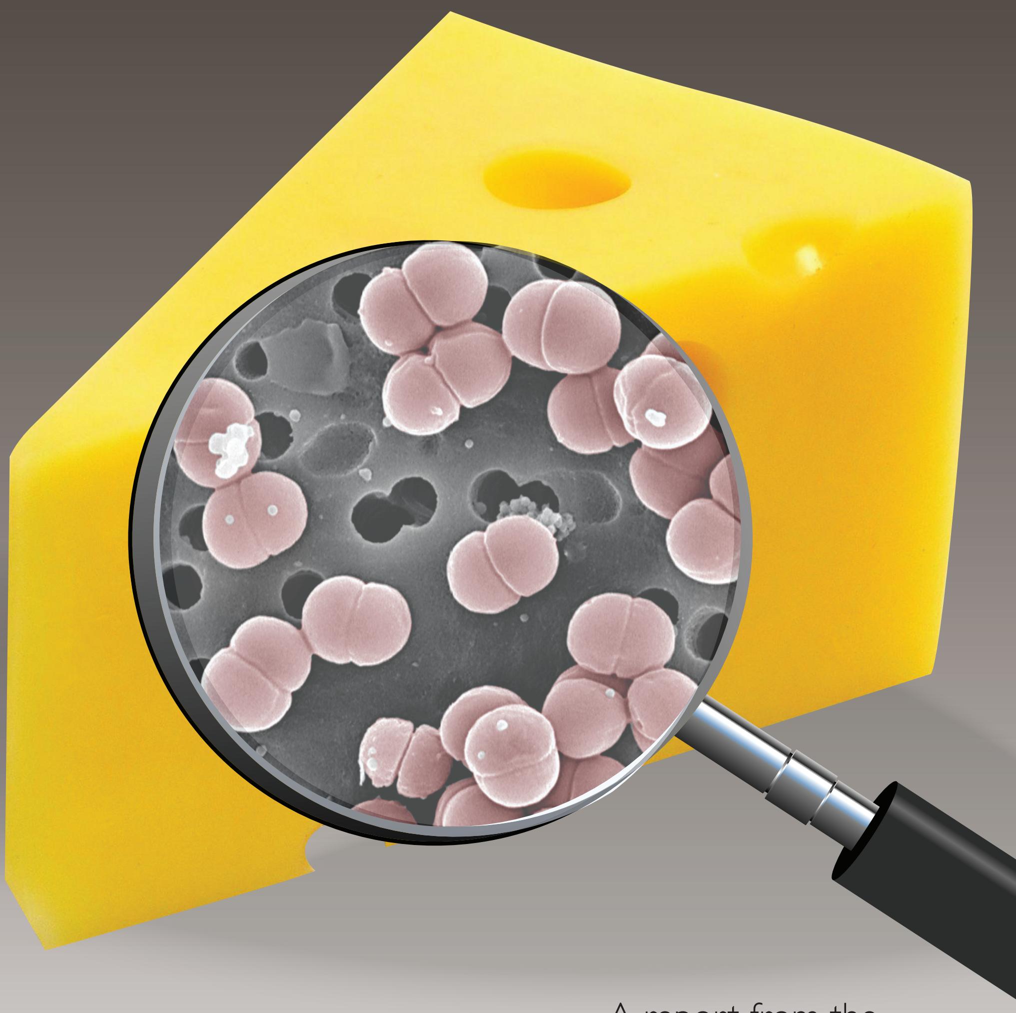
FAQ

Microbes
Make the Cheese



A report from the

AMERICAN SOCIETY FOR MICROBIOLOGY

AMERICAN ACADEMY OF MICROBIOLOGY
RECOGNIZING SCIENTIFIC EXCELLENCE

FAQ

Microbes
Make the Cheese

Report on an American Academy of Microbiology Colloquium held in Washington, DC, in June 2014

Edited by Jeffrey Fox

COLLOQUIUM STEERING COMMITTEE

Tom Beresford, Ph.D.
Teagasc Food Research Centre

Catherine Donnelly, Ph.D.
Vermont University

Rachel Dutton, Ph.D.
Harvard University

James Steele, Ph.D.
University of Wisconsin-Madison

PARTICIPANTS

John Barlow, D.V.M., Ph.D.
University of Vermont

Jeff Broadbent, Ph.D.
Utah State University

Dennis D'Amico, Ph.D.
University of Connecticut

MaryAnne Drake, Ph.D.
North Carolina State University

Mark Johnson, Ph.D.
University of Wisconsin-Madison

Mateo Kehler
Cellars at Jasper Hill

Paul Kindstedt, Ph.D.
University of Vermont

Sylvie Lortal, Ph.D.
INRA-Agrocampus Ouest

Noella Marcellino, Ph.D.
Abbey of Regina Laudis

Donald McMahon, Ph.D.
Western Dairy Center – Utah State University

Heather Paxson, Ph.D.
Massachusetts Institute of Technology

Dennis Romero, Ph.D.
DuPont Culture Development – Nutrition & Health

Ben Wolfe, Ph.D.
Harvard FAS Center for Systems Biology

ACADEMY STAFF

Marina Moses, DrPH
Director

Chelsie Geyer, Ph.D.
Colloquium Postdoctoral Fellow

Dylan Richmond
Colloquium and Public Outreach Program Assistant

Erin Seglem
Board of Governors Program Assistant

1752 N Street, NW
Washington, DC 20036
academy.asm.org

FAQ: Microbes Make the Cheese

Cheese, a traditional food incorporated into many cuisines, is used as an ingredient in cooking or consumed directly as an appetizer or dessert, often with wine or other suitable beverages. Great numbers of cheese varieties are produced, reflecting in part the versatility of the microorganisms used in cheese-making that this FAQ report will describe. Cheese is one of the few foods we eat that contains extraordinarily high numbers of living, metabolizing microbes, leading some participants to say, "Cheese is alive!" The broad groups of cheese-making microbes include many varieties of bacteria, yeast, and filamentous fungi (molds).

This report focuses on the microbiology of "natural" cheeses, those made directly from milk, including hard and soft varieties such as Cheddar, Mozzarella, and Camembert. Pasteurized process cheese, the other broad category of cheese, is made by blending natural cheeses with emulsifying agents, preservatives, thickeners, flavorings, and seasonings. "American cheese" is perhaps the classic example of a process cheese, notwithstanding recent examples of American artisanal cheese-making and changing tastes among consumers of those cheeses.

The United States (US) produces more than 1700 different cheeses, and cheese-making is an economic backbone for many states, serving as a major source of revenue (1). A 2014 report on cheese-making from the United States Department of Agriculture's (USDA) National Agriculture Statistics Services noted that Wisconsin, California, Idaho, New Mexico, and New York are the top five cheese-producing states (2). During the past decade, cheese production in the US has increased by more than one million metric tons, according to a recent report from the United States Dairy Export Council (USDEC) (3).

Front cover bacteria image courtesy of Dr. Jeff R. Broadbent, Utah State University

1. What is cheese and how is it made?

Simply defined, cheese is a food produced by removing water from milk, yielding concentrated milk proteins, fats, and other nutrients and compounds conferring flavor and aroma. Although critics of cheese point to it being high in fat, cholesterol, and salt, this food also provides many nutritional benefits. Beyond enticing the palate, cheese serves as a rich source of vitamins, minerals, and protein.

With only a few essential ingredients, including milk, microorganisms, salt, and enzymes, it is possible to create thousands of cheese varieties. All cheese-making starts with milk from one of several types of ruminant animals, mainly cows, goats, sheep, and water buffaloes. The key components of milk for cheese-making are the sugar lactose, fat, and the milk proteins known as **caseins***. In their native state,

****All bolded words throughout the text are defined on p 2.***

Key terms used throughout text

Acidification: Acidification is the production within or addition of an acid to the milk fermentation mixture, lowering its pH. Most commonly, cheese acidification is due to production of lactic acid from lactose via fermentation that depends on a starter culture containing lactic acid bacteria (LAB). Alternatively, the pH is lowered by adding food items such as citric acid, lemon juice, or vinegar, which are known as acidulants.

Casein: A milk protein that gels or solidifies during coagulation.

Coagulation: An enzymatic process that modifies the casein proteins, causing them to clump together and precipitate out of solution, creating a solid or gel-like structure.

Draining: A process that removes and separates liquid whey from curds.

Fermentation: A microbial process that depends on metabolic enzymes to break down complex substances into simpler ones that may be safer to consume and, typically, can be stored for longer than could the original material. This process also may lead to acidification of a substance as occurs when lactic acid bacteria (LAB) convert lactose sugar to lactic acid. Fermentations can also produce carbon dioxide (CO_2) and flavor compounds in cheeses or other materials.

Microbial succession: Changes that occur within and among populations of microorganisms, including bacteria, yeasts, and molds, during the ripening of cheeses.

Organoleptic properties: The sensory properties of a food or chemical, including taste, color, appearance, odor, and feel.

Pasteurization: Mild heating process, invented by Louis Pasteur, that destroys pathogens and spoilage microorganisms in foods such as milk (1).

Rennet: Rennet is an enzyme preparation from animals, plants, or microbes that breaks down casein proteins in milk, leading them to coagulate. Traditionally, rennet was extracted by bathing the 4th stomach of calves less than 12 months old (or other mammals such as camels) in brine to remove this enzyme. Currently, calf rennet is obtained by processing frozen stomachs in a production setting. Chymosin, also referred to as rennin, is the specific enzyme in rennet that is primarily responsible for degrading caseins and causing them to coagulate. Although plant and microbial rennets are used in making some traditional cheeses, calf-based rennets are widely thought to yield the highest quality cheeses (1).

