cheeses. In PDO long-ripened Italian cheese such as Parmigiano Reggiano, the NSLAB population is mainly formed by L. paracasei, Lactobacillus rhamnosus, and Pediococcus acidilactici. Lactobacillus helveticus, L. delbrueckii subsp. lactis, and L. delbrueckii subsp. bulgaricus were also detected. Continued insight into the microbial populations of traditional Italian cheeses will allow continued production of characteristic, high-quality cheeses which have been enjoyed for many centuries.

GENERAL ITALIAN CHEESE HISTORY

To understand and appreciate the great cheeses of Sicily and other parts of Italy which we enjoy today, along with the microbes associated with them, it is necessary to review the historical context surrounding the rich and deep origins of these products. The Archestrato of Gela (4th century BCE) describes Sicilian cheeses as “the best and more flavorful than caseus of Lesbo and Creta.” Many Roman writers, including Cato the Elder (234–149 BCE), Varro (ca. 116–27 BCE), Columella, and Pliny the Elder, described the cheesemaking process, the quality, and the culinary uses of cheese. Cheesemaking was well known among the Romans, who spread the knowledge of cheesemaking during the Empire period, as evidenced...
by use of the word *caseus*, the root for casein, the principal milk protein that is coagulated to make cheese. The Romans improved the cheesemaking techniques of the Greeks when they introduced the use of cow’s milk, which was uncommon at that time, since cows were used primarily to work the land and not for milk production. The Romans produced dairy products from sheep, goats, and cows, which they consumed fresh or aged. Milk left in wicker baskets spontaneously coagulated, or coagulation was quickened by continuous mixing with fig branches or the addition of fig juice or wild thistle seeds. In addition to thistle and fig, the Romans used saffron and vinegar. This composition was called *coagulum*. According to the Edict of Diocletian, milk was at that time inexpensive, approximately the same cost as a low-quality wine. In the summer, though, it was difficult to find because flocks were relocated for the transhumance toward mountain grazing.

In ancient times, all dairy products were known and consumed, except for butter (and there are some doubts on the use of fermented yogurt). Strangely, butter was not used in Rome, probably because it was not liked or it was difficult to store. Fresh curd, known as *melca* (*oxygala* in Greek), was used as a refreshing food for breakfast, obtained by the acidification of milk with vinegar and flavored with sage, onion, and leek. Whey cheese (*coagulum*) was also very common for breakfast. Colostrum, obtained from sheep directly following birthing, and milk serum from skimming were considered delicious drinks. The Romans were considered major cheese consumers and fans and used cheese in numerous recipes, as did Cato the Elder (234–149 BCE), who used it grated and mixed with honey, fruit, and flour to prepare specialty foods like *libum* (a focaccia described by Ovid), or *scribilia*, which was a dessert with honey and cheese. In the Republican period, Roman meals may have included bread, cheese, and wine for breakfast, *ientaculum*; cheeses and legumes for lunch, *prandum*; and spelt polenta and cheese with wine for dinner, *coena*. The Romans mentioned cheese as an important nutritive part of their diet, and for this reason they introduced it in the rations for legionnaires. Virgil (70–19 BCE) tells us that the daily menu for legionnaires included a pound of bread (about 320 g), 3 ounces of meat or lard (about 100 g), and 1 ounce of pecorino cheese (about 30 g), Romano type, which provided protein, energy, and salt in abundance. The famous ancient cookbook *De Re Coquinaria*, sometimes attributed to Marcus Gavius Apicius (10 BCE–23 CE), was written for aristocrats but became a recipe collection to satisfy different social conditions. Ancient confectionery was based on dough and various other ingredients, including white cheeses. Other important characteristics of Apician cooking were the grinding, crumbling, and reduction to pulp of ingredients to prepare meatballs and roulades. Residents of Rome imported cheese from countries far away, even though cheese was considered a “poor food.” Upper-class individuals who appreciated cheeses were looked down upon in contempt.

