

From Pasteur to Probiotics: A Historical Overview of Cheese and Microbes

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ABSTRACT Cheese is a food which has been produced for centuries. While cheese was originally developed as a product which extended the shelf life of milk, over time distinct cheese varieties arose, being shaped by geographic, climate, cultural, and economic factors. Global demand for artisan cheeses is creating new economic opportunities. Consumers seeking distinctive products with regional flavor, or *terroir*, are becoming connoisseurs of hand-crafted cheeses with distinctive tastes and character. These demands have spurred new inquiry into microorganisms used as starter cultures and adjunct cultures, as well as the microbiological consortia of finished cheeses. Such demands have also created new concerns for food safety and international trade. New bacterial pathogens such as *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium DT104 have emerged in the food supply, causing a reevaluation of the efficacy of traditional cheesemaking procedures to control these pathogens. Similarly, pathogens such as *Listeria monocytogenes* pose problems to susceptible human populations, and cheese can be a vehicle of transmission for this deadly pathogen. With changes in sanitary requirements due to the globalization of the food industry, governments around the world are increasingly requiring assurances of cheese safety. While many governments recognize the safety of traditional artisan cheeses manufactured from raw milk, others are demanding pasteurization of all milk intended for cheesemaking to provide assurance of microbiological safety. In response, new technologies are being proposed to increase cheese safety, but these technologies fundamentally alter the traditional artisan practices and may not enhance microbiological safety. A reevaluation of the safety of traditional artisan practices, validation thereof, and communication of the scientific principles which promote safety will be necessary to enable the continued production of traditional artisan cheeses in global commerce. This also affords the opportunity to more fully explore the microbial diversity and microbial ecology of the great cheeses of the world.

INTRODUCTION

Nowhere in the microbial world are microorganisms on more magnificent display than on the surfaces or in the interiors of the great cheeses of the world. Cheesemaking is inextricably linked to microbiology, which makes the study of cheeses, their history, and the vast science of cheese and microbes particularly fascinating. Over the past two decades, there has been explosive growth in the U.S. artisan cheese industry. The availability of artisan cheeses, made using traditional practices, has ignited renewed consumer interest in cheesemaking and cheese consumption. This affords a tremendous opportunity to educate a new population of students, scientists, cheesemakers, technologists, and cheese connoisseurs about the essential role which microorganisms play in the process of cheesemaking.

Many of the chapters in the book *Cheese and Microbes* (48) provide a scientific overview of the beneficial associations of microbes with cheese, through the lens of the numerous unique cheeses which result due to growth of bacteria, yeasts, and molds which play a crucial role in cheesemaking. Whether due to surface or internal mold, yeast, or bacterial ripening, growth, or

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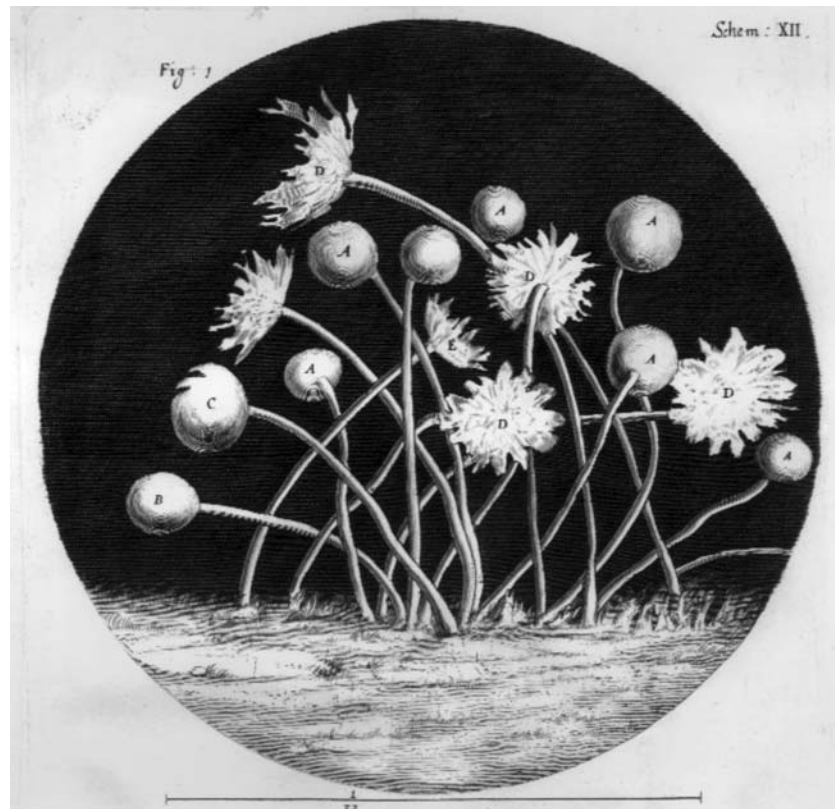
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metabolism, a vast array of products are able to be produced through transformation of a single starting material: milk. Cheeses in general are microbiologically safe foods, but there are occasional outbreaks of illness linked to cheese consumption. The chapters in *Cheese and Microbes* have been authored by scientists who are the leading researchers and experts on the various aspects of the association of microbes with traditional cheeses. Many of the authors reside in Europe, where the traditional cheeses which they study have been continuously produced for centuries. In addition to the informative overview of the science of cheesemaking and the microorganisms involved, selected photographs capture the culture, tradition, and vast array of unique cheese varieties, all of which are dependent on the action of a diverse population of bacteria, yeasts, and molds. New tools of molecular biology are informing the study of cheese microbiology in ways not previously possible, and this emerging science is providing new insights into the complexity of the microbial biodiversity of traditional cheeses. This inquiry will further advance our knowledge of some of the oldest traditional foods known to humankind.

