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Cheese | Raw Milk Cheeses

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Abstract

Raw milk carries a so far underestimated biodiversity into the cheese. Farm management practices, hygiene of milking and storage all influence the vat milk microbiota. While the applied technology and starter cultures primarily impact the product quality of fresh cheese, raw milk microorganisms fully express themselves along ripening, giving the cheese a more diversified and intense flavor. Traditional raw milk cheeses therefore remain a very popular food worldwide and especially in Europe with protected designation of origin labels. This article aims to review the ancient and recent strategies to ensure both safety and beneficial health effects of what represents a unique taste experience: raw milk cheese.

Keywords

Alkaline phosphatase, Camembert, Cheese defects, Cheese quality, Cheese safety, Emmentaler, Health aspects, Microbiota, Next generation sequencing, PDO, Raw milk Raw milk cheese, Sensory characteristics

Introduction

Raw milk cheese production by the European Neolithic farmers can be dated back to the sixth millennium BC thanks to the analysis of organic residues on Pottery strainer (Salque et al, 2013). The spontaneously occurring fermentation thereby contributed to both preserve and improve the digestibility of raw milk. Through the millennia and until the 19th century, this ancestral know-how evolved at local scale to give rise to a variety of recipes that lay the foundation for today's artisan raw milk cheeses. An empirical control over microbial communities of these traditional cheeses has been taking place through microbial colonization of equipment (especially wood) and backslopping. Backslopping consists in keeping a sample of whey of the day to inoculate the raw milk in the subsequent batch. The emergence of microbial research together with the development of mechanization during the 19th century enabled the transfer of cheese making from farmer communities up to industrial scale. With the industrialization of cheese

manufacture, the factories became larger, storage time of milk increased, and, as a consequence, the microbiological quality of the vat milk and of the corresponding cheeses often varied considerably from batch to batch. Since the 1940s, raw milk was being subjected more and more to heat treatments to improve standardization of cheesemaking as well as for food safety reasons. However, both the heat-induced changes in milk and the elimination of the raw milk microflora considerably affect the cheesemaking and cheese-ripening processes, yielding cheeses with altered sensory characteristics. It is generally accepted that cheese made from raw milk develops a more intense flavor than that made from pasteurized milk. Factors specific to each technology as well as the raw milk flora itself contribute to the hygienic safety of raw milk cheeses. A considerable amount of cheese is therefore still produced from raw milk. The present article covers the background of today's raw milk cheese production, introduces recent findings gained by next generation sequencing (NGS) studies on raw milk microbiota, and highlights the advantages and disadvantages with respect to sensory characteristics, possible cheese defects, safety, health and marketing.

Definition of Raw Milk and Raw Milk Cheese

According to the code of hygienic practice for milk and milk products (CAC/RCP 57-2004, adopted in 2004, amendments in 2007, 2009), raw milk is defined as milk that has not been heated beyond 40 °C or undergone any treatment that has an equivalent effect. The same document defines pasteurization as a microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. Pasteurization conditions are designed to destroy effectively the most heat-resistant non-spore-forming pathogens present in milk: *Mycobacterium tuberculosis*, *Coxiella burnetii* and *Listeria monocytogenes* (*see* [LIQUID MILK PRODUCTS | Pasteurization of Liquid Milk Products: Principles, Public Health Aspects](#)).

National food regulations in general define pasteurization as a rapid heating of milk to a temperature of no less than 72 °C (161 °F) and retaining it at that temperature for no less than 15 s. The same process performance can be obtained by using other temperature

and holding time combinations (e.g., heating to not less than 63 °C (145 °F) for not less than 30 min). In the early 1930s, it was noted that the enzyme alkaline phosphatase, present in raw milk, is destroyed at a time–temperature combination of 63.5 °C × 30 min, which is sufficient for the inactivation of *Mycobacterium tuberculosis* (see ENZYMES INDIGENOUS TO MILK | Phosphatases). Measurement of alkaline phosphatase activity therefore offers a means of checking whether milk has been subjected to pasteurization or not. This method has been adapted to a large number of other dairy products as well as cheese. However, its application to raw milk cheese can lead to confusing results, as raw milk cheeses encompass a high range of cheese types, from uncooked soft cheese such as Camembert to cooked hard cheese such as Emmentaler. Indeed, the typical scalding conditions applied for the manufacture of traditional raw milk hard cheeses such as Swiss Emmentaler PDO cheese (heating 52–54 °C × 40 min, dry stirring 53–51 °C × 30 min) result in an almost complete inactivation of the alkaline phosphatase in the core region (Figure 1). In contrast, clearly positive test results are obtained in samples from the rind owing to faster cooling during manufacture.

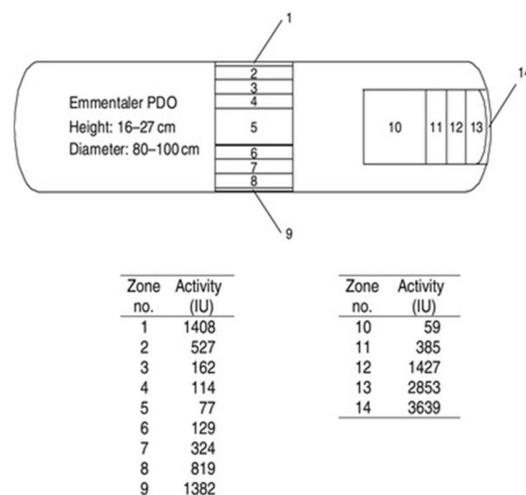


Figure 1. Residual activity of alkaline phosphatase measured in different zones of Emmentaler PDO cheese using p-nitrophenol phosphate as substrate.