Another valuable source of historic information is *De Agricultura* by Terentius Varro (116–27 BCE). Varro distinguishes the cheeses made from *caprirus* (goat’s milk), *ovillus* (sheep’s milk), and *bubulus* (cow’s milk). He describes two kinds of rennet: vegetable rennet (from yellow flowers of the galium, fig latex, thistle flowers, crocus seeds, etc.) and animal rennet (from donkey milk, dried calf stomach, lambs, goats, fawns, and hares). To make cheese, about 6½ liters of milk was added to a piece of rennet “the size of an olive.” As an alternative to rennet, vinegar was mentioned as a means of coagulating milk. Roman cheeses were made from raw milk, left to drain in wicker baskets, then salted and oiled, and placed to age in fig leaves. Varro exalted the nutritional value of cow’s, sheep’s, and goat’s milk and explained that the characteristics of the different types of milk were dependent on the pasture, the natural habitat of animals, and milking time. Varro distinguished between “soft and fresh cheese” and “aged and dry” and made reference to the best season for cheesemaking, which was from the middle of May to August: “at the rising of Pleiades until summer Pleiades.” Sometimes the cheese curd was flavored with herbs (thyme and garden savory). For salting, Varro preferred mineral salt to sea salt. Pliny the Elder (23–79 CE), in his book “*Naturalis Historia*” (Liber 28), dwelt upon milk quality used by affluent classes as beauty products and mentioned that Nero’s wife bathed in donkey milk (*Nat. Hist. libro XI, cap. 96*). Pliny argued that cow’s milk produced more cheese than goat’s milk, and that the most prized types of rennet were those from the deer, the hare, the goat, and, above all, an animal referred to as the *dasipode*, which was thought to cure dysentery. The most interesting part of this great history is when describing the delicious character of cheese, the author drew a geographic map that distinguished the dairy products coming from above the Alps from those produced in Italy, noting particularly the exquisiteness of pecorino from Agrigento. Pliny affirmed that cheeses produced in France were highly prized when consumed still fresh, while those of the Apennines
were washed with water, allowed to form a rind, and placed on shelves in an enclosed place so that the cheese remained tender. However, Columella also mentions brine salting as a method of hardening cheese. He briefly mentions the manufacture of “hand pressed” (*manu pressum*) cheese in which hot water is poured over the curds, which are then shaped by hand, a practice perhaps related to the kneading and stretching steps for pasta filata varieties. Certain cheeses were flavored with herbs and colored with smoke, practices that persist to a certain extent today. Columella dedicates some interest to the main defects of the cheeses: “full of holes” (clearly mechanical openings as the remedy recommended is increased pressing), too salty, or too dry. Columella affirms that cheese “serves to feed farmers” (*agrestis saturat*) and “to decorate elegant tables.” Cheese on humble tables was considered an important dish, indeed a primary sustenance source; on wealthy tables, cheese appeared only for decoration or as an ingredient in elaborate dishes. Cheese must have also been popular with Roman civilians, and demand exceeded supply, forcing the emperor Diocletian (who reigned from 284 to 305 CE) to fix a maximum price for cheese. Diocletian ordered that fresh cheese be sold wrapped in leaves, while the aged cheese was salted on the surface.

Literary archives from the Middle Ages present a controversial perspective on cheese, especially the medical community, which had a certain mistrust toward cheese. The treatise *Summa Lacticiniorum* affirmed a modern and innovative concept: cheese as a live product, in dynamic and constant evolution. Until the late Middle Ages, bovine breeding had a marginal role in agriculture. There were more pigs, goats, and sheep. During that period, the Sabaud Court of northern Italy appreciated cheeses (as stated in a document from 1270), while popes and lords preferred to have them as an exotic rarity and had them sent to their palaces. In the 14th century, authors of cookbooks, poets, and novelists considered cheese a delicacy, and they mention it in their books. In spite of medical prescriptions, in medieval gastronomy cheese had an important social role and was highly valued on the tables of the wealthy. In the 15th century, Pantaleone of Conflenza wrote of “reges...duces plurimos, comites, marchiones, barones, milites, nobiles, mercatores” who often ate cheese (2).

The presence of cheese on refined tables is confirmed, in these centuries, from cooking works, like that of Cristoforo di Messisbugo, chef of the Estensi court of Ferrara, which list a series of dishes including “butirro, ricotta, ricotta di butirro, cavi di latte, gioncata, panna di latte, mantichiglia; formaggio duro, grasso, tommi, pecorino, sardesco; marzolini, provature e ravogliuoli.”
At the end of the Middle Ages, the traditional superiority of sheep’s milk cheese over cow’s milk cheese created some problems. “Parmesan cheese,” which was consumed grated, is mentioned for the first time in Boccaccio’s work, while a “caciocavallo” cheese from the South appears in the works of Franco Sacchetti, a Florentine author of the 14th century. Both authors, from Florence, demonstrate that some Italian dairy products were exported to other regions in that period. Given the frequency with which cheese appears in documents, it is clear that there were few families without cheesemaking equipment. During the Middle Ages, among the grated cheeses, Parmesan was the first (but at that time Piacentino and Lodigiano were also famous). The success of Parmesan cheese is connected to the affirmation of another famous food product, pasta, and we know that both products were exported from Italy. Parmesan cheese is mentioned by Salimbene of Parma, in his work Cronaca (13th century), when he describes the friar Giovanni of Ravenna as a hearty eater of cheese lasagna. Boccaccio (1313–1375), in his work Decameron Liber VIII, describes cheese as an attraction of the utopian town of Bengodi, where macaroni and ravioli were cooked in capon stock. During the Middle Ages, monks from all over Europe became cheese supporters. The monks replaced the old earthenware or stone containers with heavy boilers, provided written cheesemaking records, and translated books from Latin to Greek. Thanks to them, we know today that, in 1000, Schabziger was cited for the first time. The monastic food model (3) is based primarily on the partial or total renunciation of meat, to be replaced by fish, eggs, or cheese. This renunciation was extended beyond the monastic area; in fact, it was imposed by ecclesiastic rules to all Christian society and involved most days of the year.

After the fall of the Roman Empire and the arrival of the Barbarians, who were major cheese consumers, cheese played an important role in the fight against hunger. Benedictines and Cistercians dedicated their activity to cheese production. During the Middle Ages, Sicily contributed to the creation and distribution of dairy products with extended shelf lives. The exportation of cheese from Palermo was relevant. At the beginning of the 14th century, the city produced more wheat and cheese than it needed. From the 1500s, the city of Trapani exported large quantities of cheese abroad. The end of the Middle Ages drew the attention of St. Lucius the martyr, the patron saint of cheese makers and herdsmen. According to tradition, he was a shepherd who tended the herds of his landlord and offered the cheese that he received as payment to the poor. When his requests for cheese increased, this angered his landlord, who killed him in a pond. During the Renaissance, cheesemakers created new and exciting varieties to augment both the traditional cheeses passed down from Roman times and the monastic ones born in the Middle Ages. In the Po Valley of Italy, large and midsize farms started to grow at the end of the 1800s, where large expanses of pasture permitted farming of large quantities of cattle. Production of cheeses of great dimension, such as Grana, commenced. These cheeses were easy to store and favored the spreading of products in different areas. In Italy, a large variety of cheeses had been produced for centuries, including Ragusano and Pecorino Siciliano. After the unification of Italy, in 1861, the Italian parliament conducted investigations to understand the socioeconomical conditions of farmers and their agricultural problems. Studies that continued into the 20th century show an extraordinary agricultural and food heritage, and, in particular, more than 400 identified cheeses. The strong connection with the territory, the tradition handed down from father to child, and the agricultural culture itself position Italy in competition with France among important places with a rooted dairy culture. In Table 1, the Italian cheeses with protected designation of origin (PDO) status are listed; we discuss a selection of them in detail.