A HISTORY OF CHEESE AND MICROBES

The development of the microscope by two pioneering scientists, Robert Hooke and Antonie van Leeuwenhoek, was an advancement which greatly informed our understanding of microbiology in general and of cheese in particular. Of important note was the very first recorded observation of microbes associated with cheese, described in 1665 by Robert Hooke in his book *Micrographia* (1). Hooke writes of “[t]he Blue and White and several kinds of hairy mouldy spots, which are observable upon divers kinds of putrify’d bodies, whether Animal substances, or Vegetable, such as the skin, raw or dress’d flesh, blood, humours, milk, green Cheese, etc....” Hooke provided the first published depiction of a microorganism, a “hairy mold” colony which microbiologists have subsequently identified as *Mucor* (Fig. 1). Shortly following Hooke’s description, in 1674 Antonie van Leeuwenhoek, in a letter to the Royal Society, affirmed Hooke’s findings, writing, “Examining this water...I found floating therein divers earthy particles, and some green streaks, spirally wound serpent-wise...and I judge that some of these little creatures were above a

FIGURE 1 Robert Hooke’s 1665 depiction of a “hairy mold” colony which was subsequently identified as *Mucor*. doi:10.1128/microbiolspec.CM-0001-2012.fl



thousand times smaller than the smallest ones I have ever yet seen, upon the rind of cheese, in wheaten flour, mould, and the like” (44).

From the earliest recognition of the role of microorganisms in cheesemaking, scientific inquiry has informed our understanding of the identities and roles of microorganisms so that cheesemaking has become a controlled, predictable activity. The majority of cheese produced around the globe today is made on an industrial scale. Industrial cheesemaking has been perfected over time to yield cheeses with consistently controlled functionality and character, largely a result of use of highly specific and defined microorganisms as starter cultures, along with controlled production and aging. Such cheeses have strayed far from their origins. In contrast, and likely in response, consumer interest in artisan cheese is experiencing a renaissance worldwide. The world of artisan cheese is truly an exciting one, particularly at this time, when global demands for cheeses and cheese products are creating new opportunities for artisan producers. Artisan cheeses are defined as cheeses made by hand on a small scale, normally using milk from heritage breed animals in a closed herd and utilizing traditional, time-honored practices such as bandage wrapping or traditional utensils (2). Artisan cheesemaking is typically characterized by small-scale production in limited volume by individual producers. There are over 1,400 named cheese varieties in the world today, yet most of these cheeses belong to one of 20 distinct cheese types which share key manufacturing conditions and compositional characteristics (35, 36). These cheese types comprise the cheese families we know today as fresh, bloomy rind, smear ripened, hard uncooked, hard cooked, and blue. The historic evolution of these cheeses was impacted by geography, climate, and cultural and economic conditions. Selection of indigenous microorganisms existing in raw milk or in the cheesemaking or aging environment became a function of the manipulation of milk by the cheesemaker. The local cheesemaking technology and environment shaped the chemistry and microbiology of local cheese, which, in turn, shaped the characteristics and identity of cheese (3). Some of the great cheeses of the world which we enjoy today, such as Parmigiano Reggiano, have been continuously produced for 700 years or longer using essentially the same production practices (Table 1) (4).

Alpine cheeses are examples of a unique family of cheeses which share similar manufacturing technologies and chemical compositions. Alpine cheeses are large cheeses with hard rinds and are commonly referred to as

TABLE 1 Years of origin of noted cheese varieties^a

Cheese variety	Yr of first documentation
Gorgonzola	879
Roquefort	1070
Grana	1200 ^b
Cheddar	1500 ^b
Parmesan	1579
Gouda	1697
Gloucester	1697
Stilton	1785
Camembert	1791

^aAdapted from reference 4.

^bDate is approximate.

Swiss cheeses. Appenzeller, Comté, Emmental, and Gruyère are among the best-known Alpine cheese varieties. Alpine cheeses are characterized by a smooth, tight knit and elastic texture with the presence of holes or eyes. Although Alpine cheeses are now produced worldwide, their production originated in the Alpine regions of Switzerland and eastern France (3). The conditions under which Alpine cheesemaking was perfected were most certainly shaped by the regional geography, which consisted of the remote mountainous regions of the Alps. It is thought that cheesemaking began in this region as early as the first century BCE. Tillable land was very limited, and thus, it was farmed intensively. The harsh Alpine winters created the need for nonperishable food. Fortunately, Alpine meadows provided suitable places to graze cows for milk production, and communal farming and cheesemaking became a necessity for farmers in these remote, isolated locales. Small-scale cheesemakers worked collaboratively to make cheese from an entire herd of cows. Copper cauldrons were used by cheesemakers as vats, and cheesemaking huts, or chalets, were built at different altitudes as cows moved up and down the mountains during the grazing season. Wooden tools and utensils were also utilized (Fig. 2) (37). This remote production dictated the cheesemaking characteristics, which required a hard, elastic durable cheese which was low in moisture and had a long shelf life and was suitable for transport down the mountains. In order to achieve these characteristics, cheesemakers developed three key innovations: the curd was cut into small particles to facilitate whey expulsion; curds were cooked at high temperatures, which further drove out moisture; and curds were pressed, which facilitated additional whey expulsion (3). The impact of these technical innovations to produce a durable cheese further shaped the distinct Alpine cheese characteristics. The slow, delayed acid



