CHAPTER 8

Nondairy Fermented Foods and Products

MEAT PRODUCTS

Fermented sausages are produced generally as dry or semidry products, although some are intermediate. Dry or Italian-type sausages contain 30–40% moisture, are generally not smoked or heat processed, and are eaten usually without cooking.[58] In their preparation, curing and seasonings are added to ground meat, followed by its stuffing into casings and incubation for varying periods of time at 80–95°F. Incubation times are shorter when starter cultures are employed. The curing mixtures include glucose as substrate for the fermenters and nitrates and/or nitrites as color stabilizers. When only nitrates are used, it is necessary for the sausage to contain bacteria that reduce nitrates to nitrites, usually micrococci present in the sausage biota or added to the mix. Following incubation, during which fermentation occurs, the products are placed in drying rooms with a relative humidity of 55–65% for periods ranging from 10 to 100 days, or, in the case of Hungarian salami, up to 6 months.[47] Genoa and Milano salamis are other examples of dry sausages.

In one study of dry sausages, the pH was found to decrease from 5.8 to 4.8 during the first 15 days of ripening and remained constant thereafter.[36] Nine different brands of commercially produced dry sausages were found by these investigators to have pH values ranging from 4.5 to 5.2, with a mean of 4.87. With respect to the changes that occur in the biota of fermenting dry sausage when starters are not used, Urbaniak and Pezacki[82] found the homofermenters to predominate overall, with *Lactobacillus. plantarum* being the most commonly isolated species. Heterofermenters such as *L. brevis* and *L. buchneri* increased during the six-day incubation period as a result of changes in pH and Eh brought about by the homofermenters.

Semidry sausages are prepared in essentially the same way as dry sausages but are subjected to less drying. They contain about 50% moisture and are finished by heating to an internal temperature of 140–154°F (60–68°C) during smoking. Cervelat, summer sausage, and Lebanon bologna are some examples of semidry sausages. "Summer sausage" refers to those traditionally of northern European origin, made during colder months, stored, aged, and then eaten during summer months. They may be dry or semidry.

Lebanon bologna is typical of a semidry sausage. This product, originally produced in the Lebanon, Pennsylvania area, is an all-beef, heavily smoked, spiced product that may be prepared by the use of

a *Pediococcus cerevisiae* starter.[19] The product is made by the addition of approximately 3% NaCl along with sugar, seasoning, and either nitrate, nitrite, or both, to raw cubed beef. The salted beef is allowed to age at refrigerator temperature for about 10 days during which time the growth of naturally occurring lactic acid bacteria or the starter organisms are encouraged and Gram negatives are inhibited. A higher level of microbial activity occurs along with some drying during the smoking step at higher temperatures. A controlled production process for this product has been studied,[52] and it consists of aging salted beef at 5°C for 10 days and smoking at 35°C with high relative humidity (RH) for 4 days. Fermentation may be carried out either by the natural biota of the meat or by the use of a commercial starter of *P. cerevisiae* or *P. acidilactici*. The amount of acidity produced in Lebanon bologna may reach 0.8–1.2%.[8,57]

The hazard of eating improperly prepared, homemade, fermented sausage was indicated by an outbreak of trichinosis: of the 50 persons who actually consumed the raw summer sausage, 23 fell ill with trichinosis.[62] The sausage was made on two different days in three batches according to a family recipe that called for smoking at cooler smoking temperatures, believed to produce a better-flavored product. All three batches of sausages contained home-raised beef. In addition, two batches eaten by victims contained pork inspected by the U.S. Department of Agriculture (USDA) in one case and home-raised pork in the other, but *Trichinella spiralis* larvae were found only in the USDA-inspected pork. This organism can be destroyed by a heat treatment that results in internal temperatures of at least 60°C or 140°F (see Chapter 29).

In the production of dry sausages, lactobacilli produce aminopeptidases that aid in the generation of amino acids from sausage proteins. The amino acids contribute to the overall flavor of dry sausages. In the case of *Lactobacillus sakei*, it produces decarboxylases that give rise to biogenic amines, and these compounds can inhibit aminopeptidases and thus reduce flavor enhancement in dry-fermented sausages (see reference 71).