<table>
<thead>
<tr>
<th>Type</th>
<th>Cheese(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semihard pressed</td>
<td>Asiago, Bitto, Bra, Caciotta d’Urbino, Castelmagno, fontina, formai de mut, Monte Veronese, Pecorino Sardo, Pecorino Toscano, Raschera, Spressa delle Giudicarie, Stelvio, Toma Piemontese, Valtellina Casera</td>
</tr>
<tr>
<td>Hard pressed</td>
<td>Canestrato Pugliese, Fiore Sardo, Grana Padano, Montasio, Pecorino di Filiano, Pecorino Romano, Pecorino Siciliano, Piacentino Ennese</td>
</tr>
<tr>
<td>Stretched</td>
<td>Caciocavallo podolico, mozzarella di bufala campana, Provolone Valpadana, Raguano, Vastedda Valle del Belice</td>
</tr>
<tr>
<td>Semicooked, not pressed</td>
<td>Parmigiano Reggiano</td>
</tr>
<tr>
<td>Soft</td>
<td>Casatella Trevigiana, Gorgonzola, Murazzano, Quartiolo Lombardo, Ricotta Romana, Robiola di Roccaverano, Taleggio, Valle d’Aosta Fromadzo</td>
</tr>
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ROLE OF MICROBES
The evolution of lactic microflora in cheeses with PDO status is of particular interest because the biochemical activities of these organisms participate in cheesemaking and may play a role in the development of organoleptic characteristics during ripening. Nonstarter lactic acid bacteria (NSLAB) constitute complex microbial associations that are characterized by the occurrence of various species and many biotypes as a result of a number of selective conditions persisting during the manufacturing process and different ecological niches (4). The NSLAB community is composed mostly of mesophilic lactobacilli such as Lactobacillus paracasei, Lactobacillus plantarum, and Lactobacillus curvatus (5). Nevertheless, Pediococcus and Leuconostoc species (6), as well as enterococci and streptococci, could also be present. Mesophilic lactobacilli are important in the maturation of cheeses, as they are able to ferment citrate and could be involved in proteolysis as well as in other enzymatic processes that occur during cheese ripening (7).

STRETCHED CHEESES
Caciocavallo Podolico
This semihard, pear-shaped, pasta filata (pulled-curd) cheese is made from cow’s milk. The zone of production includes different areas, from sea level to the mountains, and it is produced in designated areas of Italy, in the regions of Calabria, Campania, Puglia, and Basilicata. Caciocavallo has a smooth, shiny, straw-yellow rind that yellows with aging and thickens and darkens as the cheese matures. The cheese is soft, buttery, uniform, and white like milk when young, but it darkens as it matures and it separates into layers with a yellow ochre coloration.

The taste, initially sweet, buttery, and delicate (it becomes slightly spicy if it is prepared with kid rennet), is characteristic and aromatic due to the feeding regimen of the Podolica cow, which lives in the wild and consumes a diet based entirely on upland and wild grasses. The fat in the dry matter is not less than 38%.

Each cheese weighs between 1 and 2.5 kg. The unmistakable taste of well-matured caciocavallo podolico is derived thanks to the climatic conditions of the mountainous plateau of the Sila (a region located in Calabria, southern Italy). Caciocavallo podolico has a high nutritional value and can be consumed fresh or matured, grated or melted. Over time, caciocavallo in general has become one of the most important cheeses in the Mediterranean diet.

Traditional technology
Caciocavallo podolico’s technology is derived from the need to store this cheese to provide a reserve of food throughout the year for farmers. Every cheese has its typical characteristics tied to the customs of the regions where it was produced. Whole milk collected from no more than four consecutive milkings of two preceding days is used for cheesemaking. Milk is heated to 36 to 37°C; then natural animal rennet (veal, goat, or lamb) is added. After 15 to 20 min, the curd is formed, and it is broken with a “curd knife” into little grains the size of a hazelnut, collected with a cloth, and placed into a wooden vat called a tina.

After the addition of the whey, the curd is left to age for 4 to 10 h at room temperature, and then it is cut with steel knives. The cut pieces are placed in hot serum (85 to 90°C) or in a water solution made with 20% sour serum coming from the preceding day’s cheesemaking. The cheese is stretched and is completed when the curd presents the optimal parameters. The manual milk stretching consists of the formation by hand of a rope so that the external surface is smooth, without infiltration of folds or holes in the cheese interior. Therefore, this technology requires the dipping of the curd quickly in hot water at 80 to 85°C. Finally, the mass obtains the desired shape, and respecting local customs, it can be an ovoid shape with a little head or truncated cones, tied together with string.