FIGURE 2 Antique engraving by Charles-Joseph Panckoucke, 1784, depicting wooden cheesemaking tools for Gruyère production. From *Recueil de Planches de l'Encyclopédie, par ordre de matières, Tome troisième* (43) (author's collection). doi:10.1128/microbiolspec.CM-0001-2012.f2

production facilitated a high mineral content and high pH, which created a sweet cheese. Because salt was scarce, it was used sparingly in production. The low-salt, high-pH environment, in turn, selected for the bacterium *Propionibacterium freudenreichii* subsp. *shermanii*, which ferments lactate to produce CO₂, leading to the development of characteristic eyes in the cheese curd, in addition to acetate and propionate, which impart the sweet and nutty flavor characteristic of

Alpine cheeses (3, 5, 6). The classical dairy propionic acid bacteria (PAB) are important to the microbiology of Alpine cheeses. These organisms may have origins in unfermented feed, but they are rarely detected in samples other than milk and dairy products (7). Most strains isolated from cheese belong to *P. freudenreichii* subsp. *shermanii*, suggesting a high degree of heat resistance possessed by this species in comparison to other dairy PAB, which include *Propionibacterium*

jensenii, *Propionibacterium thoenii*, *Propionibacterium acidipropionici*, and *Propionibacterium cyclohexanicum* (6, 7). There is growing evidence that PAB such as *P. freudenreichii* subsp. *shermanii* may have important roles as probiotic cultures, serving as important modulators of the colon flora, and they may have a role in prevention of colon cancer (8, 9, 10).

Similar cultural and geographic forces shaped development of other cheese varieties, such as soft ripened cheeses, which include the bloomy-rind and smear-ripened/washed-rind cheeses. Bloomy-rind cheeses develop a white surface mold due to a complex ecosystem formed by the growth of *Penicillium camemberti*, *Geotrichum candidum*, *Cluyveromyces lactis*, and *Debaryomyces hansenii*, which, as ripening fungi, become major contributors to the sensory properties of cheeses such as Brie and Camembert (11). Bloomy-rind cheeses had their origins in France. Because these cheeses were produced for home consumption or sale in local villages, there was no need to withstand transportation over long distances, nor the need for these cheeses to withstand extended storage. These cheeses were small and easy to make. Following coagulation of milk with rennet, the curd was drained in small molds. Acid develops quickly and pH declines rapidly to pH 4.6, thus favoring the selection of fungi such as *P. camemberti*, which forms a white surface mold upon storage in a damp cellar (3).

Smear-ripened/washed-rind cheeses are ripened by aerobic bacteria and include notable varieties such as Muenster, Limburger, Taleggio, Beaufort, and Langres. Washed-rind/smear-ripened cheeses evolved far differently than the bloomy-rind cheeses, having origins within the monasteries of Europe. In the Benedictine order, a ban on meat consumption existed, which favored consumption of cheese, and cheese thus became an integral part of monastic life. Because cheese was produced for consumption within monasteries and thus did not require transportation, it did not need to be durable, nor did it require a long life. As cheesemaking became a source of income for the monasteries, low-temperature cooking/pressing was incorporated into cheesemaking, resulting in lower-moisture, washed-rind cheeses. In these cheeses, acid development is slow and favors surface growth of yeasts which elevate the cheese pH, which, in turn, favors growth of *Brevibacterium linens*, *Geotrichum candidum*, or *Debaryomyces hansenii* following salting (3, 6). Rea and colleagues, in studies of a washed-rind cheese (12), noted that despite inoculation of cheese milk with starter cultures, only two of five commercial cultures were subsequently found in cheese, suggesting the presence of bacteria from sources other

than inoculated cultures which become part of the dominant cheese flora during ripening. Importantly, results suggested the presence of a house microflora as evidenced by similar pulsed-field gel electrophoresis patterns isolated from cheeses produced at different times of the year (38).

Artisan cheesemaking traditions which evolved in England were much different from those in France or in the Alpine regions. English cheesemaking traditions had a tremendous impact on the establishment of farmstead cheesemaking in the United States. In England during the Middle Ages, agriculture was dominated by feudalism, with land owned by nobility or by monasteries. Small farmers secured smallholdings from landowners but also had access to common land for grazing of livestock. Farmers paid rent to landowners from proceeds of the sale of agricultural goods. Soft farmstead cheese made at home was common in this period. The bubonic plague significantly reduced the population in the 15th century, creating acute labor shortages. Tenant rights could now be purchased by peasants. Henry VIII dissolved monasteries, making land for farming available. Feudalism collapsed in the 16th and 17th centuries and was replaced by agricultural capitalism. In the 17th and 18th centuries, a few rich peasants became yeomen, chief tenants to landlords, who, in turn, raised rents and forced yeomen to become entrepreneurial (3).

