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The Basics of Cheesemaking

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ABSTRACT All cheeses have a common set of principles that involve a complex web of chemical, biochemical, and microbiological changes. These changes first transform milk into fresh or unripened cheese. Although some cheeses are consumed immediately after manufacture, most are subsequently aged or ripened for weeks to years depending on the variety. During aging or ripening, a cheese's sensory characteristics undergo multifaceted and often dramatic changes. The steps performed during the earliest days of the cheesemaking process are especially critical because they establish the chemical characteristics of the cheese at the start of ripening, and these characteristics in turn affect the ripening process. For most cheeses, the key process on the first day of cheesemaking is the fermentation of lactose to lactic acid by bacteria. The rate at which lactic acid is produced profoundly affects the initial chemical characteristics of the cheese, which selectively influence the complex microbial populations that find their way from the milk and surrounding environment into the cheese. This article discusses the basics of cheesemaking by integrating the practical steps that all cheesemakers use with the scientific principles on which those practices are based. The aim is to paint a conceptual picture in which the microbiology of cheese "fits together" with the basic practices of cheesemaking and the scientific principles that underlie them.

INTRODUCTION

All cheeses share a set of principles that involve a complex matrix of interdependent chemical, biochemical, and microbiological changes. Collectively, these changes first transform milk into fresh or unaged cheese. Although some varieties are consumed immediately after manufacture as fresh cheese, most undergo a subsequent period of aging or ripening, ranging from weeks to years depending on the variety, during which the sensory characteristics undergo multifaceted and often quite dramatic changes.

The various steps performed during the first day of cheesemaking, or first few days for cheeses that require extended salting regimens, are especially critical because they establish the chemical characteristics of the cheese at the start of ripening, which, in turn, influence the ripening process. For most cheeses, the first day of cheesemaking is centered on the bacterial fermentation of lactose to lactic acid. The rate at which lactic acid is produced profoundly shapes the initial chemical characteristics of the cheese, which, in turn, exert powerful selective influences on the complex microbial populations that invariably find their way from the milk and surrounding environment into the cheese. Of the plethora of organisms that are present in newly made cheese, some will remain viable and may even proliferate during aging, others will be suppressed completely, and others may be initially suppressed and then favored or vice versa, depending on the chemical environment to which they are subjected. To add to the complexity, the chemical environment of the cheese often changes dramatically as ripening progresses. The mix of organisms that remain viable and their population densities, as well as the timing of cell death and lysis, directly and indirectly shape the chemical and biochemical reactions that drive flavor and texture development during ripening.

All of this sounds very complicated, and indeed it is, but much of this complexity can be reduced to a handful of scientific principles that in practice can be controlled and systematically varied (even if the science is not understood) to achieve an almost limitless range of cheesemaking outcomes, for better or for worse. The historical development of distinctly different cheese

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varieties can be thought of as modulations of these basic scientific principles, or variations on a theme. Over the course of 9,000 years or so, cheesemakers in various places discovered these modulations through careful observation and trial-and-error experience, and they modified their craft and equipment as necessary to produce outcomes (i.e., wonderful cheeses) that met their needs in the time and place in which they lived. Although the science of cheesemaking is very complex and incompletely understood (despite having been the subject of systematic study for more than a century), much can be distilled down to a handful of principles. Therefore, this article presents the basics of cheesemaking by integrating the practical steps that are used by all cheesemakers with the scientific principles, in highly distilled form, upon which those practices are based. The specific goal is to paint a conceptual picture in which the microbiology of cheese "fits together" with the basic cheesemaking practices and the scientific principles that underpin those practices. It is hoped that this article will foster a better appreciation for how the bland raw material known as milk came to be transformed into the stunning array of cheese varieties that we have inherited.

THE BASICS OF MILK CHEMISTRY

Milk is the raw material from which all cheeses are produced; therefore, the basics of cheesemaking begin with the basics of milk chemistry. The following brief review of the five fundamental components of milk (i.e., water, lactose, fat, protein, and salts) lays the foundation for understanding how each component contributes to the chemistry and structure of cheese and their integration with one another.

Water

Milk is approximately 85% water; therefore, water is milk's most abundant component and serves as the continuous phase throughout which the solid components (lactose, fat, protein, and salts) are dispersed (1). Because of their strong dipolar nature, water molecules are attracted to one another and other polar molecules and ions; therefore, they tend to cluster together tightly though transient hydrogen bonding. In contrast, water molecules shun nonpolar molecules and minimize their area of interface. The solid constituents of milk remain dispersed throughout the water phase because they either are polar in nature or, in the case of milk fat and casein, are packaged within macromolecular structures that contain a polar surface layer that enables the structure to interact with water molecules. Coagulation, the pivotal first step in cheesemaking upon which all else depends, is accomplished by converting proteins in milk (or cream, or whey or buttermilk depending on the cheese variety) from their native polar form to a nonpolar form. When this occurs, the protein is forced to separate from the water phase through a process that entraps fat and minerals and, initially, all of the water and dissolved substances. This phenomenon, referred to as coagulation, and the process of syneresis (i.e., curd contraction and water [whey] expulsion) that follows coagulation give rise to discrete curd particles from which cheese is fashioned. Thus, the pivotal first step in cheesemaking centers on transforming milk proteins from a polar to a nonpolar state, thereby initiating coagulation. There are three different mechanisms by which this may occur, which give rise to three fundamentally different cheese families: rennet-coagulated, acid-coagulated, and acid/heat-coagulated cheeses (2).

Lactose

Milk contains about 5% lactose, which is a highly polar disaccharide that exists in true solution. Therefore, when the water in milk separates as whey from the curd during cheesemaking, it carries lactose with it in equal proportion. Only a small fraction (generally around 5%) of the water and lactose in milk is ultimately retained in cheese. Lactose is vital to cheesemaking, nevertheless, because it is the substrate that lactic acid bacteria (LAB) ferment to lactic acid during the manufacturing process. The small amount of residual lactose that is retained in newly made cheese also impacts ripening in a range of ways depending on the microbial players that ultimately ferment the residual lactose and the fermentation pathways that they employ.

Milk Fat

About 98% of the fat in milk consists of triglycerides (3). Triglycerides are very nonpolar and thus cannot remain dispersed in water unless they are packaged as an emulsion in the form of droplets that are coated with a polar surface layer. Milk fat exists as large triglyceride droplets or globules that are packaged in a polar phospholipid membrane that enables the globules to remain dispersed in milk (4). During cheesemaking, the milk proteins physically entrap the fat globules when the proteins separate from the water phase during coagulation and syneresis. Therefore, almost all of the fat in milk (generally 90% or more) becomes concentrated in the cheese.

Milk fat strongly influences both the flavor and texture of cheese. Texture is influenced in a highly

temperature-dependent manner because the triglycerides of milk fat possess a gradual melting range; that is, the proportion of noncrystallized (liquid) to crystallized (solid) triglycerides increases gradually with increasing temperature. At <5°C, the majority of triglycerides in milk fat globules are crystallized, forming hard solid spheres. The globules become less crystallized and progressively softer and more fluid-like with increasing temperature, attaining full liquidity around 38°C (5). In cheese, this transition translates into a softer and stickier texture. Milk fat contributes to cheese flavor by virtue of its characteristically high distribution of short-chain fatty acids within the triglycerides. When freed from the triglyceride structure through enzymatic hydrolysis (i.e., lipolysis), short-chain fatty acids (4 to 12 carbons in length) are highly volatile and possess strong piquant and pungent aroma and flavor notes that contribute to the sensory characteristics of many cheese varieties (6). The resulting flavors and aromas may range from exquisite to obnoxious depending on the concentrations and relative proportions of free fatty acids released. Bacteria, yeasts, and molds all serve as sources of esterases (i.e., lipases) capable of producing free fatty acids from milk fat; thus, the microbial ecology of cheese has profound implications for free fatty acid flavor production, for better or for worse. Free fatty acids, in turn, may serve as the substrate for the production of highly volatile and flavorful methyl ketones by mold species during aging, which may produce sensory notes ranging from fruity and floral to mushroom and musty depending on concentrations and relative proportions.