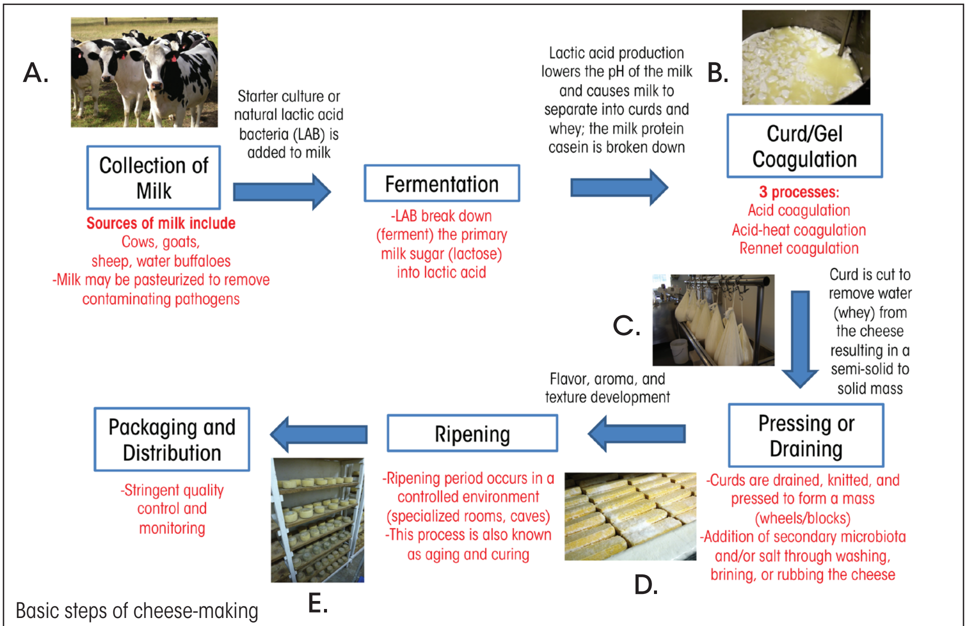
Rind: The outer layer of a cheese that can form during the ripening process.

Ripening: A maturation or aging step after initial cheese-making that imparts distinguishing flavors, aromas, and textures to particular cheeses.

Syneresis: The process that allows curds to contract and expel whey, removing water and other dissolved ingredients from the gel to achieve the desired texture and moisture content of the cheese being made.

milk proteins repulse one another, keeping them suspended in liquid. To create cheese, cheese-makers generate conditions that overcome these forces, leading the proteins to coalesce and the milk to form a semi-solid. This conversion step requires the milk to become more acidic, that is, for its pH to be lowered through the process of **fermentation**, followed by the coagulation of milk proteins to form a gel. This process is much the same as what happens during beer- and wine-making. Thus, fermentation is a means of preserving food stuffs while also profoundly changing them in ways that depend on the metabolism of microbes. Microbial fermentation is in large part an enzyme-dependent process during which protein catalysts made by the microbes break down complex substances—in this case, the proteins and sugars within milk—into simpler products that typically can be stored for longer periods than the original substance. Fermentation thus preserves milk as cheese, making it available for consumption months or years later, long after the unfermented milk would have spoiled. Fresh cheeses may be consumed immediately following the manufacturing process, whereas aged cheeses are stored for 12 months or more, during which time they undergo **ripening** to develop the distinctive flavor, smell, and texture of the particular cheese (*Figure 1*).

Figure 1



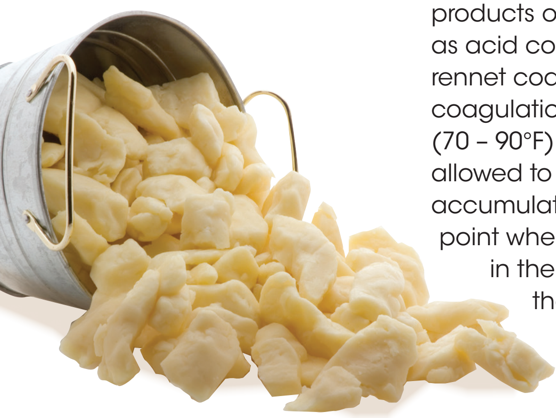
Basic steps of cheese-making

Images retrieved from A: "Cows from Lake Tyers" by Chris Howard-CC: BY-NC-SA B: Sister Noella Marcellino C: "Curd & Whey" by Todd Moon-CC: BY-NC-SA D: "Floating in the brine" by Lori Fredrich-CC: BY-NC-SA E: "Cheese maker, Altron" by Todd Wigley-CC: BY-NC-SA.

In cheese-making, not only do the milk proteins change, but so do its sugars. In particular, the milk sugar lactose is broken down into lactic acid by fermentation that depends on a group of bacteria referred to as lactic acid bacteria (LAB). Although traditional cheese-makers relied on naturally occurring LAB in milk, it is now common to inoculate milk with industrial starter cultures, defined groups of bacteria that are specifically chosen for use in cheese-making (*See p 12 for description*) and that help to ensure reliable and consistent acid production. To increase the rate of fermentation, the milk is warmed to the optimal growth temperature of microbes in the starter culture.

The acid produced during fermentation helps to form curds, also called a gel…

The acid produced during fermentation helps to form curds, also called a gel, and contributes to **syneresis** of the curd, removal of water held within the milk proteins.



Another significant step in transforming milk into cheese is converting liquid milk into a gel, consisting of a network of casein proteins whose formation depends on **acidification** of the milk and subsequent **coagulation** of those proteins. Depending on the cheese variety, three mechanisms to coagulate those proteins are drawn on to produce three very different families of cheese. The products of those three mechanisms are known as acid coagulated, acid-heat coagulated, and rennet coagulated cheeses, respectively. Acid coagulation occurs when bacteria in the warm milk (70 – 90°F) convert lactose to lactic acid, which is allowed to accumulate to high concentrations. This accumulated lactic acid lowers the milk pH to the point where the repulsive forces originally present in the milk proteins are overcome, allowing these proteins to bind to one another, forming a gel. In contrast, acid-heat coagulation yields less lactic acid from lactose but heats the mixture to a much higher temperature (around 185°F). This combination also serves to overcome the repulsive forces originally present in the milk proteins, allowing the milk proteins to gel. Finally, rennet coagulation depends on the action of these specialized enzymes that, when added to warm milk (around 90°F), can also remove the repulsive forces of the milk proteins, again resulting in gel formation.

Those three coagulation mechanisms produce gel-structures with strikingly different characteristics that then give rise to distinct families of cheese. Acid coagulated cheeses such as Cottage cheese and Cream cheese, and acid-heat coagulated cheeses such as Ricotta and Paneer characteristically have very high moisture content, making them vulnerable to spoilage and thus difficult to ripen for extended periods. Consequently, cheeses in these families are usually consumed fresh, with only a few examples of ripened acid and acid-heat coagulated cheeses. In contrast, rennet coagulation results in a gel structure with much lower moisture content, opening the door to almost unlimited possibilities for long-term ripening of such cheeses.

...curds may be knitted and pressed together to form cheese wheels or blocks of various sizes and shapes...

Once the gel reaches a required consistency, it is cut into small pieces referred to as curds, another step that facilitates the expulsion of moisture, referred to as whey, from the curds. Curds are separated from the whey via **draining**, a step in which the curds may be knitted and pressed together to form cheese wheels or blocks of various sizes and shapes according to the cheese variety being manufactured. Salt, either in crystals or in a brine solution is applied either to the curds or to the more fully formed whole cheese, respectively. This salt inhibits further growth of the starter culture, prevents growth of pathogens and spoilage microbes that are salt sensitive, selects for the growth of desirable microbes, and influences enzymes that break down protein and fat in ways that impart particular flavors and textures.

During the ripening process, a second wave of diverse bacteria and fungi (secondary microbiota) grow within the cheese and on its surface, sometimes forming a **rind**, the exterior outer layer of a ripened cheese. Microorganisms that are part of the secondary microbiota contribute enzymatic activities that affect the color, flavor, texture, and other important

Figure 2

A. B. C.

A.) The entrance to a traditional cheese cave in France (part of the caves of St. Julien in the Auvergne) used to age St. Nectaire cheese. The cheese caves are hundreds of years old and dug out of volcanic rock. **B.)** Cheese ripening in the cave. **C.)** A specially designed and controlled room for the ripening and aging of cheeses.

Images A and B retrieved from Sister Noella Marcellino via the movie, *The Cheese Nun*. Image C retrieved from Rachel Dutton.

characteristics, such as the holes in Swiss cheese, to the ripening cheese. Cheese was traditionally ripened in caves or cellars because they provided consistently cool and humid environments suitable for ripening (*Figure 2A and 2B*). In modern cheese-making plants, special ripening rooms are designed where temperature and humidity can be even more tightly controlled, leading to greater consistency in product quality (*Figure 2C*) (4). The labor-intensive process of "natural" ripening can include rubbing the cheese periodically with salt, wine, beer, fruit juices, or liqueurs to enhance flavor. Depending on the cheese variety, the ripening process is allowed to continue for extended periods, referred to as the aging process. Young cheeses that are not aged are known as fresh cheeses, some of which are spreadable. Hard cheeses are typically aged, at minimum, for 6 months to one year. The longer a cheese is aged, the sharper its flavor becomes. For example, there are mild, sharp, and extra sharp Cheddars. Mild Cheddar is aged for 2-3 months; sharp Cheddar is aged for approximately 8-12 months; and extra sharp Cheddar is aged anywhere from 18 months up to 5 years (5).

2. How did cheese-making get started and are the original techniques still used today?

Cheese was originally made to preserve and conserve the nutrients in milk…

According to the ASM publication, *Cheese and Microbes* (2014), the word "cheese" and words associated with cheese-making have ancient origins (1). The ancient Greeks called the wicker cheese basket used for draining whey from the curds a *formos,* which became *forma* to the Romans for "formed or molded." The English word "cheese" is derived from the West Germanic reconstruct *kasjus* via the Old English *cyse* and Latin *caseus.* Their roots are in the proto-Indo-European reconstruct *kwat-*, to ferment or become sour (1). Both branches of the etymology of the word "cheese" capture its essence; through the processes of fermentation, draining, and pressing, liquid milk becomes a solid food.