Yeoman dairy farmers moved away from home or local cheese production to cheese production for mass markets in London. The cheeses, made in East Anglia (Suffolk or Essex), had to be large and durable and have extended shelf life for transport to London. The manufacture of these cheeses was characterized by moderate to rapid acid production during manufacture. Curds were scalded at high temperatures and pressed for whey expulsion. Salt, which was readily available, was added to further assist whey expulsion. Such manufacturing parameters yielded a low-moisture cheese which was durable during transport. These cheeses became characterized by fundamentally different chemistry (acidity, mineral content, and salt) and microbiology than those of Alpine cheeses (3).

The microflora of Cheddar cheese is composed of both starter lactic acid bacteria (LAB) and nonstarter LAB (NSLAB). Bacterial starter cultures are well-defined and characterized strains which are utilized during cheesemaking to control the fermentation and ensure the consistency of cheese production. Starter LAB used to facilitate acid production during Cheddar making typically consist of defined mesophilic cultures such as *Lactococcus lactis* subsp. *cremoris* and *Lactococcus*

lactis subsp. *lactis*. Despite the use of defined cultures, the population of microflora emerging during Cheddar cheese aging is different in composition than the defined strains added to milk. The main microflora consists of mesophilic lactobacilli and pediococci, commonly referred to as NSLAB. The species most commonly isolated include *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus curvatus* (13). As Cheddar cheese ripens, NSLAB from milk or the cheesemaking environment develop and become important to the character of Cheddar cheese. Occasionally, strains of heterofermentative lactobacilli are identified. Isolated species vary between plants, between countries, and within cheese during ripening. NSLAB likely contribute to Cheddar cheese flavor, yet the specific role is as yet undefined. Previous authors (14, 15) have found NSLAB in association with good-quality Cheddar cheese. Somers et al. (16) determined that resident NSLAB biofilms contaminate the dairy environment, and resident niches, including floors, drains, the cheese vat, hoops, and packaging machines, serve as sources of these organisms during cheesemaking.

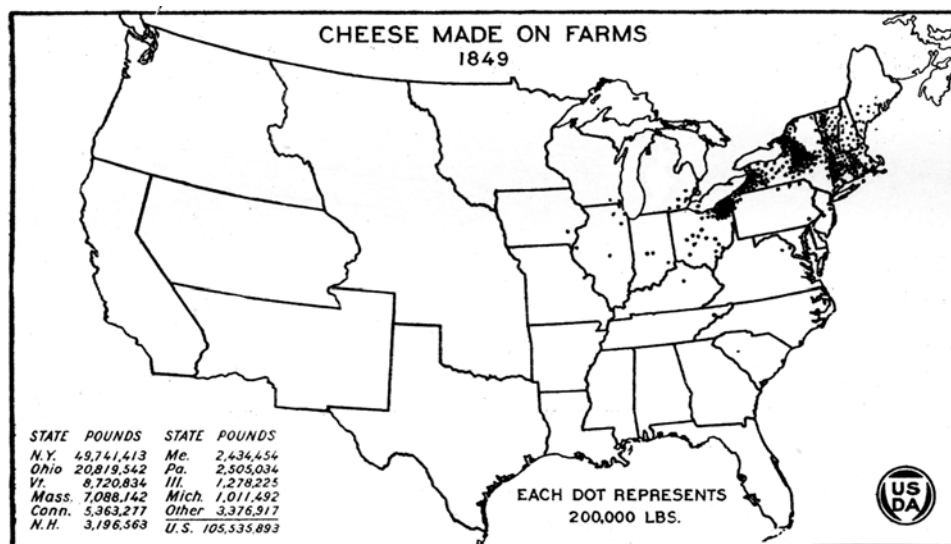
English cheesemaking techniques were brought to America by Puritan reformers and would define American cheesemaking for three centuries. By 1849, cheesemaking was well established in New England and the mid-Atlantic region of the United States (Fig. 3) (17). However, with the development of railroads, artisan cheesemaking was abandoned in the 1900s in fa-

vor of production of fluid milk for pasteurization which could be shipped great distances. By the 1940s, large-scale industrialized cheesemaking replaced artisan cheesemaking, and Cheddar cheese was almost exclusively produced in the United States. Between 1940 and 2006, the number of dairy farms in the United States declined precipitously, but the volume of milk produced dramatically increased. The 3,000 cheese plants in 1940 declined in number to approximately 400 in 2006; these plants produced 9.5 billion pounds of cheese on an industrial scale to feed an ever-expanding fast food market (http://www.nass.usda.gov/Quick_Stats/Lite/result.php?FB4AA96A-11A3-3D4D-80A1-D3F6DE2BE2EB).