Milk Protein

The protein in milk is made up of two distinct families, known as the caseins, which account for about 80% of the total protein, and whey proteins, which account for the remaining 20%. Two of the three major families of cheese (the acid-coagulated and rennet-coagulated families) arise through the coagulation of casein alone. For these cheeses, the whey proteins do not participate in coagulation and, as their name implies, are removed along with the whey during cheesemaking. In contrast, both whey proteins and caseins participate in coagulation and are incorporated into the third major cheese family, those for which a combination of acid and heat is used to initiate coagulation at the start of cheesemaking.

Caseins

The caseins consist of four major components that are designated α_{s1}-, α_{s2}-, β-, and κ-casein. They are classified as phosphoproteins, meaning that they possess up to 13 negatively charged phosphate groups that are bonded to serine residues along the amino acid backbones of the casein molecules, which range in length from 169 to 209 amino acids (7). Phosphoserine residues have the capacity to form ionic bonds with calcium ions, which are abundantly present in milk; ionically bound calcium, in turn, forms ionic complexes with inorganic phosphate ions that also are abundant in milk, thereby creating nanocrystals of colloidal calcium phosphate. Because of this feature, the vast majority of caseins in milk are packaged as large spherical macromolecular structures referred to as casein micelles. The casein micelle can be thought of as a tangled spherical mass of thousands of individual casein molecules that are bonded together in part by calcium phosphate nanocrystals through ionic linkages with phosphoserine residues on adjacent casein molecules (8). About two-thirds of the total calcium and one-half of the total phosphorus in milk are occluded within casein micelles in the form of colloidal or micellar calcium phosphate (1).

There are three characteristics of casein micelles that are essential to the making, ripening, and ultimate diversity of cheeses. First, the surface of the casein micelle is very polar due to its high concentration of κ-casein, which is unique among the caseins in that it contains highly polar carbohydrate side chains with charged acidic groups that protrude from the micelle surface and essentially form a hairy polar surface layer (1). In contrast, the interior of the micelle is comparatively nonpolar. The polar surface enables the micelle to interact with water molecules and remain dispersed in the water phase of milk. Under certain circumstances, however, the polar micelle surface can be neutralized or shaved off, thereby rendering the micelles less able to interact with water and causing them to separate in the form of a coagulum. This is the basis of coagulation, and cheese would not exist if this were not so.

Second, casein micelles have a prodigious buffering capacity (i.e., capacity to absorb hydrogen ions) because of their high calcium phosphate content. One can think of casein micelles as spherical sponges that continually absorb and neutralize a fraction of the hydrogen ions that accumulate in the water phase of the milk or cheese curd as LAB produce lactic acid during cheesemaking. As casein micelles absorb hydrogen ions, micellar calcium phosphate is converted to a soluble form and released from the micelles into the water phase (1). For some cheese varieties, the production of lactic acid during the first day of cheesemaking is characteristically rapid and extensive, which results in rapid and extensive demineralization of the curd and a final cheese that is

depleted of calcium phosphate. For other cheeses, lactic acid production is slow and limited, resulting in limited demineralization and a final cheese that is rich in calcium phosphate. The great diversity that exists among cheese varieties is partly related to differences in the rates and extents of acid production during the first day of cheesemaking and concomitant demineralization of the curd. For this reason, the first day of cheesemaking is centered on the bacterial fermentation of lactose to lactic acid. Thus, it is not surprising that dairy microbiologists have directed much research towards gaining control over the production of lactic acid by LAB through advanced starter culture technologies (9).

Finally, it is important to recognize that casein micelles possess strong water-binding and water-holding capacities. Water in cheese exists in several different physical and chemical states that fall into two broad categories: chemically bound or nonsolvent water, which is tied up and not available to support microbial growth and enzymatic processes, and bulk phase water, which is weakly immobilized or loosely held within the cheese matrix and therefore biologically available (10). The amount of bound water in cheese is directly influenced by the casein content; therefore, two cheeses with the same amount of water but different casein contents will possess different levels of casein-bound water and, therefore, biologically available water, which has implications for microbiological and enzymatic activities during ripening. The water-holding capacity of cheese, i.e., the ability to immobilize bulk phase water and prevent separation of the cheese serum (called "weeping"), is also directly influenced by casein content. Thus, for every cheese variety there is a balance between water and casein contents that must be maintained within certain ranges; some cheeses are highly sensitive to shifts in this balance and others more forgiving, but large shifts invariably affect ripening and quality.

Whey proteins

The whey proteins mostly exist as monomers or dimers that are folded into compact globular three-dimensional arrangements (11). Their native folded states enable the whey proteins to remain dispersed in the water phase of milk because the polar regions of their amino acid backbones are oriented outwards towards the water phase and shield the nonpolar regions buried beneath. Therefore, like lactose, whey proteins are removed with the whey in equal proportion to water during the making of acid- and rennet-coagulated cheeses. However, whey proteins are heat sensitive and begin to denature and unfold at temperatures above about 79°C, thus exposing their nonpolar hydrophobic regions. Whey proteins become even more heat sensitive when the pH of milk is decreased from its physiological value (around 6.7) to 6.0 or lower (e.g., due to lactic acid production by LAB or to the external addition of an acidulant). Heat-denatured whey proteins lose their capacity to interact with water molecules due to their exposed hydrophobic regions, and this causes them to aggregate with each other and with casein micelles. Casein micelles also become heat sensitive when the pH of milk is lowered, and this instability combined with whey protein denaturation and casein-whey protein interactions is the basis for acid/heat coagulation, as discussed below.

Salts

Milk contains both inorganic and organic salts, but calcium and phosphate ions in the form of calcium phosphate are by far the most important from a cheesemaking standpoint. Calcium and phosphorus combined account for about one-half of the total mineral content in milk. The chief importance of calcium phosphate lies in its association with casein micelles, as already discussed.

THE BASICS OF COAGULATION

Milk coagulation is the quintessential first step in cheesemaking because it initiates a process of selective concentration that results in the separation of most of the casein and fat in milk (along with lesser amounts of salts) as curd from most of the water and its dissolved solids as whey. Cheesemakers of old discovered three different ways to coagulate milk, which gave rise to three distinctly different cheese families: acid-coagulated, rennet-coagulated, and acid/heat-coagulated cheeses.

Acid Coagulation

Acid coagulation occurs when harmless LAB ferment lactose to lactic acid while growing and reproducing to high populations in warm (e.g., 20 to 32°C) milk. In traditional practice, LAB were naturally present in raw milk as adventitious contaminants from environmental sources such as teat surfaces and surfaces of pails, vats, utensils, etc. In modern practice, the species and strains of LAB and their population densities are determined by the starter culture that is added to the milk at the start of cheesemaking. As lactic acid is produced and the milk pH declines towards the isoelectric point of casein at pH 4.6, the accumulation of hydrogen ions essentially neutralizes the polar surfaces of casein micelles, thereby rendering them incapable of interacting with water

molecules (12). The micelles are thus forced to interact with one another in a peculiar manner that results in the formation of aggregates and chains of micelles. As coagulation progresses, the micellar chains increase in length and interlock with one another to form a three-dimensional net-like matrix that initially entraps all of the water and solid components (mainly lactose, fat, whey proteins, and salts) of the milk, as illustrated in Fig. 1. Over the course of several hours, the milk is thus transformed from a liquid state to a soft fragile gel, or coagulum. The casein matrix that gives the coagulum its structure is highly demineralized because most of the micellar calcium phosphate is dissolved by the high concentration of lactic acid and low pH needed to initiate coagulation.