Over the course of history, humans have developed creative ways to preserve foods. Cheese was originally made to preserve and conserve the nutrients in milk, typically held for consumption when milk production was reduced during some seasons. With consumption of fermented milk products such as cheese, it is easy to understand why cheese is described as "one of the primary symbols of mankind's passage into civilization" by Kamber and Terzi (2008) (6,7). This nutritious product evolved over 9000 years, and continues to provide us with a safe and flavorsome variety of cheese types. Today, cheese is not necessarily made to preserve milk but rather to produce the wide diversity of foods that people enjoy in various culinary, cultural, and snacking situations. The transformation from grass to milk to cheese continues to intrigue people, as reflected in the comment from American writer and editor Clifton Fadiman, who calls cheese "milk's leap toward immortality" (8).

There is no conclusive evidence pointing to when or where cheese-making originated. A common tale has it that some 9000 years ago, nomads stored milk from sheep and cows in pouches that were made from the stomachs of the animals. Unbeknownst to the nomads, those stomach linings contained rennet, which

Text box 1. Traditional cheese-making methods that are used today in different parts of the world

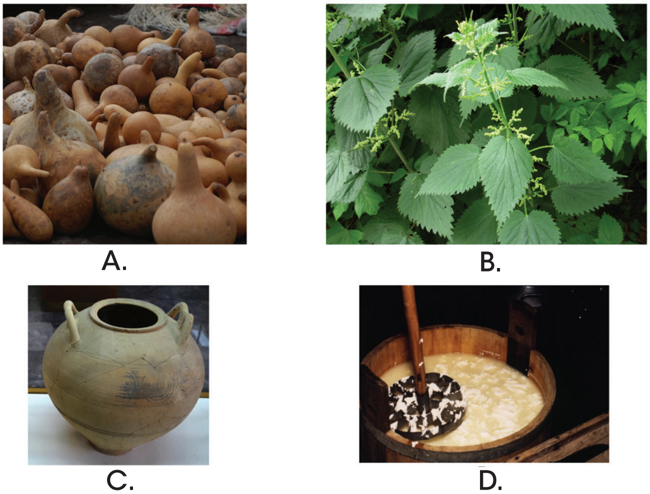
Gourds in Africa
(*Figure 3A*) – Gourds, used in Africa to collect milk from cows, also serve as containers for making butter and cheese. Once milk is collected, the gourd is placed in a warm environment for the milk to ferment and coagulate.

Stinging nettles in Pyrenees
(*Figure 3B*) – Although frequently dismissed as weeds, stinging nettles are plants that are rich in iron, calcium, and vitamin C. Additionally, stinging nettles possess antiviral, antifungal, and antibacterial properties (11). In the Pyrenees, collected milk is passed through sieves containing stinging nettles to decrease contaminating microorganisms and to allow coagulating enzymes to diffuse from the nettles into the milk.

Ceramic tools
(*Figure 3C*) – Ceramic devices continue to be used for cheese-making along the Mediterranean, in the Middle East, and in parts of Central America. Chemical analysis suggests

caused the milk to coagulate, and contaminating microbes that were naturally present in the milk converted lactose into lactic acid, thereby acidifying the product. The people of the time tasted this substance, realized it was safe to eat, and this new food began its development. There is no evidence, however, to support this account. What we do know is that Neolithic humans in Anatolia (modern Turkey) began to harvest and store milk as early as 7000 BC through analysis of fragments of pottery from that time that contain organic residues that serve as telltale molecular fingerprints for the milk that was once stored in those pots. Many of these pottery fragments contain traces of milk fat or its residues, indicating that a concentrated form of milk, most likely a simple form of cheese, was placed in those pots 9000 years ago. Some of these early cheese-makers from Turkey migrated to central Europe sometime during the next 1000 years, bringing with them their dairy animals and cheese-making expertise. By 5500 BC, these Neolithic farmers developed specialized ceramic sieves to separate curds from whey, and, here again, some of these sieves contained the telltale molecular fingerprints of milk fat. From around 3000 BC, when written language was perfected by Sumerians of southern Mesopotamia, some recovered examples of those early texts contain references to cheese. Indeed, clay tablets recovered

Figure 3



Traditional methodologies used in cheese-making. **(A)** Gourds in Africa **(B)** Stinging nettles in the Pyrenees, **(C)** Ceramic pots in the Mediterranean and Middle East **(D)** Traditional wooden tools (curd cutter and barrel) used in making farmstead *St. Nectaire* in the Auvergne, France.

Images retrieved from A: "African gourds for multiple uses" by Erik (HASH) Hersman-CC: BY-NC-SA. B: "Urtica dioica Stinging Nettle" by brewbooks-CC: BY-NC-SA. C: "Ceramics from Tarra-Pithoid amphora (2144) of the 7th c. BCE" by Dan Diffendale-CC: BY-NC-SA. D: Sister Noella Marcellino.

that ceramics or unglazed pottery found in what is now Poland served as cheese-making tools dating back nearly 7000 years ago (12).

Wooden tools
(*Figure 3D*) – Wooden tools have been used for centuries by traditional cheese-makers. These utensils include wooden vats, spoons, cream separators, molds for cheese-shaping, and shelves for cheese-ripening. Many cheese-makers think that wooden tools enhance the quality of their cheeses (1). For example, Ragusano cheese is produced primarily by farmers in eastern Sicily and requires the use of a wooden cheese vat (also known as a "tina") and a wooden staff (also known as a "ruotula"). The natural microbiota of raw milk and the wooden vat and ruotula are all used to make Ragusano cheese (1, 13).

No starter culture
For centuries and in some traditional cheese-making facilities today cheese was/is made without adding commercial starter cultures. Not only is this method more cost-effective, it also produces a cheese that more closely reflects the land from where the milk was collected (1). Cheeses made without added starter culture encompass traditional rennet coagulated cheeses that rely on natural milk microbiota to achieve the fermentation of lactose to lactic acid. If fermentation does not proceed quickly enough, this process can enable unwanted spoilage organisms to grow rapidly and overtake the process. Another technique involves adding pure acid(s) as a "non-starter" culture technique, avoiding reliance on microorganisms altogether. Paneer is an example.

from southern Mesopotamia, dated somewhat later, contained references to nearly 20 different types of cheese. Additionally, evidence recovered from Egyptian tombs dating back to 3000 BC indicates that cheese was then being made in this region as well (1).

The production of cheese in America began in the 17th century when the English Puritans brought dairy cows and their cheese-making craft with them to the New England colonies. Kindstedt (from the book, *American Farmstead Cheese*) states that the American cheese-making industry began in the Massachusetts Colony by Puritans from East Anglia, England, who arrived in North America from 1629-1640 (9). Many cheeses are known by their place of origin such as Cheddar and Cheshire, which took the name of the village and county, respectively, in England where the cheeses were first produced; Gruyère cheese was named after the town Gruyères in western Switzerland; Roquefort cheese was named after the small town of Roquefort-sur-Soulzon in France; and Muenster cheese (from the Latin Monasterium) was first created in a monastery (10). We owe many thanks to cheese-makers of earlier times for their creativity and expertise. They overcame cultural and environmental constraints to create the variety of cheeses we enjoy today.

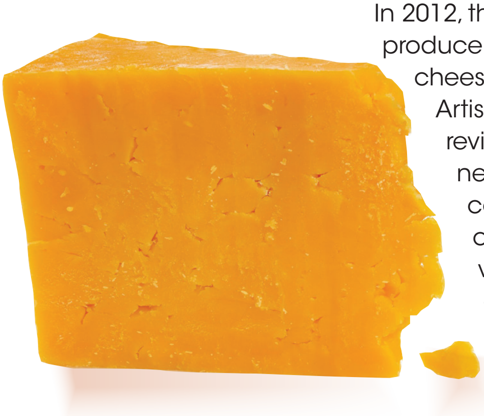
To meet worldwide demand, most cheese is now produced in large mechanized plants using advanced process control systems. Nonetheless, the basic principles underlying cheese-making remain unchanged and include: (1) removal of water from milk, (2) break-down of milk proteins (caseins) and fat, (3) addition of salt, and (4) an optional ripening period (1). Each of these steps differs, depending upon the cheese that is being created, but they all influence the final texture, aroma, and flavor. Although cheese-making is an ancient art, modern cheese production exploits our knowledge of many branches of science, including protein chemistry, organic chemistry, molecular genetics, enzymology, and microbiology, to assist in the production of consistent high quality products. Processing milk into cheese makes it possible to transform a perishable liquid into a food that has a prolonged shelf life and can be transported readily around the globe.

2A. How is cheese-making different at artisanal and mass production scales?

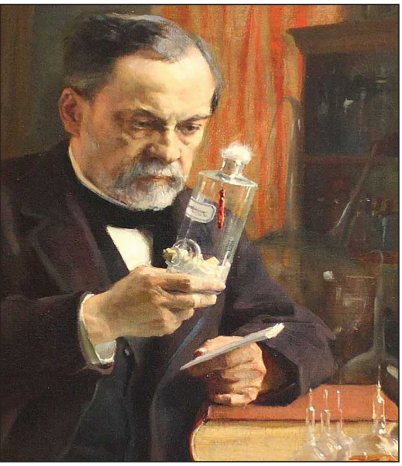
Both artisanal and mass production cheese-making methods use the same basic steps.

Both artisanal and mass production cheese-making methods use the same basic steps (*See p 2-4*). In both cases, commercial suppliers of starter cultures employ quality control measures to ensure that the bacteria introduced to the cheese-making process has consistent activity. Artisanal cheese-making typically uses minimally processed milk from livestock in a closed herd along with traditional utensils and hands-on methods. Meanwhile, mass-produced cheese is made from pooled milk from many herds and in automated, mechanized plants. Artisanal cheese-makers can adjust their techniques during production runs to accommodate changes in milk composition and fermentation rates, while variations in milk composition are minimized prior to industrial-scale production runs. Because milk and starter activity are standardized, there is no need during such runs to adjust for differences in milk composition. Consequently, the time scale for mass-produced cheese-making tends to be shorter compared to artisanal cheese-making. For example, artisanal Cheddar-making is approximately a 6-hour process while, for mass-produced Cheddar, processing takes only 3.5 hours.