MICROBIOLOGICAL SAFETY

Today we see a tremendous revival of artisan cheesemaking in the United States and elsewhere around the globe. Farmers are opting for artisan cheese production as a means of diversifying farm income. The book *The Atlas of American Artisan Cheese* profiles 345 of the 400 artisan cheese producers who were actively working in the United States in 2007 (2). As of 2012, it was estimated that there were over 800 artisan cheese producers in the United States. There is active adaptation of traditional recipes to meet present-day regulatory standards. The microbiological safety of cheese is a topic of renewed interest as global demand for cheese and cheese products continues to grow. Current regulations which govern the use of raw, heat-treated, and pasteurized

FIGURE 3 Map depicting U.S. locations of cheese made on farms in 1849. From reference 17; downloaded from Maps ETC (<http://etc.usf.edu/maps>). doi:10.1128/microbiolspec.CM-0001-2012.f3

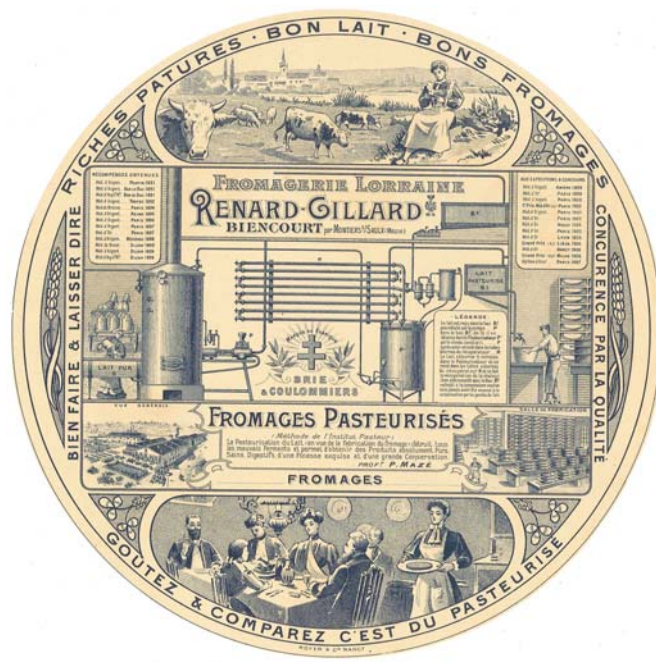


milk for cheesemaking in the United States were promulgated in 1949 (18). One of two options could be selected by cheesemakers to ensure the safety of cheese: pasteurize milk destined for cheesemaking or hold cheese at a temperature of not less than 2°C (35°F) for a minimum of 60 days (45). Research has shown that *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* can survive well beyond the mandatory 60-day holding period in Cheddar cheese prepared from pasteurized milk (19, 20, 21). Efforts have been under way in North America to examine a regulatory change requiring mandatory pasteurization of all milk intended for cheesemaking. The pathogens of concern to cheesemakers today, including *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium DT104, and *Staphylococcus aureus*, were not the same pathogens of concern as in 1949. In 1997, the U.S. Food and Drug Administration (FDA) requested that the National Advisory Committee for the Microbiological Criteria for Foods review the 60-day aging rule for cheese production. Concern was expressed that a policy revision may be necessary as 60 days of aging may be insufficient to provide an adequate level of public health protection. At the same time, in 1996 in Canada, a proposed amendment would have required all cheeses to be made from pasteurized milk or the equivalent. Health Canada ultimately withdrew this amendment because a scientific expert committee stated that the technical requirements could not be met in the manufacturing process by small-scale cheesemakers (22).

While many countries around the world view traditional cheeses made from raw milk as microbiologically safe products, other governments are demanding interventions such as pasteurization to ensure cheese safety. It is ironic that France, which has created most of the world's great raw milk cheese, was also the country where the eminent scientist Louis Pasteur developed the concept known today as pasteurization. From his home laboratory in Arbois, Pasteur conducted experiments which have revolutionized our understanding of the role of microorganisms in food fermentation. Today, in Poligny, just a few miles from Pasteur's home, contemporary scientists such as Eric Beuvier (39) are employing cutting-edge technologies to characterize a complex array of bacteria, yeasts, and both surface-growing and internal molds which impact cheese flavors and textures as well as contribute to the microbiological safety of cheese. Originally developed as a mild heat process applied to prevent spoilage of wine, pasteurization has been applied to fluid milk to eliminate bac-

terial pathogens. In the early 1900s, raw milk was a major source of human disease, including tuberculosis and scarlet fever. Numerous deaths were linked to raw milk consumption. Pasteurization has done more than any single intervention to protect public health from dangerous milk consumption. While pasteurization of milk intended for cheesemaking has also been applied to protect public health, pasteurization of cheese milk has been done largely for reasons other than safety, mainly to ensure consistency and quality of produced products. Industrial equipment for pasteurization was available as early as 1895. A large number of dairies in Denmark were using milk pasteurization in cheesemaking as early as 1908–1909, and milk pasteurization was promoted for hard cheeses produced in Denmark in order to eliminate pathogenic bacteria from milk (23). In France, Fromagerie Renard-Gillard was the first company to employ milk pasteurization for cheesemaking, using recommendations developed by Pierre Mazé of the Pasteur Institute (Fig. 4). Research in the United States on using pasteurization in the cheesemaking process began in 1907 in Wisconsin, with the primary goal of improving cheese quality, although product safety was

