The Microbiology of Traditional Hard and Semihard Cooked Mountain Cheeses

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ABSTRACT Traditional cheeses originate from complex systems that confer on them specific sensory characteristics. These characteristics are linked to various factors of biodiversity such as animal feed, the use of raw milk and its indigenous microflora, the cheese technology, and the ripening conditions, all in conjunction with the knowledge of the cheesemaker and affineur. In Europe, particularly in France, the preservation of traditional cheesemaking processes, some of which have protected designation of origin, is vital for the farming and food industry in certain regions. Among these cheeses, some are made in the Alps or Jura Mountains, including Comté, Beaufort, Abondance, and Emmental, which are made from raw milk. The principle of hard or semihard cooked cheese, produced in the Alps and Jura Mountains, was to make a product during the summer—a period during which the animals feed more and milk production is high—with a shelf life of several months that could be consumed in winter. Today, these traditional cheeses are produced according to a specific approach combining science and tradition in order to better understand and preserve the elements that contribute to the distinctiveness of these cheeses. To address this complex problem, a global approach to the role of the raw milk microflora in the final quality of cheeses was initially chosen. The modifications resulting from the elimination of the raw milk microflora, either by pasteurization or by microfiltration, to the biochemistry of the ripening process and ultimately the sensory quality of the cheeses were evaluated. This approach was achieved mainly with experimental hard cooked cheeses. Other types of traditional cheese made with raw and pasteurized milk are also considered when necessary. Besides the native raw milk microflora, traditional lactic starters (natural or wild starters) also participate in the development of the characteristics of traditional hard and semihard cooked mountain cheeses. After an initial description, their roles are described, mainly for Comté.

Traditional cheeses originate from complex systems that confer on them specific sensory characteristics (distinctiveness of the cheese). These characteristics are linked to various factors of biodiversity such as animal feed, the use of raw milk and its indigenous microflora, the cheese technology, and the ripening conditions, all in conjunction with the cheesemaker’s and ripener’s practices (know-how). In Europe, particularly in France, the preservation of traditional cheesemaking processes, of which some have a protected designation of origin, is vital for the farming and food industry in certain regions. In France, the production of raw milk cheese (excluding farmstead) represents 15% of the entire production of mature cheese, which is equivalent to approximately 170,000 metric tons.

Among these cheeses, some are made in the Alps or Jura Mountains, including Comté, Beaufort, Abondance, and Emmental, which are made from raw milk. These are big hard or semihard cooked cheeses: Comté weighs 32 to 45 kg (Fig. 1), Beaufort 20 to 70 kg (Fig. 2), Emmental 60 to 80 kg, and Abondance 7 to 12 kg. In the past, the primary objective of producing cheese in these
areas was to provide a source of protein during the winter, when the cows fed less and trade was limited by snow. The principle of hard or semihard cooked cheese, produced in the Alps and Jura Mountains, was to make a product during the summer—a period during which the animals feed more and milk production is high—with a shelf life of several months that could be consumed in winter. The only way to make such a product was to make large cheeses as dry as possible in order to significantly slow down the aging process (1). Today, these traditional cheeses are produced according to a specific approach combining science and tradition in order to better understand and preserve the elements that contribute to the distinctiveness of these cheeses. This specific approach is based on the respect of the diversity of the physicochemical composition and the milk microbiota, with the objective of modifying the raw material as little as possible so the sensory characteristics of the cheese truly reflect the land where the milk was produced (2).

These traditional cheeses have manufacturing characteristics in common, such as the following.

1. Milk production. Cattle feed must be natural and free of genetically modified organisms. Silage (fermented feed) is prohibited; this prevents or reduces the risk of the presence of butyric acid bacteria in milk and cheese, which cause late blowing.

2. Cheesemaking

a. Raw milk must be collected daily and processed within 24 h, which reduces the population of psychrotrophic bacteria in milk and cheese responsible for off-flavor defects. Additives, preservatives, and coloring agents are banned.

b. Use of a copper vat. An unpleasant taste develops in this type of cheese when using a stainless...
steel vat. Copper controls the activity of propionic acid bacteria and consequently propionic acid fermentation; the risk of gas overproduction is thus reduced (3).

c. Acidification is limited during draining (pH ≥ 6.45) and substantially during pressing, which allows a high quantity of minerals (i.e., calcium) to be retained in the cheese.