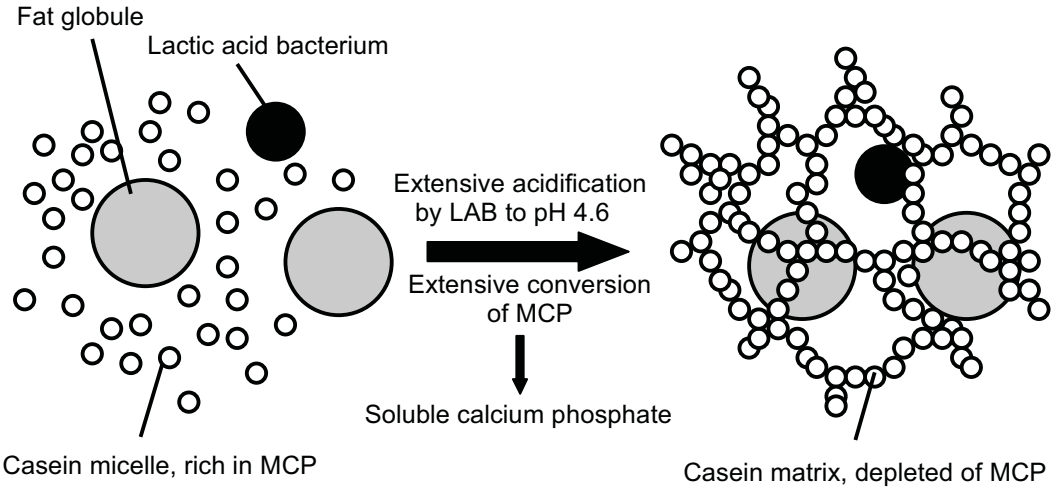
The demineralized casein matrix is very fragile and has limited ability to contract and expel whey, which impedes whey drainage during manufacture (12). Therefore, acid-coagulated cheeses are generally quite high in water content (usually around 70 to 80% moisture) and very vulnerable to microbiological spoilage, especially by yeasts and molds because of their low pH values (around pH 4.6). Therefore, most acid-coagulated cheeses are consumed fresh (unaged) as soft, high-moisture varieties. Cottage cheese, quark, fromage frais, and cream cheese are well-known modern examples of fresh acid-coagulated cheese. A small amount of rennet, for example, 1 to 10% of the dosage used for rennet coagulation, may be added to the milk shortly after the start of fermentation to increase the firmness and syneresis capacity of the curd, producing a lower-moisture product with improved texture and stability (13). There are a few ripened versions of acid-coagulated cheese, such as traditional varieties produced in the Near East that are aged in sealed clay vessels or animal skins (14). Apart from these few exceptions, however, most acid-coagulated cheeses are consumed fresh (unaged) as soft, high-moisture varieties.

It should also be mentioned that a subcategory of acid-coagulated cheese is produced by adding moderate amounts of rennet (e.g., 30 to 50% of the level used for rennet coagulation) to the fermenting milk, resulting in a hybrid acid/rennet-coagulated cheese. Hybrid cheeses are lower in moisture than acid-coagulated types, and several cheese varieties made by this technology are surface ripened with the white mold *Penicillium camemberti*. Hybrid cheeses made from goat milk are discussed in detail by Le Jaouen (15) and are not reviewed here.

Acid/Heat Coagulation

Although whey proteins are readily denatured on heating, casein micelles in fresh milk are highly heat stable and remain in colloidal dispersion at temperatures up to 140°C (11). However, milk that is moderately acidified (e.g., to pH 6.2 to 5.4), either through the production of lactic acid by LAB or through the addition of an external acidulant such as vinegar, becomes susceptible to

FIGURE 1 Diagrammatic representation of the process of acid coagulation. LAB ferment lactose to lactic acid and acidify the milk to around pH 4.6. Coagulation occurs when casein micelles aggregate to form a net-like matrix. During acidification, micellar calcium phosphate (MCP) is extensively converted to soluble form, resulting in a casein matrix that is highly depleted of MCP. doi:10.1128/microbiolspec.CM-0002-2012.f1



heat-induced coagulation at relatively low temperatures (e.g., 85°C), and this gives rise to a second, rather small cheese family known as the acid/heat-coagulated cheeses. Coagulation occurs because the whey proteins unfold and lose their ability to interact with water under the combined influence of acid and heat. Concomitantly, the polar surfaces of the casein micelles become neutralized by an incompletely understood mechanism that may involve calcium-induced destabilization (16). This causes the denatured whey proteins to attach onto the micellar surfaces and the micelles to aggregate into clusters that entrap fat globules. The resulting flocs or curd particles then float to the surface and are separated from the whey with a sieve or perforated ladle. The curds are then allowed to drain and in some cases are pressed. Ricotta is a well-known example of a drained acid/heat-coagulated cheese; queso blanco is a pressed version.

The acid/heat-coagulated cheese family is characteristically high in moisture content (around 50 to 80%), and this, in combination with their generally high pH values, renders most of these cheeses very susceptible to microbial spoilage. There are a few aged versions of acid/heat-coagulated cheeses, produced traditionally in the Near East, which are ripened in sealed clay or skin vessels (14). However, like the acid-coagulated cheeses, acid/heat-coagulated types are mostly consumed fresh.

Rennet Coagulation

"Rennet" refers to a group of aspartic proteinases that preferentially cleave κ-casein at the surface of casein micelles when added to milk, thereby initiating coagulation (17). In traditional and modern practices, rennet enzymes are derived from a variety of animal, plant, and microbial sources. Technically, the term rennet is restricted to enzymes derived from the abomasa of ruminants; coagulating enzymes from other sources are referred to simply as coagulants or rennet substitutes (18). However, in common usage "rennet" refers to any milk-coagulating enzyme used in cheesemaking, and this broader meaning is used in this article.

Calf rennet is the most widely used animal rennet, but kid and lamb rennets are also used frequently in the making of traditional cheeses from goat and sheep milk. All three animal rennets consist of a blend of the enzymes chymosin and pepsin, with chymosin dominating (18). Plant-derived rennets have also been used in traditional cheesemaking for thousands of years. Enzymes extracted from the flowers of two *Cynara* species (*Cynara cardunculus* and *Cynara humilus*), commonly known as the cardoon and the globe artichoke, are still used in the Mediterranean region for cheesemaking, especially in Spain and Portugal. In West Africa, *Calotropis procera* is used as a rennet source, and numerous other plants have contributed rennet sources in traditional practice, such as the sap of the fig tree (*Ficus carica*).

The microbial rennets, which are derived from fungal sources, are relative newcomers, developed in the second half of the 20th century. There are three major microbial rennets, derived from *Rhizomucor miehei*, *Rhizomucor pusillus*, and *Cryphonectria parasitica*, that from *R. miehei* being the most widely used (19). These fungi, which are propagated in large fermentation tanks, secrete aspartate proteinase enzymes that are harvested, purified, and standardized for milk clotting activity and then marketed as microbial or so-called "vegetable" rennets. The most recent variation on this theme, made commercially available in the early 1990s, is fermentation-produced chymosin. In the case of this product, the gene that codes for bovine chymosin is spliced into the DNA of a host microorganism, usually a yeast or a fungus, which then is propagated in large fermentation vessels to produce pure chymosin that is harvested, purified, and standardized for milk clotting activity.

Rennet coagulation occurs according to a two-step process that is characterized by an enzymatic phase and a nonenzymatic phase (19). Regardless of source, all rennet enzymes initiate the enzymatic phase by hydrolyzing κ-casein, resulting in the release of the charged, carbohydrate-rich C-terminal region of the molecule, referred to as the caseinomacropeptide. Rennet enzymes effectively clip off the carbohydrate-rich polar layer at the micellar surface. This exposes the micellar interior, which becomes nonpolar in the calcium-rich environment of milk, and this triggers the nonenzymatic phase. The spherical micelles lose their ability to interact with water molecules, forcing them to interact with one another to form micellar aggregates and chains, similar to the process that occurs during acid coagulation. As coagulation progresses, the micellar chains increase in length and interlock with one another to form a three-dimensional net-like matrix that initially entraps all of the water and solid components (mainly lactose, fat, whey proteins, and minerals) of the milk, as illustrated in Fig. 2.