During the past 15 years, interest in artisanal cheese-making has been renewed in the US and globally. Small cheese-making farms are returning to the landscape and helping to stimulate local economies while furthering time-honored traditions. In 2012, there were approximately 800 artisan cheese producers, nearly double the number of artisan cheese producers in the US in 2007 (1, 14, 15, 16). Artisanal cheese-making has the potential to revitalize farms, provide new jobs, and develop new cheese varieties with unique flavors for consumers to experience (1, 16). Artisanal cheeses are value added products that provide small dairy farmers with a viable source of income beyond what they can expect from selling milk.



3. What does microbiology have to do with cheese?



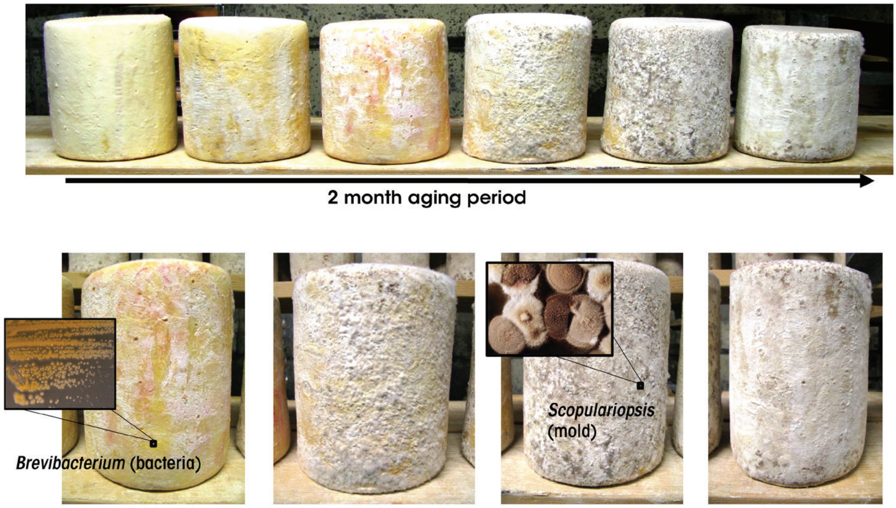
Louis Pasteur in his laboratory, from a painting by A. Edelfeldt in 1885

Louis Pasteur (1822-1895), the French microbiologist and chemist, was central in demonstrating the existence of microbes and that microorganisms are responsible for many common phenomena associated with food, including the .fermentations that produce beer, wine, and cheese. He also showed that these microscopic organisms could be inactivated by heating liquids such as milk followed by rapid cooling, a process that later became known as **pasteurization**. Pasteur's work was crucial in showing the importance of microbes to our daily lives and that by learning more about these microbes we could exert greater control over our environment and also endeavors such as medicine and trade in perishable foods. His studies on using heat to inactivate microbes revolutionized large-scale cheese-making, providing producers with a process for ridding milk of pathogens and unwanted spoilage organisms.

Without microbes, we would not be able to enjoy many wonderful and varied foods and drinks, including chocolate, tea, kimchi, bread, beer, wine, and cheese. Microorganisms transform the natural sugars and proteins in foods such as milk, vegetables, and grains into very different foods and drinks that are even more complex and interesting in flavor and taste.

Competition and cooperation among microbes (also known as microbial ecology) play a significant role in the conversion of milk to cheese. Successions of various bacterial species are required to produce all ripened cheeses. Some cheeses are produced by an orderly succession of many different microbes, a process that is like the passing of a baton between successive runners during a relay race (***Figure 4***). In most cheese varieties, the cheese microbiota is initially dominated by the starter culture; in some of the more complex cheese varieties, the starter microbiota is subsequently dominated by yeasts, and then surface mold/bacteria microbiota take over to facilitate ripening and aging of the cheese. Yeasts are known for their neutralizing activity and are com-

Figure 4



Overview of the microbial succession of a natural rind cheese.
Image retrieved from Rachel Dutton.

monly applied to the outside of washed rind cheeses to neutralize the surface pH, enabling bacteria such as *Brevibacterium linens* to grow there.

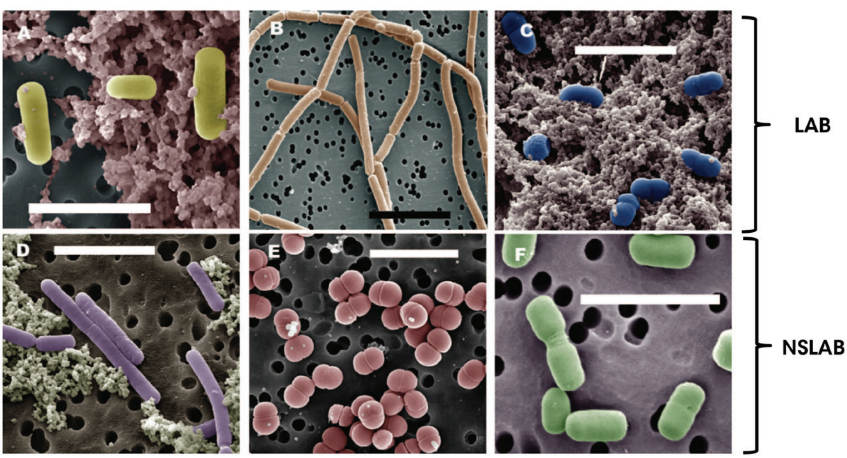
3A. What is the role of the starter culture?

The term "starter culture" is derived from the fact that these bacteria "start" the fermentation process required for cheese manufacture. The starter culture is a selectively characterized group of bacteria that are intentionally added to the collected milk. Their primary purpose is to convert lactose in milk into lactic acid. In addition, the starter culture contributes directly to flavor development through production of enzymes and metabolites. Cheese-makers can purchase industrially produced starter cultures, or they can rely on the microbes naturally present in raw milk to initiate fermentation. Reliance on industrial starter cultures usually results in more reproducible acid and flavor development.

The added starter cultures dominate the cheese microbiota, establishing conditions that select for the next microorganisms that will be capable of thriving in the changing cheese matrix. The starter culture changes the cheese microenvironment, affecting a variety of factors, including pH, redox potential, levels of organic acids such as lactate and acetate, and other nutrients. As some of the lactic acid bacteria (LAB) within the starter culture begin to die, the cells release enzymes that further break down milk proteins, mainly casein, to small peptides and amino acids. In fact, these dead starter culture cells and debris are an important food source for subsequent generations of microbes, referred to as non-starter lactic acid bacteria (NSLAB). If the starter culture is not dying, then the cheese is not growing. Precisely which organisms will comprise the second and future microbial ecologies will depend on conditions such as salt concentration and nutrients present, and which microorganisms are present (either intentionally added or contaminants) in the evolving cheese matrix.

Starter cultures are classified as either mesophilic, which means temperate-loving, or thermophilic, meaning heat-loving, based on their respective optimal temperatures for growth and acid production. These organisms, in turn, dictate the temperatures used in the cheese-making process (*See p 22*). Overall, there is a great variety of starter cultures to facilitate the making of a wide selection of cheeses (*Figure 5*) (17).

Figure 5



Colored scanning electron micrographs of representative cheese starter **(A-C)** and nonstarter **(D-F)** lactic acid bacteria (LAB). Species shown include *Lactobacillus helveticus* **(A)**, *Lactobacillus delbrueckii* subspecies *bulgaricus* **(B)**, *Lactococcus lactis* **(C)**, *Lactobacillus casei* **(D)**, *Pediococcus pentosaceus* **(E)**, and *Lactobacillus brevis* **(F)**.

Image retrieved from Broadbent, Steele 2005 (17).

3B. How do microbial communities shape the development of a cheese as it ages?

Text box 2.
Why do some cheeses melt and other cheeses do not?

Melted cheese provides us with many delightful dishes, from grilled cheese sandwiches to pizzas, fondues, and quesadillas. When melted, some cheeses become stringy and clump, whereas other cheeses will not melt at all. While Mozzarella, a cheese commonly used on pizza, melts to bubbly, gooey perfection, Paneer and Queso Fresco do not melt well at all (*Figure 6*). The flexibility of a cheese when it is heated depends upon the amount of calcium phosphate (CP) (an inorganic ingredient that binds to casein, the major protein component of milk) remaining in the cheese matrix and the extent to which casein has been broken down; two processes for which the microbes in cheese are directly responsible. High CP makes the cheese rubbery and difficult to melt, whereas low CP makes the cheese softer and easier to melt (1).

Cheese is created by orderly successions of microbial communities that produce compounds responsible for cheese flavor. For some cheese varieties such as Romano, enzymes are added to create a specific flavor. For centuries, traditional cheese-makers relied on microbes that occur naturally in milk and the cheese-making environment to produce their cheeses. Today, cheese-makers typically add specially prepared starter cultures and defined secondary cultures to their cheese mixtures. Regardless, cheese-makers strive to create controlled, reproducible environments and ecosystems for the microbes to produce the flavor compounds desired for the type of cheese being manufactured.

The metabolic activities of the cheese microbiota change conditions (e.g. microbial nutrients and concentration of lactate) within the cheese matrix. These changes allow other microorganisms to thrive and, eventually, to dominate the cheese microbiota. This process is referred to as **microbial succession** and an example is the development of the characteristic appearance of Swiss cheese (*Figure 7*). The starter

Figure 6

A.

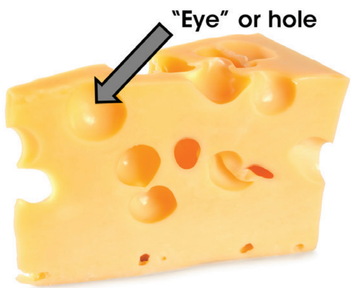
B.

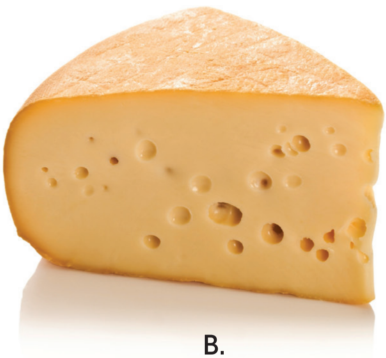
Different cheeses have different melting properties as evidenced by the grilled cheese sandwich **(A)** versus the clumpy Paneer cheese **(B)**. Acid production influences how much calcium is bound to casein, via phosphate groups. The more acid that accumulates, the less calcium phosphate (CP) is produced. High CP makes the cheese rubbery and difficult to melt; however, low CP makes the cheese softer and easier to melt.