Each cheese is pickled with a solution (40% NaCl) for 3 days and then tied up and left for aging. The typical caciocavallo podolico is naturally aged and never smoked. During the aging, each cheese is cleaned of superficial mold with a cloth. In industrial cheesemaking, each cheese is covered with paraffin. Cheese aging is variable: it can be consumed after 30 days, but a good caciocavallo requires at least 6 months of aging, and after 3 years the product is excellent. Its microstructure is typical of a pasta filata cheese, where the protein matrix and fat globules are orientated according to the stretching direction. With ripening, they lose their directionality and tend to assume an amorphous structure. Irregularly shaped lipids are due to the manufacturing process and protein matrix contraction that lead to fat globule rupture and the formation of free fat pools. The protein matrix appears compacted even though small and occasional holes (probably serum pockets) have been observed.

Studies of the principal microbial groups of caciocavallo podolico during cheesemaking have shown that the species and the concentration of bacteria change. In detail, the most representative bacteria at the end of
production are total aerobic mesophilic bacteria (6.5 log CFU/g), enterococci (5.4 log CFU/g), streptococci-fecal enterococci (4.6 log CFU/g), staphylococci (5.32 log CFU/g), mesophilic lactobacilli (6.65 log CFU/g), thermophilic lactobacilli (6.93 log CFU/g), mesophilic lactococci (7.48 log CFU/g), and thermophilic lactococci (7.97 log CFU/g). Psychrotrophic bacteria, fecal coliforms, molds, lipolytic bacteria, and propionibacteria were not present at the end of the caciocavallo podolico cheesemaking process. Studies have also been carried out to define the microbial profile of caciocavallo podolico during curd maturation, showing that the various lactic microfloras, composed of *thermofilii* (thermophilic) and *mesofilii* (mesophilic) cultures, contribute to the maturation of the curd. The rapid multiplication of the natural microfloras which are initially present leads to a rapid disappearance of undesirable microorganisms. In fact, total and fecal coliforms, lipolytic bacteria, and propionibacteria were totally absent at the beginning of ripening, while psychrotrophic bacteria disappeared after 15 days of ripening; total aerobic mesophilic bacteria, streptococci-fecal enterococci, staphylococci, and molds decreased after 15 days of ripening (data not published).

**Ragusano**

Ragusano is a brine-salted pasta filata raw-milk cheese that is aged for 3 to 12 months and is produced on farms in eastern Sicily. It is a PDO cheese made in the Hyblean region of Sicily from raw milk using traditional wood tools without commercial starters. It received the recognition of PDO in 1996. Ragusano is one of the oldest cheeses of Sicily.

**Traditional technology**

Ragusano PDO is produced exclusively from raw whole milk produced during the months of October to May. This season is characterized by green forage consisting of diverse Mediterranean plants selected by cows, e.g., *Calendula arvensis* (Asteraceae), Geraniaceae (various species), Graminaceae (various species), unspecifed short Asteraceae, and Fabaceae (various species), which contribute to the flavor profile of milk and cheese. These spontaneous pasture species comprised approximately 60% of the plant material selected by the grazing cows on a dry matter basis (8). Ragusano PDO cheese is produced traditionally by using wooden tools (*tina, ruotula, mastredda*, etc.). The wooden vat (*tina*) is characterized by a natural inoculation system due to the presence of lactic acid bacteria on the surface of the *tina*: the biofilm. Raw milk is directly placed in the wooden *tina*, and lactic acid is produced by the biofilm. The coagulation of milk takes place at 34°C by using rennet paste of lamb or kid without the addition of starter culture. A wooden staff, the *ruotula*, is used to break the curd into small grains (approximately 4 mm in size). Immediately following cutting, hot water is added to increase the temperature of the curd to 39.1 ± 1.6°C. When the cutting step is completed, the caseous mass is ready for an initial cooking time of about 11.6 min. The curd is left to drain in a plastic container, the *vascedda*, on an open-topped table (*mastredda*). The resulting whey is used to make ricotta, and the liquid from the ricotta (*scotta*) will be used for the second cooking of the Ragusano cheese curd. After draining, the mass of curd is cut into slabs about 3 cm thick and cooked again (i.e., the second cooking) in the vat for an average of 85 min by adding hot ricotta whey or hot water. The curd is then separated from the whey (or water) and left to ripen and develop acid on a wooden table for about 24 h at room temperature. Batches of about 14 to 15 kg of curd were stretched slowly with a flat wooden stick, a *manuvedda*, to obtain a spherical mass of cheese. The casein chains and clusters coalesce into long fibers that are uniformly oriented (9). As a result of stretching, whey and fat globules get an uneven distribution; they accumulate, in longitudinal channels, between the bundles of casein fibers (10, 11), resulting in a partial alignment of the fat and serum phases within the cheese (12). By turning several times in a squared-off section of the open-topped table (*mastredda*), the stretched mass is formed into the characteristic parallelepiped shape (50 by 16 by 16 cm). The cheese is left at room temperature (20 to 28°C), and when its surface is dried, it becomes a straw-yellow color that is typical of fresh cheese. The variation in color of the cheese is related to changes in the concentration of β-carotene in milk (13), which is derived from green forages. Brine salting is performed by immersing cheeses in a small basin of saturated brine at room temperature at the farm (about d/kg of cheese weight). At the end of brine salting, cheeses are aged in a ventilated room at 14 to 16°C with a relative humidity of about 80 to 90%. The cheese produced by using this traditional manufacturing is typically consumed as a table cheese from about 3 to 9 months of age (14). At the first months of ripening, Ragusano is characterized by a pleasant sweet and lightly spicy taste that becomes increasingly spicy as aging is prolonged. In the aged cheese, casein fibers lose their identity and form a compact and laminar (15) network interspersed by fat in the form of discrete globules or elongated pools (16, 17). The stretching step typical of pasta filata cheeses,
such as Ragusano PDO, induced a simplification of the raw milk profiles, allowing the persistence of only some predominant species, such as *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactococcus lactis*, and *Streptococcus macedonicus*, after the stretching step (18). During Ragusano cheesemaking, temperature and pH, mainly during stretching, have a major role in selecting the predominant natural microflora from raw milk. Whatever the initial raw milk species, thermophilic lactic acid bacteria were predominant after stretching and then decreased drastically during aging. By reverse transcriptase PCR-denaturing gradient gel electrophoresis, some authors also showed that *S. thermophilus* and *L. delbrueckii* were the only two species metabolically active after stretching (19). The viable count of thermophilic lactic acid species decreased drastically after stretching, and a 4 to 5 log reduction was evident after 7 months of ripening, indicating that this lactic flora is probably no longer metabolically active (18). Studies carried out on the biofilm of the wooden *tina* showed a natural inoculation system characterized by lactic acid bacteria (*S. thermophilus* alone or with thermophilic lactobacilli) are the most abundant. Also found in the biofilm was a variable level of yeasts, molds, and enterococci, depending on the specific *tina* and confirming the farm specificity of the microbial profile. Most importantly, acidification was quicker in the wooden *tina* than in a stainless steel vat, thus confirming the microbiological safety of this approach.

