Producing specific milks for speciality cheeses

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Protected denomination of origin (PDO) cheeses have distinctive sensorial characteristics. They can be made only from raw milk possessing specific features, which is processed through the ‘art’ of the cheesemaker. In general, the distinctive sensorial traits of PDO cheese cannot be achieved under different environmental–production conditions for two main reasons: (1) some milk features are linked to specific animal production systems; (2) cheese ripening is affected by the interaction between milk (specific) and the traditional technology applied to the transformation process (non-specific). Also, the environment for a good ripening stage can be quite specific and not reproducible. With reference to milk, factors of typicality are and/or breed, pedo-climatic conditions, animal management system and feeding. Other factors that influence cheese quality are milk treatments, milk processing and the ripening procedures. The technology applied to most cheeses currently known as PDO utilizes only raw milk, rennet and natural lactic acid bacteria, so that milk must be, at its origin, suitable for processing. The specific milk characteristics that ensure a high success rate for PDO cheeses are high protein content and good renneting properties, appropriate fat content with appropriate fatty acid composition and the presence of chemical flavours originating from local feeds. Moreover, an appropriate microflora is also of major importance. The factors that contribute to achieving milk suitable for transformation into PDO cheese are genetics, age, lactation stage, season and climate, general management and health conditions, milking and particularly feeding, which affect nutrient availability, endocrine response and health status, and also the presence of microbes and chemical substances which enrich or reduce the milk–cheese quality. Many of these factors are regulated by the Producer Associations. However, the secret of the success of PDO cheeses is the combination of modern technology and tradition, with the objective of adapting the product to market demand, without losing specificity, originality and authenticity.

**PDO cheeses: Milk traits: Milk composition: Changeable factors in milk**

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Abbreviations: LAB, lactic acid bacteria; PDO, protected denomination of origin.

*Quintilianus*

'Supervacuus foret is studiis longus labor si nihil liceret melius inventre preteritiis (Long, hard study is meaningless if it does not provide the possibility to improve existing knowledge)'
in Europe, but it is particularly high in Italy and Greece (representing 48 and 42 % for cow’s milk respectively and approximately 100 % for sheep and buffalo milks). In France artisan cheese production is also important, but it does not exceed 20 % of cow’s milk production.

The differences between various cheeses are well established, and have long been attributed to their place of origin. In a brief note the Roman poet Martial (39–104 AD) indicated the importance of the trade-name linked to the town of origin of certain Etruscan cheeses (Bonilauri, 1993). The name of Roquefort has been linked to the cheese produced in the French village of this name since 1666, and Platina (1421–1481) wrote: ‘today in Italy there are two cheeses fighting for first place: Marceolino produced in Etruria (Lazio-Toscana) . . .; and Parmesan in the Po valley (Parma) . . .’. Thus, it is not surprising that most Italian (and French) cheeses have names derived from villages (Gorgonzola, Castelmagno, Asiago etc.), towns (Parmigiano-Reggiano, Ragusano etc.) or regions (Pecorino Sardo, Pecorino Toscano etc.). The ‘denomination of origin’ or typicality of many cheeses has been regulated by law for the last 50–60 years. More recently, the EU has designated approximately 137 cheeses of ‘protected denomination of origin’ (PDO), thirty of which are Italian.

A PDO cheese is defined according to its geographical area of production, and also according to the description of the materials and of the technology used (Fig. 1); some of the latter aspects are of interest to us because it is possible to modify the milk, e.g. through species, breed and feeding conditions (Bertozzi, 1991). The designation of origin represents a means of protecting and emphasising an historic production heritage, rooted in a specific area, derived from a rural culture, and offering original characteristics. The principal aim of the PDO is not to conquer new markets, but to spread information about characteristic products relating to the specific factors of typicality that must be defined, verified and protected (Bertozzi, 1995). This type of recognition is a consequence of an EU agricultural policy that encourages the diversification and characterisation of products in Europe with the aim of achieving better market equilibrium, reducing surplus, and sometimes stimulating an extensive agricultural production system. This strategy links environmental protection and the reduction of sociological problems with the heritage and cultural diversity of traditional agricultural products (Licitra et al. 2000).

Bertozzi (1995) recently established that the factors that characterise the typicality of cheeses with PDO are animal breed, diet and rearing conditions, treatments to which the milk is subjected, processing methods and length of ageing; factors that to some extent would be linked to the geographical area. However, these cheeses are also the result of seasonal influences and the accompanying way of life, religions or family festivities and culinary traditions (Morand-Fehr et al. 1998). Nevertheless, while there is a direct bond with the soil for products such as wine and olive oil, and also for other vegetable products such as beans, peppers and asparagus, this bond becomes less strong in the case of animal products such as cheese. To reinforce the bond between the PDO cheese and the place of production, there has been an attempt to define the so-called ‘terroir’, i.e. a geographic area homogeneous for soil, climate and orography, and also for animal production systems (Brunschwig, 1998). For a more detailed definition of that bond, and following the example of wines, there have also been some attempts to define the so-called ‘cru’, i.e. the small geographical area where the PDO cheese has peculiar traits mainly related to the forage base of the cow’s diet (Coulon, 1997).

A more comprehensive approach (Monnet et al. 2000) suggests the characterization and localization of cheese georegions using two different approaches, cartographic and edaphic (sensory), as shown in Fig. 2 for Comté cheese. Many of these concepts, i.e. the importance of feeds and season for cheese quality, can be confirmed, as suggested by Addeo et al. (1998), by the production of two typical cheeses in the same Parmigiano-Reggiano zone until 1983. The cheeses differed only in the season of production; Parmigiano-Reggiano was produced in spring and summer, and Vernengo was produced in winter.
For these reasons, also, the producer associations of major cheeses, such as Parmigiano-Reggiano cheese in Italy and Comté in France, establish that the cows must be fed local forages (while the use of silages is prohibited; Bertozzi, 1991); this factor would confirm a direct link between the soil and the final product, which is the basis of every factor of typicality (Bertozzi, 1995).

