1 Introduction to fermented foods

> You can't just eat good food. You've got to talk about it too. And you've got to talk about it to somebody who understands that kind of food.
>
> Kurt Vonnegut Jr., American writer, from the novel, *Jailbird*

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FERMENTED FOODS AND HUMAN HISTORY

Long before there were microbiomes, genome sequences, pure starter cultures, and before Lister and Pasteur, there were fermented foods and beverages. Indeed, fermented foods were very likely among the first "processed" foods consumed by human beings. This was not because early humans had actually planned on or had intended to produce a particular fermented food. Rather, fermentation was simply the inevitable outcome that resulted when raw food materials were left in an otherwise unpreserved state. When, for example, several thousands of years ago, milk was collected from a domesticated cow or goat, either it was consumed within a few hours or else it would ferment. If the latter, the milk soured and curdled, turning into something we might today call yogurt. The milk could also spoil and become unpalatable, an event that must have occurred frequently. Other raw food materials likely experienced a similar fate. Juices expressed from ripe grapes and other fruits would remain sweet for perhaps only a few days before being transformed into a variety of pleasant, intoxicating, and entirely drinkable beverages.

These products provided sustenance, and they were likely appreciated, as well, for their aesthetic benefits, i.e., they tasted good. It must have also been recognized and valued early on that however imperfect the soured milk, wine, olives, and other fermented food products may have been, at least compared to more recent versions, they possessed several noteworthy advantages. They would generally have lasted longer and been less susceptible to spoilage, compared to the raw materials from which they were made. In addition, they were usually, though not always, observed to be safer.

Despite the "discovery" that fermented foods had many virtues, it took centuries for humans to figure out how to control or influence conditions to consistently produce high quality fermented food products. Remarkably, the means for producing so many fermented foods appears to have evolved independently on every continent and on an entirely

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empirical basis. Although there must have been countless failures and disappointments, small "industries", skilled in the art of making fermented foods, eventually developed. As long ago as 3000 to 4000 BCE, bread and beer were already being mass produced by Egyptian bakeries and Babylonian breweries. It is clear from historical records that the rise of civilizations, around the Mediterranean and throughout the Middle East and Europe, coincided with the production and consumption of wine and other fermented food and beverage products (Box 1.1). It is noteworthy that the types of fermented foods consumed in China, Japan, and the Far East were vastly different from those in the Middle East. Plants and seafood, rather that animal products, served as the main raw materials. It is also clear that these Asian fermentations evolved and became established around the same time.

Box 1.1 The origins of alcoholic fermentations and the drunken monkey hypothesis

Although the very first fermentations were undoubtedly accidental or inadvertent, it is just as certain that human beings eventually learned how to intentionally produce fermented foods and beverages. When, where, and how this discovery occurred are elusive questions, since written records, alas, do not exist. However, other forms of archaeological evidence do indeed exist. This has made it possible to not only establish the historical and geographical origins of many of these fermentations, but also to describe some of the techniques likely used to produce these products.

Molecular archaeology Most of the early investigations on the origins of food fermentations focused on alcoholic fermentations, namely wine and beer. Led initially by "biomolecular archaeologists" at the University of Pennsylvania Museum of Archaeology and Anthropology (http://www.penn.museum/), these researchers could not use traditional types of physical evidence, since they were often absent. Instead, they relied on chemical and molecular records, obtained from artifacts discovered in locations from around the world (McGovern et al., 2004).

Specifically, their approach involved extracting residues from ancient clay pottery jars and vessels found in excavated archaeological sites, mainly from the Near East and China. These vessels are generally porous, and any organic material would have become adsorbed and trapped within the vessel pores. In a dehydrated state, this material would have been protected against microbial or chemical decomposition. Carbon dating is used to establish the approximate age of these vessels. Then various analytical procedures, including gas chromatography-mass spectroscopy, Fourier transform infrared spectrometry, and other techniques, are used to identify the chemical constituents. The analyses revealed the presence of several marker compounds, in particular, tartaric acid that is present in high concentrations in grapes, and in wine, but generally absent elsewhere (Guash-Jané et al., 2004; McGovern, 2003). Based on these and other studies, it would appear that wine had been produced in the Near East regions around present-day Turkey, Egypt, and Iran, as long ago as the Neolithic Period (8500 to 4000 BCE).

The McGovern Molecular Archaeology Lab group has also ventured to China in an effort to establish when fermented beverages were first produced and consumed (McGovern et al., 2004). As described in Chapter 11, Asian wines are made using cereal-derived starch, rather than grapes. Rice is the main cereal used. In ancient times, other components, particularly, honey and herbs, were added. As before, the investigators analyzed material extracted from pottery vessels that were from the Neolithic Period, ca. 7000 BCE. However, the specific biomarkers would not necessarily be the same as for wine made from grapes. Rather, they would be expected to reflect the different starting materials. Indeed, the analyses revealed the presence of rice, honey, and herbal constituents, but also grapes, in the form of tartaric acid. Although domesticated grapes vines were not introduced into China until about 200 BCE, wild grapes could have been added to the wine as a source of yeast. An alternative explanation is that tartaric acid could have been derived from other native fruits and flowers. Analyses of proto-historic vessels, from ca. 1900–700 BCE, indicates that these later wines were cereal-based using rice and millet. Thus, it now appears clear that fermented beverage technology in China began around the same time as in the Near East, and that the very nature of the fermentation evolved over several millennia.

Drunken monkeys An entirely new way to estimate when humans first began consuming ethanol-containing fermented products is based on the emerging field called "paleogenetics" (Carrigan et al., 2015; Thomson et al., 2005). According to this approach, scientists work backwards in time using ancient genetic material. Then, based on Darwinian principles, they can then predict when a particular genotype emerged in a population.

Ordinarily, ethanol is metabolized in the liver by two enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). If one of these enzymes is absent, even modest consumption of alcohol will result in alcohol toxicity. For individuals that express these enzymes, if more ethanol reaches the liver than can be handled by the normal metabolic pathway, the toxic intermediate, acetaldehyde, accumulates. This results, as many college students know, in headaches and nausea, i.e., hangovers.

Recently, scientists have shown that our primate ancestors harbored an ADH that was inactive against ethanol (Carrigan et al., 2015). However, a mutation in a specific ADH gene (ADH4) led to expression of a functional ADH. This mutation, they suggest, occurred millions of years ago, and correlated to when dietary sources of ethanol would have begun to appear naturally. This would have coincided with the early days of terrestrial life when fruits that had fallen from trees were subsequently fermented. Primates, that ventured from trees to eat the fallen fruit and that could tolerate the alcohol, had a selective nutritional advantage. Consequently, these researchers concluded that hominids had learned how to "handle" their drinking long before they intentionally began to make and consume alcoholic beverages. Indeed, as one commentator suggested, one reason why alcoholic fermentative technologies evolved in the first place was because of our "adaptive predilection for ethanol" (Dominy, 2015). In other words, our ancestors' acquired ability to tolerate ethanol led, for better or worse, first to human liking of alcohol, and eventually, to the development of brewing, wine, and other ethanol technologies.