FIGURE 4 Antique Brie and Coulommiers label from Fromagerie Lorraine Renard-Gillard, located in Biencourt, near Montiers-sur-Saulx, France. Alfred Renard-Gillard worked from 1906 to 1922 with P. Mazé of the Pasteur Institute on improved techniques of cheese production, including the use of pasteurized milk for cheese manufacturing. (Author's collection.) doi:10.1128/microbiolspec.CM-0001-2012.f4



also a concern. Stevenson (24), working in New Zealand, reported on the advantages of pasteurized milk for cheesemaking, which included improved cheese flavor, superior yield, more uniformity, extended shelf life, and simplification of the cheese manufacturing process. If raw milk was of inferior quality, cheese made from pasteurized milk received preference scores during evaluation. Similar results were obtained by Hochstrasser and Price (25) when evaluating Camembert cheese manufactured from pasteurized milk. Brie cheese was first imported into the United States in 1936. Pasteurization was used to facilitate export of Brie to the United States because of the need to find a stable and safe way to distribute cheese. Pasteurized milk made it easy to produce a cheese that had a long enough shelf life for transport by ship and by rail. All of the aforementioned studies utilized holding pasteurization (145°F, 30 min). It should be noted that the pathogens of concern during these times were not the pathogens about which we have concerns today.

Many artisan cheesemakers utilize raw milk in cheesemaking and, from this raw material, manipulate the cheesemaking process to select for desirable organisms. Cheesemakers argue that raw milk is a reservoir of a diverse microflora which imparts diverse organoleptic and sensory characteristics to cheese. In many traditional cheesemaking procedures, milk is preripened (held overnight at room temperature) to select for mesophilic bacteria, which are beneficial to the cheesemaking process, facilitating the development of acidity as a result of lactic acid production during metabolism. This practice is either discouraged or not permitted in many countries, with regulations requiring instead the use of refrigeration of milk prior to cheesemaking. U.S. regulations (46) state that “if milk is held for more than 2 hours between time of receipt or heat treatment and setting, it shall be cooled to 45°F. or lower until time of setting.” Lafarge et al. (26) examined the impact of refrigerated storage of milk prior to cheesemaking on the shifts in the composition of bacterial populations in raw milk. These investigators conducted DNA analysis of bacterial populations in refrigerated versus non-refrigerated raw milk samples using temporal temperature gel electrophoresis (TTGE) and denaturing gradient gel electrophoresis (DGGE). *Lactococcus lactis* was the major raw milk species identified via TTGE in unrefrigerated milk samples, along with *Staphylococcus* species, *Streptococcus uberis*, *Listeria innocua*, *Listeria monocytogenes*, *Lactobacillus fermentum*, and *Enterococcus faecium*. DGGE analysis revealed *Klebsiella pneumoniae*, *Arthrobacter* species, and *Brevibacterium*

linens. Following incubation of raw milk samples for 24 h at 4°C, increases in psychrotrophic species, including *Listeria* (*L. innocua* and *L. monocytogenes*) and *Aeromonas hydrophila*, along with *Lactobacillus fermentum*, *Staphylococcus epidermidis*, *Pseudomonas fluorescens*, *Enterococcus faecium*, and *Serratia marcescens*, were observed. Decreases in *Lactococcus lactis*, *Brevibacterium linens*, *Lactobacillus plantarum*, and *Lactobacillus pentosus* were observed, among others. The results illustrate that employment of refrigeration to enhance milk quality and safety prior to cheesemaking may actually select for bacterial populations which pose safety and quality issues. Quigley et al. (27) recently identified the presence of several microbial genera not previously associated with cheese, including members of the genera *Faecalibacterium*, *Prevotella*, and *Helcococcus*. The authors report detection of *Arthrobacter* and *Brachy bacterium* from goat cheese. Through use of pyrosequencing of bacterial populations associated with artisanal cheeses, the authors identified 21 different genera (Fig. 5). Marcellino et al., in their groundbreaking studies on biodiversity of *G. candidum* strains, suggested that cheesemaking technologies play a role in strain selection, and the diverse strains contribute to the diversity of flavor found in artisan cheeses (40). They state that “as traditional techniques for cheesemaking are threatened or abandoned, the collection, characterization and preservation of native strains of cheese ripening microorganisms is critical.”

Bachman and Spahr (28) assessed the safety of Swiss hard and semihard cheeses made from raw milk. Approximately 80% of the cheeses made in Switzerland are manufactured from raw milk without prior heat treatment. These authors inoculated the pathogens *Aeromonas hydrophila*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella*, *Staphylococcus aureus*, and *Yersinia enterocolitica* to raw milk at levels ranging from 10⁴ to 10⁶ CFU/ml prior to the manufacture of hard and semihard cheeses. In Swiss hard cheeses, no detection of pathogens beyond 1 day was recorded. This was attributed to the cooking temperature of 53°F to which pathogens are exposed during cheesemaking. Further, the rapid decrease of the redox potential of Swiss cheese likely imparts additional inhibitory effects. Similar results have been shown for Italian Grana cheeses (29). Thus, for certain cheese varieties, the term “raw milk” cheese is a misnomer, as this term does not reflect the high curd cooking temperatures used in the manufacture of aged Swiss and Italian cheeses. The Australian Food Safety Authority concluded, in recently