Although the illustrations for acid and rennet coagulation in Fig. 1 and 2 look quite similar, there are two very important differences. First, rennet curd is more resilient (less fragile) and better able to contract and expel whey than acid curd due to structural differences that result from the two different mechanisms of coagulation. Therefore, a wider range of conditions can be

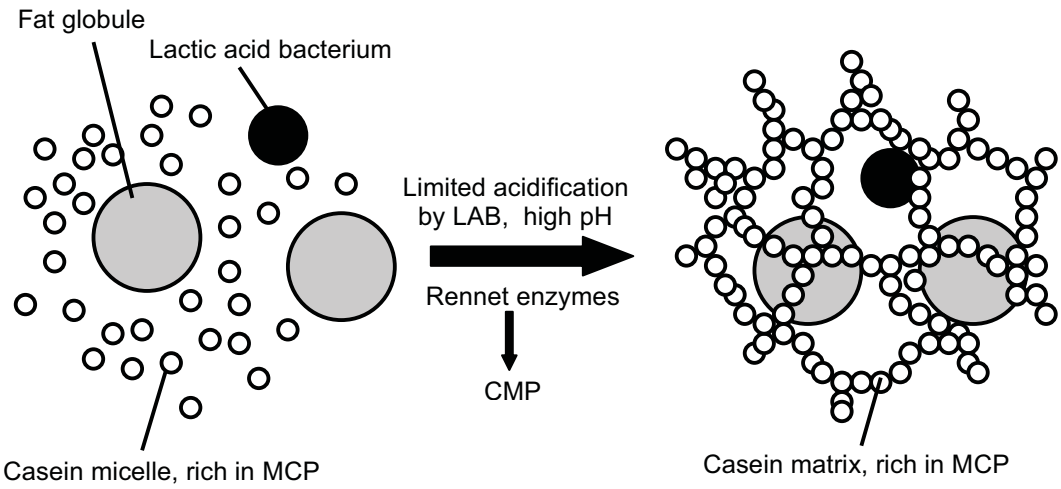


FIGURE 2 Diagrammatic representation of the process of rennet coagulation. Rennet coagulation is mediated through the action of rennet enzymes that cleave κ-casein and release caseinomacropeptides (CMP) from the casein micelles. This causes the micelles to aggregate in the form of a net-like matrix. Rennet coagulation occurs at high pH (around pH 6.6 to 6.3, depending on the variety), i.e., before extensive acidification by starter LAB. Therefore, limited conversion of micellar calcium phosphate (MCP) to the soluble form occurs before coagulation, resulting in a casein matrix that is rich in MCP. doi:10.1128/microbiolspec.CM-0002-2012.f2

applied to rennet curd to effectively promote contraction and whey expulsion (i.e., syneresis). Cheesemakers learned to exploit various conditions to enhance syneresis and produce rennet-coagulated cheeses with lower moisture contents (around 30 to 60%) than are possible with acid-coagulated cheeses (around 70 to 80%). This, in turn, opened the door to developing a much wider range of aged cheese varieties with different characteristics. Thus, the discoveries of various means to modulate whey expulsion through innovations in cutting, cooking, pressing, and salting represented great milestones in the history of the making of rennet-coagulated cheeses.

A second difference is that rennet coagulation occurs very rapidly (within 30 to 60 min) at high pH (around pH 6.6 to 6.3), whereas acid coagulation occurs over many hours (from around 5 to 48 h) and requires a very low pH (around pH 4.8 to 4.6). Consequently, rennet curd is much richer in calcium phosphate than the highly demineralized curd that forms during acid coagulation. Because of this, rennet-coagulated cheeses always possess higher calcium phosphate content and thus higher buffering capacity, sometimes much higher, depending on the rate of lactic acid production during cheesemaking. Buffering capacity is extremely important because it helps to determine the cheese pH at the start of ripening. Cheeses that are rich in calcium phosphate (due to slow acidification) retain a strong buffering capacity, which limits the pH decline and tends to establish relatively high initial values. However, cheese moisture content, or more specifically residual lactose content, also influences the initial pH, in an inverse relationship. Therefore, cheese pH at the start of ripening is a function of both the buffering capacity and the moisture content.

For example, the mineral-rich, lower-moisture alpine cheeses may have pH values as high as 5.4 at the start of ripening. Conversely, highly demineralized, high-moisture bloomy-rind cheeses may fall to pH 4.6 on the first day after manufacture. The bottom line is that it is possible to produce rennet-coagulated cheeses that range widely in both moisture content and initial pH by modulating the rates of acid production and whey expulsion during cheesemaking. Such wide compositional latitude makes possible an extraordinary diversity of ripening outcomes. Indeed, the overwhelming majority of diverse cheeses produced worldwide are rennet-coagulated types, for this reason.

THE BASIC STEPS OF CHEESEMAKING

Acid- and Acid/Heat-Coagulated Cheeses

The making of acid-coagulated and acid/heat-coagulated fresh cheeses is quite simple, at least in comparison with that of most rennet-coagulated varieties. As described above, coagulation is achieved through either

lactose fermentation and acidification to around pH 4.6 or a combination of partial acidification and heating. After coagulation is complete, the cheesemaker has only one primary objective, which is to drain enough whey from the curd to attain the desired consistency in the fresh cheese. Salt is sometimes added to acid-coagulated and acid/heat-coagulated fresh cheeses—to the former usually at levels 1% or less, while the latter may be heavily salted, as in the case of queso blanco. As noted above, it is also possible to produce aged versions of acid-coagulated and acid/heat-coagulated cheeses when the right conditions are present. Compared with rennet-coagulated cheeses, however, in acid/heat- and heat-coagulated cheeses the opportunities to increase whey expulsion from curds are limited, which limits the options for producing cheese with a low enough moisture content to permit extended aging. Therefore, most cheeses in these families are consumed fresh.

Rennet-Coagulated Cheeses

Rennet-coagulated cheeses are much more complicated to make than acid- and acid/heat-coagulated types because of the complex and unforgiving nature of the ripening process, which may take weeks, months, or years to complete. In order for ripening to proceed in the desired manner, the newly made cheese must possess a specific chemical composition, largely defined by pH and moisture and salt contents, that correctly sets the stage for the complex physicochemical, biochemical, and microbiological changes to come. In other words, the cheesemaker has three compositional targets that must be built into the newly made cheese, which means that three objectives must be achieved during the first day or few days of cheesemaking. First, the correct amount of whey must be expelled from the curd so that the target moisture content, which may range from around 60% to 30% depending on the cheese variety, is attained. Second, the rate of acidification and curd demineralization must be controlled so that the buffering capacity of the newly made cheese is compatible with the moisture content and pH target, which may range from around pH 5.4 to 4.6 depending on the variety. Finally, salt must be incorporated into the cheese at the correct rate to attain a target salt content that may vary from around 0.5% to 4.0% or higher, depending on the variety.

In short, the first day of cheesemaking can be thought of as a series of steps that are used to dehydrate and demineralize the rennet coagulum in a controlled manner, coupled with the controlled introduction of salt to the resulting curd. In practice, this can be very difficult to achieve consistently because the three chemical parameters are interrelated. For example, changes in acidification rate during cheesemaking may influence not only curd demineralization and cheese pH but also whey expulsion and cheese moisture content, which, in turn, may influence the uptake of salt during salting. Changes in salt uptake may affect not only cheese salt content but also whey expulsion and cheese moisture content, and cheese pH through its impact on the fermentation of residual lactose. In other words, seemingly small changes in key manufacturing parameters can cause a cascade of compositional changes in the resulting cheese that may influence ripening. Added to this complexity are seasonal changes in the composition of milk (especially in fat and casein contents), which affect critical operations such as whey expulsion, calcium phosphate retention and buffering capacity, and salt uptake and which may cause the targets for moisture and salt content in the final cheese to shift. Thus, the cheesemaker either has to adjust manufacturing practices across the season to deal with changing milk composition (as in traditional cheesemaking) or standardize the milk to a constant chemical composition (as in industrial cheesemaking).

The pH and moisture and salt contents at the start of ripening are critically important because collectively they shape the chemical environment within the cheese body and at the cheese surface. The chemical environment, in turn, along with the physical environment (which includes parameters such as temperature, humidity, exposure to oxygen and air movement, and physical handling such as rubbing, scraping, turning, etc.) determine which microbes (bacteria, yeasts, and molds) within the cheese and on its surface are favored and which are suppressed during ripening, as well as the timing and sequence in which they proliferate and eventually die off. The microbiological progression, in turn, profoundly affects the development of cheese flavor, aroma, texture, and appearance over time.

Similarly, the chemical environment also determines which of the many enzymes present within the cheese are switched on and which are suppressed, and sometimes their specificities. Finally, physicochemical changes, especially those that involve casein-water interactions such as structural swelling and casein solubilization, are also shaped by the chemical environment, which, along with microbiological and enzymatic changes, contributes to the transformation of the cheese during ripening. Therefore, if the moisture, acidity, or salt content falls outside of its target range, the resulting cheese cannot ripen along the intended path; its transformation during aging will be shifted in a different direction, resulting in a

different cheese with different characteristics, for better or for worse.

The basic sequence of steps used to make rennet-coagulated cheeses has not changed much over hundreds, even thousands, of years. However, cheesemakers of old (without understanding the science involved) learned to modulate moisture, acidity, and salt contents by varying the conditions at different steps using innovations in equipment and techniques to produce cheeses that were well suited for their needs. The eight basic steps of rennet-coagulated cheesemaking include setting, cutting, cooking, draining, knitting, pressing, salting, and finishing (affinage), the last also referred to as special applications (20).