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Fermentation became an even more widespread practice during the Greco-Roman era, as new raw materials, technologies, and cuisines were adopted from conquered lands, and fermented foods spread throughout the region. Due to their increased storage stability, and in some cases, ease of transport, these foods were also important for distant armies and navies. Cheese, for example, would have been a more stable and compact source of nutrients compared to milk. In addition, beer and wine were often preferred over water. No surprise there, as the latter was often polluted with fecal or other foreign material. During the Roman Empire, the means to conduct trade developed. Thus, cheese and wine, as well as wheat for bread-making, became available around the Mediterranean, Europe, and the British Isles.

Although the organized manufacture of bread had existed even in ancient Egypt, by the Middle Ages, its manufacture, as well that of beer and cheese, became the province of craftsmen and organized guilds. The guild structure involved long training and apprenticeships. Once learned, these skills were often passed on to the next generation. For some products, particularly beer and wine, these craftsmen were often monks operating out of monasteries and churches, a tradition that lasted for hundreds of years. Indeed, many of the technologies and manufacturing practices employed even today were originally developed by monks. For example, the process used to achieve carbonation in Champagne, is credited to the Benedictine monk, Dom Pierre Pérignon, who lived 300 years ago. Eventually, the manufacture of these products became more privatized, although often under some form of state control, which conveniently allowed for taxation.

From the Neolithic Period, to the Middle Ages, to the current era, fermented foods had been among the most culturally and nutritionally important foods consumed by humans (Figure 1.1). An argument can be made that fermented foods, and the subsequent development of technologies for their production, directly contributed to the dietary, cultural, and social evolution of human history. Consider, after all, how integral fermented foods are to the diets and cuisines of nearly all civilizations and cultures and how many fermented foods and beverages are consumed as part of religious customs, rites, and rituals (Box 1.2).

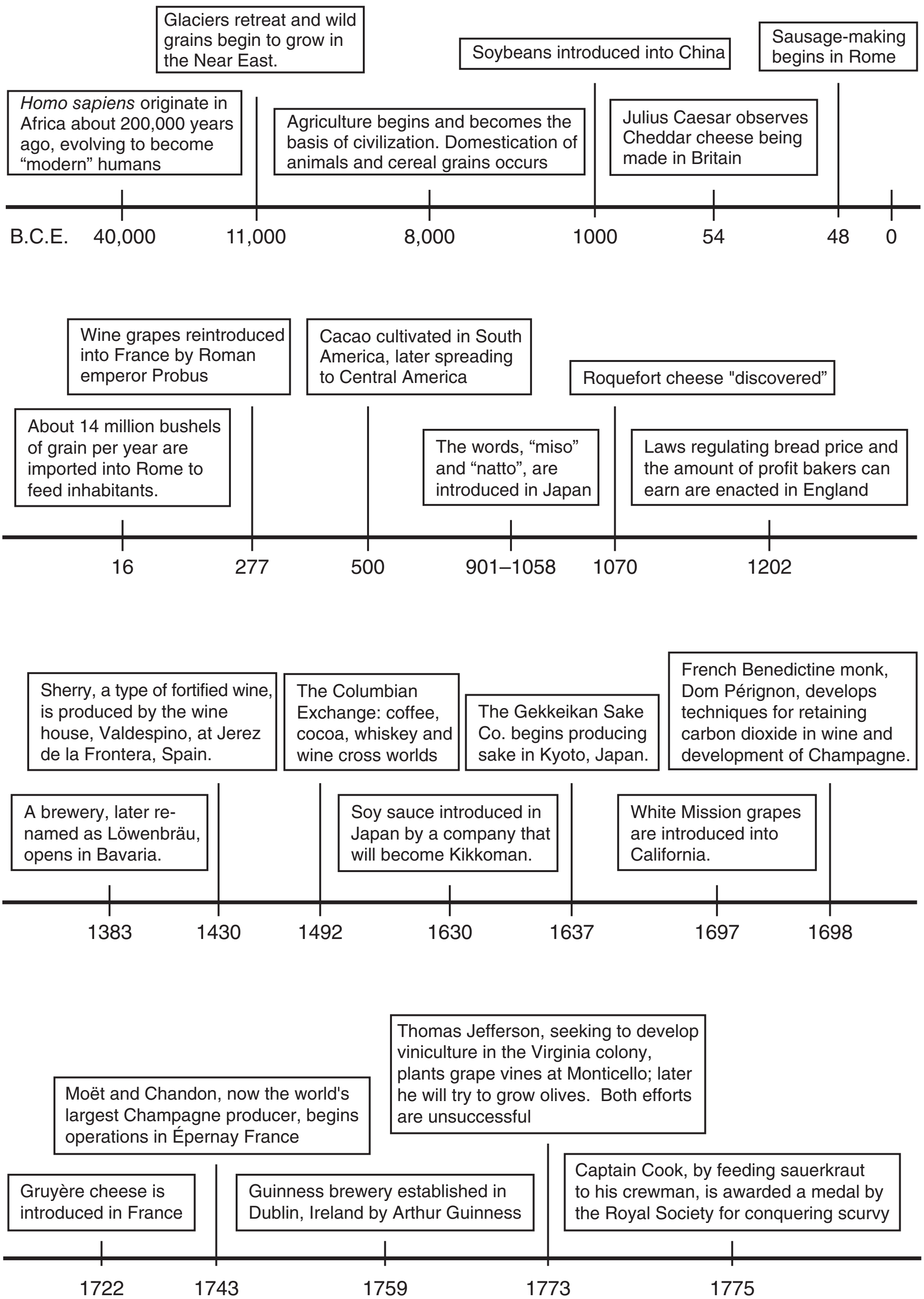

Figure 1.1 Important developments in the history of fermented foods. From Trager, 1995 and other sources.