Fermented sausages produced without the use of starters have been found to contain large numbers of lactobacilli such as *L. plantarum*.[20] The use of a *P. cerevisiae* starter leads to the production of a more desirable product.[19,36] In their study of commercially produced fermented sausages, Smith and Palumbo[77] found total aerobic plate counts to be in the 10^7–10^8/g range, with a predominance of lactic acid types. When starter cultures were used, the final pH of the products ranged from 4.0 to 4.5, whereas those produced without starters ranged between 4.6 and 5.0. For summer-type sausages, pH values of 4.5–4.7 have been reported for a 72-hour fermentation.[2] These investigators found that fermentation at 30°C and 37°C led to a lower final pH than at 22°C and that the final pH was directly related to the amount of lactic acid produced. The pH of fermented sausage may actually increase by 0.1 or 0.2 unit during long periods of drying due to uneven buffering produced by increases in the amounts of basic compounds.[90] The ultimate pH attained following fermentation depends on the type of sugar added. Although glucose is most widely used, sucrose has been found to be an equally effective fermentable sugar for low pH production.[1] The effect of a commercial frozen concentrate starter (*P. acidilactici*) in fermenting various sugars added to a sausage preparation is illustrated in Figure 8–1. *Lactobacillus gasseri*, when employed in a meat fermentation was shown to prevent enterotoxin formation by *Staphylococcus aureus* in a model sausage preparation.[6] This species was the most effective of five other *Lactobacillus* species.

Prior to the late 1950s, the production of fermented sausages was facilitated by either back inoculations, or a producer took the chance of the desired organisms being present in the raw materials. Until recently, the manufacture of these, as well as of many other fermented foods, has been more of an art than a science. With the advent of pure culture starters, not only has production time been shortened, but more uniform and safer products can be produced.[25] Although the use of starter cultures has been

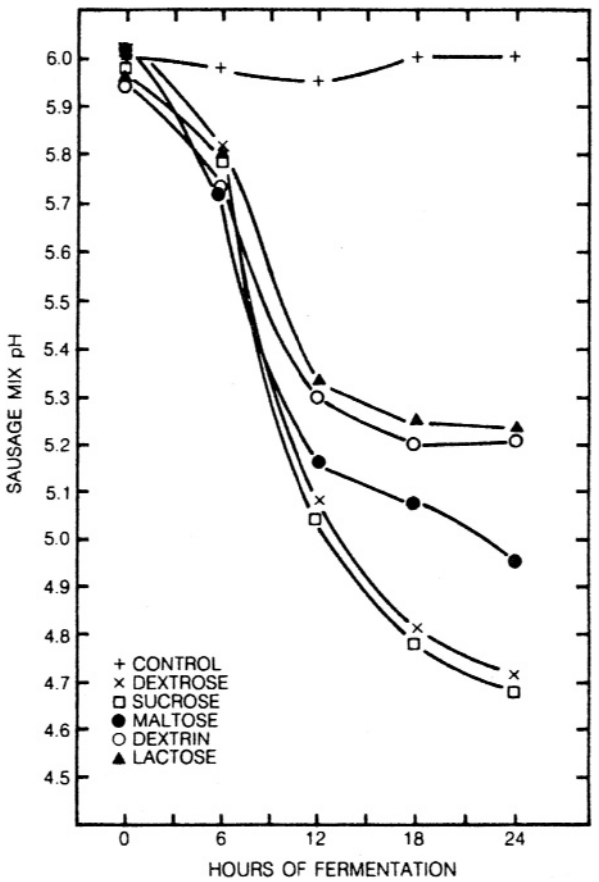


Figure 8–1 Rate of pH reduction in fermenting sausage containing 0% or 1% of various carbohydrates. *Source*: From Acton et al.[1] copyright © 1977 by Institute of Food Technologists.

in effect for many years in the dairy industry, their use in many nondairy products worldwide is a recent development with great promise. "*Micrococcus aurantiacus*" has been employed along with starters in the production of some European sausages.[47] The addition of a *Micrococcus* or a *Staphylococcus*, especially *S. carnosus*, to a lactic culture is a common practice in Europe. The nonlactic member reduces nitrates to nitrites and produces catalase that benefits the lactic culture.

Molds are known to contribute to the quality of dry European-type sausages such as Italian salami. In an extensive study of the fungi of ripened meat products, Ayres et al.[7] found nine species of penicillia and seven of aspergilli on fermented sausages and concluded that the organisms play a role in the preservation of products of this type. Fewer species of other mold genera were found. A study of the fungal biota of naturally fermented sausages in northern Italy revealed that penicillia made up 96% and aspergilli 4%.[5] The initial biota of the sausage was made up of >95% yeasts. After 2 weeks, yeasts and molds were about 50:50, but after 4–8 weeks, molds constituted >95% of the biota.[5] Fifty percent

of the mold biota was *P. nalgiovensis*. The addition of *Penicillium camemberti* and *P. nalgiovensis* during the curing of raw, dry, sausages was used in an effort to prevent the growth of mycotoxigenic house molds, and it was more successful than potassium sorbate.[11]

Country-cured hams are dry-cured hams produced in the southern United States. During the curing and ripening period of 6 months to 2 years, heavy mold growth occurs on the surfaces. Although Ayres et al.[7] noted that the presence of molds is incidental and that a satisfactory cure does not depend on their presence, it seems likely that some aspects of flavor development of these products derive from the heavy growth of such organisms, and to a lesser extent from yeasts. Heavy mold growth obviates the activities of food-poisoning and food-spoilage bacteria, and in this sense the mold biota aids in preservation. Ayres et al. found aspergilli and penicillia to be the predominant types of molds on country-cured hams.[7]

The processing of country-cured hams takes place during the early winter and consists of rubbing sugar cure into the flesh side and onto the hock end. This is followed some time later by rubbing NaCl into all parts of the ham not covered by skin. The hams are then wrapped in paper and individually placed in cotton fabric bags and left lying flat for several days between 32°C and 40°C. The hams are hung shank end down in ham houses for 6 weeks or longer and may be given a hickory smoke during this time, although smoking is not essential for a desirable product.