Setting

Setting includes the inoculation of milk with starter culture, followed by a brief period of starter incubation or ripening, the addition of rennet, and coagulation. In ancient practice, LAB entered the milk as adventitious contaminants. Their populations at the time of renneting were determined to a great extent by the length of time that the milk was stored (at ambient temperature) before cheesemaking and by the ambient temperature. When cheesemaking was performed immediately after milking, populations of LAB were relatively low and acidification occurred slowly, whereas milk stored at ambient temperature for many hours (e.g., overnight) provided ample opportunity for LAB proliferation, resulting in faster acidification during cheesemaking. Thus, one of the factors that influenced the historical development of different cheese varieties was the scheduling constraints that confronted different groups of cheesemakers, which caused the time between milk harvesting and cheesemaking to vary in different times and places (21). Superimposed on this, of course, were differences in ambient temperature conditions.

Eventually, some cheesemakers discovered that they could produce cheese more consistently by adding a small portion of whey from the previous day to the milk before adding the rennet, which essentially served as the first starter culture (22). With the advent of modern microbiology at the end of the 19th century, dairy microbiolgists quickly became interested in isolating and identifying the microorganisms responsible for the fermentation during cheesemaking. In the ensuing century, the LAB involved in cheesemaking were identified and many of their strains characterized for physiological characteristics and properties relevant to cheesemaking, such as temperature sensitivity and growth characteristics, bacteriophage sensitivity, salt tolerance, acidification rates, potential for bitterness and flavor production, etc. Highly consistent and reliable cultures were developed in frozen concentrated and freeze-dried concentrated forms, consisting of precise blends of LAB strains chosen to deliver specific predictable cheesemaking outcomes. Because of the strict requirements for milk hygiene and sanitation, and mandatory low-temperature storage, today's milk contains very few indigenous LAB; thus, the starter culture alone is the engine that drives acidification during cheesemaking except in very traditional practice, where permitted.

The LAB used in cheesemaking fall into two broad categories based on temperature sensitivity: the mesophiles (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) and the theromophiles (*Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *lactis*, and *Streptococcus thermophilus*). The mesophiles are heat inactivated at temperatures above around 40°C; therefore, the thermophiles, which can survive temperatures up to around 65°C, are used predominantly in cheeses that are cooked to temperatures higher than 40°C (22). First and foremost, the role of the starter LAB is to deliver a predictable rate of acidification during cheesemaking. Failure to do so because of impaired culture activity due to improper handling or storage, incorrect dosage or ripening time, bacteriophage infection, or any other cause invariably leads to changes in cheese quality and character. Such is the basis for the old cheesemaker adage, "The starter culture is the heart of cheesemaking."

Renneting follows the addition and ripening of the starter culture. Generally, enough rennet is added to coagulate the milk within 30 to 60 min. The firmness of the rennet coagulum can vary greatly depending on the pH of the milk and the amount of time that elapses between renneting and cutting. Delayed cutting allows a firmer curd to develop, as does renneting at a lower milk pH. Lowering the milk pH causes micellar calcium phosphate to dissolve into the water phase, thereby elevating the level of soluble calcium. Higher soluble calcium enhances the nonenzymatic phase of coagulation and gives rise to a firmer coagulum or curd. Curd firmness at cutting is important because firm curds contract and expel whey less readily than soft curds; therefore, cutting at high firmness tends to produce a higher-moisture cheese than cutting at low firmness (2). For some high-moisture, low-pH cheeses that are prone to expel too much whey during manufacture, such as the bloomy-rind types, cutting firm is used to enhance moisture retention. Conversely, highly cooked,

low-moisture cheeses that require maximum whey expulsion, such the large alpine varieties, benefit from the enhanced syneresis that results from cutting soft.

Cutting

Cutting or breaking up the coagulum is necessary to initiate the separation of whey from curd. The new surface area created when the coagulum is cut into particles acts as the gateway for whey release; the greater the surface area-to-volume ratio (i.e., the smaller the curd particle), the greater the release of whey and the lower the moisture content of the final cheese (23). Thus, cheesemakers of old developed a variety of cutting utensils and elaborate cutting techniques to produce curd particles ranging widely in size and geometry, depending on the type of cheese and the requirements for whey expulsion. For example, complex cutting techniques designed to render rice-sized curd particles were developed for Emmental and Parmigiano Reggiano cheeses, because these highly cooked low-moisture varieties require maximum whey expulsion in order to achieve their targets for low moisture content. In contrast, high-moisture bloomy-rind cheeses are traditionally cut into wide strips with far lower surface area-to-volume ratios, in order to limit whey expulsion.

Microorganisms (bacteria, yeasts, and molds) that are present in the milk become physically entrapped within the curd matrix during coagulation, and only those that occupy the cut surfaces slough off into the whey. Therefore, the majority of lactic acid produced during cheesemaking originates from inside the curd particles, where most of the LAB remain occluded. As the concentration of lactic acid within the curd particles increases, concentration gradients of lactate and hydrogen ions are created relative to the surrounding whey, which drive their diffusion (along with soluble calcium and phosphate ions that are released from the micellar calcium phosphate) from the porous curd particles to the whey.

Cooking

Cooking involves the application of heat and stirring to the mixture of curds and whey that forms after cutting. Cooking to higher temperatures for longer durations and with more stirring promotes curd contraction and whey expulsion (23). However, cooking temperature also influences the rate of lactic acid production by LAB, which, in turn, affects curd contraction and whey expulsion because curd particles contract and expel whey more readily as the pH decreases. Therefore, the effect of cooking temperature on cheese moisture content can be complex, depending on how the rate of lactic acid production is affected. Cooking temperature also influences curd demineralization and buffering capacity through its effect on the rate of acidification by starter LAB. For example, the growth characteristics of *Lactococcus lactis* subsp. *cremoris* are highly sensitive to temperatures in the range of around 34 to 38°C, such that a difference of a few degrees can cause large changes in reproduction rate and rate of lactic acid production (24). Thus, cooking schedules represent a delicate time-temperature balance designed to simultaneously (i) shrink and dehydrate and (ii) acidify and demineralize the curd particles, ultimately enabling the cheesemaker to hit the targets for pH and moisture content in the cheese at the start of ripening.

Over the centuries, cheesemakers developed a wide variety of cooking techniques as vessels of bronze, copper, and iron became available, ranging from no application of heat and no stirring (i.e., uncooked cheeses that maximize moisture retention) to high-temperature treatments (e.g., 53°C) maintained for long durations (up to 2 h) with extensive stirring (i.e., highly cooked cheeses that maximize moisture expulsion).

Dipping and Draining

Eventually it is necessary to separate the curd particles from the surrounding free whey so that the particles can fuse together and form a larger entity that will become the cheese. Two approaches were developed historically for separating the whey. The most ancient method is referred to as dipping, whereby the curd-whey mixture is scooped or poured out of the vessel that was used for coagulation (and cooking if applicable) and placed in a draining vessel such as a ceramic sieve or wicker basket. The whey then gradually drains through the perforations of the sieve or basket, leaving behind a mat of curd. Later modifications involved threading a muslin cloth along the bottom of the vessel (usually a kettle of some sort), under the curd-whey mixture, lifting the resulting cloth "bag" out of the vessel, and placing it into a mold for draining and pressing. Draining differs from dipping in that the curds remain in the vat used for coagulation and cooking, and the whey is drained off through a valve fitted with a strainer to retain the curd. Alternatively, in industrial practice the curds and whey are pumped from the vat to a draining table fitted with a perforated base that drains the whey and retains the curd.

Knitting

The knitting together of curd particles commences as the whey drains and particles contact one another and fuse

together. Knitting proceeds, often for several hours, until a continuous mass of fused curd is attained. During knitting, the curds generally continue to expel whey in a temperature-dependent manner such that higher temperature promotes greater whey expulsion. Temperature also influences the rate of acid production by LAB and therefore solubilization and loss of micellar calcium phosphate. Thus, knitting continues the process of dehydration and demineralization that began during cooking, and the temperature conditions at this stage continue to represent a delicate balance designed to simultaneously shrink and dehydrate and acidify and demineralize the curd.