**SEMIHARD PRESSED CHEESES**

**Castelmagno**

Castelmagno is a PDO semihard pressed cheese. Its origins are very ancient. Castelmagno is mentioned in documents dating to the 13th century. The area of production is confined to three communes (Castelmagno, Pradleves, and Monterosso Grana) in the Valle Grana in the province of Cuneo. The denomination of PDO was issued on 1 July 1996.

**Traditional technology**

Castelmagno is made using cow’s milk, but sheep’s and/or goat’s milk, partially skimmed for cream separation (*affioramento*), may be added. The cattle producing the milk must have been fed with fresh forage or hay from mixed meadows or pasture within the zone. This confers a particular taste to the product. The preparation of this cheese requires about 6 days. Milk from two milkings may be used: traditionally, the milk of the first milking rests overnight in stoneware or wood containers (generally, zinc or aluminum containers are used today) in a cool environment (or via immersion in cold water).

The following morning, the milk of the second milking is added. This is heated to 37 to 38°C in a copper or aluminum vat in a bain-marie or a direct flame. Milk is coagulated with liquid veal rennet. After coagulation, the curd is broken and turned with a *spannarola*. The curd is agitated for about 10 min so that it forms homogeneous grains. The curd is put in a cloth called a *reirolo* or *risolo*. A bundle is obtained, and it hangs for about 20 h, after which time it is placed in a container for about 2 days. The curd is mixed and separated in other cloths where it is hashed, salted, and finally deposited in the *fascere* for a variable period, from 1 to 3 days. In the *fascere*, the curd is first submitted to manual pressure and then pressed. The dry salting, directly on both sides, lasts 2 days. The pressing process induces whey expulsion and development of a dense and amorphous protein matrix. Fat is present in two forms: free fat pools (i.e., not globular) and coalesced fat globules (globular). The lipids act as filling particles and limit the total collapse of proteins. The aging process takes place on wooden shelves in clean and dry rooms and lasts from 2 to 5 months, during which time cheeses are periodically washed and turned. Castelmagno has a cylindrical shape (height, 12 to 20 cm; breadth, 15 to 20 cm). Each cheese weighs between 5 and 7 kg. The rind is thin, reddish yellow, and wrinkled when the cheese is aged. *L. plantarum* and *Lactobacillus paracasei* species were isolated from ripened Castelmagno PDO cheese samples with the highest frequencies. These species, generally absent in the milk, occur in dairy ecosystems and dominate the bacterial flora of many ripened semihard cheeses (5). Occasionally the species *L. lactis* subsp. *lactis*, *L. delbrueckii* subsp. *lactis*, *Lactobacillus coryniformis* subsp. *torquens*, and *Lactobacillus casei*, also originating from the dairy environment (19, 21), were isolated. *Lactococcus lactis* subsp. *lactis* was the species most frequently isolated during Castelmagno PDO manufacture, while *L. plantarum* and *L. paracasei* were isolated with the highest frequencies from ripened Castelmagno PDO cheese samples (22). The energy source used by lactobacilli for growth has not yet been clearly defined, since at the time of most active growth of lactobacilli, lactose has been exhausted (5). In Castelmagno PDO cheese, citric and lactic acids were hypothesized as potential energy sources (23). *Enterococcus faecalis* and *Enterococcus faecium* species were also isolated with high
Fiore Sardo

Fiore Sardo is a hard pressed PDO cheese. It is a pecorino-style cheese with very ancient origins, made from autochthonous Sardinian sheep’s milk in the provinces of Nuoro, Sassari, and Cagliari. Fiore Sardo cheese obtained “acknowledgement of designation” in 1955, and it was awarded PDO status in 1996.