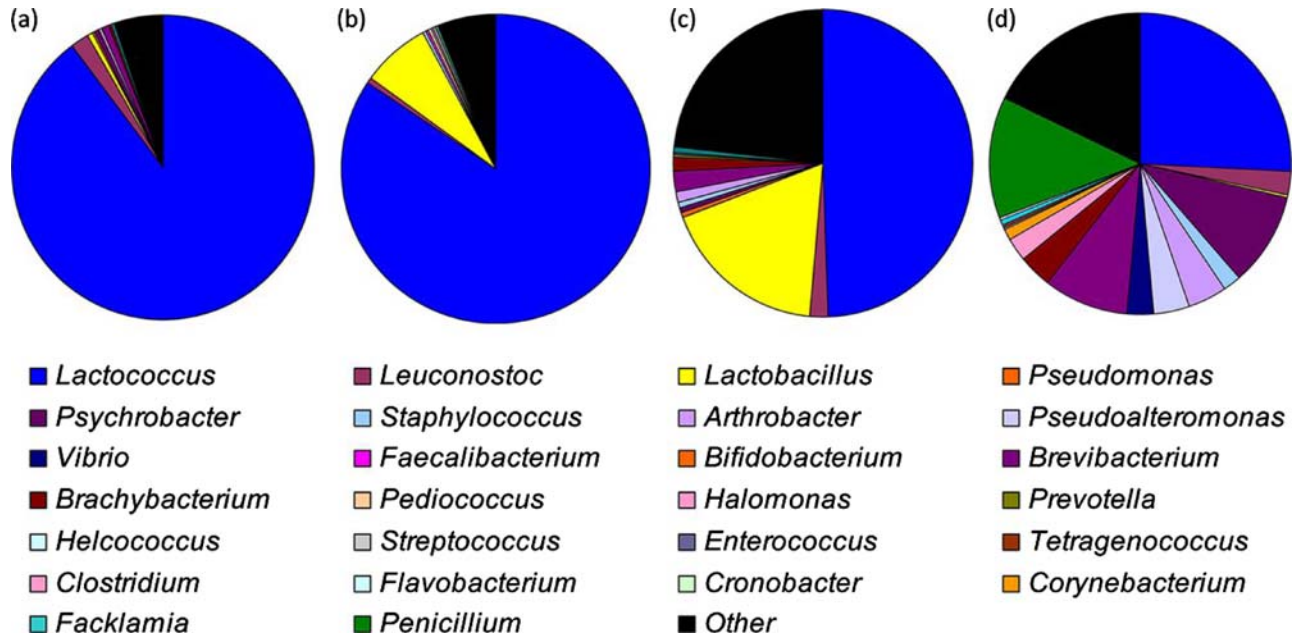


FIGURE 5 Microbial biodiversity of soft cheese (a), semihard cheese (b), hard cheese (c), and cheese rinds (d). Reproduced with permission from reference 27. [doi:10.1128/microbiolspec.CM-0001-2012.f5](https://doi.org/10.1128/microbiolspec.CM-0001-2012.f5)

completed comprehensive risk assessments, that raw milk hard Swiss cheese varieties, including Emmental, Gruyère, and Sbrinz, and extra hard grating cheeses, including Parmigiano Reggiano, Grana Padano, Romano, Asiago, and Montasio, had microbiological safety equivalent to that of cheeses made from pasteurized milk due to manufacturing and aging parameters (30).

In tracing the origins of pasteurization in the United States, in 1924, the Public Health Service implemented the Standard Milk Ordinance to assist states in the voluntary adoption of programs to control milk-borne disease. In 1939, milk pasteurization was adopted in the United States for the first time and was defined in a milk ordinance and code (31). In 1950, the U.S. Surgeon General invited regulatory agencies to establish procedures for a voluntary Interstate Milk Shipper Certification Program. The Grade A Pasteurized Milk Ordinance established national uniform standards. Products covered under the Grade A Pasteurized Milk Ordinance included creams, concentrated milks, yogurts, and low-fat and skim milk. The FDA is also responsible for additional regulations to protect the safety of cheese, but these regulations are not part of the grade A program. Milk can be grade A or grade B, with grade A milk meeting the sanitary standards for fluid milk products (and usable for any dairy product). Grade B is

considered a manufacturing grade. Milk in the United States is also classified, with classification used for pricing systems. Producers may participate in the Market Order Program, which establishes prices according to milk uses. Class I is of the highest price and is used for fluid milk products. Class II is used for soft milk products, like yogurt, cottage cheese, and ice cream. Class III is used for hard cheeses, and class IV is used for butter and for milk products in dried form. The U.S. Code of Federal Regulations (47) states, “No person shall cause to be delivered into interstate commerce or shall sell, otherwise distribute, or hold for sale or other distribution after shipment in interstate commerce any milk or milk product in final package form for direct human consumption unless the product has been pasteurized or is made from dairy ingredients (milk or milk products) that have all been pasteurized, except where alternative procedures to pasteurization are provided for by regulation, such as in part 133 of this chapter for curing of certain cheese varieties.” In the U.S. Code of Federal Regulations, cheese has been defined as belonging to one of four groups: very hard, hard, semisoft, or soft. The type of cheese depends on the type of milk used, the methods used for coagulation of the curd, the cooking and forming of the curd, the type of culture used, the salting method, and the ripening conditions. For instance, a soft cheese, like

cottage cheese, is an unripened cheese with 80% moisture. Parmesan and Romano are very hard cheeses, referred to as grated or shaker cheeses.