3. Ripening
   a. The wheel of cheese is pressed for several hours and then placed on spruce planks. The microbial consortium is stable on spruce planks, which has an impact on undesirable microorganisms, i.e., Listeria monocytogenes (4).
   b. The cheesemaker turns the wheels of cheese over and rubs them with salt and morge (a sticky film on the surface of the rind containing surface microorganisms such as Arthrobacter or Brevibacterium linens).

A major characteristic of raw milk cheese is the diversity of microorganisms in terms of microbial groups (genera) as well as species and even strains. This diversity of microorganisms, important agents in the cheesemaking process, is not generally found in cheese made with pasteurized milk. It is recognized that this microbial diversity is responsible for the diversity of flavors and aromas found in traditional and artisanal raw milk cheeses. Due to the diversity of the microflora and its evolution throughout the ripening process, it is difficult to study one specific microorganism, not to mention the effects of possible interactions between the different microfloras. The conditions of the medium, which change throughout the ripening process, can “awaken” the activity of microorganisms that have remained inactive at a given time during ripening. All these phenomena contribute to the complexity of studying the ripening process of cheese.

To address this problem, a global approach to the role of the raw milk microflora in the final quality of cheeses was initially chosen. The modifications resulting from the elimination of the raw milk microflora, either by pasteurization or by microfiltration, to the biochemistry of the ripening process and ultimately the sensory quality of the cheeses were evaluated. This approach was achieved mainly with experimental hard cooked cheeses. Other types of traditional cheese made with raw and pasteurized milk are also considered when necessary.

Besides the native raw milk microflora, traditional lactic starters (natural or wild starters) also participate in the development of the characteristics of traditional hard and semihard cooked mountain cheeses (5). After a description of this, their role is described mainly for Comté cheese.

To go even further in the determination of the role of the microflora, it is important to specify the nature of the microorganisms which make up the microbial ecosystem of raw milk cheeses and to monitor their development from the milk to the fully ripened cheese. This work was carried out on a raw milk cheese with a protected designation of origin, Comté. Only microorganisms in the heart of the cheese were studied. Other types of traditional cheese have also been considered when necessary.

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This chapter is based essentially on the results obtained by the INRA research unit for dairy technology and analyses in Poligny, Jura, France, alone or in collaboration.

**ROLE OF NATURAL MILK MICROFLORA IN BIOCHEMICAL AND SENSORY CHARACTERISTICS OF CHEESES**

During the ripening of cheese, various biochemical transformations of the caseins (proteolysis, i.e., degradation of the casein matrix into a selection of peptides and free amino acids), fat (lipolysis, i.e., liberation of free fatty acids), amino acids, citrates, and lactates (fermentation) take place. These transformations lead to the formation of volatile compounds that are extremely important in the development of flavor compounds. The cheese will thus “mature” and develop its organoleptic characteristics.

Numerous phenomena are involved in these biochemical transformations, which depend on the initial physicochemical and biochemical compositions of the milk, the cheesemaking process (including milk preparation processes [milk maturation, heat treatment, etc.]), the manufacturing auxiliaries (lactic starters, coagulating enzymes, etc.) involved in the various stages of coagulation and draining, the physical technological factors, the physicochemical composition of the curd, its
structure, and the ripening conditions. The equilibrium between these different parameters can vary and be more or less stable according to the dairies, without our really being able to explain why.

During ripening, cheese behaves like a fermentor in which the microbial populations from the milk, the starter culture, and the environment develop and lead to biochemical modifications.

Transformations of the constituents of the curd will thus enable the cheese to acquire a characteristic texture (mainly as a result of proteolysis) and a rich and characteristic taste as a result of proteolysis, fermentation, lipolysis, and various biochemical transformations such as decarboxylation and deamination of amino acids (6, 7).

To study the role of the raw milk microflora on the biochemical and sensory characteristics of cheese, the experimental cheesemaking plant at INRA in Poligny was used (Fig. 3). At this plant it is possible to make model cheeses, leading to a better comprehension of the phenomena occurring in cheeses.