Authentication methods based on metagenomics using NGS technologies (Kamilari et al., 2019) should become valid alternative methods in the near future. The prerequisites

are a careful sampling according to a well-defined scheme associated with catalogues of microbiome biodiversity recorded for each cheese type. The cheese microbiota is originating from multiple sources, including raw milk, starter cultures as well as the equipment that the cheese comes in contact with through its production. While the applied technology and starter culture strongly select the microbiota that is able to colonize a given cheese type, both shift in major components of the microbiota as well as increased biodiversity at subdominant level has been observed in raw milk cheeses compared to pasteurized cheeses (Delcenserie et al., 2014).

For food safety reasons and to prevent undesirable fermentations, thermization of the cheese milk at 57–68 °C for 10–20 s has been introduced in the manufacture of various semi-hard and soft cheeses (*see* [HEAT TREATMENT OF MILK | Thermization of Milk](#)). Such heat treatments of raw milk reduce markedly the number of bacteria, with a minimum of collateral heat-induced changes in milk. Depending on the manufacturing prescriptions of the individual cheese association, substitution of raw milk with 0–100% thermized milk became common for the production of a large number of traditional soft and semi-hard cheese varieties. In practice, thermization is often applied only to the evening milk prior to storage, whereas the freshly collected morning milk is made into cheese without heat treatment. This example illustrates that a correct definition or declaration of raw milk cheese is difficult to achieve. Nowadays, partial or full use of raw milk for the manufacture of cheese has to be declared in most countries, whereas a declaration of the heat treatments (thermization, pasteurization) of cheese milk is voluntary. A recent study suggests that gas chromatography-mass spectrometry untargeted metabolomics may be a way to discriminate between cheeses produced from raw versus thermized milk (Caboni et al., 2019).

The Tradition of Raw Milk Cheeses

Raw milk cheeses must have a history that dates back thousands of years as it probably developed soon after the emergence of animal husbandry in Western Asia in the early Neolithic. The records of monasteries are the first written documents mentioning the manufacture of cheese from the eleventh century CE in different areas of Europe.

According to those documents, the use of rennet was established as a major curdling technique around the sixteenth century. Most of today's popular cheese varieties made from bovine milk, such as Cheddar, Parmigiano Reggiano, Gruyère, Gouda, Camembert de Normandie, and Emmentaler, were first mentioned in records of the seventeenth and eighteenth centuries. From the 17th century, the tradition of raw milk cheeses in all its diversity and richness has been repeatedly introduced to North and South America by European immigrants. Industrialization from the early 20th century have led to a more or less drastic diminution of raw milk cheese production all over the world with various outcomes. The long history of cheesemaking in several European countries is a major reason for the still prevailing tradition of consuming raw milk cheeses. Since 1996, around 200 cheese varieties have obtained a registration as products with a protected designation of origin PDO (Giview, accessed 28 December 2020). Most of these registered cheeses originate from a few countries with a long tradition of cheesemaking such as Italy (53), France (46), Spain (26), Greece (22), Portugal (11) Switzerland (10) and the United Kingdom (10), and were initially manufactured from raw milk.

The use of high-quality milk, the use of adequate starter cultures, the application of appropriate manufacturing and ripening conditions, and last but not least the know-how of the cheesemaker are important requirements for the manufacture of high-quality raw milk cheeses. The existing know-how has been passed down from generation to generation. During the past decades, the procedures of cheesemaking have been greatly modified for almost all cheese varieties: In the Mediterranean areas, several traditional sheep and goat milk cheeses were progressively substituted by analogues made from cow's milk; the use of traditional whey cultures was replaced to a great extent by well-defined cultures; and in the course of the last 50–60 years, several milk pretreatments such as thermization, pasteurization, bactofugation, and microfiltration were introduced in order to obtain better process control and a higher standardization. Nowadays, the protocols of a large number of registered PDO cheeses allow such treatments and do not require explicitly the use of raw milk. Nevertheless, there are numerous well-known cheese varieties in Europe still being made exclusively from raw milk ([Figure 2](#)). The two best-selling PDO cheeses in France, Comté and Roquefort, but also Camembert de

Normandie and Brie de Meaux, or Parmigiano Reggiano in Italy, as well as Swiss cheese varieties such as Emmentaler and Gruyère, and various alpine cheeses (e.g., L'Etivaz and Berner Hobelkäse) are still made fully from raw milk. Statistics of the year 2018 show that around 30% of Swiss as well as Italian cheeses and 11 % of French cheeses are produced from raw milk.



Figure 2. Examples of European cheese varieties made exclusively from raw milk.

In the United States, which is the second largest cheese producer after Europe, the intensification of both milk production and processing led to the pasteurization of almost all milk for cheesemaking as a safety and quality control measure. However, artisan cheese is being increasingly produced in the US nowadays, making up 1 % of the total production, with raw milk accounting for 0,4 % (American Cheese Society, 2016). In recent years, it has been recognized that artisanal raw milk cheese constitutes an important economic niche in which its producers enjoy competitive advantage. Besides the aspects of product quality, sociocultural factors also are of growing importance for the marketing of raw milk cheeses. Raw milk cheeses meet the expectations of an increasing number of consumers who prefer minimally processed foods and natural

products without additives as well as authentic and artisanal specialties with well-defined origins.