Traditional technology

Fiore Sardo is a hard cheese made exclusively from raw, fresh whole sheep’s milk and is normally coagulated with lamb rennet or, occasionally, kid rennet. It is still made on pastoral farms using the traditional methods which have evolved over many centuries, with knowledge handed on from father to son. The milk used for the production of Fiore Sardo is not heated at high temperatures. Raw milk from one milking is filtered and poured into a tinned copper boiler where the coagulation occurs.

The boiler is covered to prevent the cooling of the milk. Milk is coagulated at a temperature of 32 to 35°C; but during the winter or on cold days, it is coagulated to the highest temperature. In this case, if necessary, milk is brought to temperature by heating using a direct flame. In the spring or in warm weather, where previous verification of the temperature has occurred, milk is coagulated without thermal intervention. The rennet used for cheesemaking is often produced at home. Milk is sometimes inoculated with natural lactic cultures typical of the area of production. Once coagulation is obtained, the caseous mass is broken into curds the size of a grain of rice, which are then washed and immediately transferred to cheese molds described as truncated cones.

At this point, the scottatura practice starts; the molds containing the cheeses are immersed in hot water to help give an external finishing of the cheeses and to develop the thick outer rind. The salting is mixed (in a brine solution and dry), and it has a variable duration, according to the dimensions of the cheeses.

At this point, a first maturation of the cheese occurs (it is placed on a trellis-type mat made of rushes which is suspended in the smoky area above the fireplace) in the shepherd’s hut. The maturation takes place when the cheeses are transferred in a contiguous place or in the roof. When the warm season arrives, the cheeses are transferred to underground cellars generally situated in mountain areas. During this phase, the cheeses are periodically turned and greased with olive oil, sometimes mixed with sheep lard. The maturing and aging process lasts 6 months. The cheeses can be described as two truncated cones joined at their wider base, with a height of 12 to 15 cm and a diameter from 12 to 20 cm. The weight varies from 1.5 to 4 kg. The rind varies from deep yellow to dark brown in color, and the taste of the typically compact and pale yellow or white paste is slightly spicy depending on the maturing.

Fiore Sardo is a semihard cheese banded by a hard, black, natural rind. It has a paste more golden than is usually found in sheep’s milk cheese. Its microstructure appears to be made of a fine protein matrix where fat globules are entrapped. Even though most of them seem to have maintained their globular native structure, truly they are coalesced fat globules resulting from the blend of many smaller lipids. By the rind, the structure becomes completely different, with a compact protein matrix and some free fat pools.

Previous studies (24, 25, 26) showed that the dominant microbial flora is comprised of lactococci (L. lactis) and that these bacteria are always associated with enterococci (E. faecium), which was the main microbial group colonizing this cheese. Nevertheless, Mannu et al. (27, 28) confirmed that a high number of mesophilic lactobacilli in Fiore Sardo cheese were important during the ripening process. Other researchers confirmed similar results in other types of cheeses (29, 30, 31). PCR identification allowed identification of the main species isolated in Fiore Sicano cheese: L. plantarum and the L. casei (L. paracasei) species. It has been shown that, during ripening, the microflora of the cheese is “controlled” by L. paracasei that could be involved in proteolysis and in other enzymatic processes of cheese ripening.

Pecorino Romano

Pecorino Romano is a PDO, hard pressed cheese. The best known is the Pecorino Romano; other popular pecorinos are from Tuscany, Sardinia, and Sicily. Pecorino is a generic term, and it can be used to describe all cheeses made from the milk of sheep. And in fact, in Italy there are a great variety of pecorino cheeses; each one is characteristic of a particular area of production or of a specific breed of sheep.

Pecorino Romano, whose ancient zone of origin is the Agro Romano territory, is one of the most ancient cheeses in the world. A number of ancient authors, such
as Varro, Pliny the Elder, Hippocrates, and Columella, describe minutely in their books the ancient method of Pecorino Romano production, and comparison with the modern methods reveals few substantial changes. And in fact, still today, although it is called Roman, this pecorino is mostly produced in the Lazio region surrounding Rome, where its cheesemaking technology was spread by some enterprising Roman and Neapolitan farmers.

Pecorino Romano has attained great commercial success and is exported from Italy, especially to the United States. Areas of production include the province of Rome, Rieti, Viterbo, Lazio, Grosseto, Cagliari, Nuoro, Oristano, and Sassari. The PDO status was obtained on 12 June 1996.

**Traditional technology**

Pecorino Romano is a pressed white cheese, aged from 8 to 12 months. Fresh milk is filtered and heated to eliminate undesirable microorganisms. It is put in a vat, and natural starter cultures, the *scotta-innesto*, are added to start the lactic acid fermentation. Later, the lamb rennet is added. Following coagulation, the curd is broken into the size of a grain of corn or rice. Subsequently, the mass obtained is submitted to cooking at 45 to 48°C, and then it is poured out in a drain vat to obtain the mass and the first drainage of serum.