Italian-type country-cured hams are produced with NaCl as the only cure. Curing is carried out for about a month, followed by washing, drying, and ripening for 6–12 months or longer.[33] Although halophilic and halotolerant bacteria increase as Italian hams ripen, the biota, in general, is thought to play only a minor role.[56] In Europe, molds are critical in the production of safe and high quality products such as salami and hams (Parma from Italy and Solano from Spain). For more detailed information on meat starter cultures and formulations for fermented sausages, along with cure ingredients for country-style hams, see references 6 and 57.

FISH PRODUCTS

Fermented fishery products are rather widespread in parts of Asia where marine sources contribute more protein to the human diet than is the case in the Western world. More on fermentation can be found in Chapter 7. Only two classes of fermented seafood products are noted below–sauces and pastes.

Fish sauces are popular products in Southeast Asia, where they are known by various names such as *ngapi* (Burma), *nuoc-mam* (Cambodia and Vietnam), *nam-pla* (Laos and Thailand), *ketjap-ikan* (Indonesia), and so on. The production of some of these sauces begins with the addition of salt to uneviscerated fish at a ratio of approximately 1:3, salt to fish. The salted fish are then transferred to fermentation tanks generally constructed of concrete and built into the ground or placed in earthenware pots and buried in the ground. The tanks or pots are filled and sealed off for at least 6 months to allow the fish to liquefy. The liquid is collected, filtered, and transferred to earthenware containers and ripened in the sun for 1–3 months. The finished product is described as being clear, dark-brown in color with a distinct aroma and flavor.[70] In a study of fermenting Thai fish sauce by the latter investigators, the pH from start to finish ranged from 6.2 to 6.6 with the NaCl content around 30% over the 12-month fermentation period.[70] These parameters, along with the relatively high fermentation temperature, result in the growth of halophilic aerobic spore formers as the predominant microorganisms of these products. Lower numbers of streptococci, micrococci, and staphylococci were found, and they, along with the *Bacillus* spp., were apparently involved in the development of flavor and aroma. Some part of the liquefaction that occurs is undoubtedly due to the activities of fish proteases. Although

the temperature and pH of the fermentation are well within the growth range of a large number of undesirable organisms, the safety of products of this type is due to the 30–33% NaCl.

Fish pastes are also common in Southern Asia, but the role of fermenting microorganisms in these products appears to be minimal. Among the many other fermented fish, fish-paste, and fish-sauce products, are the following: *mam-tom* of China; *mam-ruoc* of Cambodia; *bladchan* of Indonesia; *shiokara* of Japan; *belachan* of Malaya; *bagoong* of the Philippines; *kapi*, *hoi-dong*, and *pla-mam* of Thailand; *fessik* of Africa; *nam-pla* , pla-ra, *pla-chom*, and *pla-com* of Thailand. A fermented shrimp product of Thailand is *kung-chom*.

Soy sauces are fermented condiments of various plant materials. Typically, the plant material first undergoes a fungal fermentation followed by a brine fermentation in which *Tetragenococcus* spp. are active. In Chinese soy sauce only soy beans are used, whereas in Japanese both wheat and soy beans are used. *T. halophilus*, which can tolerate 18% NaCl, is active in the brine of the soy sauces noted.[68] Another *Tetragenococcus* sp., *T. muriaticus*, has been isolated from fermented squid liver sauce.[72] This species can grow in 1–25% NaCl, and it produces histamine. Some soy sauces are made by acid hydrolysis of soy beans.