South American countries host a high diversity of traditional artisan cheeses (González-Córdova et al., 2016). Safety considerations have led the authorities to globally prohibit the use of raw milk for cheese production from the mid of the 20th century. Since then, pasteurized version of the traditional artisan cheeses stands alongside informal selling of raw milk cheeses at local scale. However, regulatory exceptions started emerging that enable the legal production of raw milk cheeses in a defined region for a given technology. One example is the production of Coalho cheese in Pernambuco, a Northeast Region of Brazil (Kamimura et al., 2019). Intensive scientific research is currently being conducted on the cheese microbiome and cheese safety of traditional raw milk cheeses from South America to help the authorities taking science-based decisions.

Managing the sources of microbial diversity as a way to ensure the quality and safety of raw milk cheeses

The manufacture of raw milk cheeses is a challenging task. Both the production of high-quality raw milk and its accelerated processing into cheese are essential requirements for a successful manufacture of raw milk cheeses. The microbiota of raw milk intended for the production of raw milk cheeses has to get the right balance between beneficial bacteria that will contribute to both sensory characteristics and preservation, and alteration or pathogenic bacteria that may spoil the cheese or endanger food safety. The microbiological composition of the raw milk ready to process depends on a number of factors: animal health and management on the farm (feed, housing type) with potential seasonal differences, milking conditions and procedures, cleanliness of equipment, temperature control, processing place (on-farm or in a collective plant), duration of intermediate storage, and appropriate conditions for transport (*see* [MILKING AND HANDLING OF RAW MILK | Effect of Storage and Transport on Milk Quality](#); [MILKING AND HANDLING OF RAW MILK | Milking Hygiene](#)). The emergence of NGS studies will enable a more informed management of raw milk quality by unraveling the major contributing factors in milk production. Doyle et al. (2017) showed that outdoor *vs* indoor

habitat was a significant driver for milk microbiota composition, increasing the proportions of soil-associated microbes and host/gut bacteria, respectively. Teat skin followed by feces are the major sources of raw milk microbiota (Frétin et al., 2018). Carafa et al. (2020) further showed that the microbial levels of alpine milk are significantly higher than those of milk from permanent lowland grasslands. Strains belonging to species well known for their technological or probiotic activities (*Lactococcus lactis* subsp. *lactis* / *cremoris*, *Lacticaseibacillus paracasei*, *Bifidobacterium crudilactis* and *Propionibacterium* sp.) have been isolated from alpine milk in 4% to 13% higher frequencies than from lowland milk. Likewise, the grazing system (extensive vs semi-extensive) of dairy farm is associated, *via* the teat microbiota, with the microbiota of the milk and cheese (Frétin et al., 2018): 85% of operational taxonomic units identified in raw milk and 27% of those identified in ripened cheese were also found on teat skin. The routes and factors of dissemination of food-borne pathogens from animals, especially their udder, and from their environment to milk, have been extensively studied (Castro et al., 2018). However, few studies investigated the drivers of the balance between commensal microbiota and food-borne pathogens at the farm level. Monsallier et al. (2012) have shown that certain practices, such as the use of straw bedding and moderate milking hygiene (unsystematic disinfection of the teat), tend to eliminate pathogens and preserve microbial populations of technological interest.

For the production of raw milk cheese, the milk is usually processed within 18 h after milking. Milk that is not processed immediately after milking must be cooled to 4-12°C. Kable et al. (2016) found that the bacterial populations in tanker-truck raw milk arriving at two dairy processing facilities in California were highly diverse and differed according to season. They were vulnerable to modification upon transfer of the milk to storage silos. Distinct milk bacterial communities dominated either by *Streptococcus* spp. or by *Acinetobacter* were selected depending on silos, suggesting that processes other than just cold storage might have determined the community development. Fast controls of the quality of raw milk and full traceability back to the individual milk suppliers are mandatory requirements. Owing to such logistic constraints, raw milk cheeses are preferably produced in small, peripheral plants, which in general are certified according

to the international standard Food Safety System Certification (FSSC) 22000 or management systems with comparable requirements. Good agricultural practice (GAP) and good manufacturing practice (GMP) are overarching principles for the production, processing, and handling of all milk products. However, the requirements for the production of raw milk cheeses go far beyond those for pasteurized milk cheeses: Advanced hygienic practices throughout the whole production and processing chain as well as additional control measures from raw material production to the point of consumption are essential for ensuring a good product quality and food safety. Different growth patterns of pathogens in cheese were observed depending on the raw milks used. Out of 36 raw milks provided by 6 farms, 11 milks originating from 2 farms were found to be less favorable to the growth of *E. coli* O26: H11 in semi-hard cheeses (Frétin et al., 2020). Those milks were characterized by a greater relative abundance of bacterial genera assigned to lactic acid bacteria (*Lactococcus*, *Lactobacillus*, *Leuconostoc*) but also to Gram-negative bacteria (*Acinetobacter*, *Serratia*, *Hafnia*) and to *Macroccoccus*. On top of that, dairy specific in-house microbiota located at the milk processing site, in brines and/or in the cheese cellar will positively contribute to typicality and safety of raw milk cheeses (Montel et al., 2014). Detrimental pathogenic or spoilage organisms that sometimes establish in facilities have to be tracked down and eradicated. A period of unsettled quality may follow such a sanitation procedure until the beneficial in-house microbiota is restored.