The caseous mass is transferred to molds and then pressed and placed in the *fascere*, where the branding is done. The branding allows identification of the manufacturing dairy and the month and year of production. This cheese is made by a special method called “rummaging curd” which consists of introducing a reed in the curd, in the phase of pressing cheese, to facilitate the exit of the serum. The dry salting is done on several occasions and for 2 months in special places, known in the past as *caciare*. The cheese should age for at least 8 months. It is a cheese of cylindrical shape, and each cheese weighs between 22 and 33 kg. The rind is straw-yellow or light or deep brown if it is rubbed with fat and amber resin or other protective material (*cappatura*). The surface, white or straw yellow, is generally compact or pierced slightly. The aroma is fragrant and characteristic, and the taste is typically spicy. *L. lactis* and *S. thermophilus* have been detected in Pecorino Siciliano cheese (32). The presence of these species in artisanal cheese indicates that the manufacturing process and ripening conditions allow the occurrence of these species in the final product. This could be due to the high resistance of thermophilic bacteria to cooking temperature and the adaptation of these species to the cheese environment during ripening (33). The strains dominating the first stages are not necessarily present in the later phases of ripening. Indeed, many of the strains of *L. plantarum* were abundant during the early stage of ripening and then decreased during ripening, whereas the number of *L. paracasei* increased or remained constant until 90 days. Strains of *L. curvatus* were found only in the early stages of ripening (34). The composition of NSLAB species was affected by farm environment and technology, as confirmed by numerous studies based on comparison of different types of Italian cheese made with different farm technologies. For example, *Lactobacillus brevis* isolates were found mainly in cheeses produced with a high temperature during curd cooking, a high concentration of salt in moisture, and a long ripening time, such as Pecorino Romano cheese.

Pecorino Romano PDO is a hard, salty Italian cheese made out of sheep’s milk. Its crumbly texture is confirmed by the structure that is made of a quite open protein matrix. At ×20 magnification, large fat globules have been observed. It is interesting that both Pecorino Romano cheese and Fiore Sardo cheese are characterized by large globular lipids encompassed by a fine protein matrix. This feature is not observed in any of the other cheeses discussed here. At ×40 magnification, the protein matrix often appears to be interrupted by empty areas.

**Parmigiano Reggiano PDO**

Parmigiano Reggiano is a semihard cheese produced in the north of Italy. It is made from partially skimmed raw milk. The cheese has a hard consistency and granular structure and contains crystals of tyrosine. Even though grainy, its microstructure is more compact than that of Castelmagno cheese, maybe due to the loss of moisture during the ripening period (Parmigiano cheese is ripened for a longer time than Castelmagno cheese). Lipids are dispersed within the protein matrix nearly homogeneously except for those areas occupied by the curd grain junction. Here, fat globules may have been released into the whey during the cheesemaking process. Lipid size seems to be smaller than in the other cheeses described in this chapter.

In Parmigiano Reggiano, the number of starter bacteria (thermophilic lactic acid bacteria [LAB]) commonly exceeds 10⁷ CFU/g of cheese when ripening begins. NSLAB populations, whose initial numbers are typically below 10⁴ CFU/g, begin to grow and eventually plateau at cell densities of 10⁷ to 10⁹ CFU/g after 3 to 9 months of aging (35, 36). Therefore, starter LAB (SLAB) are dominant until the second month of ripening. After
brining, species coming from milk (NSLAB) are able to grow, while SLAB cells undergo autolysis \(^{(37)}\). When NSLAB cell numbers increase, most of the residual lactose in cheese has been utilized by SLAB; therefore, NSLAB seem to better adapt to the absence of lactose in cheese than SLAB. This is dependent on their ability to utilize the substrate potentially available like milk components, bacterial metabolites, and cell lysis products \(^{(38)}\). In a PDO long-ripened Italian cheese such as Parmigiano Reggiano, NSLAB population is mainly formed by \(L.\ paracasei\), \(Lactobacillus\ rhamnosus\), and \(Pediococcus\ acidilactici\). \(Lactobacillus\ helveticus\) and \(L.\ delbrueckii\) subsp. \(lactis\) and \(L.\ delbrueckii\) subsp. \(bulgaricus\) were also detected \(^{(39)}\).

**Pecorino Siciliano PDO**

Pecorino Siciliano is a pressed cheese which has a sponge-like structure. The globular casein micelles join each other to form clusters and chains with no defined orientation \(^{(40)}\). By pressing, curd granules fuse together, and whey and fat globules are uniformly distributed within the cheese matrix \(^{(10)}\). At the end of pressing, the cheese already has a loose, spongy structure, formed by large casein clusters; during ripening, the initial structure is transformed into a compact and homogeneous sponge structure \(^{(16, 17, 41)}\). Randazzo et al. \(^{(32)}\) demonstrated the presence of \(L.\ lactis\) and \(S.\ thermophillus\) in Pecorino Siciliano cheese. The presence of these species in artisanal cheese indicates that the cheesemaking process and ripening conditions allow the occurrence of these species in the final product. This could be due to the high resistance of thermophilic bacteria to cooking temperatures and their preferential adaptation to the cheese environment during ripening \(^{(33)}\). The strains dominating the first stages are not present in the later phases of ripening. Indeed, many of the strains of \(L.\ plantarum\) were abundant during the early stage of ripening and then decreased during ripening, whereas the number of \(L.\ paracasei\) increased or remained constant until 90 days. Strains of \(L.\ curvatus\) were found only in the early stages of ripening \(^{(34)}\). The composition of NSLAB species was affected by farm environment and technology, as confirmed by numerous studies based on comparison of different types of Italian cheese made with different farm technologies. For example \(L.\ brevis\) isolates were found mainly in cheeses produced with a high temperature during curd cooking, a high concentration of salt in moisture, and a long ripening time, as in Pecorino Romano cheese \(^{(42)}\).