Levels of the Raw Milk Microflora and Biochemical and Sensory Characteristics of Cheeses

In a study by Beuvier et al. (8), microfiltration (passage of skim milk through a ceramic membrane with 1.4-μm pores) and pasteurization (72°C for 30 s) of the milk led to decreases in the total viable count of 99 and 90%, respectively, to reach levels of around 500 and 5,000 CFU/ml. The raw milk total viable count was adjusted to approximately 50,000 CFU/ml by addition of enough retentate, obtained by microfiltration, to raw skim milk and raw cream. The direct epifluorescence filter technique, a rapid microscopic method enabling enumeration in 30 min, was used to determine the population of microorganisms in the retentate. A fourth procedure was included which consisted of adding the microfiltration retentate containing the raw milk microflora to pasteurized milk; the total viable count was also adjusted to approximately 50,000 CFU/ml. Cheeses of 1 kg were then made from the different milk samples, wrapped in wax in order to overcome the problem of a surface microflora, and ripened for 3 months.

At the end of ripening, the mini cheeses made with raw milk and pasteurized milk with added retentate had a more intense aroma and were sharper than the other two types of cheese. These characteristics were correlated with higher populations of facultative heterofermentative lactobacilli (10⁸ CFU/g) (Fig. 4), propionic acid bacteria (10⁸ CFU/g), and enterococci (10⁶ CFU/g). These cheeses contained higher levels of small peptides and amino acids (estimated from the phosphotungstic acid-soluble nitrogen fraction) and also acetic, propionic, and isovaleric acids. A few differences were observed between the cheeses made from pasteurized or microfiltered milk, and they were also very different from the other two types of cheese. The cheeses made from pasteurized milk had a lower pH than the cheeses.
made from microfiltered milk and contained a higher proportion of γ-caseins due to the activation of plasmin (native milk enzyme). Furthermore, the cheeses made from pasteurized milk were more acidic. The addition of the raw milk microflora (retentate) to the pasteurized milk helped restore part of the biochemical and sensory characteristics of the raw milk cheeses evaluated in this study (Fig. 4). Finally, the first principal-component analysis axis, which explains 55% of the variation in the composition of the cheeses, enables the cheese with a milk microflora (R and P + bact) to be distinguished from the cheeses made with the microbiologically purified milk (P and MF). The second axis, which explains only 17% of the variation in the composition of the cheeses, differentiates the cheeses made with heated milk (P and P + bact) from those made with unheated milk (R and MF), excluding the pasteurization of the cream (approximately 10% of the milk used to make the cheese) in the case of the manufacture with microfiltered milk. As the distribution of the cheese samples in Fig. 4 shows, the cheeses made with milk containing the native microflora have a wider variety of characteristics (more heterogeneous distribution).

In another study on a hard cooked cheese, when the raw milk microflora was eliminated using microfiltration (9) whilst varying the inoculation with lactic acid bacteria and after 4 months of ripening, a decrease in the intensity of the taste (5.1 instead of 5.9/10) and the “distinctiveness” (3.6 instead of 4.9/10) was observed in comparison with the same cheeses made with raw milk.

These results were confirmed on experimental semi-hard Morbier-type cheeses (10). The raw milk cheeses had a more intense aroma than the pasteurized milk cheeses and were characterized by animal, spicy, garlic, rancid, and sharp aromas. The pasteurized milk cheeses differed, with fresh milk and fruit aromas. These sensory differences were compared with the differences in the profiles of the aromatic molecules. The raw milk cheeses contained more sulfur compounds and alcohols, whilst the pasteurized milk cheeses were richer in ketones (Fig. 5).

The effects of the indigenous raw milk flora on the chemical composition of Bergkäse (an Austrian alpine hard cooked cheese) were evaluated by means of repeated cheesemaking experiments using raw or

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**FIGURE 4** Distribution of physicochemical, microbiological, and flavor criteria (additional variables in italics and bold) in R (raw), P (pasteurized), MF (microfiltered) and P + bact (pasteurized plus microorganisms contained in retentate) milk using principal-component analysis. MesoLb, mesophilic lactobacilli; Entero, enterococci; PAB, propionibacteria; C2, acetic acid; C3, propionic acid; iC5, isovaleric acid; PTA, phosphotungstic acid-soluble N; CN, casein. Adapted from reference 8. doi:10.1128/microbiolspec.CM-0006-2012.f4
Pasteurized milk \((11, 12, 13)\). Proteolysis was affected by milk pasteurization. Degradation of \(\alpha_s1\)-casein was found to be delayed in cheeses made from pasteurized milk due to effects resulting from the heat treatment and partial elimination of the raw milk flora. An enhanced cleavage of \(\beta\)-casein was also found in these cheeses due to an increase in plasmin activity. Moreover, differences in the citrate metabolism and in propionic acid fermentation, which occurred in raw milk cheeses only, could be attributed to the occurrence of facultatively heterofermentative lactobacilli and propionic acid bacteria, respectively. The sensory characterization of the smell and aroma as well as the basic taste categories of mature Bergkäse samples showed that pasteurization led to lower intensities of the typical Bergkäse flavor but significantly higher bitterness scores. Finally, the authors concluded that it is essential to use raw milk to produce Bergkäse cheese with satisfactory properties.