Pressing

Pressing is closely related to knitting and involves the application of external pressure to the curd during knitting or, in some cases, after knitting is completed. In ancient practice, pressing was accomplished by pressing down on the curd by hand or by placing a weight such as a stone on the top of the curd as it drained and knitted. Some cheeses are referred to as "unpressed" because gravity draining alone is used to knit the curd particles together. Pressing helps to expel whey and promotes more complete fusion of the curd particles, resulting in a more closed cheese texture with fewer mechanical openings. Later, cheesemakers developed a variety of pressing devices that could apply much higher pressures to the curd, thereby enabling the production of large cheeses with very closed and compact textures and tight surfaces, the former being essential for the entrapment of microbiologically produced gas in the form of round "eyes" and the latter being necessary for the development of tough impervious rinds on large brine-salted cheeses.

Salting

The addition of salt to cheese curd creates an osmotic driving force that draws whey to the surface, where it is released (24). Thus, salting is still another step used to expel moisture from the cheese. The greater the uptake of salt, the greater the release of whey. Three different approaches were developed in the past to incorporate salt into cheese. Dry salt can be rubbed onto the surface of the finished cheese, where it dissolves into the water phase and gradually diffuses into the interior; concomitantly, moisture is drawn from the surface and evaporates. Cheesemakers of old learned to apply salt repeatedly with mildly abrasive rubbing to develop a smooth, dense, impervious rind that rendered the cheese quite durable and long-lived. Although dry salting works well for small cheeses, the technique becomes problematical with large cheeses because dry salting dehydrates the surface too quickly, creating a thin dense rind that impedes further diffusion of salt from the surface to the interior (25). Therefore, it is difficult to transfer adequate salt to the body of large cheeses by dry salting before the surface closes up and restricts further inward migration of salt.

Salt can also be applied to the surface by submerging the cheese in concentrated salt brine. Brine salting is particularly useful in the making of large rinded cheeses because it allows for greater salt absorption while gradually dehydrating the surface in preparation for rind formation. Brine salting results in large persistent gradients of salt and moisture extending from the surface to center of the cheese, which affect the microbial ecology in a zonal manner; salt-sensitive microbes may occur more abundantly in the low-salt center, salt-tolerant types at the high-salt surface.

A third method of applying salt is to mix the salt directly with the curd particles before the curd is pressed together to form the final cheese (24). Using this technique, it is possible to incorporate high concentrations of salt uniformly throughout the curd mass and to uniformly and efficiently expel whey from the curd in the process. Thus, the resulting cheese lacks the large gradients of salt and moisture found in dry salted and brine-salted varieties. The very efficient expulsion of whey achieved by this salting method also allowed cheesemakers of old to produce low-moisture varieties like Cheddar without having to resort to high cooking temperatures, a great practical advantage. On the other hand, it is much more difficult to develop a rind on a large cheese that is salted before pressing because the gentle surface dehydration that occurs during brine salting must be replaced by other approaches, such as through bandaging and application of a semipermeable lipid layer (e.g., lard).

In summary, during the steps from cutting to salting there are multiple opportunities to force out more or less whey from the curd to produce a finished cheese with a lower or higher moisture content. Similarly, there are various conditions, such as the length of time and temperature at each step, that can speed up or slow down the fermentation of lactose to lactic acid by LAB, thereby modulating the mineral content and pH of the cheese at the start of ripening. Over the centuries, cheesemakers in different times and places discovered various modifications that produced cheeses that were better suited for their needs. Often such modifications were beneficial because they had a desirable effect on moisture

expulsion, the rate of lactic acid production, the incorporation of salt, or all three simultaneously. Thus, cheesemakers developed signature manufacturing schedules and techniques that created characteristic profiles of pH and moisture and salt contents in their cheeses at the start of ripening.

Finishing (affinage)

Finishing, or affinage, is the process through which the unaged or "green" cheese (comprised of bland, uninspiring curd) is transformed or "ripened" to its fully mature state. Finishing often requires a specific combination of environmental conditions (temperature, humidity, physical surroundings, presence of specific indigenous microflora, etc.) and physical manipulations (rubbing, scraping, turning, washing, etc.) that are carried out by the cheesemaker or affineur (a person who specializes in the art of finishing). Finishing may require weeks, months, or years depending on the cheese.

The ripening process that occurs during finishing can be broadly divided into two distinct zones, the body (or interior) of the cheese and the surface. Within the cheese body, subzones of ripening may also develop as a result of salt and moisture gradients that form during brine salting or pH gradients that develop during aging. From a microbiological standpoint, the cheese body and surface usually represent radically different environments, the former becoming highly anaerobic during ripening and the latter remaining aerobic. An important exception to this is when cheese is prewrapped and ripened in an oxygen-impermeable barrier film, in which case both the body and surface remain anaerobic.

Interior ripening

Obligate aerobes such as molds cannot grow in the cheese interior unless the integrity of the surface is compromised and oxygen is able to diffuse into the body. For most varieties, mold growth in the interior is a defect to be avoided, but for blue-veined cheeses such as Roquefort, prolific internal mold growth is requisite to ripening. For this reason, blue-veined cheeses are often pierced with needles at the start of ripening to create shafts that allow carbon dioxide to vent out and oxygen to diffuse in (Fig. 3). Furthermore, these varieties are made without pressing, which favors incomplete curd particle fusion and an open texture that facilitates the diffusion of oxygen throughout the body of the cheese. Also, citrate-fermenting bacteria such *Leuconostoc cremoris* and *Lactococcus lactis* subsp. *lactis* bv. diacetylactis are often used as adjunct cultures to enhance the open texture of the cheese through their production of carbon dioxide gas (26). In traditional practice, spores of the blue-green mold *Penicillium roqueforti* were naturally present in milk and cheese as adventitious contaminants. In modern practice, adequate mold growth is achieved by inoculating the milk before renneting or by spraying the curds before knitting with a spore suspension of *P. roqueforti*.

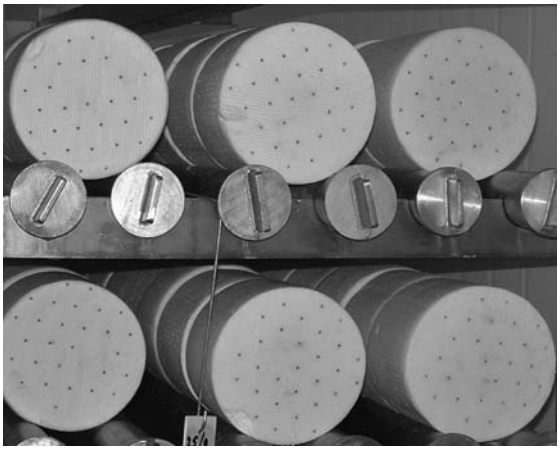


FIGURE 3 Blue-veined cheeses ripening on racks in a cool (ca. 13°C), humid (ca. 90% relative humidity) environment. Note the needle shafts that have pierced the body of each cheese to permit oxygen diffusion into the interior. doi:10.1128/microbiolspec.CM-0002-2012.f3

In addition to their oxygen requirement, blue-veined cheeses must possess an initial chemical composition that favors the growth of the *P. roqueforti*. Composition varies considerably from one variety to the next, but in general, moderate to high moisture (around 38 to 50%) and salt (2 to 5%) contents and low pH (4.6 to 5.0) characterize the blue-veined cheeses. Within these broad ranges for moisture, salt, and pH, the selective pressures that are exerted on the cheese microflora can vary considerably, which contributes to some of characteristic differences among different blue-veined varieties. Cool storage temperature (around 5 to 10°C) is necessary for selective mold growth, as is a high relative humidity (around 85 to 90%) to prevent excessive dehydration, especially at the surface. Finally, blue-veined cheeses must be turned regularly to prevent moisture accumulation and rind rot and to promote uniform surface growth. Until modern times, underground caves and cellars were the only microenvironments that maintained the consistently cool and humid conditions necessary for ripening blue-veined cheeses. Therefore, varieties in this family often developed in regions where natural climate-controlled subterranean chambers were found.