Nevertheless, it is worth emphasising that the typicality of cheese is not only linked to the type of agriculture, and consequently to the milk, but also to the processing technology. This concept has been clearly demonstrated by Martin & Coulon (1995) and Martin et al. (1997) in trials carried out on Reblochon cheese from six farms in the Haute-Savoie area of France; technological variables had to be adapted to milk characteristics in order to obtain good cheese. It becomes obvious, however, that simply applying traditional cheesemaking technology outside the regulation area does not result in PDO cheese production.

Another important aspect, the source of some controversy, is whether and to what extent technology can be innovated. First, it may be useful to have better definition of the problem; despite having demonstrated a relationship between traditional animal breeding and the Mediterranean landscape, Laureano (2000) suggested that tradition does not exclude innovation. In fact, tradition is not static and unchangeable. A good contribution to the argument is that of Salvadori del Prato (1997), who showed that the techniques involved in traditional technology, which must be respected, can be modern. These concepts have been well described by Bottazzi & Battistotti (1997) for Parmesan and by Bianchi Salvadori & Rottigni (1997) for Gorgonzola, while Blanc’s (1981) statement explains that: ‘the culture of these differences was tenaciously defended also in the last century, since the end of the eighteenth, when many technological aids: i.e. centrifuge, lactobacillus cultures, pasteurisation, continuous cheesemaking etc. were introduced. In fact, despite the tendency towards the enlargement of the dairies and many improvements in the materials and tools, some peculiarities were frequently maintained: raw milk, natural culture, discontinuous cheesemaking, slow salting and ripening; in other words, the technology of the traditional background was used to maintain the best traits of typical cheeses’.

Thus, considering that the edaphic criteria are of major importance, they must guide the choice of any innovation, as suggested by Addeo et al. (1998): ‘Technological changes allow manufacturers to solve current technical and social problems. Such changes, however, should be accepted and evaluated in relation to their influence on the product quality’.

Finally, it should be recognised that the sensory traits of typical cheeses have changed with time. For example, in Le Cuisinier et le Médecin (Lombard, 1860; cited by Alberini, 1993), Parmesan is defined as ‘Cheese originally from the Duchy of Parma, in Italy. It is sold in cylindrical wheels weighing 30 kilos. It has a dry consistency and is salty; saffron powder is used to give it a sulphur color. It is made much like gruyère is’. Today saffron is no longer used and the taste is not salty but ‘gentle’, for reasons discussed in detail by Alberini (1993).

How to obtain and guarantee ‘protected denomination of origin’ cheeses

From the point of view of technology, a large proportion of the cheeses currently known as PDO utilise only raw milk, supplemented with natural lactic acid bacteria (LAB) cultures. Thus, milk can only be slightly modified in order to achieve the properties required for any cheese: renneting, bacteriology, gross compositional traits etc.; i.e. milk must be adequate for processing at its origin. This factor therefore explains the tendency to introduce at farms fairly restrictive rules for milk production, in order to obtain appropriate protein and fat contents, good renneting, quick and efficient fermentation by suitable bacterial strains while inappropriate bacteria must be absent or inhibited (i.e. clostridia, coliforms etc.); when necessary, also, creaming activity must be effective. More recently, additional rules have been introduced in order to maintain the natural properties (i.e. avoiding animal-derived feedstuffs) and increase safety precautions, particularly in relation to mycotoxins and other undesirable substances (e.g. pesticides). Nevertheless, some of these rules often appear excessive, while others, although useful, do not provide a complete solution to the problems.

There are so many factors affecting milk traits (Bertoni, 1996a) that only the ‘real art’ of the cheesemaker allows successful processing (Carini & Lodi, 1997). It is surprising that the same concept can be recognised in the response of an artisan cheesemaker from Cork (Anonymous, 2000): ‘and that is what cheesemaking is all about: the expression of a personality. Singers must sing, musicians must play, painters must paint, and it seems to me that cheesemakers must make cheese.’

The changing and expanding market has led to an increasing need to establish objective and verifiable variables to certify the typicality of all production, as well as to differentiate the authentic product from imitations. The various national organisations controlling the application of the standards that govern the production of cheeses with PDO have supported numerous studies to define the physical, chemical and sensorial variables necessary to define and verify, in an objective way, the intrinsic characteristics of these products (Bertozzi, 1995).

An example of this important effort is the research of Moio & Addeo (1998), who demonstrated that the olfactometric profile of Grana Padano cheese aroma is more complex than that of an imitation cheese. Another example is the study of Mucchetti et al. (2000), who found that pyroglutamic acid is present in many cheese varieties, and in particularly high amounts (0.5 g/100 g cheese) in extensively-ripened Italian cheeses (Grana Padano and Parmigiano-Reggiano) that are produced with thermophilic LAB as starters, i.e. when a natural whey starter has been used (Coppola et al. 1995). Also of interest is the evaluation of free amino acids and biogenic amines (Krause et al. 1997). However, in the future, attention will be directed towards methods allowing the identification of the specific sensorial traits (Moio, 1998; Valfré et al. 1999) that are frequently associated with natural pasture (Wilson & Keen, 1998; Balza et al. 1999; Gresta et al. 1999), and therefore
could require molecular markers (Addeo et al. 1998; Mayol et al. 2000; Versini et al. 2000).

Of course, typicality does not necessarily imply high safety and quality (Casati, 1991). Thus, these factors must be among the objectives of any typical cheesemaker or, preferably, of the whole production chain from the field (or barn) to the table. There has been some discussion between the EU and the supporters of PDO cheese in relation to hygiene. The 92/46 EC directive (European Union, 1992) was very restrictive with respect to hygiene. Nevertheless, it was received in Italy with some derogation, particularly for cheeses ripened for at least 60 d, which can be considered as traditional (historically recognised or made according to processes systematically coded in a country of the EU; Radogna, 1997). This apparent inconsistency with general rules aimed at guaranteeing safety has been justified by Panari & Pecorari (1999), who stated, with particular reference to Parmigiano-Reggiano: ‘These processing systems are continually aiming at improvements in quality and only the best technologies persist through the centuries; furthermore they have accepted corrective practices (as in the modern Hazard Analysis and Critical Control Points protocols) suitable for customers’ requirements, including hygienic traits’.

**Main features of milk suitable for typical cheeses**

In general, the specific traits of PDO cheeses are linked to technology as well as to milk differences which are related to animal species or breed, protein content and renneting properties, fat content and its fatty acid composition, or other chemical flavours originating from specific feeds, and finally the original microflora. Each cheese is associated with a specific technology that will not be considered here. However, we will discuss factors associated with the milk, and how these factors change.