BREADS

San Francisco *sourdough* bread is similar to sourdough breads produced in various countries. Historically, the starter for sourdough breads consists of the natural biota of baker's barm (sour ferment or mother sponge, with a portion of each inoculated dough saved as starter for the next batch). The barm generally contains a mixture of yeasts and lactic acid bacteria. In the case of San Francisco sourdough bread, the yeast has been identified as *Saccharomyces exiguus* (*Candida holmii*[80] and the responsible bacteria are *Lactobacillus sanfranciscensis*, *L. fermentum*, *L. fructivorans*, some *L. brevis* strains, and *L. pontis*.[89] The key bacterium is *L. sanfranciscensis*, and it preferentially ferments maltose rather than glucose and it requires fresh yeast extractives and unsaturated fatty acids.[34] The souring is caused by acids produced by these bacteria, and the yeast is responsible for the leavening action, although some CO_2 is produced by the bacterial biota. The pH of these sourdoughs ranges from 3.8 to 4.5. Both acetic and lactic acids are produced, with the former accounting for 20–30% of the total acidity.[40] *Lactobacillus paralimentarius* is another of the sourdough bacteria.[15]

Sourdoughs are placed into three groups and each has its unique fermentation consortium. Type I sourdoughs are fermented at 20–30°C and the two primary organisms are *L. sanfranciscensis* and *L. pontis*. Type II doughs employ baker's yeast as a leavening agent, and the dominant lactics are *L. pontis*, *L. panis*, and from one to nine other lactobacilli. Type III doughs are dried products of traditional fermentations (see reference 21). Greek wheat sourdoughs belong to Type I, and the fermentation consortium in the traditional wheat product consists of *L. sanfranciscensis*, *L. brevis*, *L. paralimentarius*, and *Weissella cibaria.*[21] Among other organisms found in some sourdough fermentations are *Candida humilis*, *Dekkera bruxellensis*, *Saccharomyces cerevisiae*, and *Saccharomyces uvarum*.

Idli is a fermented bread-type product common in southern India. It is made from rice and black gram mung (urd beans). These two ingredients are soaked in water separately for 3–10 hours and then ground in varying proportions, mixed, and allowed to ferment overnight. The fermented and raised product is cooked by steaming and served hot. It is said to resemble a steamed, sourdough bread.[78] During the fermentation, the initial pH of around 6.0 falls to values of 4.3–5.3. In a particular study, a batter pH of 4.70 after a 20-hour fermentation was associated with 2.5% lactic acid, based on dry grain weight.[46] In their studies of idli, Steinkraus et al.[78] found total bacterial counts of 10^8–10^9/g after 20–22 hours of fermentation. Most of the organisms consisted of Gram-positive cocci or short

rods, with *L. mesenteroides* being the single most abundant species, followed by *E. faecalis*. The leavening action of idli is produced by *L. mesenteroides*. This is the only known instance of a lactic acid bacterium having this role in a naturally fermented bread.[46] The latter authors confirmed the work of others in finding the urd beans to be a more important source of lactic acid bacteria than rice. *L. mesenteroides* reaches its peak at around 24 hours, with *E. faecalis* becoming active only after about 20 hours. Other probable fermenters include *L. delbrueckii* subsp. *delbrueckii*, *L. fermentum*, and *Bacillus* spp.[69] Only after idli has fermented for more than 30 hours does *P. cerevisiae* become active. The product is not fermented generally beyond 24 hours because maximum leavening action occurs at this time and decreases with longer incubations. When idli is allowed to ferment longer, more acidity is produced. It has been found that total acidity (expressed as grams of lactic acid per gram of dry grains) increased from 2.71% after 24 hours to 3.70% after 71 hours, whereas the pH decreased from 4.55 to 4.10 over the same period.[65] (A review of idli fermentation has been made by Reddy et al.[65])

PLANT PRODUCTS

Sauerkraut

Sauerkraut is a fermentation product of fresh cabbage. The starter for sauerkraut production is usually the normal mixed biota of cabbage. The addition of 2.25–2.5% salt restricts the activities of Gram-negative bacteria, while the lactic acid rods and cocci are favored. *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, and *Leuconostoc fallax* are the three most dominant lactics in sauerkraut production, with the two *Leuconostoc* spp. having the shorter generation time and the shorter life span. The activities of the cocci usually cease when acid content increases to 0.7–1.0%. The final stages of kraut production are effected by *L. plantarum* and *L. brevis. P. cerevisiae* and *E. faecalis* may also contribute to product development. The final total acidity is generally 1.6–1.8%, with lactic acid at 1.0–1.3% and pH in the range of 3.1 to 3.7.

The microbial spoilage of sauerkraut generally falls into the following categories: soft kraut, slimy kraut, rotted kraut, and pink kraut. Soft kraut results when bacteria that normally do not initiate growth until the late stages of kraut production actually grow earlier. Slimy kraut is caused by the rapid growth of *Lactobacillus cucumeris* and *L. plantarum*, especially at elevated temperatures. Rotted sauerkraut may be caused by bacteria, molds, and/or yeasts, whereas pink kraut is caused by the surface growth of *Torula* spp., especially *T. glutinis*. Due to the high acidity, finished kraut is generally spoiled by molds growing on the surface. The growth of these organisms effects an increase in pH to levels where a large number of bacteria can grow which were previously inhibited by conditions of high acidity.