In another study, Cornu et al. \((14)\) manufactured Cantal-type cheese (Cantal is a hard uncooked cheese from Auvergne, France) with either raw or pasteurized milk from cows fed either pasture or a hay-and-concentrate diet. The cheese aroma was rather mild in the pasteurized milk cheese and rather strong in the raw milk cheese, and it was little affected by the cows’ feeding patterns. The intensity of one of the major odor-active compounds, butanoic acid, was much stronger in raw milk cheeses than in pasteurized milk cheeses \((14)\).

All these results show that the raw milk microflora plays a paramount role in the development of the taste characteristics of cheese. The intensity of the flavor and its characteristics (distinctiveness and aromatic richness) are to a large extent determined by the action of the milk microflora in the cheese during ripening.

The results of the above-mentioned research were obtained with matching pairs of experimental cheeses. However, Chambers et al. \((15)\) obtained similar results with commercial French cheeses. Using experienced, highly trained sensory panelists, they observed that all the cheese types tested (Brie, Coulommiers, Camembert, Saint-Nectaire, goat cheese, and blue cheese), except Munster, showed similar changes in sensory attributes as a result of the use of pasteurized rather than raw milk. Raw milk cheeses (Brie, Coulommiers, Camembert, Saint-Nectaire, goat cheese, and blue cheese) had stronger odor properties and a higher overall aroma intensity and somewhat different flavors than pasteurized milk cheeses.

**Nature of Raw Milk Microflora and Sensory Specificity of the Cheeses**

Within the context of Yann Demarigny’s doctoral research \((16)\) carried out at INRA in Poligny, France, we were able to show that beyond the quantity of microorganisms present in the milk, the nature of the microflora played a determining role in the formation of
the sensory quality of the cheese. In order to dissociate the effect of the physicochemical and biochemical composition of the milk from that of the microflora, microfiltration was again used. From a common physicochemical and biochemical base, corresponding to a mixture of three milk samples of different origins, microfiltered with the addition of the corresponding pasteurized cream, three retentate samples containing the microflora of the three raw milk samples were added. In order to highlight a “nature” effect of the microflora, the total viable count was again adjusted in the reconstituted milk to 50,000 CFU/ml thanks to the rapid determination of the microbial populations in the retentate using the direct epifluorescence filter technique. Mini-cooked pressed cheeses (1 kg) were made, in winter and autumn, using these three mixtures of milk, and also using microfiltered milk without the addition of a microflora, which was used as a control.

After 12 weeks of ripening, it was possible to differentiate the minicheeses according to the origin of the microflora on the basis of fermentation criteria. At the end of ripening (24 weeks), differences were perceptible, particularly concerning proteolysis (Fig. 6a). This discrimination was confirmed by the sensory analysis (Fig. 6b). Considering the results obtained, a certain stability of the milk microflora could be noted, as the three cheeses for each different microflora corresponding to three separate experiments over 2 weeks were closely grouped.

This study also enabled knowledge concerning the evolution of the different microfloras in mini-raw milk hard cooked cheeses to be acquired. It was observed that the evolution of the dominant microflora during ripening in these minicheeses was similar whatever the origin of the raw milk.

This approach (comparison of raw milk versus pasteurized milk) has been used by other researchers for other types of cheese. For instance, Callon et al. (17) observed differences in sensory characteristics of Salers-type cheeses (hard uncooked cheeses from the Auvergne Mountains) manufactured with the same pasteurized

**FIGURE 6** Influence of the level and origin of the raw milk microflora on the microbiological, biochemical, and sensory characteristics of Gruyère-type cheeses. MF, microfiltered; A, B, and C, dairies from which the microfloras reincorporated in the milk originated; Lb, lactobacilli; C3 and iC5, volatile fatty acids; NS, water-soluble nitrogen; NPT, phosphotungstic acid-soluble nitrogen. Adapted from reference 18. doi:10.1128/microbiolspec.CM-0006-2012.f6
milk reinoculated with three different microbial communities from three different microfiltered milk filtrates (milk was sampled from wooden vats called gerles). Indeed, adding different microbial communities to specimens of the same (biochemically identical) pasteurized milk led to the production of cheeses with different sensory properties: cheeses with fresh cream, hazelnut, and caramel attributes and cheeses with fermented cream, chemical, and garlic flavors.