Sensorial Characteristics of Raw Milk Cheeses

Raw milk microflora is considered to be responsible for the wide diversity of aromas of traditional cheeses. A significant comparison of cheeses made from raw milk with similar cheeses made from pasteurized milk is difficult to achieve, because in practice pasteurization of cheese milk is usually only one among many differing factors during processing. As an example, the production of a generic Emmental (see CODEX STAN 269-1967; [see POLICY SCHEMES AND TRADE IN DAIRY PRODUCTS | Codex Alimentarius](#); [POLICY SCHEMES AND TRADE IN DAIRY PRODUCTS | Standards of Identity of Milk and Milk Products](#)) and Camembert cheese in a large-scale factory may

differ considerably from the artisanal manufacture of an Emmentaler PDO cheese and a Camembert de Normandie PDO cheese, respectively, as shown in Tables 1 and 2. Treatments such as pasteurization, thermization, bacto-fugation, and microfiltration allow additional control of bacteriological cheese milk quality, offer more flexibility regarding the storage time and transportation distances, and thus enable the production of cheese in large-scale factories.

Table 1. Typical differences in the manufacture of Swiss Emmentaler PDO made from raw milk and generic Emmental cheese made from pasteurized milk

Cheese characteristics	Swiss Emmentaler PDO cheese	Generic Emmental cheese
Production of milk	Within a radius of 20 km around the cheese factory, about 10–30 milk suppliers	Larger number of milk suppliers and longer transportation distances
Feeding of cows	Without silage. The feed ration must be based on roughage.	With silage. No requirements on the proportion of roughage
Intermediate storage of milk	Processing takes place a maximum of 24 hours after milking	Transformation frequently within 72 h after milk collection
Heat treatments of cheese milk	Not allowed	Pasteurization
Other milk treatments	Not allowed	Bactofugation or microfiltration
Additives	Not allowed	• Colors
		• Preservatives (e.g., lysozyme, nitrate, nisin)

Cheese characteristics	Swiss Emmentaler PDO cheese	Generic Emmentaler cheese
		• Acidity regulators
		• Ripening enzymes
Equipment for cheesemaking	Copper vessels or copper vats	Stainless-steel vats
Heating of the curd	52–54 °C for 30–60 min	Typically 48–50 °C
Dimensions of cheese loaves	Wheels of 75–120 kg	Typically wheels or blocks of 40 kg or more
Firmness type	Hard cheese (dry matter >620 g kg ⁻¹)	Semihard or hard cheese (dry matter >600 g kg ⁻¹)
Ripening	Only dry ripening; minimal ripening time is 4 months for mild cheese, >8 months for fully flavored cheese, and >12 months for cave-aged cheese	Usually ripening in a film, normally 6–8 weeks
Size of cheese factory	2–4 persons producing 100–200 tonnes yr ⁻¹	Usually large-scale factories producing >10 000 tonnes yr ⁻¹

Table 2. Typical differences in the manufacture of Camembert de Normandie PDO made from raw milk and generic Camembert cheese made from pasteurized milk

Cheese characteristics	Camembert de Normandie PDO cheese	Generic Camembert cheese
Production of milk	Normande cows required in herd (minimum 50%)	No requirement

Cheese characteristics	Camembert de Normandie PDO cheese	Generic Camembert cheese
Feeding of cows	Pasture minimum 6 months per year	No requirement
Intermediate storage of milk	Transformation within 72 h after milk collection	Transformation up to 96 h after milk collection
Heat treatments of cheese milk	Not allowed	Pasteurization, thermization
Other milk treatments	Not allowed	Microfiltration
Equipment for cheesemaking	Coagulation in vats of 330L maximum	Continuous coagulation processing
Processing	Primary ripening of milk	Facultative primary ripening of milk with less mesophilic LAB starter or no primary ripening
	Slight stabbing of the curd or none	More stirring of the curd
Molding	Ladle molded, generally 5 fillings	Multimolds system, 1 filling
Curd type	More acidic curd	More rennet curd
Green cheese characteristics	Calcium/nonfat dry matter: 0.6–0.7%	Calcium/nonfat dry matter: 1.0–1.5%
	Dry matter: 39–40%	Dry matter: 42–43%
	pH 4.6–4.7	pH 4.9

Cheese characteristics	Camembert de Normandie PDO cheese	Generic Camembert cheese
Secondary starter	<i>Geotrichum candidum</i> (GC) and <i>Penicillium camemberti</i> (PC)	GC+PC and other secondary starter (bacteria and yeasts)
Ripening	12 days minimum in the ripening room at 10–18 °C;	Usually more quickly ripened (15 days before sale)
	Minimal ripening time is 21 days before sale	
Package	Paraffin film	Multilayer film
Size of cheese factory	Small factories with slight mechanization	Usually large-scale factories with large-scale mechanization

The real contribution of raw milk to sensory properties of cheeses has been evaluated via experiments in which only heat treatment of milk varied, despite the conditions being not really the same as in commercial cheeses, where adaptations are made to the processing of pasteurized milk. Available data are presented in [Table 3](#).