Olives

Olives to be fermented (Spanish, Greek, or Sicilian) are done so by the natural biota of green olives, which consists of a variety of bacteria, yeasts, and molds. The olive fermentation is quite similar to that of sauerkraut except that it is slower, involves a lye treatment, and may require the addition of starters. The lactic acid bacteria become prominent during the intermediate stage of fermentation. *L. mesenteroides* and *P. cerevisiae* are the first lactics to become prominent, and these are followed by lactobacilli, with *L. plantarum* and *L. brevis* being the most important.[87]

The olive fermentation is preceded by a treatment of green olives with 1.6 to 2.0% lye, depending on the type of olive, at 21–25°C for 4–7 hours for the purpose of removing some of the bitter principal. Following the complete removal of lye by soaking and washing, the green olives are placed in oak barrels and brined so as to maintain a constant 28°–30° salinometer level. Inoculation with *L. plantarum* may be necessary because of destruction of organisms during the lye treatment. The fermentation may take as long as 6–10 months, and the final product has a pH of 3.8–4.0 following up to 1% lactic acid production.

Among the types of microbial spoilage that olives undergo, one of the most characteristic is *zapatera spoilage*. This condition, which sometimes occurs in brined olives, is characterized by a malodorous fermentation. The odor is due apparently to propionic acid, which is produced by certain species of *Propionibacterium.*[61] In addition to propionic acid, formic, butyric, succinic, isobutyric, *n*-valeric, and cyclohexacarbolic acids, as well as methanol, ethanol, 2-butanol, and *n*-butanol may be produced (see reference 31).

A softening condition of Spanish-type green olives has been found to be caused by the yeasts *Rhodotorula glutinis* var. *glutinis*, *R. minuta* var. *minuta*, and *R. rubra.*[88] All of these organisms produce polygalacturonases, which effect olive tissue softening. Under appropriate cultural contions, the organisms were shown to produce pectin methyl esterase as well as polygalacturonase. A sloughing type of spoilage of California ripe olives was shown by Patel and Vaughn[55] to be caused by *Cellulomonas flavigena*. This organism showed high cellulolytic activity, which was enhanced by the growth of other organisms such as *Xanthomonas*, *Enterobacter*, and *Escherichia* spp.

The production of some biogenic amines has been shown to occur primarily in Spanish-style green olives during the brining process.[32] The amines found were cadaverine, histamine, tyramine, tryptamine, and putrescine with the latter being in highest concentration after 3 months of brining. The others were found in samples taken after 12 months.[32]

Pickles

Pickles are fermentation products of fresh cucumbers, and as is the case for sauerkraut production, the starter culture generally consists of the normal mixed biota of cucumbers. In the natural production of pickles, the following lactic acid bacteria are involved in the process in order of increasing prevalence: *L. mesenteroides*, *E. faecalis*, *P. cerevisiae*, *L. brevis*, and *L. plantarum*. Of these, the pediococci and *L. plantarum* are the most involved, with *L. brevis* being undesirable because of its capacity to produce gas. *L. plantarum* is the most essential species in pickle production, as it is for sauerkraut.

In the production of pickles, selected cucumbers are placed in wooden brine tanks with initial brine strengths as low as 5% NaCl (20° salinometer). Brine strength is increased gradually during the course of the 6- to 9-week fermentation, until it reaches around 60° salinometer (15.9% NaCl). In addition to exerting an inhibitory effect on the undesirable Gram-negative bacteria, the salt extracts water and water-soluble constituents from the cucumbers, such as sugars, which are converted by the lactic acid bacteria to lactic acid. The product that results is a salt-stock pickle from which pickles such as sour, mixed sour, chowchow, and so forth may be made.

The general technique of producing brine-cured pickles has been in use for many years, but it often leads to serious economic loss because of pickle spoilage from such conditions as bloaters, softness, off-colors, and so on. The controlled fermentation of cucumbers brined in bulk has been achieved, and this process not only reduces economic losses of the type noted, but leads to a more uniform product over a shorter period of time. The controlled fermentation method employs a chlorinated brine of 25° salinometer, acidification with acetic acid, the addition of sodium acetate, and inoculation with