**Animal species and breed**

Some milk characteristics are obvious, while others need explanation. In fact, some of the typical traits of cheeses can be attributed to the species, i.e. cheeses obtained from goat’s, ewe’s, buffalo’s or cow’s milk can be distinguished; however, identifying their precise differences in order to prevent adulteration with milk from other species would require very sophisticated methods based on electrophoresis, immunochrometry (Aranda et al. 1988; Rodríguez et al. 1990; Feligni et al. 1999) or specific chemical indices (Pellegrino et al. 1996), and more recently on the oligosaccharide profile (Beccati et al. 1999).

With respect to breed, the differences are much less evident, but still important in achieving some specific traits in the milk, and therefore in the cheese. In Italy, for Fontina only a particular breed of cattle, Valdostana, can be used; there are other examples in France (Roquefort with Lacaune sheep and Cantal with Saler cattle). The effect of breed can be found in the gross composition of the milk (fat and protein contents), but very often the differences are associated with the rennetability and/or the suitability for particular production systems (marginal areas, mountains etc.), and hence large differences in feeding and management.

With respect to rennetability, in Italy there is the case of Parmigiano-Reggiano cheese, mainly produced using indigenous breeds (Reggiana and Modenese) until the end of the 1800s, then gradually substituted with Alpine (Swiss) Brown and finally with Friesian (at present Italian Friesian). The original breeds have almost disappeared, nevertheless there is renewed interest in them, particularly Modenese (Mariani, 1985) but also Reggiana, because their milk has better rennet–coagulation properties (Table 1) which seem to be attributed not only to a higher casein content, but also to differences in casein fractions (the κ fraction is higher and the κ1 fraction is lower; Mariani et al. 1998). Moreover, when comparing the Alpine Brown with the Italian Friesian, the former seems to ensure a better milk renneting property, and therefore the resulting Parmigiano-Reggiano cheese has better texture, taste and flavour characteristics (Bonato et al. 1987a; Mariani et al. 1993). However, the same authors (Bonato et al. 1987b) have demonstrated that the milk of well-managed Friesian cows also shows good cheesemaking properties. Furthermore, Bettencourt et al. (1998) confirmed for Portuguese serpa cheese (sheep) that breed cannot be considered of major importance for the quality of typical cheeses.

Nevertheless, a further (and maybe essential) explanation for the effect of breed could be different adaptability to the environment, which is undoubtedly the case for three similar breeds in the Valle d’Aosta, Aosta Red Pied, Aosta Black Pied and Aosta Brown. The cows are of small to average body size, with low milk yield (2500–3800 kg/lactation) and average milk composition (Bianchi et al. 1992), but they spend 6 months per year grazing mountain pastures up to 1200–1800 m above sea level (Ubertalle et al. 1992) with obvious positive effects on milk and cheese (Fontina) quality. Another example is Caciocavallo, of which a special type is produced using Podolica milk; Podolica is a dual-purpose breed which grazes throughout the year on the hills and mountains of some southern areas of Italy (R Rubino, personal communication).

These particular factors are of great interest because they have socio-economic relevance and can influence some breeding choices; i.e. the extent of pasture, the possibility of substituting local forages with imported forages and the amount of permitted concentrates (Coulon et al. 1997) can in turn influence the choice of breeds and their productivity.

**Table 1.** Titratable acidity and rheological variables measured on 595 individual milk samples from four breeds of cattle (from Pecorari et al. 1987)

<table>
<thead>
<tr>
<th>Breed</th>
<th>Italian/Friesian</th>
<th>Italian/Brown</th>
<th>Reggiana</th>
<th>Modenese</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Titratable acidity</strong></td>
<td>3.29</td>
<td>3.45</td>
<td>3.33</td>
<td>3.59</td>
</tr>
<tr>
<td><strong>Clotting time</strong></td>
<td>16.5</td>
<td>16.3</td>
<td>16.0</td>
<td>17.9</td>
</tr>
<tr>
<td><strong>Curd firming rate</strong></td>
<td>14.1</td>
<td>8.90</td>
<td>7.70</td>
<td>10.5</td>
</tr>
<tr>
<td><strong>Curd firmness</strong></td>
<td>22.2</td>
<td>28.2</td>
<td>30.7</td>
<td>25.4</td>
</tr>
</tbody>
</table>

*SH, ml NaOH N/4 per 50 ml.*
Fat and protein contents

Fat and protein contents are often considered important, since they are useful for increasing cheese yield, although yield, for typical cheeses, is not the main objective. However, they are still of great interest; fat because it can modify the creaming activity and also the flavour, especially in goat’s milk cheese and the cheeses obtained from grazing animals, and protein because it is a factor of renneting rate and curd features (Remeuf et al. 1991).

There has been less interest in fat and creaming activity since the introduction of centrifuges. Nevertheless, it remains an essential step for some PDO cheese technologies, i.e. Parmigiano-Reggiano and Grana Padano in Italy, as well as Gruyère in France. Interest obviously goes beyond reduction of fat content and includes an effect on bacterial contamination; bacteria and spores are in fact significantly reduced after good creaming activity (Bottazzi et al. 1968; Ducruet et al. 1980). Many factors are responsible for the variations in creaming activity, but globule characteristics and fatty acid composition appear to be the main factors (Speroni & Bertoni, 1984; Piccioli Cappelli et al. 1990).

Furthermore, fatty acid composition and the minor chemical flavours are also responsible for the special flavour nuance typical of the pasture (or dry forage) of any land (Wilson & Keen, 1998); it is also influenced by the season and the stage of development of the grass (Urbach, 1998) and can be evaluated by sensory analysis or chemically as previously described.

The renneting properties are of major importance for the rheological characteristics of curd and then for the chemico-physical and structural properties of cheese; these properties are characterised as rennet-clotting time, coagulum strength and syneresis rate (Losi & Mariani, 1984). They influence whey drainage, which affects the moisture content of the curd, fundamental for the appropriate start of the cheese-ripening processes which are in turn linked to fermentation activity (Pecorari et al. 1988). However, there are many milk traits that can modify this property (Bertoni & Calamari, 2000), particularly protein (casein) content (Brule, 1991), Ca and P contents (Remeuf et al. 1991), pH or titratable acidity (Mariani et al. 1981; Remeuf & Hurtaud, 1991) and the small size of casein micelles (Losi & Mariani, 1984; Remeuf et al. 1991).