**MOLECULAR CHARACTERIZATION OF PREDOMINANT NSLAB IN SOME ITALIAN TRADITIONAL CHEESES**

The evolution of lactic microflora in cheeses with PDO status is of particular interest because the biochemical activities of these organisms participate in cheesemaking and may play an acknowledged role in the development of organoleptic characteristics during ripening. NSLAB constitute complex microbial associations that are characterized by the occurrence of various species and many biotypes as a result of a number of selective conditions persisting during the manufacturing process and different ecological niches \(^{(4)}\). The NSLAB community is composed mostly of mesophilic lactobacilli such as \(L.\ paracasei\), \(L.\ plantarum\), and \(L.\ curvatus\) \(^{(5)}\). Nevertheless, pediococcus and leuconostoc species \(^{(6)}\), as well as enterococcus and streptococcus, can also be present. Mesophilic lactobacilli are important in the maturation of cheeses, as they are able to ferment citrate and could be involved in proteolysis as well as in other enzymatic processes that occur during cheese ripening \(^{(7)}\). Presumptive mesophilic lactobacilli represented a predominant microbial group in Fiore Sardo cheese until the third month of ripening, and then they slowly decreased after 7 months. The majority of the isolates gave the specific amplification product when amplified with primers for \(L.\ plantarum\). In the last period of ripening, the majority of the isolates detected in the cheese were represented by \(L.\ paracasei\), and only one \(L.\ plantarum\) isolate was detected at 5 months. In the last period of maturation, from 2 to 6 months, \(L.\ paracasei\) predominated among the isolates. The evolution of different species during ripening showed that, when present, \(L.\ paracasei\) persists and dominates the microflora of the cheese in the last period of ripening, suggesting that this species, more resistant to the constraints of the mature cheese, could be involved in proteolysis and in other enzymatic processes occurring during cheese ripening \(^{(27)}\). Indeed, the stretching step typical of pasta filata cheese, such as Ragusano PDO, induced a simplification of the raw milk profiles, allowing the persistence of only some predominant species, such as \(S.\ thermophillus\), \(L.\ delbrueckii\) subsp. \(lactis\), \(L.\ lactis\), and \(S.\ macedonicus\), after the stretching step \(^{(18)}\). Whatever the initial raw milk species, thermophilic LAB were predominant after stretching and then decreased drastically during aging. By reverse transcriptase PCR-denaturing gradient gel electrophoresis, some authors also showed that \(S.\ thermophillus\) and \(L.\ delbrueckii\) were the only two species metabolically active after stretching \(^{(19)}\). The viable count of thermophilic lactic acid species decreased
drastically after stretching, and a 4- to 5-log reduction was evident after 7 months of ripening, indicating that this lactic flora is probably not metabolically active anymore (18). L. plantarum and L. paracasei species were isolated from ripened Castelmagno PDO cheese samples with the highest frequencies. These species, generally absent in the milk, occur in dairy ecosystems and dominate the bacterial flora of many ripened semihard cheeses (5). Occasionally, the species L. lactis subsp. lactis, L. delbrueckii subsp. lactis, L. delbrueckii subsp. torquens, and L. casei, characteristic of the dairy environment (19, 21), were also isolated. L. lactis subsp. lactis was the species most frequently isolated during Castelmagno PDO manufacture, while L. plantarum and L. paracasei were isolated with the highest frequencies from ripened Castelmagno PDO cheese samples. The energy source used by lactobacilli for growth has not yet been clearly defined, since at the time of most active growth of lactobacilli, lactose has been exhausted (5). In Castelmagno PDO cheese, citric and lactic acids could be hypothesized as potential energy sources (22). E. faecalis and E. faecium species were isolated with high frequencies. Although their presence in manufacturing samples is mainly associated with poor hygienic conditions during milk collection and storage, their occurrence in ripened cheese could be related to an intense lipolytic activity (23).

During cheesemaking, the presence of different levels of LAB, both starter and nonstarter, is the result of their ability to grow in milk and curd and of bacterial cell autolysis (43). In Parmigiano Reggiano, a PDO long-ripened Italian cheese, the number of starter bacteria (thermophilic LAB) commonly exceeds $10^9$ CFU/g of cheese when ripening begins. NSLAB populations, whose initial numbers are typically below $10^2$ CFU/g, begin to grow and eventually plateau at cell densities of $10^7$ to $10^9$ CFU/g after 3 to 9 months of aging (35, 36). Therefore, SLAB are dominant until the second month of ripening. After brining, species coming from milk (NSLAB) are able, like milk components, bacterial metabolites, and cell lysis products (38). In PDO long-ripened Italian cheese, such as Parmigiano Reggiano, NSLAB population is mainly formed by L. paracasei, L. rhamnosus, and P. acidilactici. L. helveticus, L. delbrueckii subsp. lactis, and L. delbrueckii subsp. bulgaricus were detected too (39). Continued insight into the microbial populations of traditional Italian cheeses will allow continued production of characteristic, high-quality cheeses which have been enjoyed for many centuries.

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REFERENCES
18. Licitra G, Ogier JC, Parayre S, Pediliggeri C, Carnemolla TM, Falentin H, Madec MN, Carpin S, Lortal S. 2007. Variability of bacterial cell lysis products (38). In PDO long-ripened Italian cheese, such as Parmigiano Reggiano, NSLAB population is mainly formed by L. paracasei, L. rhamnosus, and P. acidilactici. L. helveticus, L. delbrueckii subsp. lactis, and L. delbrueckii subsp. bulgaricus were detected too (39). Continued insight into the microbial populations of traditional Italian cheeses will allow continued production of characteristic, high-quality cheeses which have been enjoyed for many centuries.