Thus, on the basis of the origin of the microflora, the cheeses did not have the same organoleptic specificities. The influence of the raw milk microflora was independent of when the cheeses were made (18). Finally, it can be said that the nature of the milk microflora ensures the organoleptic diversity of raw milk cheeses. This diversity in the sensory characteristics is of major interest in the production of traditional hard and semihard cooked mountain cheeses and traditional cheeses in general.

**Conclusion**

The raw milk microflora plays a determining role in the transformation of the components of the milk in the cheese, with significant consequences for the sensory characteristics of the cheeses. This microflora participates in the diversity of the cheeses in terms of both its population and its nature. The diversity of cheeses, for a given type, is linked to the complexity of the raw milk microflora.

**ROLE OF NATURAL OR WILD STARTERS IN THE BIOCHEMICAL AND SENSORY CHARACTERISTICS OF CHEESES**

Lactic starters are widely used in dairies, even in traditional cheesemaking processes. If these starters confer great regularity on the cheeses, they can also make them lose part of their specific characteristics, as these starters are predominantly produced on an international scale. Work is therefore currently being undertaken by the traditional cheese industry to create collections of local bacterial strains isolated from milk, whey, or cheeses derived from the production area. Lactic starters, grown in whey or sometimes milk, have been developed from these local strains. However, whey starters without the addition of strains of lactic acid bacteria are also used.

**Natural or Wild Starters: Example of Comté Cheesemaking**

The use of whey starters is a peculiar feature of Comté cheesemaking. The “natural” starters are prepared in each dairy using the whey recovered at the end of the cheesemaking process, called “raw” whey; i.e., they undergo no additional heat treatment for the preparation of the starters. Different types of starters (the list below is not exhaustive) exist (19).

One kind of starter is the mesophilic starter (with thermized whey), such as the following:

- whey, incubated with a commercial mesophilic starter at 25°C for 20 h, reaching a final acidity of 35 to 45°D
- whey, incubated with a commercial mesophilic starter at 30°C for 10 h, reaching a final acidity of 40 to 45°D
- whey, incubated at 30°C for 10 h without a commercial mesophilic starter, reaching a final acidity of 40 to 45°D; this starter contains mainly thermophilic streptococci

An “ambient” starter also exists. In this case, the whey from the cheesemaking process is not thermized or inoculated with commercial strains. It is incubated at ambient temperature until the morning after the removal of the whey. This starter is composed of lactic acid bacteria, mainly thermophilic, but can also contain lactococci (20).

Sterilized or skimmed ultrahigh-temperature-processed milk can also be used to prepare mesophilic starters; in this case the milk is inoculated with commercial strains.

Another kind of starter is the thermophilic starter (with “raw” whey):

- whey, incubated with a commercial thermophilic starter (*Streptococcus thermophilus*) at 42°C for 4 h, reaching a final acidity of 35 to 45°D; 5 g of skim milk/liter is sometimes added to the whey
- whey, incubated at 42°C for 4 h, without a commercial thermophilic starter, reaching a final acidity of 35 to 45°D
- whey, incubated at 42°C for 10 h with dried calf abomasum, maintained at ambient temperature for a further 10 h, reaching a final acidity of 90 to 110°D
- In *recuite*, proteins are precipitated out of the whey by heating (92°C) and acidification. The whey thus obtained is incubated at 42°C for 10 h with dried calf abomasum and then for a further 10 h at ambient temperature, reaching a final acidity of 110 to 130°D.

The last two thermophilic starters contain mainly thermophilic lactobacilli (*Lactobacillus helveticus* and
Lactobacillus delbrueckii subsp. lactis), while the other two thermophilic starters contain mainly thermophilic streptococci. Recuite is used to simultaneously supply clotting enzymes and thermophilic lactic acid bacteria.