During ripening, *P. roqueforti* secretes extracellular lipases that release high concentrations of free fatty acids

from the triglycerides of fat globules (27). Adventitious yeasts within the cheese body also contribute to lipolysis, though to a lesser extent. The volatile short-chain fatty acids contribute to piquant flavor notes, but their impact is dampened through their subsequent conversion to methyl ketones by *P. roqueforti* and through their conversion to nonvolatile calcium salts as the cheese pH rises. The pH rises substantially during ripening because *P. roqueforti* catabolizes lactic acid to carbon dioxide, which deacidifies the curd. The mold also secretes extracellular proteolytic enzymes that contribute to the production of ammonia. Therefore, pH increase is greatest in regions of the cheese where mold growth is most prolific. Adventitious yeasts, starter LAB, and nonstarter bacteria also contribute to proteolytic changes in the cheese body that affect flavor and texture development. Clearly, many microbial players are involved in the ripening of blue-veined cheeses, and there is ample opportunity for the desired sequence of events to shift in different directions when the cheese composition deviates from the target ranges.

Apart from the blue-veined varieties, most other ripened cheeses quickly develop anaerobic conditions in the interior unless the surface rind is compromised. Under anaerobic conditions, indigenous yeasts usually contribute little to ripening in the cheese interior unless their populations are abnormally high due to poor sanitation during milk harvesting and cheesemaking. In contrast, both starter LAB and nonstarter bacteria play central roles in flavor and texture development in the anaerobic interior of many cheeses, such as aged Cheddar and Gouda, primarily through their participation in cascading proteolytic sequences that are mediated by residual rennet (i.e., rennet that is retained in the cheese in active form), starter culture proteinases and peptidases, and nonstarter peptidases. Such proteolytic cascades result in the conversion of casein to peptides and ultimately free amino acids, which, in turn, are catabolized by starter LAB and nonstarter bacteria into flavor compounds (28). Proteolytic cascades are very sensitive to the chemical environment that exists within the cheese; abnormal pH or moisture or salt content can affect proteolytic rates and specificities at different stages to render atypical flavors, or off-flavors (e.g., bitterness), or less intense typical flavor. Abnormal chemical composition can also trigger undesirable fermentations by nonstarter bacteria, such as *Propionibacterium freundenreichii* or *Clostridium tyrobutyricum*, that are usually suppressed, resulting in abnormal flavors and gas production.

Although the production of carbon dioxide and propionic and acetic acids by *Propionibacterium freundenreichii* is considered a defect in some cheese varieties (e.g., Cheddar), it is essential to others, especially the so-called "Swiss" or alpine cheeses such as Emmental. Propionibacteria are salt and pH sensitive; therefore, alpine cheeses are intentionally made with low salt contents (1% or less) and under conditions of delayed acidification which yield a mineral rich, highly buffered cheese that maintains a high initial pH (around pH 5.0 to 5.4). When alpine cheeses are stored at warm temperatures (e.g., 20 to 24°C) for several weeks, their low-salt, high-pH environment strongly selects for the growth of propionibacteria, which ferment lactate to carbon dioxide and propionic and acetic acids. Traditional alpine cheeses are made with a dense rind at their surface that slows the diffusion and venting of CO_2 to the atmosphere, thus enabling carbon dioxide to accumulate and supersaturate the water phase of the cheese as gas bubbles (29). As the gas bubbles expand in volume, the protein matrix expands elastically around them and they become embedded within the cheese body as round holes or "eyes." The protein matrix is able to expand elastically, rather than fracturing into cracks and splits, because the body of alpine cheeses is very closed and free of mechanical openings (due to the pressing conditions employed). Furthermore, the high mineral content and pH of the curd render the structure very resilient and able to expand elastically without fracturing under the pressure of accumulating gas (30). At the cheese surface, the rind also expands elastically to accommodate the increase in cheese volume, creating a concave exterior (Fig. 4).

Carbon dioxide is odorless and flavorless; however, propionic and acetic acids are highly volatile acids that serve as base flavor compounds for the alpine cheeses.

FIGURE 4 Alpine (Swiss)-style cheeses ripening on racks in a warm (ca. 22°C) environment. The cheeses occupying the top shelves have remained in the warm room longer than those on the lower shelves. Note the development of concave surfaces with increasing time in the warm room due to eye formation and volume expansion of the cheese. doi:10.1128/microbiolspec.CM-0002-2012.f4

Thus, active propionibacteria are essential to the ripening of alpine cheeses, and adjunct cultures of *P. freundenreichii* are added to the milk along with the starter culture in modern practice to ensure adequate levels in the cheese. It goes without saying that abnormally high salt content and/or low pH wreaks havoc on alpine cheeses by shutting down the propionibacteria and thus the production of carbon dioxide and propionic acid and acetic acid, rendering a "blind" cheese that lacks typical flavor.

Surface ripening

Ripening at the cheese surface differs from that which takes place in the body by virtue of the aerobic environment and the inadvertent or deliberate transfer of the environmental microflora to the surface. The specific microfloras that dominate the surface depend on the organisms present; the chemical composition of the cheese; the temperature, humidity, and oxygen levels of the ripening environment; and the physical manipulations of the cheese such as rubbing, turning, and washing. Surface-ripened cheeses can be divided into two broad categories based on the initial pH at the cheese surface: low pH (pH < 5.0) and high pH (pH > 5.0).

Low-pH surfaces, in combination with cool temperatures (11 to 13°C) and high relative humidity (90%), favor the sequential growth of yeasts followed by molds, which opens the door to the production of bloomy-rind cheeses such as Brie and Camembert. Bloomy-rind cheeses are traditionally made under conditions of rapid acidification that lead to extensive demineralization and low initial pH values (e.g., pH 4.6 to 4.7). Bloomy-rind-making procedures are also designed to maximize whey retention, producing high initial moisture content (e.g., ca. 50% or higher). The resulting high-moisture, low-pH cheese surface serves as a hospitable platform for the growth of the white mold *Penicillium camemberti*, which in former times was present as an adventitious environmental contaminant but in modern practice is either added to the cheese milk before renneting or sprayed onto the surface before ripening as a spore suspension (Fig. 5).

A variety of yeasts, such as *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, and *Debaromyces hansenii*, as well as the yeast-like mold *Geotrichum candidum*, are typically the first to colonize the surface of bloomy-rind cheeses as adventitious contaminants (26). In modern practice, yeasts and *G. candidum* are often added to the cheese milk as adjunct cultures. By raising the pH slightly through lactic acid fermentation and by producing peptides through the secretion of extracellular proteinases,

FIGURE 5 Spraying of a suspension of *Penicillium camemberti* mold spores onto the surfaces of Camembert cheeses at the start of ripening. doi:10.1128/microbiolspec.CM-0002-2012.f5

the yeasts and *G. candidum* stimulate the growth of *P. camemberti*. At this early stage of ripening, salt and moisture contents at the surface play a critical role through their influence on competing microbial populations (31). If the salt content is too low or the moisture content too high, *G. candidum* may outcompete the white mold and form a surface defect known as "toad skin"; alternatively, undesirable *Rhizomucor* molds may gain a foothold. On the other hand, if the salt content is too high, the growth of *G. candidum* will be suppressed and *P. camemberti* may grow excessively in the absence of competition. Excessive white mold growth, in turn, leads to excessive proteolysis and bitterness defects.

P. camemberti aggressively catabolizes lactic acid, resulting in rapid deacidification and pH rise at the cheese surface, which approaches pH 7.0 by the end of ripening. This has three important implications for ripening. First, when the pH rises above 5.8 to 6.0, the surface becomes favorable to the growth acid-sensitive strict aerobic bacteria known as the coryneforms, which include *Brevibacterium*, *Corynebacterium*, *Arthrobacter*, and *Microbacterium* species (32). Coryneforms are often present at the cheese surface as adventitious environmental contaminants but may also be added to the cheese milk as adjunct cultures. These orange- and yellow-pigmented organisms are highly proteolytic and prone to produce volatile sulfur compounds and extensive ammonia, which contribute to flavor and aroma and further elevate the surface pH. Thus, traditional bloomy-rind cheeses sometimes display orange-like patches of growth overlaying the lawn of white mold. (Similar coryneform growth may occur on the surface of well-ripened blue-veined cheeses, particularly if the cheese is on the higher end of the moisture range and lower end of the salt range.)

Second, rising pH at the surface creates an environment that is very favorable to the growth of the pathogen *Listeria monocytogenes*, thus rendering the cheese highly vulnerable to contamination from the environment. Finally, high surface pH causes dissolved calcium phosphate to crystallize there, which sets up the migration of calcium and phosphate ions from the cheese center to the surface along concentration gradients, where they continue to crystallize out. The end result is a casein matrix that is depleted of calcium phosphate and very sensitive to the pH gradient that develops as *P. camemberti* deacidifies the surface (33). Under these conditions, the attainment of high pH strongly promotes casein-water interactions and thus a softening at the cheese surface, which gradually works its way towards the center during ripening as the interior pH increases. Consequently, bloomy-rind cheeses display a characteristic zonal pattern of ripening from surface to center.