Image A retrieved from "Grilled cheese, So simple, yet so delicious" by Matthew James-CC: BY-NC-SA. Image B retrieved from © iStockphoto.com/helovi.

culture used in Swiss cheese-making reduces the pH to a target value that is on the high end of the pH range for rennet coagulated cheeses. The salt level for such cheese is also deliberately kept low. These conditions (high pH and low salt) and adjustments made by the cheese-maker during ripening set up a milieu ideal for the addition of a secondary culture containing the specific bacterial species, *Propionibacterium freundenreichii*. In this environment, the *P. freundenreichii* bacteria convert some of the lactic acid produced by the starter culture into propionic acid, which contributes to the unique flavor of Swiss-type cheeses, along with acetic acid, and carbon dioxide gas (CO_2). This gas collects at weak spots in the cheese matrix, building up pressure until holes form (1, 18). If for some reason the *P. freundenreichii* fail to grow in the cheese and these "eye" holes do not form, the cheese is referred to as being "blind."

Figure 7





Swiss cheese and its characteristic holes created by the action of *Propionibacterium freundenreichii* bacteria. *P. freundenreichii* grows during ripening at 24°C and ferments lactate to acetate, propionate, and CO_2. The propionate and acetate provide the sweet and nutty flavor of the cheese and the CO_2 helps in "eye" creation. Generally, the longer the Swiss is aged, the larger the eyes will be **(A)**. Additional conditions that affect eye formation include acid content and temperature which can be altered to control the size of the eye. True Swiss has eyes that are cherry to walnut-sized. Swiss manufactured in the US, however, tends to have smaller eyes as a result of a shorter aging process **(B)**.

Image A retrieved from © iStockphoto.com/Givaga. Image B retrieved from © iStockphoto.com/Joe Biafore.

3C. How do microbes affect the flavor, aroma, texture, and color of different kinds of cheese?

Milk composition, steps and techniques used during the process, and the varieties of microbes being used dictate the flavor, aroma, texture, and color of the final cheese product. The microbes are particularly important because their metabolic activity produces hundreds of compounds from the protein and fat components in milk that affect the flavor, aroma, texture, and color of cheese.

FLAVOR

Flavor is perceived by the mouth and, more specifically, receptors on the tongue. The complex flavor characteristics of a cheese typically reflect the activities of several or many strains and species of microorganisms. For example, the white surface mold of a bloomy rind cheese such as Brie or Camembert is due to a complex ecosystem involving at least four different microbes, *Penicillium camemberti, Geotrichum candidum, Kluyveromyces lactis,* and *Debaryomyces hansenii* (***Figure 8***). These microbes impart the flavors and aromas that are characteristic of bloomy rind cheeses. One of them, *G. candidum* stimulates sulfur flavor, reduces bitterness, and influences the thickness and texture of the rind. The white, bloomy appearance of the rind arises mainly from

Figure 8

Classic, French style Brie. This cheese possesses a thin, bloomy rind with a gooey middle core. Many flavors such as roasted nuts and cauliflower are elicited by this cheese during peak ripeness.
Image retrieved from Sister Noella Marcellino and Jasper Hill Farms.

the activities of *P. camemberti* (18, 19). The overall flavor is produced as a result of the combined metabolic activities of these microbial species.

AROMA

The distinct aromas of cheese, detected in the nose, are due mainly to a special set of volatile organic compounds, or VOCs, that result from microbial metabolic activities and enzymatic reactions during cheese-making and ripening (18). For example, the distinctive aroma of Cheddar cheese is due to more than 500 different compounds produced by the microbes that are used in producing this cheese. Cheeses elicit a smorgasbord of aromas that range from lightly aromatic to bold and strongly overpowering. The smell of a cheese depends on microbial and enzymatic activities changing the casein and fats in milk and also on the microbiota associated with a cheese variety further refining those aromas as the cheese ripens. In general, the more extensively the casein and milk fat are broken down, the stronger the aroma of the final cheese product. The smell of a cheese is also a direct result of the ripening process and length of time the cheese is given to age. Depending on the type of cheese being made, ripening can range from a few months to several years. The smell of a cheese is a key factor in enjoying its flavor. Reflecting the importance of those aromas as well as their complexity, cheese vendors and connoisseurs depend on a broad variety of descriptive terms to describe the smells of cheese (*Figure 9*). See the list on page 19 (*Table 1*) for smell descriptors paired with cheese examples.

The smell of a cheese is a key factor in enjoying its flavor.

SENSE OF SMELL

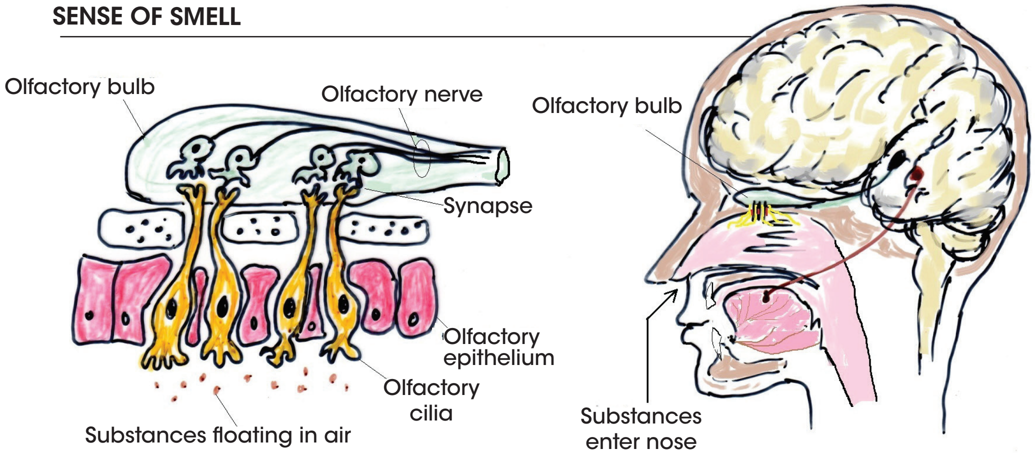
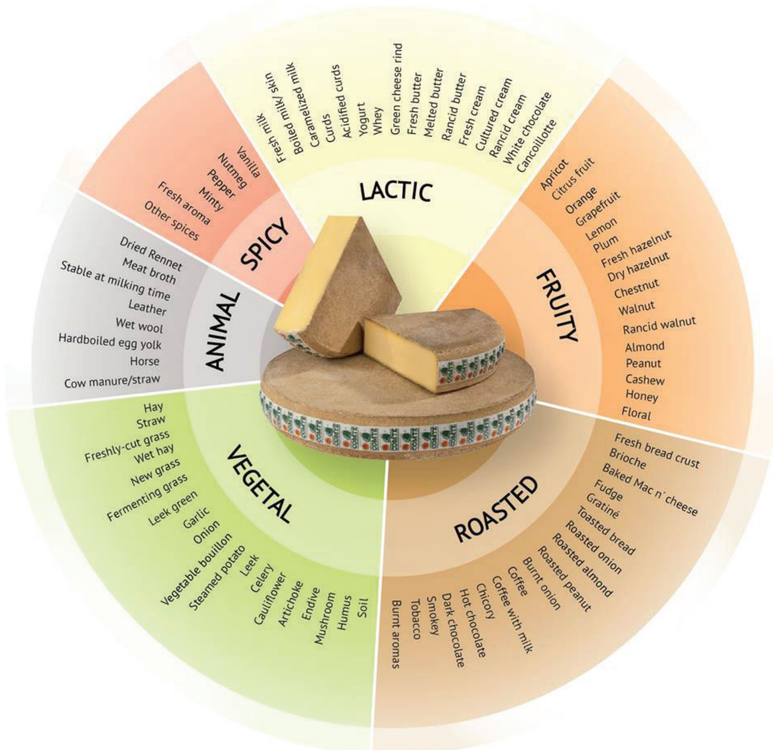


Figure 9

Text box 3.
CIGC (Comité Interprofessionnel du Gruyère de Comté), the professional organization based in Poligny, France is responsible for Gruyere de Comte, France's largest AOC (controlled designation of origin) cheese in terms of production. AOC is a French certification based on "terrior." The concept of terrior hypothesizes that a regions' landscape, climate, soil, people, and animals delivers a geographically distinct property to the cheese (1). The wheel was developed by Florence Bérodier, a very respected scientist who works for CIGC. To see how this wheel developed, please see the further reading section.



Common descriptors of cheese divided into six families and displayed in a flavor wheel (not all flavors/aromas are displayed). These characteristics are a result of the complex ecosystems created among the microbes (bacteria, yeast, and filamentous fungi) during cheese development, in addition to the type of animal from which the milk was collected (cow, goat, sheep), the breed of the animal, and the animal's diet. This image was created on the base of a scientific study published by the CIGC.
Image retrieved from Comté Cheese Association/CICG.