This rennet preparation is made from whey and calf abomasum. (The abomasum is part of a ruminant’s stomach. For rennet, the abomasum of a young calf that has fed on grass only is used. After being collected, abomasas are inflated, dried, and then stored flat.) In a study carried out at INRA-URTAL in Poligny in 1982 on 10 samples of traditional rennet from six Comté cheese plants (unpublished results), it was observed that the amount of added calf abomasum varied from 2.5 to 6.5 g/liter, with an average of 5.3 g/liter; the amount of chymosin from 5.4 to 32.6 mg/liter, with an average of 16.5 mg/liter; the amount of pepsin from 3.1 to 4.8 mg/liter, with an average of 4.3 mg/liter; and the chymosin/pepsin ratio from 3.1 to 4.8, with an average of 3.9.

The cheesemaker generally uses three types of starter, in quantities that vary from 0.2 to 1.5‰.

At the end of the 1980s, the Comté industry decided to create a collection of local strains of lactic acid bacteria, selected according to their technological and organoleptic aptitudes, to be used regularly in the manufacturing of Comté. This decision was part of the measures taken to prove the specificity of Comté through its organoleptic qualities as well as its bond with the land, justifying its registered designation of origin.

Work has thus been carried out with INRA-URTAL in Poligny leading to the constitution of a collection of strains of thermophilic and mesophilic lactic acid bacteria regularly used in around 70 dairies. The use of these strains isolated from the land enables, among other things, better control of the acidification, which results in the production of a quality cheese (21).

Natural or wild starters are also used in the making of other traditional raw milk cheeses such as Beaufort (a French alpine hard cooked cheese) (use of recuite only), Abondance (a French alpine semihard cooked cheese) (Fig. 7), Parmigiano Reggiano and Grana Padano (Italian hard cooked cheeses), Gruyère (a Swiss hard cooked cheese), and Bergkäse (an Austrian hard cooked cheese) (11, 19, 22–25).

**Lactic Starter Cultures and the Biochemical and Sensory Characteristics of Comté Cheese**

Over the past 10 years, tests have been carried out in order to evaluate the effect of the nature of the starter (“wild” lactic starter cultures or selected Comté lactic starter cultures), as well as the interactions with raw milk, on the quality of Comté (21). Thus, on the same manufacturing site, Comté cheeses were made using raw milk and starters of various origins. This project brought to light the effect of wild lactic starter cultures on the aromatic quality of Comté. Indeed, with the “wild” starters, the intensity of the aroma of Comté, the persistence and the aromatic richness, was very significant (Fig. 8); the nature of the “roasted” note also changed (more “roasted” with the wild lactic starter cultures), and, finally, a change from “fresh lactic” to “melted butter” was observed.

Interactions between the nature of the starters and the raw milk were observed, notably concerning the eyes in Comté. These effects were due to, among other things, the increase in the intensity of proteolysis observed in the Comté cheeses made with starters of different composition.

In conclusion, whey starter cultures (wild) amplify the influence of the raw milk on the sensory properties of Comté by “refueling” the milk with a natural microflora (21).

Similar results have been found in other studies of goat and sheep milk. Three starters made of wild strains

**FIGURE 7** Addition of starter during the manufacture of Abondance cheese, Alpage Le Gouly, Abondance, France. (Source: © Eric Beuvier/INRA.) doi:10.1128/microbiolspec.CM-0006-2012.f7
of lactic acid bacteria (*Lactococcus lactis*, *Lactobacillus paraplantarum*, and *Leuconostoc mesenteroides*) were tested in raw goat milk and raw sheep milk used for Venaco cheese (a soft Corsican raw milk cheese). The starter strains inoculated in the milk, just like the indigenous lactic acid bacteria (notably strains of *Lactococcus lactis*), were found in the cheese during ripening; it thus turns out that a certain equilibrium is established between this microflora and the starter strains. Variations in the physicochemical composition of mature cheeses leading to cheeses with significantly different sensory properties were observed (26).

Finally, the work carried out on Comté or Venaco showed that the wild starters or those made with wild strains preserved the diversity of the indigenous raw milk microflora and reinforced the intensity of the aroma of cheeses made with these starters, thus helping preserve the bond with the land.