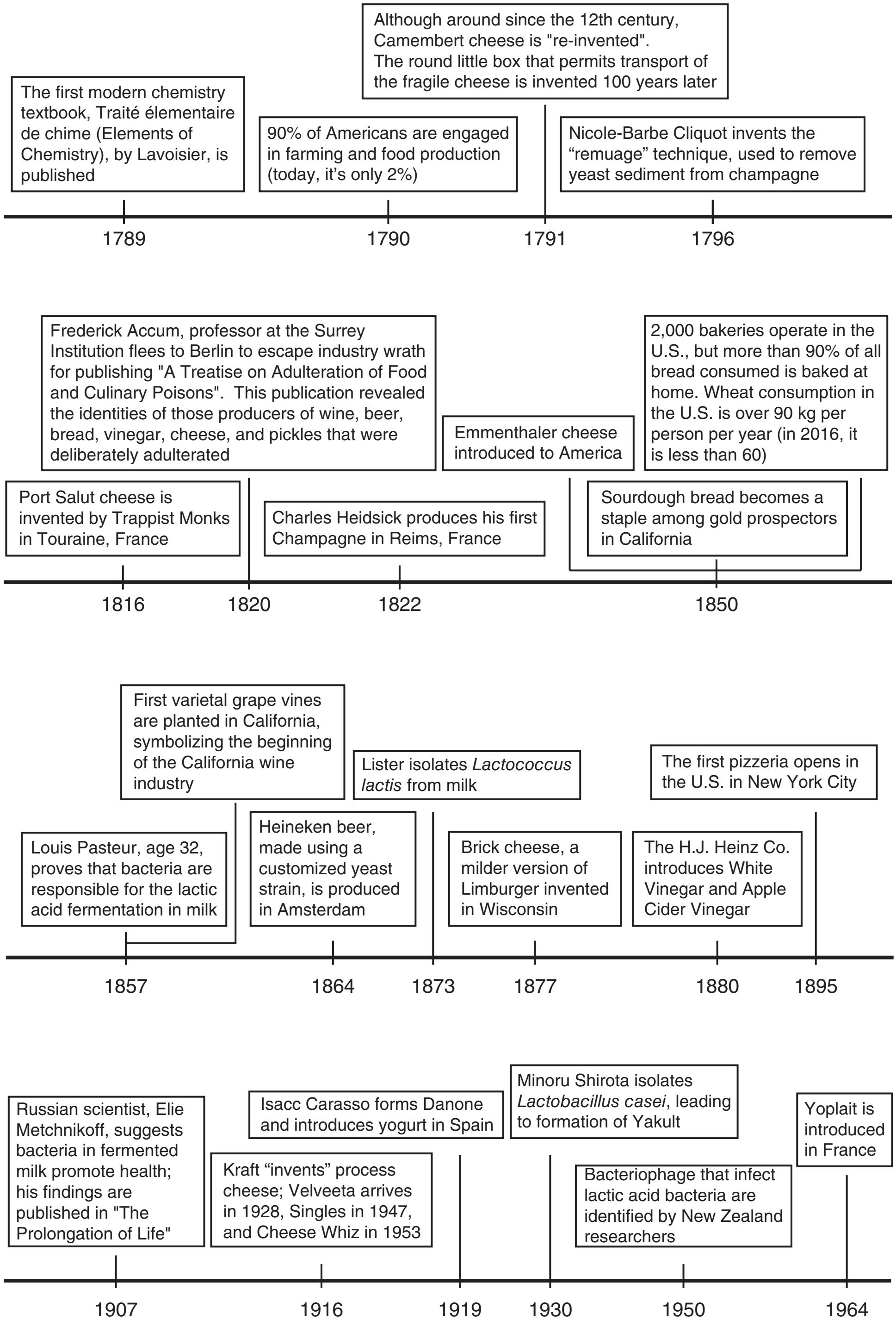

Figure 1.1 (*Continued*)

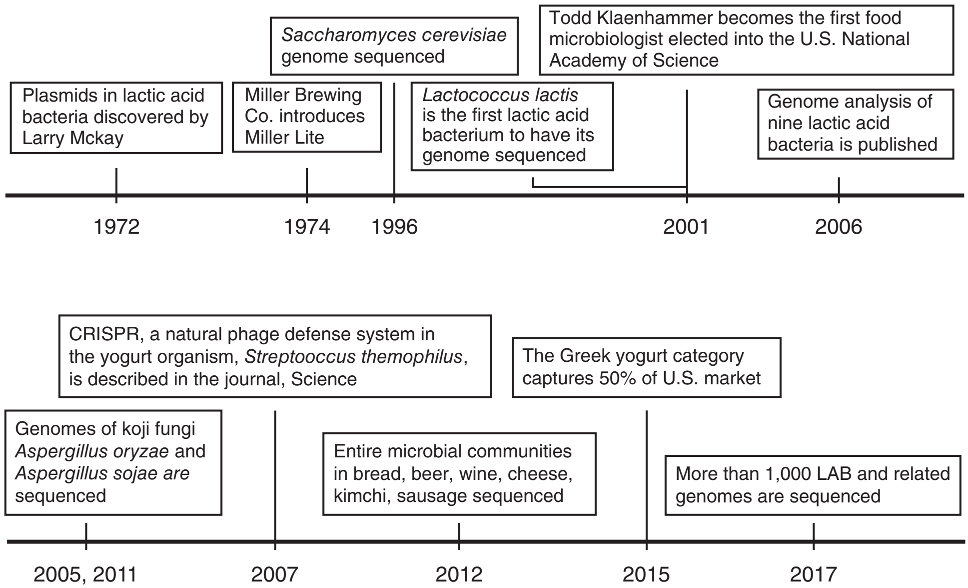

Figure 1.1 (*Continued*)

Box 1.2 Fermented foods and ancient texts

It is not much of an overstatement to argue that fermented foods and beverages have had a major impact on the cultural history of human societies. After all, bread – the "staff of life", and wine, "which cheers man's heart" – are among the earliest fermented foods. No wonder that references to these and other fermented foods are common in the early records of human history and civilization. Notably, the Bible, both Old and New Testaments, and other religious tracts, contain numerous references to fermented foods. In some sections, as indicated below, fermented foods are even the focal point.

Fermented foods, apart from their mention in the Bible, have also served a major role in ancient Eastern and Western mythologies. There were no scientific explanations to account for the unique sensory and often intoxicating properties of fermented foods. Therefore, these products were often described as "gifts of the gods". In Greek mythology, Dionysus was the god of wine, and Bacchus held this position in Roman mythology. The Iliad and the Odyssey, classic poems written by the Greek poet, Homer, in about 1150 BCE, also contain numerous references to wine, cheese, and bread. A hemisphere away, Korean and Japanese mythology also refers to the gods that provided miso and other Asian fermented foods (see Chapter 14).

Fermented foods and the Bible From the Genesis story of Eve and God's warning not to eat from "the tree of knowledge", to the dietary laws described in the books of Exodus and Leviticus, food serves a major metaphoric and thematic role throughout the Old Testament. Fermented foods, in particular, are often mentioned in biblical passages. Clearly, these foods must have already been well known to those cultures and civilizations that lived during the time the bible was written. In Genesis (Chapter 9: Verse 20), the first action taken by Noah, after the flood waters had receded and he "went forth from the ark", was to plant a vineyard. It is instructive to note in the very next line that Noah drank enough wine to become intoxicated.

Just a few chapters later (Genesis 18:8), Abraham receives three strangers, presumably, angels, to whom he offers various refreshments, including milk "curds". The latter has been popularized as being yogurt, and perhaps Abraham's regular yogurt consumption accounted for his longevity of 175 years. Probably the most relevant reference to fermentation in the Bible is the story of Passover. The events described in Exodus culminate with the hasty departure of the Hebrew slaves (Chapter 12:39). Once Moses had secured their freedom from Pharaoh, they were "thrust out of Egypt, and could not tarry". There was not enough time for microorganisms to ferment, so the bread dough could not rise or become "leavened". Instead, it had to be baked in its "unleavened" state. This product, called matzoh, is still eaten today by people of the Jewish faith to commemorate, symbolically, the Hebrew exodus.

Ritual consumption of other fermented foods is also prescribed in Judaism. On the weekly Sabbath, the egg bread, Challah, is to be eaten, and either grapes or wine is drunk, preceded by appropriate blessings of praise.

Fermented foods are also featured prominently in the New Testament. At the wedding in Cana (John 2:1–11), Jesus' first miracle is to turn water into wine. Later (John 6:1–14), another miracle is performed when five loaves of bread, and two fish, provide sustenance for five thousand men. During the crucifixion, Jesus is given drops of vinegar. In Catholicism, the Sacrament of Holy Communion, described by Jesus during the Last Supper, is represented by bread and wine.