In addition to these traits, other important traits involved in the renneting of milk are: (a) the casein fractions, because high κ-casein (Losi & Mariani, 1984; Marziali & Ng-Kwai-Hang, 1986), as well as β-casein (Remeuf & Hurtaud, 1991) and perhaps αs2-casein (Christian et al. 1999) improve the renneting property; (b) a high Cl– content tends to slow κ-casein hydrolysis, and therefore renneting. Moreover, it is an index of mastitis that causes a reduction in normal casein available for the proteolytic activity associated with the higher plasmin content (Politis et al. 1989); as a consequence there is an increase in γ-casein and an impairment of the renneting activity.

There is no doubt that a good renneting property in milk is of major importance for cheese yield (Losi & Mariani, 1984; Mariani & Pecorari, 1987; Remeuf et al. 1991; Fossa et al. 1994) and also for cheese quality (Parmigiano-Reggiano), both for texture and colour, and for organoleptic properties such as flavour and taste (Green & Grandison, 1993; Martin et al. 1997; Pecorari et al. 1995). Nevertheless, it cannot be considered separately from other aspects of milk properties (i.e. bacteriology) and technology; i.e. for PDO cheeses Martin & Coulon’s (1995) statement is interesting: ‘a strong relationship exists between the technological parameters and the milk traits’. This finding could also explain the conclusions drawn by Grandison et al. (1985) that ‘the composition and texture of farmhouse Cheshire cheeses depend on the individual manufacturer regardless of variations in milk composition within the ranges studied’.

In fact, when considering farmhouse and/or PDO cheeses, where milk treatments are not allowed, curd properties depend only on milk composition and clotting characteristics, which are subject to wide fluctuations over the year owing to the cow’s stage of lactation and nutritional factors (Martin et al. 1997). These variations often result in seasonal differences in the chemical or sensory properties of the cheese, which are sometimes difficult for the scientist to understand, but they are well-known and managed by good manufacturers in order to maintain acceptable uniformity and a high standard. A good example is given in Table 2, which shows the effects on the dairy process of a higher casein content of milk. These technological changes are based on empirical observations linked to the sensorial evaluations consequent to the dairyman’s personal experience, which could be accomplished only in part by chemical or physical determinations (Mora, 1985).

However Zantige’s (1999) conclusion, which is obviously valid for industrialised cheese production, is in direct contrast, i.e. in the case of PDO cheese processing, milk can be extremely different for many reasons, and it is only the ‘art’ of the dairyman which allows appropriate technological changes to be made in order to obtain similar (not identical), but still good cheeses (Carini & Lodi, 1997). According to Barillet et al. (1998), it is impossible to standardize milk traits and the dairyman is therefore obliged to adjust the process technology from the beginning to the end.

<table>
<thead>
<tr>
<th>Table 2. Variations consequent to an increase in milk protein content (from Mora, 1985)</th>
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<tbody>
<tr>
<td><strong>Milk</strong></td>
</tr>
<tr>
<td>Acidity ↑</td>
</tr>
<tr>
<td>Defatted DM ↑</td>
</tr>
<tr>
<td>Curd firmness ↑</td>
</tr>
<tr>
<td>Renneting time ↓</td>
</tr>
<tr>
<td>Curd hardening ↑</td>
</tr>
<tr>
<td>Resistance to the cutting ↑</td>
</tr>
<tr>
<td>Syneresis during cutting ↑</td>
</tr>
<tr>
<td>Losses in the whey ↓</td>
</tr>
<tr>
<td>Reactivity to the heating ↓</td>
</tr>
<tr>
<td>Dehydration – cohesion ↑</td>
</tr>
<tr>
<td>Cooking temperature ↑</td>
</tr>
<tr>
<td><strong>Cheese on table</strong></td>
</tr>
<tr>
<td>Elasticity ↑</td>
</tr>
<tr>
<td>Firmness ↑</td>
</tr>
<tr>
<td>Permeability ↑</td>
</tr>
<tr>
<td><strong>Cheese</strong></td>
</tr>
<tr>
<td>Yield ↑</td>
</tr>
<tr>
<td>Quality ↑</td>
</tr>
</tbody>
</table>

↑, Increase; ↓, decrease.
of the production season (ewe’s cheese), and this factor contributes to the refining of the dairyman’s ‘art’, and above all avoids uniformity of industrial processes. Technological aids can, of course, be of some help to the dairyman, but they are not always available or economically suitable, and they must be within the regulations for the specific cheese.

Bacteriological traits

Finally, a major factor in the success of PDO cheeses is the correct management of bacteriological traits; Martin & Coulon (1995) have emphasised that variations in organoleptic qualities are mainly due to technological factors, and among them to the kinetics of acidification during cheesemaking. A good example of this factor can be the influence of the degree of acidification on the process technology, as described by Mariani & Pecorari (1987) for Parmigiano-Reggiano: (a) when renneting is mainly of the enzymic type, the micelle complex maintains its properties, the losses of Ca are moderate and consequently the curd has modified and the Ca losses are more substantial. Thus, the curd is less elastic and firm, so whey drainage is reduced.

The microflora and its management is therefore of great importance as previously noted (Alais, 1984); this fact has been well known since the beginning of the century when Gorini (1907; cited by Mucchetti et al. 1998) suggested that the differences between the two Italian Parmesans (Parmigiano-Reggiano and Grana Padano) were due to the effects of forage, climate and technology on the microbes. The effects of these and other local factors on the microbial activity in milk has been confirmed recently (Limsowtin & Powell, 1996). This finding supports the idea that typical cheeses require indigenous microflora (Bouton & Grappin, 1995; Demarigny et al. 1997; Freitas & Malcata, 2000) or, preferably, a natural whey culture such as that used for Parmigiano-Reggiano and buffalo’s milk Mozzarella (Coppola et al. 1990; Mucchetti et al. 1998). The importance of the starter is particularly relevant when milk lacks useful bacteria or has too many undesirable (and sometimes pathogenic) microbes; it modulates the fermentation and reduces defects in the cheese (Mucchetti et al. 1998; Bottazzi et al. 1999). However, it is debatable whether these natural starters are really linked to the general environment (Bouton & Grappin, 1995; Rossi, 1998) or simply to the technological process (Mucchetti et al. 1998; Bottazzi et al. 1999); in the latter case selection of these starters has to consider the process technology and not the geographical region.