**DESCRIPTION OF THE MICROBIAL ECOSYSTEM OF TRADITIONAL HARD AND SEMIHARD COOKED MOUNTAIN CHEESES: THE EXAMPLE OF COMTÉ CHEESE**

To go even further in the determination of the role of the microflora in the formation of the sensory quality of cheese, it is important to specify the nature of the microorganisms which make up the microbial ecosystem of raw milk cheeses and to monitor their development from the milk to the fully ripened cheese. Indeed, the work described previously was based on a global approach to the microbial ecosystem and did not show any qualitative or quantitative differences in the major microbial groups and genera present in various cheeses. The microbial ecosystem of a raw milk hard cooked registered denomination of origin cheese, Comté, was described.

The microbial diversity in the heart of Comté is a result of, among other factors, the diversity of the microflora found in the raw milk and the lactic starters used in the manufacturing process. Heating in the vat at 53 to 56°C for 20 to 30 min, as well as the subsequent acidification during pressing, regulates the microbial balance at the start of the ripening process of Comté. Finally, a long ripening period, with different time-temperature cycles, guides the growth of the microflora in the Comté.

Figure 9 provides a fairly representative idea of the evolution of the dominant microflora in Comté (average of 20 cheeses).

The results from work carried out at INRA-URTAL are used to illustrate this section. The research concerned two Comté cheeses from two dairies located in two different geographic areas (located at altitudes of 790 and 950 m) and generally producing Comté cheeses (called Comté 1 and Comté 2) with very distinctive sensory characteristics (27, 28).

So, with the help of molecular biology tools involving PCR (repetitive extragenic palindromic PCR [Rep-PCR] and species PCR), it was shown that the diversity of the two microfloras, the mesophilic lactobacilli and the propionic acid bacteria, dominant during ripening (10^6
to $10^8$ CFU/g of two Comté cheeses with different tastes (and from different regions), was at the strain and not the species level, each cheese having its own strain profile with its own growth dynamics.

The two species of mesophilic lactobacilli dominant in Comté are *Lactobacillus paracasei* and *Lactobacillus rhamnosus*. *Lactobacillus plantarum*, found in the milk used to make the cheese, is rarely found during ripening of Comté, as in other hard cooked cheeses such as Gruyère (23) and Parmigiano Reggiano (29), yet it is a dominant species in Camembert (30). This species is obviously not adapted to cooked cheeses; cooking notably involves heating in the vat to 54 to 56°C and subsequent acidification during pressing. Only one species of propionic acid bacteria—*Propionibacterium freudenreichii*—has been identified during ripening; the species *P. jensenii* has been found only in the milk used to make the cheese. The same species were therefore found; however, quantitative differences exist at the species level between the two Comté cheeses throughout the ripening process.

*Lactobacillus paracasei* showed the greatest diversity during ripening, 11 to 15 strains, whereas there were only 4 to 7 strains of *L. rhamnosus*. Among these two species of mesophilic lactobacilli, only 3 to 4 strains in each Comté accounted for the majority of the strains present. Moreover, it was not necessarily the most abundant strains of each species in the vat milk that were dominant during ripening. Figure 10 illustrates the growth dynamics of the strains of the species *Lb. paracasei* and *Lb. rhamnosus* in the two Comté cheeses studied.

There were up to 40 different strains of *Propionibacterium freudenreichii*, the only species of propionic acid bacteria found in these two Comté cheeses, and contrary to the lactic acid microflora, no strain really got the upper hand during ripening. Figure 11 illustrates the growth dynamics of the strains of *P. freudenreichii* in the two Comté cheeses studied. The majority of strains of mesophilic lactobacilli and propionic acid bacteria in these two Comté cheeses came from the raw milk.

Another important microbiological component in the manufacturing and ripening of Comté is the thermophilic lactic acid bacteria provided by the starters, which in the case of Comté are mostly raw whey starters—that is to say, they do not undergo any additional heat treatment.
other than that of the manufacturing process to prepare the starters, or thermization, undergoing an additional heat treatment. These thermophilic lactic acid bacteria belong to the species *Streptococcus thermophilus* (Fig. 12), *Lb. helveticus*, and *Lb. delbrueckii* (Fig. 13). There again, the same species were found in the two Comtés; the growth dynamics of each species and the composition of strains differed in each Comté. Only one to three dominant strains per species were represented during ripening.