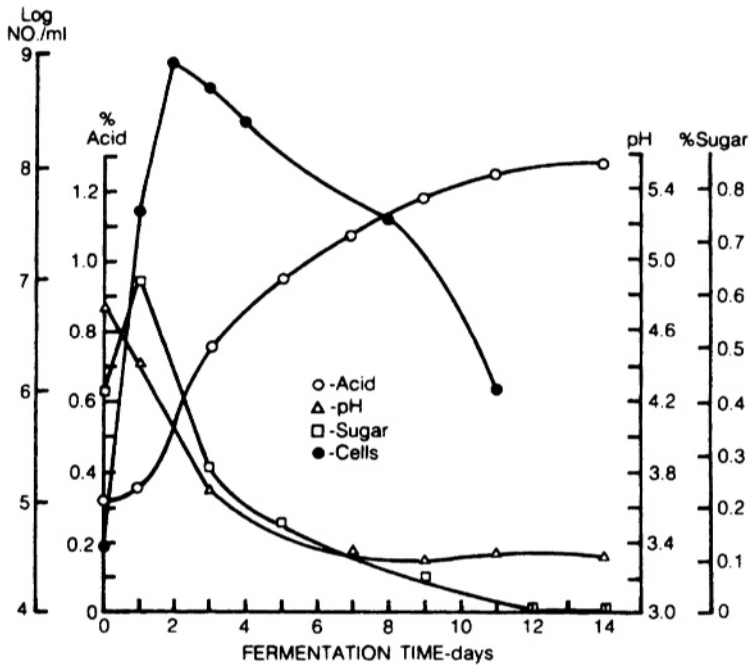


Figure 8–2 Controlled fermentation of cucumbers brined in bulk. Equilibrated brine strength during fermentation, 6.4% NaCl; incubation temperature = 27°C. *Source*: From Etchells et al.[24]; copyright © 1975 by Academic Press.

P. cerevisiae and *L. plantarum*, or the latter alone. The course of the 10- to 14-day fermentation is represented in Figure 8–2.

With a final pH of ~4.0, pickles undergo spoilage by bacteria and molds. Pickle blackening may be caused by *Bacillus nigrificans*, which produces a dark water-soluble pigment. *Enterobacter* spp., lactobacilli, and pediococci have been implicated as causes of a condition known as "bloaters," produced by gas formation within the individual pickles. Pickle softening is caused by pectolytic organisms of the genera *Bacillus*, *Fusarium*, *Penicillium*, *Phoma*, *Cladosporium*, *Alternaria*, *Mucor*, *Aspergillus*, and others. The actual softening of pickles may be caused by any one or several of these or related organisms. Pickle softening results from the production of pectinases, which break down the cementlike substance in the wall of the product.

BEER, ALE, WINES, CIDER, AND DISTILLED SPIRITS

Beer and Ale

Beer and ale are malt beverages produced by brewing. An essential step in the brewing process is the fermentation of carbohydrates to ethanol. Because most of the carbohydrates in grains used for brewing exist as starches, and because the fermenting yeasts do not produce amylases to degrade the

starch, a necessary part of beer brewing includes a step whereby malt or other exogenous sources of amylase are provided for the hydrolysis of starches to sugars. The malt is first prepared by allowing barley grains to germinate. This serves as a source of amylases (fungal amylases may be used also). Both β- and α-amylases are involved, with the latter acting to liquefy starch and the former to increase sugar formation. In brief, the brewing process begins with the mixing of malt, malt adjuncts, hops, and water. Malt adjuncts include certain grains, grain products, sugars, and other carbohydrate products to serve as fermentable substances. Hops are added as sources of pyrogallol and catechol tannins, resins, essential oils, and other constituents for the purpose of precipitating unstable proteins during the boiling of wort and to provide for biological stability, bitterness, and aroma. The process by which the malt and malt adjuncts are dissolved and heated and the starches digested is called mashing. The soluble part of the mashed materials is called *wort* (compare with *koji*). In some breweries, lactobacilli are introduced into the mash to lower the pH of wort through lactic acid production. The species generally used for this purpose is *L. delbrueckii* subsp. *delbrueckii*.[39]

Wort and hops are mixed and boiled for 1.5–2.5 hours for the purpose of enzyme inactivation, extraction of soluble hop substances, precipitation of coagulable proteins, concentration, and sterilization. Following the boiling of wort and hops, the wort is separated, cooled, and fermented. The fermentation of the sugar-laden wort is carried out by the inoculation of *S. cerevisiae*. Ale results from the activities of top-fermenting yeasts, which depress the pH to around 3.8, whereas bottom-fermenting yeasts (*S.* "*carlsbergensis*" strains) give rise to lager and other beers with pH values of 4.1–4.2. A top fermentation is complete in 5–7 days; a bottom fermentation requires 7–12 days. The freshly fermented product is aged and finished by the addition of CO_2 to a final content of 0.45–0.52% before it is ready for commerce. The pasteurization of beer at 140°F (60°C) or higher, may be carried out for the purpose of destroying spoilage organisms. When lactic acid bacteria are present in beers, the lactobacilli are found more commonly in top fermentations, whereas pediococci are found in bottom fermentations.[39]