Table 1. Various aromas that cheese can elicit

Aroma	Description	Cheese Example/Additional Information
Ammonia-like	Sweaty or chlorine-like; An ammonia smell can also indicate an overripe or improperly ripened cheese	Brie
Barnyardy	Hay/straw, barn filled with animals	Goat's cheese
Buttery	Butter	Havarti – As this cheese ages it produces more of a hazelnut smell.
Delicate	Faint, not overpowering nor pungent smell	Mozzarella – This cheese is most commonly identified as a pizza topping and can be paired with tomatoes and basil.
Earthy	Wet leaves, dirt	Cave aged Cheddar – When melted, this cheese produces an earthy aroma and flavor that closely mimics a wet basement. Eating this cheese with mushrooms brings about its earthy flavor.
Fruity	Apple, peach, melon	Aged Asiago
Gamey	Meaty or similar to salami	Taleggio
Human feet	Old gym socks	Limburger
Mushroomy	Field of mushrooms and can be exhibited by soft and semi-soft cheeses	Camembert, Brie
Nutty	Hazelnut	Swiss and Dry Jack – This aroma is commonly produced by hard cheeses
Peppery	Tangy, strong	Blue – A common salad topper
Salty	Sea-coast	Romano – This is one of the oldest Italian cheeses and is commonly grated over pastas and salads.
Sharp	Pungent	Aged Cheddar
Smoky	Camp-fire	Pavarti, Provolone – Both elicit a light smoky smell
Sweet	Salted caramel candies, toffee, butterscotch, sweet cream	Jarlsberg – This cheese is easily melted and commonly found on sandwiches or in fondues; Caciocavallo Podolico is another example
Tangy	Sharp, pungent aroma with a bit of a spice	Fromage blanc, Aged Ragusano
Zesty	Spicy, lively	Pepper Jack – Receives its spice from the jalapenos/Habanero hot peppers incorporated in the cheese

Perceptions of how cheeses smell, taste, look, and feel can differ markedly from one individual to another. These characteristics are known as **organoleptic properties**, those features of food that stimulate the senses (1).

COLOR

Cheese can range in color from white to ivory, yellow, golden, orange, gray, and blue-streaked. The color of cheese may be due to added colorants or natural pigments such as carotene that come from the grasses that the milk-producing animal eats. Additionally, the microbes involved in the ripening process can affect the color of a cheese, producing pigments as part of the microbial interactions with ingredients in the cheese (*Figure 10A*). The creation of blue cheese, for example, requires human intervention during the aging process (*Figure 10B*). Thus, the blue veins of this cheese are due to the growth of a mold, known as *Penicillium roqueforti,* that will not grow without oxygen. Thus, cheese-makers use long needles to pierce wheels of the ripening cheese, allowing oxygen into the interior of the cheese and enabling *P. roqueforti* to grow in those channels. Meanwhile, the orange-tinted bacterium *Brevibacterium linens* gives the color and aroma to the rinds of Limburger and other surface ripened cheeses (1).

Furthermore, non-toxic bleaching/coloring additives such as titanium dioxide, hydrogen peroxide, benzoyl peroxide, and annatto can be added to milk to alter the color or otherwise improve the appearance of some cheeses. Depending upon the season and breed of cow, the milk produced contains a vari-

Figure 10

A.

B.

A) Oma, a wash rind cheese with a thin orange rind and occasional white flora. The orange-whitish rind is produced by *Brevibacterium linens* and the yeast-like fungus *Geotrichum candidum.* **B)** Baley Hazen Blue cheese, highlighting the blue-green veins. Cheese-makers intentionally manipulate this type of cheese by piercing the product with needles to allow the mold to grow and form the blue appearance.

Images retrieved from Sister Noella Marcellino and Jasper Hill Farms.

able amount of carotene content that can result in Mozzarella cheese appearing yellow. However, when titanium dioxide is added to cow's milk, the resulting Mozzarella is whiter, making it look more like water buffalo Mozzarella. Water buffalo milk is naturally white because it contains little or no carotene (20).

TEXTURE

The texture of a cheese depends on the initial process during which the milk acidifies, the duration of ripening, and the changing moisture content of the cheese. These factors control how much calcium will be retained in the cheese and the extent to which the milk proteins break down. Typically, the older the cheese, the softer or creamier its texture will become, although this varies and depends on the cheese type. For example, Camembert is ripened for a relatively short time but is soft and creamy, while Parmesan is ripened for two years and is very hard and brittle. The amount of moisture remaining in a cheese determines whether it will be creamy, smooth, firm, or hard. Harder cheeses contain less moisture in comparison to softer cheeses. Therefore, natural cheeses can be categorized according to their degree of hardness or softness (*Table 2*).

Typically, the older the cheese, the softer or creamier its texture will become…

A cheese's texture can also be affected by crystal formation. The ratio of salt to moisture influences the final pH that can cause crystals to form on and within the cheese (21) (*Figure 11*). Crunchy crystals can form in aged, hard cheeses. In some cheeses, such as Comté and Parmigiano-Reggiano, this characteristic can be regarded as desirable. However, gritty crystals are considered a costly defect in other cheeses such as Cheddar.

Table 2. Five categories of cheese based on texture

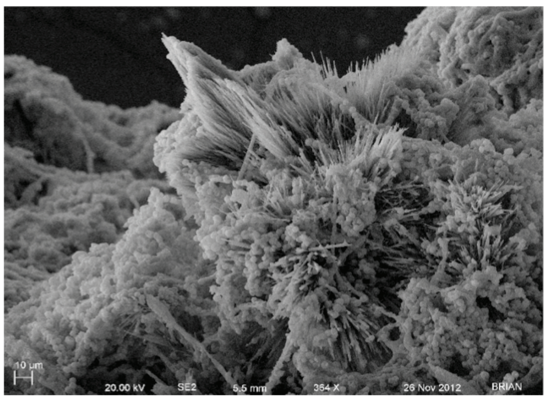
Texture	Cheese Examples
Creamy	Mascarpone; Teleme; Crescenza; Mexican style soft cheeses, including Queso Blanco and Queso Fresco
Soft	Brie, Camembert, Ricotta, Cottage
Semi-soft	Monterey Jack, Feta (a characteristic feature of feta is that it crumbles), Blue, Havarti, Muenster, Provolone, Mozzarella
Hard	Cheddar, Gouda, Colby, Edam, Swiss
Very hard	Parmesan, Romano

3D. Are certain types of microbes associated with specific kinds of cheese?

The identity of most cheese types is based on the microbial communities that make the cheese. Bacterial species that withstand temperatures as high as 132°F during fermentation are referred to as thermophilic. Thermophilic species such as *Streptococcus thermophiles, Lactobacillus helveticus*, and *Lactobacillus delbrueckii ssp.* are associated with Swiss- and Italian-type cheeses. Bacterial species can also be classified as mesophilic because these microorganisms ferment lactose only at 105°F or less. Examples of mesophilic species include *Lactococcus lactis* and *Leuconostoc mesenteroides*, both of which aid in Cheddar and Gouda production (1, 22).

The identity of most cheese types is based on the microbial communities that make the cheese.

Figure 11

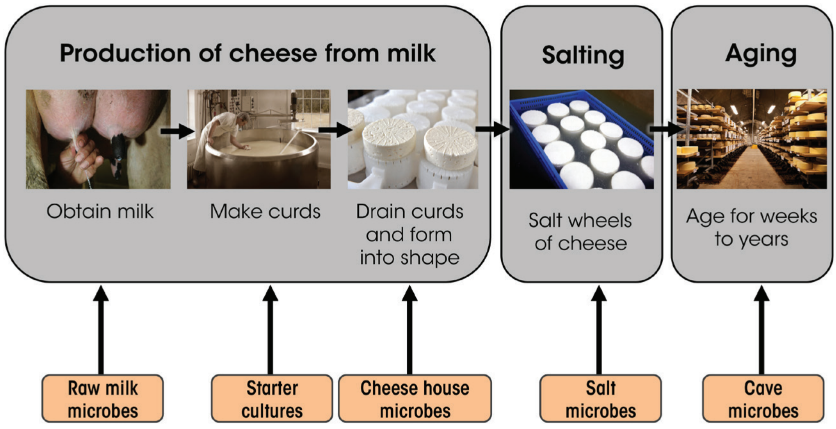


Electron micrograph of Bayley Hazen Blue showing crystals of calcium phosphate interspersed with *Penicillium* spores.
Image retrieved from Jasper Hill Farms.

4. Where do all of these microbes come from and how are they introduced during the cheese-making process?

Microbes associated with cheese production can be introduced deliberately (i.e., added as a starter culture) or unintentionally (i.e., by people, animals such as insects and rodents, and production equipment) (*Figure 12*). Not surprisingly, virtually all the starter cultures used in cheese manufacture are species that were originally isolated from cheese or milk. Microorganisms that can inadvertently enter the cheese-making process can come from the soil, feces, bedding materials, and feed (i.e., silage). These microbes can attach to the milking animals' teats and enter into the milk upon collection. There are also environmental microbes from the cheese house and aging facilities that can settle on the cheese as the product is being made and as it ages. In fact, some aging facilities develop their own desirable microbial communities symbiotically with the cheese types they produce. Even with thorough sanitation practices, however, some unwanted microorganisms (e.g., NSLAB) can remain on the surfaces of milking and processing equipment and can enter into the product during manufacturing.

Figure 12



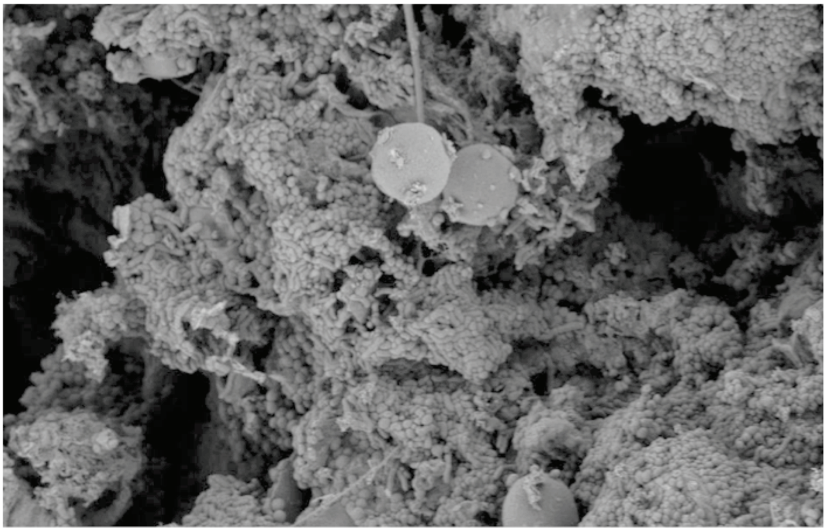
Migration and introduction of microbes throughout cheese production.

Image adapted from Rachel Dutton.