The microbial ecology of surface-ripened cheeses that have a high pH (>5.0) at the start of ripening, primarily the washed-rind or smear-ripened varieties, displays both similarities to and differences from that of the low-pH bloomy-rind cheeses. In contrast to bloomy-rind cheeses, washed-rind varieties are produced under conditions of slow acidification that lead to a highly buffered cheese with pH values at the start of ripening ranging from around 5.4 to 5.0. Washed-rind varieties are ripened under cool (13 to 15°C) and very humid (95 to 98% relative humidity) conditions to favor coryneform growth. As with bloomy-rind cheeses, yeasts and *G. candidum* are usually the first to colonize the surface of washed-rind cheeses, where they catabolize lactic acid and cause the pH to increase (32). Within a few days, the pH rises to 5.8 to 6.0 and the surface becomes favorable to the growth of coryneform species that in traditional practice were present at the cheese surface as adventitious environmental contaminants. In modern practice, adjunct cultures of coryneforms are often applied to the surface along with dilute salt brine during washing or smearing, though adjunct coryneforms do not necessarily dominate the surface flora during ripening (35).

Washing and smearing involve rubbing the cheese surface with dilute salt brine (Fig. 6) to favor the growth of coryneforms over competing molds that may be present (34). During washing, which is usually repeated every few days for a couple of weeks, the coryneforms are spread across the entire surface, creating a continuous bacterial lawn of orange-yellow growth. As noted above, the coryneforms are highly proteolytic and produce volatile sulfur compounds and extensive ammonia, resulting in strong pungent flavor and aroma and further elevating the surface pH. As with the bloomy-rind cheeses, the high surface pH of washed-rind cheeses creates a zonal pattern of softening and ripening and renders these varieties highly susceptible to the growth of *Listeria monocytogenes*.

FIGURE 6 Washing of the surface of a washed-rind cheese with dilute salt brine containing an adjunct culture of coryneform bacteria doi:10.1128/microbiolspec.CM-0002-2012.f6

Coryneforms are quite versatile and can be coaxed into growing on cheese surfaces that are very low in moisture and high in salt, such as that of Gruyère cheese. Thus, their use as a surface-ripening flora extends to not only high-moisture soft and semisoft varieties but also hard and semihard cheeses.

SUMMARY AND CONCLUSION

This article represents an incomplete and admittedly cursory view of the great complexity and diversity of manufacturing practices, aging conditions, and ripening phenomena that apply to traditional cheeses, especially the rennet-coagulated varieties. Clearly, many varieties of cheese and many aspects of ripening have not been considered here, and the reader is directed to other chapters in *Cheese and Microbes* (36) for greater depth and breadth. Nevertheless, it is hoped that the examples included in this article have illustrated the relationship that exists between the steps that are used on the first day to make cheese, the resulting chemical composition that is built into the cheese at the start of ripening, and the specific ripening pathways that subsequently unfold over weeks, months, or years under controlled storage conditions. With that in mind, one can distill the basics of the making of rennet-coagulated cheese down to a few key components, as described below and illustrated schematically in Fig. 7.

1. Cheesemakers use an eight-step process to produce aged rennet-coagulated cheese varieties. The first seven steps occur during the first day or few days of cheesemaking and include setting, cutting, cooking, dipping

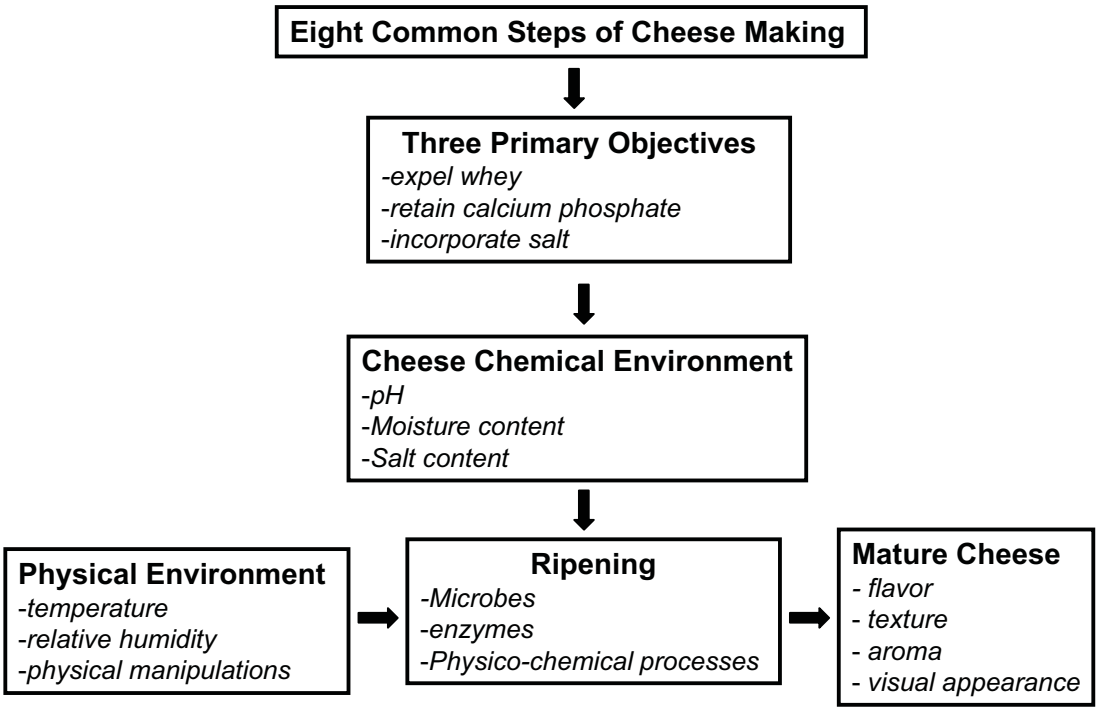


FIGURE 7 Simplified summary of the making of rennet-coagulated cheese. See text for explanation. doi:10.1128/microbiolspec.CM-0002-2012.f7

(or draining), knitting, pressing, and salting. The eighth and final step, affinage (finishing), involves an extended period of controlled storage when ripening takes place. The tools and techniques employed at each step may vary depending on the cheese variety, but the basic sequence of eight steps is common to all aged cheeses.

2. Cheesemakers have three primary objectives during the first seven steps, namely, to expel the correct amount of whey from the curd through controlled syneresis, to retain the correct level of calcium phosphate in the curd through controlled production of lactic acid by LAB, and to incorporate the correct amount of salt into the final cheese. Successful execution of these objectives results in a newly made cheese with a chemical composition that falls within specific target ranges for pH and moisture and salt contents.

3. The pH and moisture and salt contents of the cheese at the start of ripening are critically important because collectively they determine the initial chemical environment within the body and at the surface of the cheese.

4. The chemical environment, in combination with the surrounding physical environment, shapes the microbial ecology within the body and at the surface of the cheese during ripening and influences enzymatic and physico-chemical processes as well. The physical environment during ripening must be controlled appropriately with respect to temperature, relative humidity, and manual manipulations (rubbing, scraping, turning, washing, etc.) that are carried out by the cheesemaker or affineur.

5. The microbiological, enzymatic, and physico-chemical changes that occur within the cheese and at the surface during ripening in response to the chemical and physical environments collectively transform the flavor, aroma, texture, and visual appearance of the cheese over time to the desired mature state.

Deviations from the appropriate target ranges for cheese composition (moisture, pH, and salt) at the start of ripening will alter the course of ripening and thus the characteristics of the final cheese, for better or for worse. Such deviations, stumbled upon in the distant past, occasionally led to the development of new cheese varieties, but they undoubtedly more often resulted in disappointing or inedible batches of cheese, much to the chagrin and bewilderment of the cheesemaker. The great diversity of traditional cheeses that we enjoy today is a testament to the tenacity and ingenuity of cheesemakers throughout history to adapt their tools and practices to the constraints of local climate, geography, culture, and economy to produce cheeses that satisfied their needs and, indeed, enriched their (and now our) lives.

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