Independent of the cheese variety, raw milk cheeses have a more diversified and ‘stronger’ flavor than pasteurized milk cheeses, which gives them typical characteristics. On the one hand, raw milk cheeses present a higher degree of maturity at the same age; on the other hand, pasteurized milk cheeses fail to develop certain flavors throughout ripening. Besides the overall flavor intensity, pungency is often associated with raw milk cheeses, and so are acid, sour, rancid, and animal attributes. These flavor characteristics are linked to the earlier and more extensive development of raw milk microflora, to differences in the patterns of proteolysis, to higher lipolysis, and to a generally larger extent of formation of volatile compounds (*see* [CHEESE | Biochemistry of Cheese Ripening](#)). Changes in proteolytic and lipolytic activities involve nonmicrobial as well as microbial enzymes. Both earlier production and higher levels of volatile compounds occur

in raw milk cheeses. In particular, end products such as volatile fatty acids and esters are more abundant in raw milk cheeses in all cases, and alcohols and sulfur compounds in most cases. Methyl ketones and aldehydes have a variable distribution because they are intermediary metabolites and the balance between their production and degradation depends on the ripening stage at which the cheeses are analyzed. Diacetyl and/or acetoin are higher in pasteurized milk cheeses, because they are present in young cheeses and are further degraded during ripening in raw milk cheeses.

Differences in texture are less obvious, because texture settling is dependent more on water retention and binding, calcium equilibrium, and primary proteolysis due to nonmicrobial enzymes, and less on the activity of raw milk microflora. Raw milk cheeses seem in general more firm and grainy, and less elastic or rubbery, which indicates a higher maturity at the same age of consumption.

Table 3. Sensory characteristics and composition of raw compared to pasteurized milk cheeses (compilation of 11 studies)

	Hard cheese (Comté, Bergkäse)	Cheddar	Semi-hard cheese	Hard ovine cheese (Roncal, Idiazabal)	Lactic goat cheese
Flavor	+ Overall intensity, pungent - Acid, bitter ± Salty	+ Overall intensity, pungent, acid, sour, bitter, creamy, fruity, sweet, sulfur, rancid, unclean	+ Intensity, acid milk, rind, spiced, animal, garlic, chemical, rancid, bitter, pungent - Fresh milk, fruit	+ Characteristic, overall intensity, aftertaste, pungent, salty, acid, animal, fruit, cream - Bitter, sour, sweet, roasted, toasted ± Sour, milk	+ Overall flavor and odor, defects
Texture	+ Firm, granular	+ Crumbly, grainy		+ Firm, grainy - Elastic, creamy	

	Hard cheese (Comté, Bergkäse)	Cheddar	Semi-hard cheese	Hard ovine cheese (Roncal, Idiazabal)	Lactic goat cheese
		- Rubbery			
Lipolysis		+	=		+
Volatile compounds	+ Acetic, propionic, isovaleric acids	+ Esters - Acetoin ± Methyl ketones, aldehydes, alcohols, sulfur	+ Esters, sulfur - Ketones, aldehydes	+ Volatile fatty acids, ketones, alcohols, esters, sulfur ± Aldehydes	
Proteolysis	+ β -casein/ PTASN - α_{S1} -casein/ FAA ± WSN = TCASN/ PTASN	+ WSN4.6/ FAA - TCASN ± PTASN = α_{S1} -casein/ β -casein/ WSN4.6	= α_{S1} -casein/ β -casein/ WSN/ PTASN	+ α_{S1} -casein/ β -casein/FAA - WSN/TCASN	

+: Raw milk > Pasteurized -: Raw milk < Pasteurized ±: + or – depending on the study
=: no differences.

α_{S1} -casein, β -casein: native caseins, indicators of less primary proteolysis.

WSN (water-soluble nitrogen), WSN 4.6 (pH 4.6-soluble nitrogen), TCASN (trichloroacetic acid-soluble nitrogen), PTASN (phosphotungstic acid-soluble nitrogen), FAA (free amino acids): nitrogen fractions characteristic of secondary proteolysis.

The determinants of flavor differ according to the cheese variety, due to different structures, physicochemical compositions, and microorganisms involved, so variations due to pasteurization may differ between cheese varieties. One example of a variation in the sensory profile is bitterness: Hard cheeses, unlike others, are more bitter when made

from pasteurized rather than raw milk, which shows that proteolysis patterns are affected by pasteurization. This may be explained by the fact that pasteurization activates plasminogen to plasmin and thereby could increase the formation of bitter peptides.

Raw milk cheeses contain a higher microbial diversity, at family, genus, species and strain levels (Montel et al, 2014). NGS allows to follow the dynamics of most microbial species during cheese processing and ripening (Afshari et al, 2020a). A microbial selection occurs from milk to cheese, according to the cheese process. In all cheese varieties, LAB constitute the dominant microbiota of the cheese core. In hard cheeses, where the high temperature in the vat reduces the number of species able to survive, NSLAB from raw milk become dominant during ripening, together with propionibacteria if the ripening temperature is high enough. In semi-hard cheeses, many genera other than LAB develop, including yeasts, with more bacterial genera detected for raw milk cheeses (Salazar et al, 2018).