5. Are the microbes in cheese good for me?

Humans have been consuming cheese microbes for centuries. Each piece of cheese that we eat may contain as many as 10^{10} microbes (that amounts to 10,000,000,000 or 10 billion microbes, more microbes than there are people on Earth) (***Figure 13***). Although the microbes found in cheeses are considered safe to eat, these organisms are considered neither particularly healthful nor harmful. Nevertheless, cheese, like other foods, can become contaminated with other microorganisms, some of which may be harmful for consumers, if it is improperly made or stored. Additionally, consuming certain cheeses can be risky for immunocompromised individuals and for pregnant women when pathogens such as *Listeria* are present (***See p 27 for more information***).

Figure 13



Analysis (electron micrograph) of a cheese rind highlight the incredible number of microbes that are involved in its formation. There are nearly 10 billion cells per gram of cheese rind.
Image retrieved from Rachel Dutton.

6. What kinds of things can go wrong in cheese-making?

A great deal of both science and craft goes into making cheese.

A great deal of both science and craft goes into making cheese. Anything that disturbs this process can lead to defects in quality, some with food-safety implications. It is therefore important that cheese manufacturers are adequately trained and aware of the importance of strictly following time honored processes associated with cheese manufacture. There are training courses for cheese-makers to increase their skills and knowledge base to create consistently high-quality, safe cheese products. The key to quality cheese manufacture is maintaining control over the process regardless of the scale of operation. Parameters such as initial milk composition, temperature, moisture level, pH, the size of curd particles at cutting, stirring rates, and levels of salt addition all influence the cheese-making process and the quality of the resulting cheese. The activities of microbes during every step of the process are of paramount importance to the production of quality cheese, and controlling these microbial activities is in many ways the most difficult challenge faced by the cheese-maker. The activity of the starter culture is particularly important as the rate and level of acid production contributes to a very large extent in defining the final composition of the cheese and the subsequent communities that help to create it. Inadequate process control encompassing all details of the manufacturing process is the primary problem that can lower the quality of cheese. Therefore, large-scale cheese-makers must employ adequate testing, and small-scale cheese-makers must pay close attention to consistency with their particular cheese-making procedures. Additionally, bacteriophages, viruses that infect only bacteria but not humans, can also contaminate the microorganisms involved in cheese-making and inhibit the initial fermentation step (1). These viruses are hardy, and may be found naturally on our skin and within our intestines as well as in the environment, water supply, and in many foods (*See p 28 for more information*).

6a. Can pathogens grow in cheese and can they make me sick?

The occurrence of pathogens in cheese that cause disease in humans is very rare…

The occurrence of pathogens in cheese that cause disease in humans is very rare, especially if the cheese is properly made. However, some pathogens can survive and grow in cheese when particular production standards are not met. How they contaminate the cheese varies widely but sources of those pathogens include the milk, the processing environment, and people working in the cheese plant. Consumers also need to be considered when weighing these risks because each consumer is different and carries a different degree of vulnerability when encountering pathogens in cheese. For example, because fresh, non-ripened cheeses such as Queso Fresco and wash rind cheeses have higher moisture contents and a higher pH than do other cheeses, these products are more prone to be contaminated with *Listeria* and to support its growth (***See more on Listeria on p 27***). Contaminated soft cheeses may therefore pose a risk to people that are particularly vulnerable to *Listeria* infections, including pregnant women, individuals who are immunocompromised, and the elderly. *Listeria* contamination can be a consequence of improperly pasteurized milk, improper cooling of the milk, or post-pasteurization contamination in the cheese plant or in the homes of consumers. Cheese-makers make every attempt to manage these and other safety risks associated with cheese production. Interestingly, there are some cheeses such as Parmigiano-Reggiano, English Cheddar, and Gruyère, that do not support the growth of pathogens if correctly manufactured. These cheeses are dry, acidic (low pH), and have a high salt content, all traits that inhibit pathogen growth.

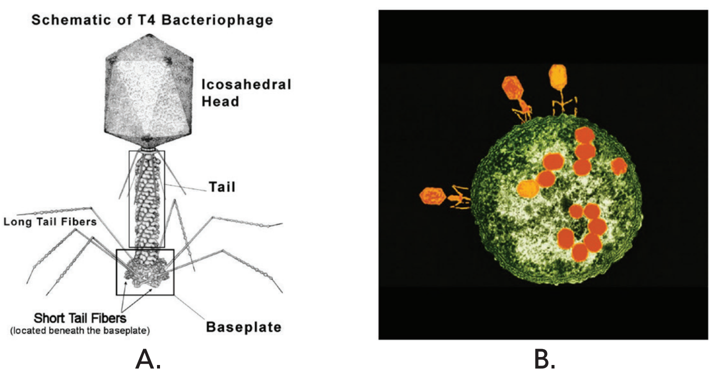
6b. How can contaminating microbes affect the cheese outcome?

Both desirable and undesirable microbes are introduced to cheeses during production. The desirable microbes include LAB in the starter culture and the secondary microbiota that dominate during cheese ripening and aging. Depending on the characteristics of the individual contaminants, they can accelerate typical flavor development, have no direct impact on flavor development, or can lead to development of off-flavors. Undesirable contaminating microbes are any microbes that are not intentionally introduced during the cheese-making process that lead to development of off-flavors such as bitterness and rancidity, poor texture, unappetizing appearance, or pose health risks from consuming the cheese produced.

6c. How are contaminating microbes controlled during cheese-making to preserve both the flavor and safety of cheese?

Listeria monocytogenes is one of the main undesirable microbial contaminants which the cheese industry must address. It can cause a very serious illness known as listeriosis that has a mortality rate of 20-30% (23). *L. monocytogenes* is widespread and can survive for extended periods in the environment among soils, vegetation, and water, and can also be found on the surfaces of equipment, walls, and floors in farms and cheese-production facilities, sometimes hiding in the nooks and crannies of milking and cheese-making devices. Because *Listeria* is associated with vegetables, it can also hide in home refrigerators. Many measures have been implemented by cheese-making regulators to prevent contamination. As a general rule of thumb to prevent cheese-making contamination, the "**3Ks**" must be followed: **K**eep the

Figure 14



Bacteriophages (phages) are a source of pathogen contamination that can kill starter cultures used in cheese-making, resulting in discarded batches. More recently, phage are being implemented as a contamination control measure in cheese-making **A.** General schematic of a bacteriophage. **B.** Bacteriophages attached to the surface of a bacterium.

Image A retrieved from "bacteriophage" by Nano Transformation-CC: BY-NC-SA. Image B retrieved from "T2 bacteriophage virus (seen in orange) attacking an *Escherichia coli* bacterium. Each phage consists of a large, DNA-containing head and a tail composed of a tube-like central sheath with several fibres." by Sarah Jensen-CC: BY-NC-SA.

Text box 4.
Technologies used for contamination control in cheese-making

Use of phages for food safety: Bacteriophages (phages) are the most common microorganisms on the planet and are essential for life on Earth (*Figure 14A and 14B*). These microbes do not directly affect humans, plants, or animals but specifically target bacterial hosts, making them the natural nemesis of bacteria. It is estimated that phages kill nearly half of all the bacteria on Earth every two days (23). In cheese-making, phages can be sources of unwanted contamination or can be helpful for controlling contamination. As unwanted contaminants, phages can attack bacteria in the starter culture, thereby preventing acid production and coagulation, resulting in a low-acid, low-quality cheese.

However, phages can also be used to kill specific, unwanted bacterial strains and pathogens that may cause problems for cheese-makers (1). For example, some FDA (Food and Drug Administration)-approved phage cocktails have a specific preference for *L. monocytogenes*. These cocktails are generally applied as a topical spray to foods such as cheese and the surrounding environment in which they are being produced, and can be used to prevent *Listeria* contamination during the production of meats, vegetables, fruits, and cheeses. The phages specifically target and kill *Listeria*, but will not affect other bacterial species used to produce cheese. Use of this technology does not affect the taste, texture, or other properties of the treated cheese or other foods.

Nisin-producing cultures: Nisin is an antimicrobial peptide known as a bacteriocin that is produced by particular types of starter bacteria. The natural antimicrobial properties of nisin inhibit the growth of many potentially contaminating microorganisms, including important foodborne pathogens such as *Listeria, Staphylococcus, Escherichia coli, Salmonella, Campylobacter,* and *Clostridium*.

pathogens (undesirable microbes) out (sanitation), **K**ill them (with heat or antimicrobial treatments), and **K**eep them from growing (temperature, salt content). Extensive post-processing monitoring of cheeses along with compositional testing analysis also helps to control contamination. There are also several new technologies that help with contamination control (*See Text box 4*).

6d. How is cheese-making regulated?

In the US, 15 federal agencies share responsibilities for regulating food safety (24). Among them, the Food and Drug Administration (FDA) has regulatory jurisdiction over cheese and stipulates that cheese be made in one of two ways (1):

1. Use milk that is pasteurized to FDA standards.
2. If made from raw (unpasteurized) milk, cheese must be aged for a minimum of 60 days at a temperature no less than 35°F.

Cheeses produced in the US must meet strict sanitation and quality standards that also are specified by the US FDA. From the initial step of milking animals to the shipment of cheeses to consumers, the product is subjected to rigorous monitoring, quality assurance standards, and specific tests. It is estimated that approximately 30% of the world's cheese production comes from more than 450 cheese-makers in the US, making the US the largest cheese-producer in the world (1). In addition to FDA oversight, state agencies, which may collaborate with federal agencies, also play an active role in ensuring that cheese safety is enforced in their respective states. Many professional societies worldwide support cheese safety and sustainability by offering training courses and annual certification exams for cheese-makers, helping them to become more competent in their trade.

The US cheese industry is also expanding its exports, reflecting in part the high quality and safety of the cheeses produced in the US. According to the

United States Dairy Export Council (USDEC), cheese exports dramatically increased from 12,000 metric tons in 1991 to more than 123,000 metric tons in 2008 (25). In 2011, the FDA implemented the Food Safety Modernization Act (FSMA) which aims to prevent food contamination and enforce stricter risk-based food safety standards. Additionally, the FSMA states that all cheese imported to the US must conform to the FDA's standards (1).