It can be supposed that both factors are important, as recently shown for the Bergkäse; i.e. in cheese produced with raw compared with pasteurised milk there is different development of organic acids during maturation (Eliskases-Lechner et al. 1999), different lipolytic activity (Ginzinger et al. 1999b) and better intensity of the typical flavour, and therefore the use of raw milk is essential for the production of authentic Bergkäse (Ginzinger et al. 1999a). Similar conclusions have been suggested by Cavaza et al. (2000) in relation to typical mountain cheeses. In fact, the general environment, and particularly the botanical composition of the pasture, have been recognised as a factor in flavour enrichment of cheese not only per se (terpenes, sesquiterpenes etc.), but also for compounds of microbial origin (Buchin et al. 1999).

Microbial aspects of cheese production are not only related to ripening success, but also to health issues, both of which are of great importance as we consider that in milk, and for different reasons, the following undesired bacteria can be recognised: pathogens, like Listeria, Brucella, Staphylococcus aureus, Mycobacterium tuberculosis, some Enterobacteriaceae, etc.; those that are unsuitable for cheese production like clostridia spores, or simply competitors for the desired LAB.

When raw milk is used, as generally occurs with PDO cheeses, the bacterial quality can be optimised according to the following procedures: prophylactic and hygienic means of eradicating human transmissible diseases, to minimise faecal contamination and to guarantee the cleanliness in the milking plant; reduction in active bacteria and spores through the physical effect of fat globules rising during natural creaming as previously described. With particular reference to the spores of clostridia, another possible method is bactofugation, as suggested in the past and more recently by Bottazzi & Corradini (1987). Also useful to improve the bacterial quality is inoculation of natural starters (i.e. acidified whey as used for buffalo’s milk Mozzarella, Parmigiano-Reggiano, Grana Padano etc.); moreover with respect to Grana Padano cheese, lysozyme can also be added as an anti-clostridial agent because of its lytic activity, but mainly because it is considered a normal component of milk, particularly of human milk (Carini & Lodi, 1997). There are in fact attempts to obtain transgenic cows for lysozyme expression in milk (Bawden et al. 1994, cited by Karatzas & Turner, 1997).

Some of these procedures are part of the technology, but have some links with milk traits; starter production, particularly natural starter production, is also influenced by fermentation ability and original bacteria contamination. Thus, another important factor in the variation between milks is the so-called bacterial fermentation ability or acidification rate (Marth, 1974), particularly in the case of raw milk. Raw milk is in fact also used as a special feed for the purpose of reducing enteric infections (Reiter, 1978; Group F19-IDF, 1991); in this case different anti-microbial mechanisms are involved, one of which is nutrient availability, particularly small-molecular-weight N compounds (amino acids, nucleic acid derivatives), essential fatty acids, trace elements and vitamins (Rašic & Kurmann, 1978). Nutrient availability could explain the positive effect of yeast extract on lactic fermentation of raw or pasteurised milk (Chazaud & Larpont, 1980), and also the positive effect of Fe³⁺ and Zn²⁺ that could affect the redox potential (Maianti et al. 1995), as well as the effects of the addition of small amounts of Mn²⁺ which suppress the inhibitory effect of aeration on Leuconostoc mesenteroides (Bellengier et al. 1997); however, in trials with an amino acid mixture or with cysteine supplements there was no increase in the acidification rate of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus (MG Maianti, unpublished results). In similar studies with amino acids, Dave & Shah (1998) obtained an improvement.
with *Lactobacillus acidophilus*, *Bifidobacteria* spp. and *S. thermophilus*. A possible explanation for this difference could be the proteolytic activity of *L. delbrueckii subsp. bulgaricus* that allows the release of essential amino acids.

A second important group of bacterial activity modifiers is that of known anti-microbial agents: lysozyme, lactoferrin, lactoperoxidase system, immunoglobulins, vitamin-binding proteins. Their effects on the inactivation of pathogens or saprophytic micro-organisms, as well as on the LAB, need further studies (Group F19-IDF, 1991); nevertheless, the results of our trials relate to one of these mechanisms, i.e. that antioxidants (vitamin C and E) improve raw-milk acidifying activity while heat treatments show different effects according to temperature (Fig. 3; Bertoni *et al.* 1992; Maianti *et al.* 1993).

It should, however, be emphasised that some complex interference could occur because: colostrum, which is very rich in immunoglobulin and lactoferrin (Ye & Yoshida, 1995), is acidified more quickly than milk (Maianti *et al.* 1992); mastitic milk, with a high leucocyte content, is also better acidified (MG Maianti, unpublished results); perhaps because, according to Okello & Marshall (1986), the increase in somatic cells results in stimulation of *S. thermophilus* due to higher proteolysis; lactation phase modifies the acidification rate, with the lowest values in the 2nd–4th month of lactation (Calamari *et al.* 1986; Maianti *et al.* 1992); milk from small farms has, in general, better rates (L Calamari, unpublished results).

It is worthwhile remembering that a higher rate of acidification after the coagulation phase during cheese-making (Table 3) plays an important part in the control of gas-producing microflora, and could be a useful way of reducing the blowing problems of milk obtained from silage-fed cows (Bottazzi *et al.* 1992). The importance of this statement is increased when it is considered that since the early 1970s we demonstrated that milk from maize-silage-fed cows is slowly fermentable (Battistotti & Bertoni, 1974) and also that the milk of large herds, where more concentrates and silage are fed, are characterised by slower acidification (Calamari *et al.* 1986).

Finally, we want to highlight the link between the renneting properties and the microbial activity in the very early stage of cheese ripening (24 h), which in turn influence the global maturation of cheese. According to Mocquot *et al.* (1954; cited by Remeuf *et al.* 1991), slow-renneting milk produces a soft gel with poor drainage, whereas fast-renneting milk produces a solid gel with good drainage and consequently curd with a satisfactory texture and moisture content. This process is important in order to obtain a structurally homogeneous cheese mass, uniformly and adequately dehydrated throughout, which is a fundamental condition for the normal start of fermentation and for the optimal development of the whole cheese ripening process (Mariani & Battistotti, 1999).