In the 21st century, the global demand for artisan cheeses is creating new economic opportunities. Consumers seeking distinctive products with regional flavor, or *terroir*, are becoming connoisseurs of hand-crafted products with distinctive tastes and character. Such demands have created new concerns for food safety and international trade. In response, new technologies, such as microfiltration, are being proposed to increase cheese safety, but these technologies fundamentally alter the traditional artisan practices and may not enhance microbiological safety. European cheesemakers have protected their artisan cheese practices through programs such as protected designation of origin and *appellation d'origine contrôlée* (AOC). AOC establishes the authentication of content, method, and origin of production of a French agricultural item. In 1935, the Institut National des Appellations d'Origine (INAO) was created as a government branch developed to administer and manage the AOC process for wines. The INAO's responsibilities were later broadened to protect other artisanal and traditional products, such as cheese. Every AOC product has its own set of regulations based on the product's unique history, area of production, and locally recognized practices. There are currently 44 AOC cheeses representing approximately 15% of the more than 600 cheeses produced in France. Since 1996, the European Union Protected Designation of Origin system has also protected regional foods, wines, and spirits on a European level.

Camembert de Normandie is an AOC product that must be made with unfiltered raw milk produced in Normandy from cows fed under strict conditions and have a fat content of 38%. Corroler et al. (32) conducted an ecological study to determine the effect of geographic origin of specific strains on the manufacture and ripening of a traditional Camembert de Normandie cheese. The consistent and specific presence of wild-type strains of *Lactococcus lactis* subsp. *lactis* strains isolated from raw milk produced within the AOC Camembert region confirmed the dairy significance of the Camembert registered designation of origin region. As stated by the authors, "It is well known that traditional cheeses made with raw milk ripen faster and develop a more intense flavor than cheeses made with pasteurized or microfiltered milk." Understanding the biodiversity of the microbial population associated with artisan cheese affords a look into the uniqueness which artisan production contributes to a biodiverse microflora which, in

turn, imparts unique sensory attributes. A variety of culture-dependent and culture-independent and molecular methods have been utilized for microbial characterization, but many of the traditional approaches are cumbersome and may miss unique strains which are difficult to culture and characterize. New advances in molecular biology offer some innovative approaches for rapid and comprehensive characterization of microbial communities (41).

As of 2013, it appears that efforts to require mandatory pasteurization of milk for cheesemaking are being abandoned in favor of a risk-based approach to ensure cheese safety. This is due, in part, to a redefinition of pasteurization which occurred as part of the 2002 Farm Security and Rural Investment Act (33). The U.S. legal definition of pasteurization is "[a]ny process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage" (33). At the University of Vermont, research has been conducted to examine the fate of pathogens in cheeses legally manufactured under the 60-day aging rule (42). Microbiological risk varies depending on the specific characteristics of the cheese being manufactured. Of highest risk are the bloomy-rind soft cheeses, for which high-pH and high-moisture conditions facilitate growth of pathogens. The U.S. Code of Federal Regulations (21 CFR 133.182) permits manufacture of soft ripened cheeses from raw milk provided that these cheeses are aged for 60 days or longer at a temperature of not less than 35°F (34). Due to renewed interest in specialty cheeses, artisan and farmstead producers are manufacturing soft mold-ripened cheeses from raw milk, using the 60-day holding standard to achieve safety. Lower-moisture soft ripened cheeses to be held for 60 days supported the growth of very low levels of *L. monocytogenes* as a postprocess contaminant independent of the milk type used for manufacture. The safety of cheeses within this category must be achieved through control strategies other than a 60-day holding period, and revision of current federal regulations is warranted (34).

The study by D'Amico et al. (34) is of particular interest to the FDA and Health Canada as they embark upon a joint soft cheese risk assessment. The FDA and Health Canada have documented associations between consumption of certain soft cheeses and onset of listeriosis. They are therefore continuing to evaluate the safety of soft ripened cheeses, particularly those made from raw milk, and will do so through a joint FDA/

Health Canada risk assessment. This risk assessment will assess the public health impact of *L. monocytogenes* in soft ripened cheese through focusing on sources of contamination, the impact of various manufacturing and processing steps, and the effectiveness of intervention strategies, including new technologies. The impact of consumer handling practices will also be evaluated and a model developed to assess predicted risk associated with manufacturing processes, interventions, and handling practices. It is important to note that the majority of cheese-related outbreaks are caused by postprocess recontamination of cheese; thus, employment of pasteurization of milk does not address this problem. A reevaluation of the safety of traditional artisan practices, validation thereof, and communication of the scientific principles which promote safety will therefore be necessary to enable the continued production of traditional artisan cheeses in global commerce.

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