The microbial diversity inherited from raw milk may enhance the expression of diverse metabolic functions in cheeses (Montel et al, 2014). NGS and correlations between different omics techniques will allow to unravel the participation of the various microbial species in the production of flavour compounds (Afshari et al, 2020b). As an example, there is evidence from Cheddar cheese showing a link between lactobacilli and the release of glutamic acid, and between *Streptococcus thermophilus* and the presence of succinic acid, both compounds bearing umami flavour.(see [LACTIC ACID BACTERIA | Lactic Acid Bacteria in Flavor Development](#)).

In surface-ripened cheeses, a major contribution of the surface microbiota to sensory characteristics has been repeatedly shown. Recent NGS studies highlighted striking similarities between the microbiota of teat skin, raw milk and cheese surface. It is likely that intense sensory characteristics associated with raw milk cheeses are partly linked to a more diverse cheese surface microbiota inherited from raw milk.

As raw milk cheeses ripen more rapidly than pasteurized milk cheeses, due to enhanced enzymatic activities, the optimum age of consumption is more critical than for pasteurized

milk cheeses, especially in high-moisture varieties with faster ripening. Moreover, as raw milk microflora diversity and levels are not standardized, raw milk varieties present a higher sensory diversity between cheeses than exhibited by pasteurized milk varieties (*see* [CHEESE | Cheese Flavor](#)). Sensory differences between raw and pasteurized milk cheeses are modulated by technological aspects such as the starter composition or the temperature of ripening. Various attempts have been made to overcome the sensorial disadvantages of pasteurized milk cheese that tend to have a milder and more uniform flavor, by the addition of adjunct cultures. However, application of such NSLAB adjunct cultures is not common practice.

Cheese Defects Observed in Raw Milk Cheeses

A number of defects occurring in cheeses made from raw and pasteurized milk are directly related to insufficient milk quality. However, traditional raw milk cheeses are especially susceptible to cheese defects due to the lack of corrective milk treatments that reduce the number of total bacteria and spores or inactivate indigenous milk enzymes. The time of appearance and the symptoms of most cheese defects that occur frequently are quite characteristic and allow quick diagnosis. In addition, analytical determination of important metabolites such as short-chain carboxylic acids helps to distinguish unambiguously between various types of faulty fermentations even in the early stages of ripening. For example, the defect of early gas blowing occurs already within 2 days after cheese production and is characterized by the formation of numerous small holes in the curd. This defect is more common in cheeses with low scalding temperatures and results from a combination of weak acidification by the starter and poor bacteriological quality of the raw milk. A slow and incomplete degradation of lactose by lactic acid bacteria enables the rapid multiplication of coliform bacteria and yeasts that produce CO₂ and H₂. Furthermore, elevated concentrations of formic and acetic acid are found in the curd at an early stage. Poor hygiene at milking and of plant equipment is considered to be the main reason for contamination with coliforms and yeasts.

The natural presence of propionic acid bacteria in raw milk is not unusual for cheese varieties such as Comté cheese. However, the production of raw milk cheeses without

propionic acid fermentation requires the concentration of propionibacteria in raw milk below 30 cfu ml⁻¹. Strains of *Propionibacterium freudenreichii* are rather heat-resistant and survive even harsh scalding conditions. During prolonged ripening, the undesired growth of colonies of propionibacteria leads to the formation of reddish-brown spots in the curd, an atypical sweet flavor, opening defects, and a poor storage quality in semi-hard, hard, and extra-hard cheeses such as Raclette, Gruyère, and Sbrinz, respectively. Similarly, for the production of Emmentaler PDO, too, concentrations of propionibacteria in raw milk are preferably kept low, because the naturally present strains in raw milk may progressively replace the added culture strains during ripening and reduce storage quality at advanced stages of ripening.

The incidence of elevated concentrations of biogenic amines in cheeses made from raw milk is higher than that in cheeses made from pasteurized milk. Enterococci and non-starter lactobacilli such as *Lentilactobacillus parabuchneri* play an important role in the formation of biogenic amines in raw milk cheese (*see* BIOGENIC AMINES). The most abundant biogenic amines in cheese are tyramine and histamine, whereas elevated concentrations of cadaverine and putrescine are characteristic of contamination with enterobacteria and occur mainly in raw milk cheeses with obvious flavor and opening defects or in pasteurized milk cheeses in case of serious recontaminations. However, raw milk with low total bacterial counts (<5000 cfu ml⁻¹) sporadically contains tyramine- and histamine-producing strains in low concentrations (<100 cfu ml⁻¹) yielding cheeses with high contents of biogenic amines in the range of 1000–2000 mg kg⁻¹ after ripening periods of 6–12 months. The formation of high amounts of biogenic amines may cause adverse toxic reactions and considerably affects the storage quality of cheese due to the continuous decarboxylation of amino acids and the related production of CO₂. Such cheeses typically exhibit slits and cracks and a strongly unpleasant burning and pungent taste. However, in raw milk cheeses with high scalding temperatures such as Gruyère (56–58 °C) or Comté (54–56 °C), only low concentrations of biogenic amines were detected even after a ripening period of 12 months.