**How to obtain specific milk for speciality cheeses**

PDO cheeses are a considerable resource in Italy (as well as in other countries), where approximately 50 % of the milk produced is processed for this purpose at significantly higher prices than imported milk. This policy also ensures continued animal production in marginal or problem areas, which in turn ensures a population presence in these areas. The social and economic consequences are quite obvious, and they are made possible because the typical cheeses, as well as other similar foods, have particular sensory traits that can provide customer satisfaction and are acceptable despite the higher price.

These particular traits cannot be obtained under other environmental–production conditions for two main reasons (Corradini, 1992): some aspects of milk quality are linked to that animal production system; the ripening, generally...

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**Fig. 3.** Acidification rate of raw (●●●) and pasteurised milk (80°C for 10 min; ■■■) during lactation. Sterilised milk has a ‘constant’ acidification rate of 10–11 ml NaOH n/4 per 50 ml.
prolonged, is affected by the interaction of previous milk traits (specific) and the applied technology (not specific). Furthermore, the environment for a good ripening stage can also be quite specific and not reproducible.

It can be easily understood that the required traits of milk may change to a great extent according to the cheese; however, they generally relate to fat and protein contents, the renneting ability, the microbial population of useful or possibly harmful types and its growth potential, and also the creaming activity (if the milk is naturally skimmed). Furthermore, because the permitted milk treatments for PDO cheeses are few and mild, these traits must be an original feature of the milk itself. The critical stage for the real success of cheeses can be considered to be the achievement of optimal curd properties, which determine the retention of fat and moisture and, thus, cheese yield and composition (Green & Grandison, 1993). Milk coagulation is affected by many aspects of milk itself, i.e. high protein (or preferably casein) content, low pH (or good titratable acidity) and a normal somatic cell count, because mastitis causes casein hydrolysis. All these variables are in fact included in the criteria used to establish the price of milk (Table 4), and are also included in another variable describing milk quality, the lactodynamographic index which assesses rennet coagulation properties, including clotting time, clot firming rate and curd firmness (Zannoni & Annibaldi, 1981).

Microbiological quality is the second most important aspect of milk quality for many reasons: risks of pathogenic forms that can be avoided using prophylactic, therapeutic and hygienic methods; contaminating bacteria, coming from teat and udder surfaces, milking and collecting machines, milkers, etc., that can be subdivided into desirable and undesirable microflora. While the undesirable microflora, i.e. propionibacteria, enterobacteria (particularly coliforms) and clostridia spores, should be as low as possible (Table 4), the desirable microflora or LAB are undoubtedly useful for the required extent of acidification to facilitate the rennet response, and for the rapid acidification of the curd. However, an excess of LAB can shift the coagulation towards a lactic–rennet type which can alter the moisture content of the curd (Mariani & Pecorari, 1987). Thus, the total microflora should not exceed 200,000–400,000 colony-forming units/ml (for cheese with a ripening period longer than 60 d), but should not be too low because the LAB in the milk represent one of the links between the cheese and the local environment (Mariani & Battistotti, 1999).

The quality of the milking procedure is of major importance with respect to reducing contamination of milk; however, a natural means of removing bacteria from milk is achieved through the creaming activity, in that the bacteria and spores are trapped by the floating fat globules so that the level of contamination of the vat milk can be two to four times lower. Furthermore, many undesirable bacteria (coliforms and clostridia) are competitively inhibited when the LAB (added with natural starters) are grown quickly.

Thus, it can be concluded that a milk can maximise successful PDO cheese production if: the protein content is high and can ensure optimal renneting properties (appropriate titratable acidity, low somatic cell count and good lactodynamographic indices); the fat content is high and the fatty acid composition is appropriate for the release of good flavours and for effective creaming activity (if required); the microbial population is pathogen ‘free’ and the desirable bacteria are dominant, but not in excess, and can easily grow in the milk. Moreover, extra value for PDO cheese is guaranteed if the animal production system leads to the production of milk with a ‘high’ content of the chemicals which give the individual aroma to the cheese (i.e. aromatic herbs, fatty acids from fat hydrolysis etc.).

The factors affecting these characteristics of milk are numerous, and some of them cannot be fully controlled; therefore, milk composition and quality can change to some extent. Nevertheless, success depends on these variations not exceeding the skill of the cheesemaker, and therefore not
requiring technological adaptations beyond the established rules for any specific cheese. The present paper will not consider the required traits of milk for any particular PDO cheese, but we are confident that the ‘ideal’ features of milk for Parmigiano-Reggiano cheese are similar to those needed for any PDO cheese shown in Table 4.

At this point it would seem worthwhile to give some general advice on how to obtain the best milk at its origin, and in particular how to avoid the so-called disgenic milks (low titratable acidity, slow and difficult coagulation, low acidification rate and inadequate coagmeling activity; Corradini, 1992).

Bertoni (1996a) identified three classes of milk traits, grouped according to the ease with which they can be modified:

(a) those that are unchangeable, i.e. species and breeds that have already been established and can be monitored (buffalo for buffalo’s milk Mozzarella and sheep, goat or cattle for other speciality cheeses; in Italy the breed for Fontina is mandatory, i.e. Valdostana breeds);

(b) those that cannot be modified or can only be modified slowly and with difficulty, i.e. age, stage of lactation, season and climate, and genetic background. Their effects can be managed to some extent, i.e. age and lactation stage can be overlapped with a mixed herd (animals of different ages and lactation stages);

(c) those that can be modified by human activity, such as breeding systems (intensive or extensive) in which many aspects can be changed, including welfare conditions, health conditions (with special attention to the mammary gland and therefore milking techniques and reduction in mastitis), and finally the feeding, that in part could be influenced by the breeding system (i.e. seasonal grazing), but is also affected by the forage storage system, the type and amount of concentrates and the method of feed administration (total mixed ration, autofeeders etc.).

Some of these factors are fairly rigidly established in protocols (i.e. species, breed, breeding and feeding rules etc.), while others can only be included in a definition of better milk production through aid to the farmers to manage their farming system and as a result of accurate monitoring of farms and of milk characteristics (Dubof et al. 1997).