The industrial spoilage of beers and ales is commonly referred to as beer infections. This condition is caused by yeasts and bacteria. The spoilage patterns of beers and ales may be classified into four groups: ropiness, sarcinae sickness, sourness, and turbidity. *Ropiness* is a condition in which the liquid becomes characteristically viscous and pours as an "oily" stream. It is caused by *Acetobacter*, *Lactobacillus*, *Pediococcus cerevisiae*, and *Gluconobacter oxydans* (formerly *Acetomonas*).[26,64,91] *Sarcinae sickness* is caused by *P. cerevisiae*, which produces a honeylike odor. This characteristic odor is the result of diacetyl production by the spoilage organism in combination with the normal odor of beer. *Sourness* in beers is caused by *Acetobacter* spp. These organisms are capable of oxidizing ethanol to acetic acid, and the sourness that results is referable to increased levels of acetic acid. *Turbidity* and off-odors in beers are caused by *Zymomonas anaerobia* (formerly *Achromobacter anaerobium*) and several yeasts such as *Saccharomyces* spp. The growth of bacteria is possible in beers because of a normal pH range of 4–5 and a good content of utilizable nutrients.

Some Gram-negative obligately anaerobic bacteria have been isolated from spoiled beers and pitching yeasts, and the six species are represented by four genera:

Megasphaera cerevisiae	*Selenomonas lacticifex*
Pectinatus cerevisiiphilus	*Zymophilus paucivorans*
P. frisingensis	*Z. raffinosivorans*

All but *M. cerevisiae* produce acetic and propionic acids, and *S. lacticifex* also produces lactate.[73] Although *M. cerevisiae* produces negligible to minor amounts of acetic and propionic acids, it produces large quantities of isovaleric acid in addition to H_2S.[23] *P. cerevisiiphilus* was the first of these to be

associated with spoiled beer when it was isolated from turbid and off-flavor beer in 1978.[43] It has since been found in breweries not only in the United States, but also in several European countries and Japan. Among the unusual features of these organisms as beer spoilers is their Gram reaction and obligately anaerobic status. Previously, the typical beer spoilers were regarded as being either lactic acid bacteria or yeasts. *Megasphaera* and *Selenomonas* are best known as members of the rumen biota. In addition to the organic acids noted above, *Pectinatus* spp. also produce H_2S and acetoin. The beers most susceptible to their growth are those that contain <4.4% alcohol.

With respect to spoiled packaged beer, one of the major contaminants found is *Saccharomyces diastaticus*, which is able to utilize dextrins that normal brewers' yeasts (*S*. "*carlsbergensis*" and *S. cerevisiae*) cannot.[39] Pediococci, *Flavobacterium proteus* (formerly *Obesumbacterium*), and *Brettanomyces* are sometimes found in spoiled beer.

Wines

Wines are normal alcoholic fermentations of sound grapes followed by aging. A large number of other fruits such as peaches, pears, and so forth may be fermented for wines, but in these instances the wine is named by the fruit, such as peach wine, pear wine, and the like. Because fruits already contain fermentable sugars, the use of exogenous sources of amylases is not necessary, as it is when grains are used for beers or whiskeys. Wine making begins with the selection of suitable grapes, which are crushed and then treated with a sulfite such as potassium metabisulfite to retard the growth of acetic acid bacteria, wild yeasts, and molds. The pressed juice, called *must*, is inoculated with a suitable wine strain of *S*. "*ellipsoideus.*" The fermentation is allowed to continue for 3–5 days at temperatures between 70°F and 90°F (21°C and 32°C), and good yeast strains may produce up to 14–18% ethanol.[58] Following fermentation, the wine is racked–that is, drawn off from the lees or sediment, which contains potassium bitartrate (cream of tartar). The clearing and development of flavor occur during the storage and aging process. Red wines are made by initially fermenting the crushed grape must "on the skins" during which pigment is extracted into the juice; white wines are prepared generally from the juice of white grapes. Champagne, a sparkling wine made by a secondary fermentation of wine, is produced by adding sugar, citric acid, and a champagne yeast starter to bottles of a previously prepared, selected table wine. The bottles are corked, clamped, and stored horizontally at suitable temperatures for about 6 months. They are then removed, agitated, and aged for an additional period of up to 4 years. The final sedimentation of yeast cells and tartrates is accelerated by reducing the temperature of the wine to around 25°C and holding for 1–2 weeks. Clarification of the champagne is brought about by working the sediment down the bottle onto the cork over a period of 2–6 weeks by frequent rotation of the bottle. Finally, the sediment is frozen and disgorged upon removal of the cork.

Table wines undergo spoilage by bacteria and yeasts with *Candida valida* being the most important yeast. Growth of this organism occurs at the surface of wines, where a thin film is formed. The organisms attack alcohol and other constituents from this layer and create an appearance that is sometimes referred to as *wine flowers*. Among the bacteria that cause wine spoilage are members of the genus *Acetobacter*, which oxidize alcohol to acetic acid (produce vinegar). The most serious and the most common disease of table wines is referred to as *tourne disease*. Tourne disease is caused by a facultative anaerobe or an anaerobe that utilizes sugars and seems to prefer conditions of low alcohol content. This type of spoilage is characterized by an increased volatile acidity, a silky type of cloudiness, and later in the course of spoilage, a "mousy" odor and taste caused by *Brettanomyces* spp. commonly found in Bordeaux wines.