Quick Facts about Cheese

- Cheese-making is an art that has been in practice for more than 9000 years. The cheeses that we enjoy today are a testament to cheese-makers throughout history for their persistence, originality, and ability to modify their skills based on their culture, climate, geography, and available technology and equipment.
- Cheese is a living food and microbes are essential for its production; hence, "Microbes Make the Cheese."
- Cheese, which is produced by concentrating and changing the proteins (caseins) and fats in milk, depends on the actions of microbes to drive this transformation.
- The manufacturing and ripening of cheeses results from microbial successions that can include bacteria, yeasts, and filamentous fungi (molds).
- The key steps in the manufacturing of cheese include milking, fermentation, coagulation, curd and whey separation, salting, and ripening.
- Microbes contribute to the final flavor, smell, texture, and color of cheese. Specific microbes impart the characteristics of particular cheeses (i.e., holes in Swiss cheese).
- Extensive contamination control and quality assurance tests are maintained throughout every step of the cheese-making process for all US cheese-makers. Newer technologies are available to help in minimizing and eliminating contamination.
- In the US, the FDA, state, and local regulators oversee the safety of cheese.

Text box 5.
Future research needs and questions in cheese microbiology

As with any topic of scientific inquiry, the more we learn about cheese, the more questions that are raised.

1. What are the pre- and pro-biotic properties, if any, of the microorganisms in cheese?
2. How do the microbes within cheese interact with the natural microbiota of the human gut and can cheese be used to assist in maintaining a healthy gut microbiota?
3. What is the biogeography of cheese microbes? Studies have shown that microbial communities are not identical across a given space (1). Understanding how microbial communities are structured over large biogeographic scales could help in understanding whether microbes aid in flavor development in specific biogeographic regions. It is possible that regional differences in microbial communities could make a cheese in Vermont different than the same cheese produced in France.
4. Are all microbes in cheese metabolically active even though some of these organisms cannot be cultivated under normal laboratory conditions?
5. By shepherding microbes in different ways, could new cheese varieties be produced? What new flavors are still out there to discover or, indeed, have all the possibilities already been identified after 9000 years of cheese-making?
6. With new DNA-based methodologies, can faster detection of contaminating pathogens in cheese be achieved? How can we apply these newer methods to enhance the safety of cheese?

Further Reading and Information

1. **ASM Microbiology of Cheese Broadcast June 11, 2014.** https://www.youtube.com/watch?v=nmXKhVsIltU

2. **Caldwell G**. 2012. *Mastering Artisan Cheesemaking: The Ultimate Guide for Home-Scale and Market Producers*. Chelsea Green Publishing.

3. **Cheese and Microbes (Microbe World Video 28) with Dr. Catherine Donnelly** http://www.microbeworld.org/podcasts/microbeworld-video/archives/429-cheese-and-microbes-mwv28.

4. **Donnelly CW.** 2014. *Cheese and Microbes*. ASM Press.

5. **Fletcher J.** 2007. *Cheese & Wine: A Guide to Selecting, Pairing, and Enjoying*. Chronicle Books.

6. **Harbutt J**. 2009. *The World Cheese Book*. DK Publishing.

7. **Herbst ST.** 2007. T*he Cheese Lover's Companion: The Ultimate A-to-Z Cheese Guide with More Than 1,000 Listings for Cheeses and Cheese-Related Terms*. William Morrow Cookbooks.

8. **Kindstedt P**. 2012. *Cheese and Culture: A History of Cheese and its Place in Western Civilization.* Chelsea Green Publishing.

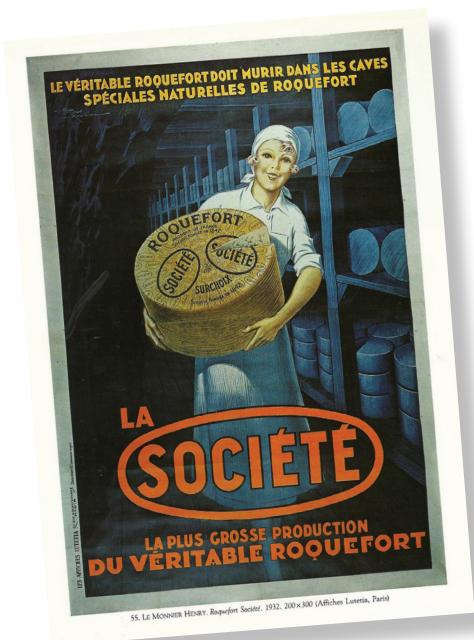
9. **McCalman M.** 2009. *Mastering Cheese: Lessons for Connoisseurship from a Maître Fromager.* Clarkson Potter.

10. **McCalman M**. 2005. *Cheese: A Connoisseur's Guide to the World's Best*. Clarkson Potter.

11. **Monnet JC, Berodier F, Badot PM.** 2000. *Characterization and Localization of a Cheese Georegion* Using Edaphic Criteria (Jura Mountains, France). Journal of Dairy Science 83:1692-1704.

12. **Roberts J.** 2007. *The Atlas of American Artisan Cheese*. Chelsea Green Publishing.

13. **Teubner C**. 2009. *The Cheese Bible*. Chartwell Books, Inc.



References

1. **Donnelly CW.** 2014. *Cheese and Microbes*. ASM Press, Washington, D.C.
2. **Natural Agricultural Statistic Services. United States Department of Agriculture.** 2014. http://www.nass.usda.gov/. Accessed 16 June 2014.
3. **United States Dairy Export Council**. http://www.usdec.org/home.cfm?navItemNumber=82205. Accessed 17 June 2014.
4. **The Cheese Nun: Sister Noella's Voyage of Discovery.** 2006. PBS Home Video. Alexandria, VA.
5. **Clark S, Drake M, Costello M, Bodyfelt F.** 2009. *The Sensory Evaluation of Dairy Products*. Springer Science New York, NY.
6. **Kamber U, Terzi G.** 2008. The traditional cheeses of Turkey: Central Anatolian Region. *Food Rev Int* 24:74-94.
7. **Curry A.** 2013. Archaeology: the milk revolution. Nature 500:20-2.
8. **LeMay, E.** 2010. *Immortal Milk: Adventures in Cheese.* Simon & Schuster, New York, NY.
9. **P. Kindstedt with The Vermont Cheese Council**. 2005. *American Farmstead Cheese: The Complete Guide To Making and Selling Artisan Cheese*. Chelsea Green Publishing Company, White River Junction, VT.
10. http://www.cheese.com/. Accessed 17 June 2014.
11. **Ilhami G, Küfrevioglu ÖI, Oktayb M, Büyükokuroglu ME**. 2004. J Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica L.*). *J Ethnopharmacol* 90:205-15.
12. **Dunne J, Evershed RP, Salque M, Cramp L, Bruni S, Ryan K, Biagetti S, di Lernia S**. 2012. First dairying in green Saharan Africa in the fifth millennium BC. *Nature* 486:390-4.
13. **Licitra G, Ogier JC, Parayre S, Pediliggieri C, Carnemolla TM, Falentin H, Madec MN, Carpino S, Lortal S.** 2007. Variability of Bacterial Biofilms of the "Tina" Wood Vats Used in the Ragusano Cheese-Making Process. *Appl Environ Microbiol* 73:6980-7.
14. **Johnson MA, Law BA**. 1999. The origins, development and basic operations of cheesemaking technology, p 1-32. In Law BA (ed), *Technology of Cheesemaking*. CRC Press, Boca Raton, FL.
15. **Andren A.** 2003. Rennets and coagulants, p 281-286. In Roginski H, Fuquay JW, Fox PF (ed), *Encyclopedia of Dairy Sciences*, vol 1. Academic Press, San Diego, CA.
16. **Paxson H.** 2013. *The Life of Cheese: Crafting Food and Value in America.* University of California Press, Berkeley, CA.
17. **Broadbent JR, Steele JL**. 2005. Cheese flavor and the genomics of lactic acid bacteria. ASM News 71:121-8.
18. **Button JE, Dutton RJ.** 2012. Cheese microbes. *Curr Biol* 22:R587-9.
19. **Marie-Hélène L, Viel C, Boyle B, St-Gelais D, Labrie S**. 2014. Metatranscriptome analysis of fungal strains *Penicillium camemberti* and *Geotrichum candidum* reveal cheese matrix breakdown and potential development of sensory properties of ripened Camembert-type cheese. *BMC Genomics* 15:235.
20. **Kosikowski FV, Brown DP.** 1969. J Dairy Science 52:968-70.
21. **Agarwal S, Powers JR, Swanson BG, Chen S, Clark S.** 2008. Influence of salt-to-moisture ratio on starter culture and calcium lactate crystal formation. *J Dairy Sci* 91:2967-80.
22. **Johnson ME, Steele JL.** 2007. Fermented Dairy Products, p 767-781. In Doyle MP (ed), Beuchat LR (ed), *Food Microbiology: Fundamentals and Frontiers* (3rd Ed). ASM Press, Washington, D.C.
23. **Foodborne illness: Who monitors our food?** http://www.brandeis.edu/investigate/foodborne-illness/who-monitors-food.html. Accessed 18 June 2014.
24. **Oskar A, Meydani SN, Russell RM.** 2004. Yogurt and gut function. Am *J Clin Nutr* 80:245-56.
25. **United States Dairy Council.** 2014. https://www.usdec.org/home.cfm?navItemNumber=82205. Accessed 17 June 2014.

Images retrieved from: p 2) James McKelvey; p 4) goldenagecheese.com; p 5) Imen McDonnell "Farmette"; p 6) Roma 2011, Timbres Italy, Italia 2011, Postzegels Italy; p 7) Wikipedia Creative Commons "Cheese-making, Tacuinum sanitatis Casanatensis (14th century)" -CC: BY-NC-SA; p 10) Katherine Rosploch; p 17) Wikipedia Creative Commons "The organ of smell " by bioxeocotobade.pbworks.com -CC: BY-NC-SA; p 22) DAIRYBELLE Gouda Round; p 25) Jennifer Smith "Moldy Cheese" -CC: BY-NC-SA; p 30) Henry Le Monnier (1893 - 1978)

American Academy of Microbiology
1752 N Street, NW
Washington, DC 20036

academy.asm.org