Given that fermented foods were a major part of the human diet during the biblical era, it is no surprise that the Old and New Testaments often refer to these staple foods.

FERMENTED FOODS: FROM ART TO SCIENCE

It may be difficult for the twenty-first century reader to imagine that fermented foods, whose manufacture relies on the intricate and often subtle participation of microorganisms, could have been produced without even the slightest notion that living organisms were involved. Likewise, neither could the early manufacturers of fermented foods and beverages have appreciated the science involved in their production, since it was only in the last 150 to 200 years that microorganisms and enzymes were "discovered". In fact, up until the middle of the nineteenth century, even many of the most prominent members of the scientific community still believed in the concept of spontaneous generation. The very act of fermentation was a subject for philosophers and alchemists, not biologists. Although the Dutch scientist, Antonie van Leeuwenhoek, had first observed microorganisms in his self-made microscope in 1675, the connection between Leeuwenhoek's "animalcules" and their biological or fermentative activities was only slowly realized. It was not until later in the next century when scientists began to address the question of how fermentation occurs.

Initially, chemists were among the first scientists to study the scientific basis for fermentation. With the exception of van Leeuwenhoek, microbiologists, weren't yet on the scene. By the late 1700s and early 1800s, the French chemists Antoine Lavoisier and Joseph Louis

Gay-Lussac had independently described the overall equations for the alcoholic fermentation.

Meanwhile, improvements in microscopy, had allowed Theodor Schwann and others to observe the presence of yeast cells in fermenting liquids, including beer and wine. These observations led Schwann to propose, in 1837 (as recounted by Barnett, 2003), that, "It is very probable that, by means of the development of the fungus, fermentation is started." Still, the suggestion that yeasts were actually responsible for fermentation was not widely accepted. Rather, it was argued by several of Schwann's prominent contemporaries that fermentation was caused by aerobic chemical reactions, with the involvement of suitable catalysts. Yeasts, they claimed, were merely inert precipitates, and had nothing to do with fermentative processes.

The debate over the role of microorganisms in fermentation was brought to an unequivocal conclusion by another chemist, Louis Pasteur. Writing in 1857, Pasteur stated that, "Fermentation, far from being a lifeless phenomenon, is a living process," that "correlates with the development of…cells and plants which I have prepared and studied in an isolated and pure state" (Schwartz, 2001). In other words, fermentation could only occur when microorganisms were present. The corollary was also true – that when fermentation was observed, growth of the microorganisms occurred. In a series of elegant, and now famous publications, Pasteur described details on lactic and ethanolic fermentations, including those relevant to milk, wine, and beer. He also identified the organism that causes the acetic acid (i.e., vinegar) fermentation that was responsible for wine spoilage.

The behavior of yeasts during aerobic and anaerobic growth also led to important discoveries in microbial physiology. For example, it was observed that when yeasts were exposed to air or oxygen during fermentation, glycolytic metabolism was abruptly inhibited, a phenomenon appropriately termed the Pasteur Effect. Eventually, the recognition that fermentations (and spoilage) were caused by microorganisms led Pasteur to work on other microbial problems, in particular, infectious diseases. Nonetheless, it was readily apparent from Pasteur's research that food spoilage and fermentation were both instigated by microorganisms. Future studies on food and beverage fermentations would be left to other scientists who had embraced this new field of microbiology.

Once the scientific basis of fermentation was established, microbiologists quickly realized the value in identifying and cultivating the yeasts and bacteria responsible for performing specific fermentations. Emil Christian Hansen, working at the Carlsberg brewery in Denmark, adopted the techniques and recommendations of Pasteur, Lister, Koch, and others to isolate a specific yeast strain from wild beer cultures. He used this strain to produce beer having consistent quality characteristics. The advantages of this approach were clearly evident, and by the end of the nineteenth century, breweries throughout Europe, the Americas, and Asia were using pure yeast strains.

Other fermented food industries that also relied on cultures appreciated the benefits of pure culture technology. Soon cultures for butter, cheese, and other dairy products became available. One of the main advantages of these cultures was their consistent performance, in contrast to the unpredictable outcomes that occurred when using so-called back-slopping techniques (Chapter 4). However, as the scale of these industries grew, the availability of concentrated cultures became important as well. Eventually, the dairy industry became one of the largest users of commercial cultures.

The specialized dairy culture supply "houses", formed at the turn of the century, not only sold cultures, they also sold enzymes, colors, and other products necessary for the manufacture of cheese and cultured milk products. Many cheese factories continued to propagate their own “in-house” cultures throughout the first half of the twentieth century. However, as factory size and product throughput increased, the use of commercial dairy starter cultures eventually became commonplace. Likewise, commercial cultures for bread, wine, beer, and fermented meats became the norm for industries producing these products.

THE MODERN FERMENTED FOODS INDUSTRY

Like other segments of the food processing industry, the fermented foods industry has changed dramatically in the past several decades. Not only has the average size of a typical production facility increased many-fold, so has the rate, or throughput, at which raw materials are converted to finished product. Nonetheless, many small, traditional-style facilities still exist and not just in developing countries. They are common throughout Europe where traditional manufacturing procedures are often required or mandated for a variety of fermented foods. Small-to-medium scale manufacturing of these products has also made a comeback in the US, as is evident by the many craft breweries, boutique wineries, and artisanal bakeries and cheese operations that have opened in recent years. Despite these trends, however, the fermented foods industry is dominated by high-volume producers with large production capacity.

As the size and scale of the fermented food industry have increased in the past century, so has the fundamental manner in which fermented foods are produced (Table 1.1). For example, up until the past 60 or so years, most cheese produced world-wide was made using raw milk of Manufacturing or Grade B quality. Now, at least in the US, Canada, New Zealand, Australia, and parts of Europe, pasteurized Grade A milk, meeting higher quality standards, is more commonly used. Open, copper-lined cheese vats still exist, but modern manufacturing tanks and vats are often enclosed and constructed from stainless steel or other materials that facilitate cleaning and even sterilization treatments. From the outset, modern facilities are specifically designed with an emphasis on sanitation requirements so exposure to air-borne microorganisms and bacteriophage is minimized. In fact, the entire plant design, air-handling, and personnel access is considered in order to prevent inadvertent cross-contamination.

Many of the unit operations are now highly mechanized or automated. Other than a few keystrokes from a control panel, computer, or smart phone, modern manufacture of fermented foods often involves minimal human contact. More than ever before, fermented food

Table 1.1 The fermented foods industry: past and present.

Traditional	Modern
Small scale (craft industry)	Large scale (in factories)
Non-sterile medium	Heat-treated medium
Open and exposed	Closed and aseptic
Manual	Automated
Insensitive to time	Time-sensitive
Varying quality	Consistent quality
Safety not a major concern	Safety a major concern
No culture knowledge	Extensive culture knowledge

production is now subject to time and scheduling demands. In the not too distant past, if an industrial cheese or yogurt fermentation was slow or sluggish, it simply meant that the workers (who may have been family members) would be late for supper, and little else. In the current high-volume production environment, a slow or delayed fermentation could mean that workers would need to stay beyond their shift, and be paid overtime. In many cases, the entire production schedule might be affected, since the production vats would not be emptied and refilled as quickly as needed.