Genetic background

Genetic properties can be considered a changeable factor for the breed of choice, as the levels of some casein fractions in milk can be increased, thus improving renneting properties. Russo & Mariani (1978a,b) demonstrated the positive influence of the BB genotype on both κ-casein and β-lactoglobulin. This relationship has been studied extensively in Italy with respect to higher yield and quality of Parmesan cheese produced using milk from BB cows (Losi & Mariani, 1984; Aleandri et al. 1990).

Moreover, some breeds can adapt readily to grazing in marginal areas, with lower milk yield, but higher milk quality for PDO cheese production (e.g. the Valdostana breed for Fontina cheese).

Using selection in order to increase the fat and protein contents of milk (Seegers & Grimard-Ballif, 1994) does not require any comment, except the acceleration that will be possible through the introduction of biotechnological techniques which increase the efficiency of selection using the study of quantitative trait loci in the genome of the animals.

Physiological factors

Although age is not so important, the stage of lactation has an enormous effect on milk composition and properties (fat and protein contents, renneting, creaming and acidification rate are less favourable between days 60 and 120 of lactation, with gradual variations before and after this period; Maianti et al. 1992). Of course, these factors cannot be appreciably modified; they particularly affect semi-intensive systems that are linked to the seasonal availability of pasture. Nevertheless, the changes are always within a substantial suitability range, resulting in some variation in the cheese (Coulon et al. 1997, 1998), and requiring different technology to maintain a good product (Martin & Coulon, 1995).

Season and climate

These factors are unavoidable, but it should be noted that longer daylight hours are associated with a tendency to higher milk yields and lower fat and protein contents (Coulon, 1994). However, particularly for typical cheeses linked to extensive or semi-intensive systems, the natural feed resources (as well as the stage of lactation) may change substantially, with effects on many milk traits. In addition, climate can change according to season, with a fluctuating effect on the milk yield and composition. Nevertheless, many of these effects are small. Only for high temperatures (a long period above 28–30°C during the day combined with high humidity) have there been well-defined studies (Bernabucci & Calamari, 1998; Calamari & Mariani, 1998) which have shown that heat stress causes a sometimes dramatic yield reduction, while fat and protein contents are less affected. Moreover, the cheesemaking properties are negatively affected, with a lower titratable acidity and an impairment of the rheological properties. Considering that some microbiological properties are less easy to check, the risks of higher quantities of waste cheese are greatly increased in the warm season. However, in intensive systems a considerable improvement can be achieved using shade, artificial ventilation with or without water nebulisation, and other devices aimed at reducing body temperature (Cappa, 1998).

Milkling

Milking is a well-known factor of milk yield and quality. Proper maintenance and cleanliness of the equipment, and a milking procedure in which attention is given to physiological and hygienic aspects guarantee the expected milk yield and composition, and also an acceptable level of microbial contamination. Also linked to these considerations is the risk of mastitis, which can affect the cheesemaking properties not only directly (there is a negative relationship between somatic cell count and the rheological properties of milk), but also indirectly (the risks of inhibitory residues).
Health status and animal welfare

These factors can be discussed together because of their strong interactions (Bertoni, 1999a). In addition to mastitis, there are many disease situations that are less compatible with milk production and its suitability for cheese production, i.e. parasitism, lameness, metritis, inflammations etc. These factors can reduce milk yield and most of all they impair the renneting properties (Bertoni, 1995a); furthermore, it appears that the inflammatory cytokines (interleukins 1 and 6 and tumour necrosis factor) could have a great effect (Bertoni et al. 1996), but further studies are required. It is obvious that impaired health status causes a reduction in well-being and is termed ‘disease stress’; nevertheless, there are many other stress factors, in addition to the previously mentioned climatic conditions, e.g. trauma, fear, pain and overcrowding. Acute stress is known to modify milk composition, by lowering fat content (Varner et al. 1983) and increasing titratable acidity (Bertoni et al. 1985). Chronic stress factors combine to cause digestive problems, diseases and reduction in fertility (Bertoni, 1999a), which also have negative effects on milk quality (Calamari et al. 1992).

Feeding

Feeding is a factor of major importance and liable to human intervention. Fat content is raised when rumen fermentation favours acetic (and butyric) acid, and also when more fatty acids (saturated and/or protected from the rumen) can be absorbed. It has been reported (McClymont & Vallance, 1962) that insulin level could be responsible for milk fat variations, by preferential partitioning of fat precursors to the mammary gland or body tissues (Hart, 1983). Subsequently, Kalscheur et al. (1997) stated that the results of new studies do not preclude the theory that the trans-C18:1 FA (fatty acids) that are formed in the rumen are associated with milk fat depression; this theory has been strongly supported by McGuire et al. (1995) and Griinari et al. (1998). Fig. 4 reviews the factors affecting milk fat content, and confirms that in order to obtain a good milk fat content the previously well-known rules regarding rumen fermentation have to be complemented with a careful distribution of meals (Bertoni, 1999b) and appropriate use of fat supplements (since these can also modify the fatty acid content, and therefore the cheese characteristics).

The optimal creaming globules are those richer in oleic acid (early lactation, recombinant bovine growth hormone-treated animals, tallow supplements etc.; Piccioli Cappelli et al. 1990) and those with a lower aspartate transferase content. The higher of this enzyme indicates a higher presence in the fat globule of cytoplasm, a watery material that would reduce the density difference between the two milk phases (F Romanello, unpublished results).

With respect to milk protein content, it is well known that high-energy diets are more effective in increasing protein content than high-protein diets (Kaufmann & Hagemeister, 1987). According to Bertoni (1996b), the higher insulin level associated with a high-fermentable diet would modify the partition of amino acids between catabolism and mammary synthesis in favour of the latter. However, the balance with lactose synthesis is also important because a simultaneous increase in protein and lactose leads to higher milk and protein yields, but the contents remain almost constant. In addition to an adequate (not excessive) protein supply, a high-fermentable diet increases the protein content of milk, particularly after the 4th–5th month of lactation (E Trevisi, unpublished results).