Rancid flavor of cheese originates from hydrolysis of milk fat by lipases and subsequent accumulation of n-butyric acid and n-caproic acid in the product. In raw milk cheese,

indigenous milk lipoprotein-lipase (LPL) is usually responsible for the development of rancid flavor (*see* [ENZYMES INDIGENOUS TO MILK | Lipases and Esterases](#)). Elevated LPL activity in milk has been related to short milking intervals, malnutrition or hormonal disorder of the cow, high somatic cell count, and late lactation. In addition, physical damage of milk fat globules makes the milk fat accessible for the enzyme and results in an increased rate of lipolysis. As LPL is heat labile, high-cooked cheese and cheeses made from thermized milk are less prone to rancidity. Moreover, in highly proteolyzed cheese (e.g., Blue cheese), rancidity is less perceptible due to the high pH and is masked by other aroma components. Cheeses undergoing butyric acid fermentation may develop a similar off-flavor, but that can be distinguished from rancidity on the basis of molar ratio of n-butyric acid to n-caproic acid, which exceeds 4:1 in case of butyric acid fermentation.

Food Safety Aspects

Besides beneficial microorganisms, raw milk may contain a variety of pathogenic bacteria and bacteria having a controversial safety status, for example, enterococci exhibiting antibiotic resistances and bacteria producing biogenic amines (*see* [MICROORGANISMS ASSOCIATED WITH MILK](#)). Survival of pathogens present in raw milk is greatly dependent on the technology applied to cheese processing. The acidification rate and the temperature–time profile of the whole process including ripening as well as the moisture and salt content constitute the main factors affecting the survival and growth of pathogens in cheese (*see* [CHEESE | Public Health Aspects](#)). As a consequence of their prevalence in raw milk, and their survival and growth potential during cheesemaking and cheese ripening (*D*-value, growth temperature range, tolerance to acids and salt), the pathogens most frequently found in raw milk cheeses are enterotoxin-producing *Staphylococcus aureus*, verotoxin-producing *Escherichia coli* (VTEC), *Listeria monocytogenes*, and *Salmonella* spp. (*see* [PATHOGENS IN MILK | *Escherichia coli*](#), [PATHOGENS IN MILK | *Listeria monocytogenes*](#), [PATHOGENS IN MILK | *Salmonella* spp.](#); [PATHOGENS IN MILK | *Staphylococcus aureus* – Molecular](#)). This is a great safety concern, as consumption of even low numbers of *Salmonella* spp. or pathogenic *E. coli* (10-100 cfu per serving) and medium numbers of *L. monocytogenes* (10'000-100'000 cfu

per serving) may induce disease. In particular, surface-ripened cheeses are the most susceptible to the psychrotrophic *L. monocytogenes* contamination on their surface (due to the pH close to neutral), during both ripening and storage at cool temperatures. Contamination of milk with *S. aureus* is usually associated with udder infections. Poor-quality raw milk may contain several thousand per milliliter, and, consequently, fresh curd produced from such milk may have an *S. aureus* count of more than 100 000 g⁻¹ due to growth and passive enrichment in the cheese matrix. If the count of *S. aureus* exceeds 100 000 cfu g⁻¹, significant amounts of staphylococcal enterotoxins (SET) may be formed, which will persist in the curd for months, though the bacteria themselves substantially decrease in number during cheese ripening. The European Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs has set down process hygiene criteria for coagulase-positive staphylococci, requiring examination of the product at the times during the manufacturing process when the number of staphylococci is expected to be highest. In 2019 Commission Regulation (EU) 2019/229, amending EC Regulation (EC) No 2073/2005, has been published.

Extra-hard and hard cheeses made from raw milk, such as Parmigiano Reggiano, Gruyère, Comté, and Emmentaler, are considered to be free from viable pathogens, as hard cheeses combine a series of hurdles that prevent their survival, namely, high temperature applied during cooking and pressing, low pH and moisture content, and minimum ripening periods in the range of 4–18 months. Nevertheless, on the surface of extra-hard and hard cheeses, a recontamination with pathogens (e.g. *L. monocytogenes*) must be prevented.

Semi-hard cheeses are more susceptible to contamination with pathogens as the temperatures applied during production are closer to their growth optimum. Pathogens such as *S. aureus*, *E. coli*, or *Salmonella* spp. can be controlled if the contamination of the vat milk is kept low and if rapid acid production is ensured by the addition of an active starter. *Listeria monocytogenes*, which may be present in raw milk, is hardly inactivated during the manufacture and ripening of semi-hard cheese. Therefore, regular examination of milk as well as of cheese rind samples is an important measure to control *L. monocytogenes* in semi-hard raw milk cheese.

Soft cheeses are very susceptible to contamination by *L. monocytogenes*, *Salmonella* spp., *S. aureus* and *E. coli*. Soft cheeses come along as short-ripened small loaves, making them prone to extensive deacidification by surface microflora. Together with the fact that soft cheeses may contain residual sugars depending on the technology applied, this establishes inviting conditions for these pathogens, with better survival on the rind compared to the cheese core, and even growth . Because soft cheeses are generally consumed with their rind, monitoring of both raw milk and end product at an increased frequency compared to other cheese types is a key element of the strategy ensuring food safety of these products.