Traditional manufacturing practices may not have always yielded products having consistent quality characteristics, although lot sizes were generally small. Thus, economic losses due to an occasional misstep were not likely to be too serious. Besides, it was probably often the case that for every cask of wine or wheel of cheese considered inferior, or not quite up to the expected standard, there would have been an equally spectacular lot compensating for the ones that turned out badly. Consider the absolute worst case scenario – a food poisoning outbreak occurs due to an improperly manufactured product. Such an event, however costly, would likely have been limited in geographical scope, as well as number of cases, due to the narrow distribution range and small production volume. Continuing these practices today, however, is simply beyond consideration for most major food manufacturers. A day's worth of production may be worth tens, if not hundreds of thousands of dollars, in raw materials cost alone. There is simply no way a producer could adsorb or tolerate such losses, even if they occurred only sporadically.

Food safety, in particular, has become an international priority. Minimizing risks associated with food borne pathogens, or other food safety issues, is a major goal for the fermented foods industry. Depending on the country or region, there may be zero tolerance for pathogens and other hazards in fermented foods. Quality assurance programs now exist throughout the entire food industry with the major goal being to produce safe food products on a consistent and predictable basis.

In essence, the fermented foods industry has evolved from a mostly art- or craft-based practice to one that relies on modern science and technology. The emphasis on safety, sanitation, quality, and consistency, applies to all processed foods, not just fermented foods. What is uniquely different about fermented foods, compared to other foods, is that the manufacture of fermented foods relies on biologically alive ingredients, namely the cultures. Therefore, it is the only food processing industry in which product success depends on the presence, growth, and activity of microorganisms. If a particular trait is somehow lost, the cells become less viable, or the culture is infected by viruses or inhibited by other environmental factors, the fermentation may fail and product quality and safety may suffer. For fermented products for which food safety is a concern, many are made from raw substrates without the benefit of a thermal process or "kill step". Although salt and other anti-microbial agents may be added, control of pathogens is primarily achieved biologically. Thus, the implications are highly significant.

In contrast to flavoring, nutritional, or functional food ingredients, microorganisms used to initiate fermentations are not easily standardized. This is because their viability, biochemical activity, and even their concentration (number of cells per unit volume) may fluctuate from lot to lot, or during storage. Although custom-made starter cultures standardized for cell number and activity are widely available, many industrial fermentations still rely on the presence of naturally-occurring microorganisms. The composition and biological activities of this indigenous microbiota are often subject to considerable variation. In addition, microorganisms are often exposed to a variety of inhibitory chemical and biological agents in the food or environment that can compromise their viability and

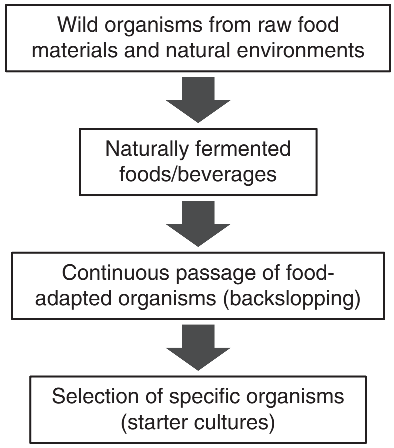

Figure 1.2 Domestication of cultures, from wild to modern. Adapted from Gibbons and Rinker, 2015, with permission.

activity. Since the culture organisms, whether added or indigenous, are often the main means by which spoilage and pathogenic microorganisms are controlled in fermented foods, their role in ensuring food safety and quality cannot be overstated. If the culture fails to perform in an effective and timely manner, the finished product will be subject to spoilage, quality defects, or worse. Thus, the challenge confronting the fermented foods industry is to manufacture products whose very production is subject to inherent variability, yet still satisfy the modern era demands of consistency, quality, through-put, and safety. It is no surprise that fermentation organisms (and starter cultures) have essentially been domesticated such that their performance is predictable and reliable (Figure 1.2). The co-evolution of *Saccharomyces* and fermented foods is a perfect example of microbe domestication, given that similar but distinct strains are used in bread, beer, and wine (Box 1.3).

Box 1.3 Yeasts – Man's best friend

The domestication of plants and animals for the purpose of producing food was arguably one of the most important events in human history. Without the plentiful food supply provided by agriculture, human civilizations would not have developed. Fermented foods, particularly bread, beer, and wine, were among the foods first produced by early civilizations some 10,000 years ago. As will be described in subsequent chapters, all of these products require yeasts, namely *Saccharomyces cerevisiae*, for their production. That the manufacture of these products depends on specific yeasts suggests that relevant strains may have co-evolved in parallel with human activities. Recent research on the evolution of *S. cerevisiae* suggest that domestication of wild strains emerged at nearly the same time when plants and animals were domesticated, and when fermented foods were first produced by humans (Sicard and Legras, 2011; Steensels and Verstrepen, 2014). These foods would rely entirely on natural or "spontaneous" fermentations. Eventually, the practice of backslopping, intentional passage of "cultures" from one batch to the next, was adopted. This would have led to selection of adapted strains that were truly "domesticated" (Gibbons and Rinker, 2015).

Researchers have also been able to trace the spread of *S. cerevisiae*, geographically and temporally, by comparing the genotypes of hundreds of bread, beer, and wine strains collected around the world (Legras et al., 2007). In this study, genotypes were based on the sequences of microsatellite DNA, regions of yeast DNA that serve as molecular fingerprints. These sequences are also useful for establishing relatedness of different strains. The results showed there were more than 550 distinct genotypes among the 651 strains, but there was considerable relatedness, as well. Nonetheless, strains could still be clustered based on their origin. Thus, it's possible to consider S. cerevisiae strains as bread strains, beer strains, or wine strains, albeit with some overlap. There were also geographical differences, suggesting that some strains had been domesticated locally.

Beyond describing the differences between different strains of *S. cerevisiae*, researchers have also been interested in the evolution of *S. cerevisiae* (Liti et al., 2009). That is, how and when did *S. cerevisiae* strains become specialized or adapted to the different habitats with which they are associated? Researchers have suggested that the phenotypic properties of different extant, i.e., contemporary yeasts strains reflect their adaptation to different environments (Liti et al., 2009). Genetic rearrangements and duplications lead to new alleles and ultimately new phenotypes that are well-suited for growth in specialized habitats (Ibáñez et al., 2014). Although these present-day strains sort within well-defined lineages, they also have a "mosaic" genetic structure. That is, they harbor lots of genes or sequences from many sources, reflecting centuries of cross-breeding.

Ultimately, by practicing fermentation science for several millennia, humans have essentially shaped the genome of *S. cerevisiae* (Ibáñez et al., 2014). In fact, the domestication of wild yeast is not unlike the domestication of man's "other" best friend.

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PROPERTIES OF FERMENTED FOODS

As suggested in the previous discussion, fermented foods were likely among the first "processed" foods produced and consumed by humans. The reasons for their popularity, more than 5000 years ago are, for the most part, the same reasons why they continue to be popular today (Table 1.2).