The main role of proteins, particularly for the PDO cheeses, is their renneting ability (more protein does not necessarily mean better coagulation). Changes in diet affect protein content and also titratable acidity (Calamari et al. 1983), and consequently rennetability of milk. However, this relationship appears to be more complex, and studies

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**Fig. 4.** Main causes of changes in milk fat content. Dietary factors (and suggested mechanism) can positively (+) or negatively (−) influence the mammary synthesis of fatty acids or the availability of triacylglycerols. ↑, High values; ↓, low values; SFA, saturated fatty acids. (From Bertoni & Piva, 1999.)
involving substantial feeding changes have shown an improvement in the coagulation ability when animals are transferred from a winter diet to a spring diet (Grandison et al. 1984; Coulon et al. 1988), and when a shortage of grass results in the use of additional concentrates (Mackle et al. 1999; O’Brien et al. 1999).

On the other hand, there are studies showing inconsistency in the relationship between feeding and rennetability (Martin & Coulon, 1991; Coulon et al. 1995; Agabriel et al. 1999). However, our field trials have suggested the possibility that there is a link between feeding and coagulation other than due to protein content; the rheological properties of milk were improved when the diet was modified to reduce excess fermentability and to supply some rumen and intestinal buffers (Calamari et al. 1992).

With respect to the microbes in milk, we have previously shown that contamination is of major importance, particularly for some bacteria spores. Nevertheless, the suitability for bacterial growth is a property of milk that can modify the quantity and type of bacteria. All these aspects can be affected by feeding factors; unfortunately, there is insufficient experimental evidence.

While the ‘undesirable’ bacteria can be neutralized with a good natural starter, at least for the cooked and hard cheeses, the main problem is associated with the spores of Clostridium spp. (C. sporogenes, C. butyricum and particularly C. tyrobutyricum) which can cause the blooming of cheese (Bottazzi et al. 1982). The most studied of these species is C. tyrobutyricum, which causes late blooming and is particularly influenced by the feeding regimen: i.e. the type and amount of silage in the diet (Baraton, 1985).

Depending on the cheese, the deleterious effects of spores can be avoided by the use of technological procedures described earlier, i.e. better creaming activity or bactofugation, or treatment with lysozyme, nitrates or bacteriocins (Thuault et al. 1991); however, we must aim to reduce the number of spores in milk to a safe level of less than 200 spores per litre.

The spores are in the faeces, and any means of achieving a clean udder (buildings, bedding, available area, season etc.) and high milking hygiene (parlour, washing and drying teats etc.; Rasmussen et al. 1991) is of great importance (Pecorari, 1984). Nevertheless, the number of spores in the faeces can be modified by a number of factors, including silage in the diet, since the spores are in the soil and can germinate and grow in the silage (Leibensperger & Pitt, 1987; Spoelstra, 1990). However, the clostridia contamination of silage can be quite variable (Emaldi et al. 1977), and the presence of spores in faeces can be affected by factors other than silage contamination (Bani et al. 1991; Colombari & Fantuzzi, 1991).

The number of spores in milk is a major factor in late blooming of hard cheeses; however, other factors such as chemico-physical traits of the curd can modify their germination potential (Pecorari, 1984). This factor could explain the higher proportion of wastage in different areas of Grana Padano production: i.e. higher in Cremona district v. Picenza district despite similar breeding conditions and similar or lower spore numbers in milk (B Battistotti, personal communication). We have previously shown that renneting properties and milk fermentability can contribute greatly to the features of curd (Remeuf et al. 1991) which affect spore germination; also relevant are the previously discussed causes of differences in acidification rate of milk.

Furthermore, we have also suggested that feeding regimens could influence, directly or indirectly, the appearance of mastitis (and other diseases) but studies are very difficult to conduct (Bertoni, 1995b). Thus, the effects of feeding on the cheesemaking properties of milk may be greater than previously reported (see Martin & Coulon, 1995; Coulon et al. 1997; Mordenti & Zotti, 1998).

Feeding regimens based on pasture can be of great importance with respect to some of the factors discussed, and also to the presence in milk and cheeses of volatile organic compounds that can significantly contribute to the PDO cheese aroma (Moio et al. 1996; Fedele et al. 2000), and in relation to antioxidant substances (Pizzoferrato et al. 2000). Moreover, forage, particularly that of local origin, can substantially affect the microbial population, and therefore the cheese quality (Pirisi et al. 1999), as well as being the link to the locality (Mordenti & Zotti, 1998; Cavazza et al. 2000), which is one of the most distinctive traits of the PDO cheeses.

Conclusions

It seems clear to us that PDO cheeses have particular traits, and that they are made possible by some specific milk characteristics and to the ‘art’ of the cheesemaker. Furthermore, it appears that the increase in available information could lead to substantial improvement in milk quality and innovative technologies to facilitate the dairyman’s job and to increase his success rate.

It is interesting to ascertain how the findings of scientific research can explain what centuries ago was written about the ‘Lodigiano’ cheese (now Grana Padano): ‘The cheesemaker begins his day by asking the herdsmen: which kind of good forage has been fed; whether the whole herd was treated for any reason; whether milking was normal and without anxiety factors. Then he examines the milk with his senses . . . Only after satisfactory answers is milk processed to . . . ‘Lodigiano’. Since then, the success of cheese processing has been based on experience and art (and perhaps St Lucio, the dairyman’s patron in Italy).’ Thus, there is no doubt that today science and technique can help the PDO cheese production system, but this must be done without losing their distinctive characteristics. In other words, can innovation (at farm and dairy level) be accepted? We agree with Laureano (2000) who, having shown a relationship between traditional animal breeding and the Mediterranean landscape, suggests that tradition does not exclude innovation.

Some difficulties could arise concerning the criteria to be used to judge the acceptability of innovation. However, a good approach is that suggested by Morand-Fehr et al. (1998): ‘In the future, as was the case in the past, typical dairy and meat products will only be able to maintain their markets or develop them, unless they are capable of holding their commercial ground and adapting to the market’s needs and demands without losing their specificity, originality and authenticity’.

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However, it is now also time for a Quality Liability System which could guarantee traditional traits linked to the locality and cultural roots, as well as good quality features based on the standards required to satisfy consumer expectations (Panari, 1996). Nevertheless, the unique features of these production systems have to be taken into account to avoid any excessive changes that could contribute to the loss of these “inimitable treasures”.

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