Thermophilic whey starters, sometimes called wild starters, participate in the development of sensory qualities and help amplify the influence of the raw milk by refueling the milk with a natural microflora (21). However, with the generalized use of selected starters, capable of lastingly establishing themselves in the cheese, there is a risk of reducing the diversity of the strains of thermophilic lactic acid bacteria present during ripening of Comté and consequently decreasing the aromatic diversity of these cheeses, which is why the Comté cheese industry recommended, in its decree issued in 2004, using at least one raw whey starter, thus providing a natural microflora (21).

Finally, these results underline the relevance and richness of the microbial biodiversity in Comté cheeses. Each Comté from a different land showed a specific microflora in the center throughout ripening, in terms of both the nature of the strains and the numbers of each species. This detailed description of the microflora at the species level reinforces the idea that the sensory diversity of raw milk cheese is partially linked to the diversity of the microorganisms in these cheeses. In the case of Comté, the microbial diversity is provided not only by the raw milk but also by the natural whey starters.

Overall, artisanal cheeses have a higher microbial diversity than industrially manufactured cheeses regardless of the type of cheese. This pattern undoubtedly reflects the use of raw milk and undefined starters (or natural whey starters) in artisanal cheeses (31).

**CONCLUSIONS**

Work carried out over the past few years undoubtedly shows that raw milk cheeses have a greater sensory richness than cheeses made with pasteurized milk. This richness is linked to the quantity and the nature of the microorganisms in the raw milk cheeses.

In the case of hard and semihard cooked mountain cheeses, notably Comté, it has been shown that the sensory diversity is linked to the diversity of the microflora in the cheese during ripening. This microbial diversity comes in various forms linked to a form that is common to all the cheeses of this variety. It comes from the genetic variability between strains, which constitutes...
the first level of diversity between microorganisms and translates for strains of the same species, and *a fortiori* for strains that are more different genetically, by differences in the adaptation to their environment leading to differences in growth kinetics in the cheese. It is due to the use of raw milk and starters which provide different strains and species from various ecological niches at the start of the manufacturing process. It is also due to the diversity of the environment of the microflora between cheeses and during the transformation of the milk into mature cheese which enables a diversity of microbial strains.

**FIGURE 11** Growth dynamics of the strains of *P. freudenreichii* during the ripening of Comtés 1 and 2 (28). Temperatures at the top are ripening temperatures. d, days. doi:10.1128/microbiolspec.CM-0006-2012.f11


growth kinetics (5, 21). In other research concerning European raw milk cheeses, significant diversity of microfloras at the genus level and at the species level within a genus was observed (32).

For all that, the practices of making traditional cheeses evolve and change (sometimes to make things easier) and can affect the cheeses’ sensory characteristics. These evolutions take place at different levels: at the farm level with farming practices that can induce changes in the composition of the milk, at the processing level through cheesemakers’ practices, and, finally, at the ripening process level. Thus, work needs to be carried out today in order to maintain the sensory specificity of traditional cheeses. This involves, amongst other steps, the preservation of the raw milk microflora, notably on the farm. A few results obtained over the past few years have shown that it is possible to act positively from the outset on the raw milk microbial flora (33). It has been shown that the cows’ teats are a reservoir for microfloras of technological interest (34). Moreover, links have been brought to light between practices that influence the microflora in the animals’ environment, notably in the stables, and the milk microflora. Furthermore, the presence of identical strains in the animals’ environment and the milk reinforce the hypothesis of a flow of microorganisms from the environment to the milk (35, 36). It would therefore seem possible in the future to facilitate the inoculation of the milk with this microflora by the controlled cleaning of the teats and/or promotion of influential practices.

In addition, the cheesemakers’ practices can also be a source of sensory diversity in hard and semi-hard cooked mountain cheeses, notably through the use of lactic starters and traditional coagulants. Work still needs to be undertaken today in order to understand these starters and traditional coagulants better (how to prepare them), to know how they interact with the components of milk so that the raw milk microflora can express its aromatic potential during the ripening of the cheeses and thus retain a strong bond with the land.

Finally, the production of approximately 700,000 metric tons of traditional raw milk cheeses in Europe/year (approximately 10% of the total cheese production) represents not only an economic issue but also a cultural issue: raw milk cheeses develop flavors and aromas that are characteristic of their land of origin; they are an integral part of the regional gastronomic heritage.

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ASMscience.org/MicrobiolSpectrum

The Microbiology of Traditional Hard and Semihard Cooked Mountain Cheeses