Preservation

Fermented foods were observed to be well preserved, at least compared to the starting materials. This was obviously important thousands of years ago, when few other preservation techniques existed. A raw food material such as milk or meat had to be consumed shortly after it was collected or it would soon spoil, especially in temperate climates. Although salting, smoking, or drying could have been used for some foods, fermentation must have been an attractive alternative, due to other desirable features, noted below. Preservation was undoubtedly one of the main reasons why fermented foods became such an integral part of the human diet and contributed to the cuisines of nearly every culture on every continent. However, even today, preservation and enhanced shelf-life remain as important properties of fermented foods. As described in subsequent chapter, cultures now exist that not only perform the normal fermentation, but that also produce specific antimicrobial agents in the food. Such cultures have the added benefit of providing an extra margin of food safety and extended shelf-life.

Nutrition

Most fermented foods are made from nutritionally-rich raw materials, such as milk, meat, rice, soybeans, and wheat. Additional nutritional value has long been attributed to fermented foods, even though the scientific basis for many of the nutritional claims have, until recently, not been well established. Evidence that fermentation may enhance nutritional properties now exists for several fermented products.

The nutritional case for yogurt is particularly interesting. In much of the world, fluid milk is not regularly consumed because most people are unable to produce the enzyme β-galactosidase which is necessary for digestion of lactose, the main sugar present in milk. Lactose malabsorption is the physiological condition that occurs when individuals deficient in β-galactosidase production consume lactose. A variety of unpleasant, though non-life-threatening symptoms are associated with this disorder. These include mild-to-severe intestinal distress, bloating, diarrhea, and cramps. This condition is especially common among Asian, African, and Middle Eastern populations, although adult Caucasians may also

Table 1.2 Properties of fermented foods.

- Enhanced preservation
- Enhanced nutritional value
- Enhanced functionality
- Enhanced organoleptic properties
- Uniqueness
- Increased economic value

be lactose intolerant, and many individuals from these regions consume dairy products without ill effects. Interestingly, numerous studies have revealed that lactose-intolerant subjects can consume yogurt without any untoward symptoms and can therefore obtain the nutritional benefits (e.g., calcium, high quality protein, and B vitamins) contained in milk. The metabolic and physiological hows and whys to explain these observations will be described in more detail in Chapter 3.

Wine is another great example of a fermented product that appears to be nutritionally superior compared to the raw material from which it was derived. There is now a large volume of literature showing that wine contains components, other than ethanol, that may contribute to enhanced health (Chapter 10). Specifically, resveratrol and several other phenolic compounds have been identified in red wine and shown to have anti-oxidant activities that may reduce the risk of heart disease and cancer. Wine, as well as other fermented foods, are widely consumed in Mediterranean countries where mortality rates due to heart disease are generally lower than in other regions. This has led to the suggestion that a "Mediterranean diet" may be ideal for human health.

Finally, it has been suggested that there may be health benefits associated with consumption of fermented foods that extend beyond the macronutrients or metabolic end-products produced by fermentative microorganisms. Specifically, the very microorganisms that perform the actual fermentation are now thought to contribute to human health, especially in the gastrointestinal tract. Yogurt and cultured dairy products have attracted the most interest as carriers of desirable microorganisms. Many other fermented foods, including fermented vegetables, cereals, and soy products may also harbor beneficial organisms, as well. Even extra-intestinal benefits, including mental health, cognition, and acuity, have been suggested to result from consumption of fermented foods (Box 1.4). The nutritional importance of fermented foods has become so widely appreciated that their consumption is now being recommended as part of a healthy diet. Perhaps the US MyPlate, Canada's Food Guide, the Chinese Food Guide Pagoda, or other guideline will eventually include fermented foods (Figure 1.3). As recently suggested by one group of nutrition researchers, "knowing the general benefits of traditional and supplemented fermented foods, they should be a daily item on most national food guides" (Bell et al., 2017).

Box 1.4 Fermented foods, mental health, and the gut–brain axis

As noted elsewhere in this chapter, there are many reasons accounting for why fermented food have remained popular from ancient times to the present. In particular, there are many nutritional advantages humans have gained by consuming fermented foods. Fermented foods, like cheese, sauerkraut, and sausage provide a stable source of vitamins, minerals and other nutrients. Wine and other fermented beverages are enriched in polyphenol compounds that have antioxidant and other biological activities. Yogurt, miso, and kimchi contain populations of live microorganisms that contribute to intestinal health.

Recently, several research groups have suggested that the nutritional benefits of fermented foods extend beyond physical health (Selhub et al., 2014; Dasha et al., 2015; Hilimire et al., 2015; Logan et al., 2015). Specifically, they have proposed that depression, mood, anxiety, cognition, and behavior are affected by diet and that fermented foods, in particular, can improve mental health. Data to support this hypothesis is based, in part, on epidemiological studies and self-reported symptoms. Several studies have shown that traditional or Mediterranean diets that are naturally rich in fermented foods reduces the risk of depression, compared to high sugar, high fat Western diets (Lai, et al., 2014; Skarupski et al., 2013; Jacka et al., 2010).

There is also physiological data showing that the molecules produced by the gut microbiota may act as neurological signals to the brain. This gut–brain axis has emerged as one of the most intriguing and exciting new areas in food science and gut biology. It should be noted that the diet–gut–brain connection is not entirely new – suggested linkages date all the way back to Metchnikoff (Orla-Jensen et al., 1945). What is new is that this hypothesis is now supported by considerable evidence, and several possible mechanisms may explain how the microbiota, and fermented food, could influence mental health and well-being (Dinan and Cryan, 2017; Kennedy et al., 2016).

First, fermentation may result in formation of biologically active end-products, *in situ*, including those having anti-oxidant activity (Wang et al., 2006; Wang et al., 2014). Depending on the substrate (i.e., soy, bran, or dairy), fermented foods may also contribute particular dietary components. In other products, it's not the fermented foods, per se, that are responsible for the suggested mental health benefits, but rather the ingested microorganisms that reach the colon. This would include bacteria that are either added directly as starter cultures, like in yogurt, or those present as part of the normal microbiota, like in fermented vegetables. Germ-free mice, i.e., raised without a microbiota, have reduced anxiety-like behavior (Foster and McVey Neufeld, 2013). Bacteria added to fermented foods for their probiotic activities could also contribute to mental health benefits (Tillisch et al., 2013). Likewise, prebiotics naturally present or added to fermented foods could shift the microbiota toward a stress-reducing phenotype (Burokas et al., 2017).

Studies have shown that bacterial products produced in the gut could activate the central nervous system or modulate the immune system. For example, the neurotransmitter, gamma-aminobutyric acid (GABA) is produced by *Lactobacillus* and *Bifidobacterium* species, resulting in lower stress levels in mice (Bravo et al., 2011). Recently, another mechanism has been hypothesized to account for how the gut microbiota affects mental health, and autism, in particular (Reddy and Saier, 2015; Thakur et al., 2014). Briefly, these authors suggested that an altered gastrointestinal microbiota may affect gut epithelial barrier function. Specifically, a dysbiotic microbiota can lead to increased gut permeability, or leaky gut syndrome. Ultimately, microbial end-products reach the bloodstream, resulting in metabolic endotoxemia and inflammation. Leaky gut syndrome has been associated with gastrointestinal disorders, as well as several neurological conditions, including autism.