As a primary means of food safety, milk is often treated by pasteurization, thermization, or microfiltration in order to eliminate or reduce the concentration of pathogenic bacteria present in the vat milk. However, the incidence of outbreaks associated with cheese consumption and the occurrence of contaminated cheese on the market are not higher for raw milk cheeses than for pasteurized milk cheeses. In the United States, all listeriosis outbreaks leading to fatal outcomes were linked to recontamination of pasteurized cheeses (Costard et al., 2017). In Europe, the high safety level is primarily achieved by the implementation of comprehensive HACCP concepts in cheese factories processing raw milk and monitoring programs based on legally defined food safety criteria and process hygiene criteria (*see* HAZARD ANALYSIS AND CRITICAL CONTROL POINTS | HACCP Total Quality Management and Dairy Herd Health). Some countries disregard the fact that a high food safety standard can be obtained in raw milk cheeses, and require the labeling of all cheeses made from unpasteurized milk with special health alerts (Figure 3). In the United States, the regulations of the Food and Drug Administration (FDA) require all cheeses made from unpasteurized milk to be aged at least 60 days before being sold. As a consequence, traditional soft cheeses such as Brie de Meaux and Camembert de Normandie are available on the US market in the form of analogues made from pasteurized milk.

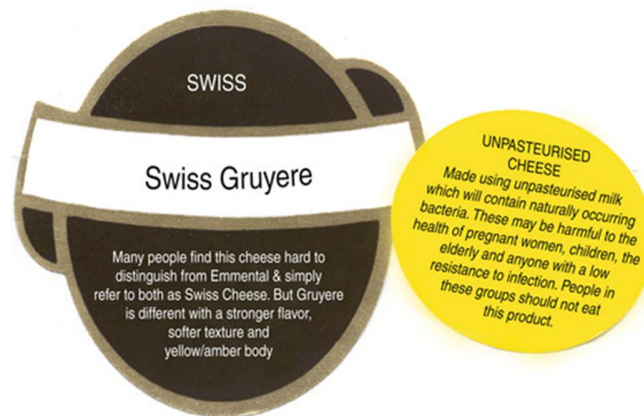


Figure 3. Example of labeling of raw milk cheeses with health alerts.

Heat treatments reduce the risk of occurrence of pathogens in the vat milk but simultaneously reduce the biodiversity of the flora present in the final cheese. Raw milk cheeses harbor a complex flora acting as a protective barrier against pathogens (Montel et al., 2014), whereas pasteurized milk cheeses are much more susceptible to post-pasteurization contamination, for example to contamination by *E. coli* and *L. monocytogenes*, typically occurring in ripening facilities or during cutting at the retail premises. Moderate heat treatments such as thermization may select for heat-resistant microorganisms (e.g., Enterococci, *M. tuberculosis*, *Coxiella burnetii*), by enabling their survival while reducing the competitive flora.

Health aspects

Today, we know that the bacteria in the human gut affect us in a number of ways: in addition to performing an immunological function, synthesising vitamins, and breaking down dietary fibre, they possess metabolic properties involved in the prevention of obesity and cardiovascular diseases. They even influence our mental health. For all these functions, a gut microbiome as diverse as possible is essential. Any diet that supports this diversity also encourages good health. Because of their high microbial diversity, raw milk and raw-milk products such as raw milk cheeses have a positive effect on gut microbiome diversity.

The primary aim of heating milk is to reduce its bacterial content and ensure microbiological food safety. As a side effect of the heating process, however, heat-sensitive components of the milk are altered in a variety of ways. Because of the reduced microbial diversity and changes in the heat-sensitive components, various positive health effects of the raw milk, such as protection from allergies, are weakened or even completely lost. Production-related differences such as e.g. the higher omega-3 fatty acid content should not be underestimated. Higher bacterial diversity leads to a higher number of bioactive peptides that could be beneficial for consumers (Egger et al, 2021). The effects on our health are long-term and multifactorial in nature, making them very difficult to document and quantify. The risks, however, often involve rather short-term effects with an obvious cause, such as e.g. microbial food poisoning. It is important not to overestimate the negative effects and underestimate the positive effects.

See also

[BIOGENIC AMINES. CHEESE | Biochemistry of Cheese Ripening; CHEESE | Camembert, Brie, and Related Varieties; CHEESE | Cheese Flavor; CHEESE | Public Health Aspects; CHEESE | Swiss-Type Cheeses. ENZYMES INDIGENOUS TO MILK | Lipases and Esterases; ENZYMES INDIGENOUS TO MILK | Phosphatases. HAZARD ANALYSIS AND CRITICAL CONTROL POINTS | HACCP Total Quality Management and Dairy Herd Health. HEAT TREATMENT OF MILK | Heat Stability of Milk; HEAT TREATMENT OF MILK | Thermization of Milk. LACTIC ACID BACTERIA | Lactic Acid Bacteria in Flavor Development. LIQUID MILK PRODUCTS | Pasteurization of Liquid Milk Products: Principles, Public Health Aspects. MICROORGANISMS ASSOCIATED WITH MILK. MILKING AND HANDLING OF RAW MILK | Effect of Storage and Transport on Milk Quality; MILKING AND HANDLING OF RAW MILK | Milking Hygiene. PATHOGENS IN MILK | *Escherichia coli*; PATHOGENS IN MILK | *Listeria monocytogenes*; PATHOGENS IN MILK | *Salmonella* spp.; PATHOGENS IN MILK | *Staphylococcus aureus* – Molecular. PLANT AND EQUIPMENT | Safety Analysis and Risk Assessment. POLICY SCHEMES AND TRADE IN DAIRY PRODUCTS | Codex Alimentarius; POLICY](#)

Further Reading

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