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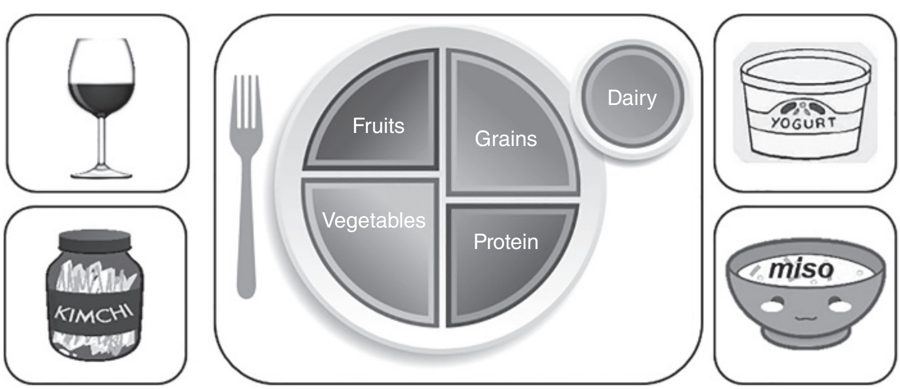

Figure 1.3 Possible future nutritional guideline: "Fermented foods, including those that contain live microorganisms, should be included as part of a healthy diet". Adapted from the USDA's MyPlate promotional program.

Functionality

Most fermented foods differ nutritionally from the raw materials of which they are made. Also, most are dramatically different with respect to their functional properties. Cheese, for example, differs in function, form, and flavor from milk. Functional enhancement is perhaps most evident for bread and beer. When humans first began some 10,000 years ago to use wheat as food there was little they could do with the flour, except to make simple doughs and flat breads. Yet allowing the dough to ferment and leaven prior to baking, would transform the flour into flavorful and expansive breads.

Barley was another widespread grain in the ancient world and was often used in foods. However, compared to wheat, low gluten content of barley limited its functionality in bread. Barley did become important in another fermented product – beer (and later whisky). Given that barley is the main ingredient, other than water, in the manufacture of beer, it can reasonably be argued that beer is the best example of enhanced functionality due to fermentation.

Organoleptic

Although preservation, nutrition, and functionality were likely responsible for the early adoption and eventual spread of fermented foods throughout the world, there was another important reason for the success of these foods. Simply, fermented foods, must have tasted, smelled, and looked dramatically different from the starting raw materials. Many of these differences would have certainly been duly appreciated, then as now. While individuals that do not particularly care for aromatic Limburger cheese, fermented fish sauce, or a viscous soybean product called natto might argue that those differences are for the worse, there is little argument that fermented foods have aroma, flavor, and appearance attributes that are quite unlike the raw materials from which they are made. For those individuals who partake and appreciate Limburger cheese, the sensory characteristics between the cheese and the milk make all the difference in the world.

Uniqueness

With few exceptions (see below), there is simply no way to make fermented foods without the fermentation process. The hole or eyes, as well as complex flavors, of Swiss and Gouda cheese cannot be formed mechanically or by any other means, other than fermentation. Likewise, dry cured salami, Champagne, Pilsner beer, Balsamic vinegar, and kimchi cannot be produced any other way. For many fermented products, even the procedures used for their manufacture are unique, requiring strict adherence (Box 1.5). Parmesan cheese, for example, must be made in a defined region of Italy, from milk obtained from specific breeds of cows, manufactured according to traditional and established procedures, and then aged under specified conditions. The finished product must also meet quality requirements. Any deviation results in forfeiture of the name Parmesan.

It is possible, nonetheless, to manufacture "fermented" foods, such as certain fresh cheeses, sausages, and even soy sauce, without fermentation. The manufacture of these products generally involves direct addition of acids, enzymes, or flavors to simulate the activities normally performed by fermentative microorganisms. These products, which the purist might be inclined to dismiss from further discussion, generally lack the flavor and overall organoleptic properties of their traditional fermented counterparts.

Box 1.5 Fermented foods of protected origins

For thousands of years, the manufacture of fermented foods was based on experience, skill, art, and sometimes luck. Science, and microbiology and biochemistry, in particular, did not enter the scene until the nineteenth century. From identifying the microorganisms responsible for fermentation, to establishing fermentation pathways, to sequencing entire microbial communities found in fermented foods, much has been learned in just over a century. Moreover, engineering and processing applications have led to automation, robotics, and other technologies for producing fermented foods on a large scale with minimal human labor.

Despite these remarkable advances, many fermented foods and beverages are still made via centuries old manufacturing practices. In fact, traditional manufacture of fermented food is codified in several regions, most notably in Europe (see below). Thus, the milk used for traditional cheeses is usually raw, fermented sausages are dried, not cooked, and ingredients are limited to an essential few. For some products, starter cultures cannot even be used. Geography is also of utmost importance, and is often the major determinant for these products.

Official origins The official criteria for such foods depends on the region. In the European Commission, three categories exist. To obtain "Protected Designation of Origin" (PDO) status, the product must be "produced, processed and prepared in a given geographical area using recognized know-how". Products that have Protected Geographical Indication (PGI) status are "closely linked to the geographical area" and "at least one of the stages of production, processing or preparation takes place in the area." Finally, "Traditional Speciality Guaranteed" (TSG) products simply have "traditional character, either in the composition or means of production". To achieve one of the designations, manufacturers must follow a rather long and detailed application process, which is subsequently subject to careful review by experts. Nonetheless, hundreds of products have received PDO status. Foods or beverages that are made similar to a PDO product, but either with production deviations or made outside the designated areas cannot be labeled as the PDO product. Thus, Parmesan cheese can only be made in the designated Parma-Reggio regions in Italy.

The case of wine There are also country-specific designation in Europe and elsewhere. In France, for example, the wine industry has long followed strict regulations, called the "appellation d'origine controlee" (AOC), for naming wine. These regulations are based on region and method of manufacture. Similar systems exist in Italy ("denominazione di origine controllata or DOC") and throughout the EC. In the US, wines can be geographically labeled, and in Tennessee, bourbon whiskey is also a protected name ("Tennessee whisky"), provided it is made in Tennessee according to specified procedures.

Although it might strike twenty-first century readers as rather odd for manufacturers to make cheese, sausage, beer, and wine without taking advantage of modern science and engineering, there are several reasons why these industries, as well as consumers and governments, support the PDO system. First, the PDO system provides consumers with the confidence that the product was made according to strict quality standards. For example, the Parmesan cheese mentioned above, must be made in small copper-lined vats using whey as the culture and calf rennet as the coagulant. The cheese must be aged at least 12 months, and wheels are inspected and graded. Thus, consumers have a pretty good idea what to expect when they buy Parmesan cheese.

Likewise, PDO, PGI, and TSG designations provide manufacturers (and the region and country) with considerable market advantages. The value for products with terroir or "geographical indications" (GI) is over $50 billion (Capozzi and Spano, 2011). Consider, for example, the branding associated with the aforementioned Parmesan, or Roquefort and Feta cheeses, Black Forest ham, or lambic beer. In the wine industry, there is hardly a brand more valuable than "Champagne". Importantly, in addition to regional protections, many of these GI products are also recognized internationally by treaty or trade agreements.

Terroir The concept of terroir is central to the GI designation, and refers to the collection of environmental factors that contribute to product quality. Temperature, rain, elevation, soil composition, cultivar or breed, and human interventions are among the elements that account for terroir. In the past decade, researchers have proposed that the microbial community residing in foods is a major contributor to terroir, as well (Capozzi and Spano, 2011; Felder et al., 2012; Bokulich et al., 2014). This so-called microbial terroir is as specific to province and as influential to product quality as are other environmental factors. Thus, not just wine, but cheese and other fermented foods have also been suggested to possess a microbial terroir (Paxson and Helmreich, 2014). Recently, researchers examined the microbial communities from cheeses obtained from disparate regions (Wolfe et al., 2014). Even though the microbiotas were similar and reproducible, human interventions, i.e., handling of the cheese by different cheese makers, apparently account for the variations observed in these products.

Ultimately, the tools of molecular ecology can be applied to even traditionally-made fermented food products, providing a basis for understanding and improving product quality (Cocolin and Ercolini, 2015).

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ECONOMIC VALUE

For food scientists, "value-added" refers to foods to which inexpensive or "low value" starting materials are converted or processed to yield "higher value" products. Thus, corn plus extrusion, plus sugar, plus other ingredients, becomes corn flakes. Fermented foods, made primarily by the addition and manipulation of the appropriate microorganisms, are the original members of the "value-added" category. While milk is milk, one can add the relevant culture and enzymes, manipulate the curds in a particular manner, age it for just the right time, and the result may be a fine cheese that fetches a price well above the combined costs of the raw materials, labor, and other expenses. Grapes are grapes, but if they are cultivated, harvested, and crushed in a particular environment under precise conditions, and then the juice is allowed to ferment and mature under optimal conditions, then voila – the finished product will be worth far more than the original grapes. In reality, the economic value of fermented foods, especially fermented grapes, can reach dizzying heights. As noted in Chapter 11, there are wines listed on various wine-buying internet sites with price tags of more than $20,000 a bottle. In 2010, a bottle of Cheval Blanc 1947, from the Bordeaux region of France sold for over $300,000. Granted, it was an "imperial" bottle that is the equivalent of eight normal bottles. There are even some specialty vinegars (Chapter 12) that sell for over $200 for just 100 ml.

Not all fermented foods command such a high dollar value. The fermented foods market is just as competitive, and manufacturers are under the same market pressures as other segments of the food industry. For the most part, fermented foods are made from inexpensive commodities – wheat, milk, meat, grapes – and most commercial products have modest profit margins. Some products, such as non-aged cheese, are sold on commodity markets, with very tight margins. Other products have long aging periods with uncertain outcomes. The challenge in making fermented foods a profitable enterprise is perhaps best epitomized by the well-known joke about the wine business – "How do you make a million dollars in the wine industry? Easy, first you start with two million dollars."

Finally, not only is there an economic incentive for both small and large manufacturers of fermented foods, but on an industry-wide basis, fermented foods may have a substantial economic impact on a region, state or even a country. In California, for example, the wine industry was reported to contribute nearly 800,000 jobs and more than $110 billion to the US economy in 2016 (according to a Wine Institute report; www.wineinstitute.org). A similar analysis of the US beer industry (www.beerinstitute.org) reported a combined overall annual impact of more than $350 billion to the US economy in 2016.

FERMENTED FOODS IN THE TWENTY-FIRST CENTURY

Archaeological evidence has shown that for 10,000 years, humans have consumed fermented foods. As noted above, throughout human history, fermentation provided a means for producing safe, nutritious, and well-preserved foods. Even today, fermented foods are still among the most popular type of food consumed. No wonder that about one-third of all foods consumed are fermented. In the United States, beer is the most widely consumed fermented food product, followed by bread, cheese, wine, and yogurt (Table 1.3). While global statistics are incomplete, it can be estimated that alcoholic products are the most popular fermented foods in much of the world. In Asia, soy sauce production and consumption ranks at or near the top. Collectively, the sales volume of fermented foods, on a global basis, exceeds a

Table 1.3 Consumption of selected fermented foods and beverages[1].

Product	United States	International
Wine[2]	11 L	44 L (Croatia)
Beer[3]	75 L	142 L (Czech republic)
Cheese[4]	16 kg	27 kg (France)
Yogurt[5]	7 kg	36 kg (Netherlands)
Fermented meats[6]	0.3 kg	5.5 kg (Germany)
Bread[7]	25 kg	120 kg (Turkey)
Table olives[8]	0.6 kg	1 kg (Albania)
Kimchi[9]	–	35 kg (Korea)
Soy sauce[10]	1 L	6 L (Japan)

[1] Per person, per year.
[2] Wine Institute (updated 2015).
[3] Kirin Beer University Report for 2015.
[4] International Dairy Federation and USDA (data from 2014).
[5] Euromonitor 2013, AC Nielsen.
[6] Lücke and Zangerl, 2014 (data from 2008).
[7] International Association of Plant Bakers Bread Market Report (data from 2013) and the National Association of Manufacturers of Pan (Adepan), 2012.
[8] Data for 2015, International Olive Council.
[9] Data for 2015, Park, J., and H.-J. Lee, 2017. Korean J. Community Nutr. 22:145–158.
[10] Japan Federation of Soy Sauce Manufactures Cooperatives; Nakadai, 2015, and Mikio Bakke, personal communication, data for 2014.

trillion dollars. When jobs, tax revenues, and the effects on rural and agricultural communities are also considered, the overall economic and social impacts are even greater.

Fermented foods have been part of the human diet for thousands of years. As the world becomes more multicultural and cuisines and cultures continue to mix, it is highly likely that fermented foods will assume an even more important dietary and nutritional role. It may surprise younger readers, but prior to the 1940s, yogurt was unknown nearly everywhere, except Europe and the Middle East. It is now one of the most popular fermented food products consumed throughout the world. Foods such as kimchi (from Korea), miso (from Japan), fish sauce (from Thailand), and kefir (from Eastern Europe) are fast becoming part of the Western cuisine. Certainly, the desirable flavor, sensory, and nutritional attributes of traditional, as well as new-generation fermented foods, will drive much of the interest in these foods.

Consumption of these products also will likely be increased as the potential beneficial effects of fermented foods on human health become better established, scientifically and clinically. As noted above, compelling evidence now exists to indicate that red wine may reduce the risk of heart disease and that live bacteria present in cultured milk and other products may positively influence gastrointestinal health. Perhaps the most important development in the past decade has been the availability of genomic information on the microorganisms involved in food fermentations. The composition of entire microbial communities can now be quickly and inexpensively measured and described. The functional role of specific members involved in fermentations can also be assessed. Likewise, the field of metabolomics has advanced to the point where the metabolic products of fermentation, even those produced at very low levels, can be identified and quantified. Thus, it is now becoming possible to link the metabolic activities of individual or collections of microorganisms with their organoleptic contributions. These technological developments may provide opportunities for researchers to custom-produce fermented foods, not only with predictable flavor and other functional characteristics, but that also impart nutritional benefits to consumers.

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