Malo-lactic fermentation is a spoilage condition of great importance in wines. Malic and tartaric acids are two of the predominant organic acids in grape must and wine, and in the malolactic

fermentation, contaminating bacteria degrade malic acid to lactic acid and CO_2:

$$\text{L(}-\text{)-Malic acid} \xrightarrow{\text{“malo-lactic enzyme”}} \text{L(}+\text{)-Lactic acid} + CO_2$$

L-Malic acid may be decarboxylated also to yield pyruvic acid.[41] The effect of these conversions is to reduce the acid content and affect flavor. The malo-lactic fermentation (which may also occur in cider) can be carried out by many lactic acid bacteria, including leuconostocs, pediococci, and lactobacilli.[63] Although the function of the malolactic fermentation to the fermenting organism is not well understood, it has been shown that *Oenococcus oeni* is actually stimulated by the process.[60] The decomposition in wines of tartaric acid is undesirable also, and this process can be achieved by some strains of *Lactobacillus plantarum* in the following general manner:

$$\text{Tartaric acid} \longrightarrow \text{Lactic acid} + \text{Acetic acid} + CO_2$$

The effect is to reduce the acidity of wine. Unlike the malo-lactic fermentation, few lactic acid bacteria break down tartaric acid. Sluggish or stuck alcoholic fermentations of wines is caused by *Lactobacillus kunkeei* and *L. nagelii*.

The bacterium *Oenococcus oeni* is an acidophile that can grow in grape must and wine at pH 3.5–3.8, and actually prefers an initial growth pH of 4.8.[22] It can grow in the presence of 10% ethanol but requires special growth factors found in grape or tomato juice. For a review, see reference 44.

Cider

Cider, in the United States, is a product that represents a mild fermentation of apple juice by naturally occurring yeasts. In making apple cider, the fruits are selected, washed, and ground into a pulp. The pulp “cheeses” are pressed to release the juice. The juice is strained and placed in a storage tank, where sedimentation of particulate matter occurs, usually for 12–36 hours or several days if the temperature is kept at 40°F (4.4°C) or below. The clarified juice is cider. If pasteurization is desired, this is accomplished by heating at 170°F (76.7°C) for 10 minutes. The chemical preservative most often used is sodium sorbate at a level of 0.10%. Preservation may also be effected by chilling or freezing. The finished product contains small amounts of ethanol in addition to acetaldehyde. The holding of nonpasteurized or unpreserved cider at suitable temperatures invariably leads to the development of cider vinegar, which indicates the presence of acetic acid bacteria in these products. The pathway employed by acetic acid bacteria is summarized in Chapter 7, Figure 7–1F, G.

In their study of the ecology of the acetic acid bacteria in cider manufacture, Passmore and Carr[53] found six species of *Acetobacter* and noted that those that display a preference for sugars tend to be found early in the cider process, whereas those that are more acid tolerant and capable of oxidizing alcohols appear after the yeasts have converted most of the sugars to ethanol. *Zymomonas* spp., Gram-negative bacteria that ferment glucose to ethanol, have been isolated from ciders, but they are presumed to be present in low numbers. *Saccharobacter fermentatus* is similar to *Zymomonas* in that it ferments glucose to ethanol and CO_2.[92] It was isolated from agave leaf juice, but its presence and possible role in spoiled ciders have yet to be determined. *Zymobacter palmae* is an ethanol fermentor isolated from palm sap.[51] It produces ethanol from mannitol.[37]

Following several illness outbreaks traced to apple cider, the microbial load of finished ciders produced in the state of Iowa was investigated, and in apples from 21 producers, APC ranged from 15 to $> 1.1 \times 10^5$/ml; coliforms from <1 to 2.1×10^3; and *E. coli* was <10/ml.[18] The fate of *E. coli* 0157:H7 in fermenting apple cider was studied by Semanchek and Golden[75] who found that 6.4 $\log_{10}$ cfu/ml of this organism were reduced to <0.5 $\log_{10}$ cfu/ml after 3 days at 20°C, while in nonfermenting

ciders, the initial number was reduced only to 2.9 $\log_{10}$ cfu/ml after 10 days at 20°C. Although the pH of the two products was not significantly different, ethanol concentration in the fermenting products reached 6.1% after 10 days at 20°C. The investigators believed the combination of low pH and the ethanol were important in the reduction of the pathogen. In another study of *E. coli* 0157:H7 survival in four apple varieties (Golden Delicious, Red Delicious, Rome, and Winesap), the organism behaved essentially the same in each variety.[28]

Distilled Spirits

Distilled spirits are alcoholic products that result from the distillation of yeast fermentations of grain, grain products, molasses, or fruit or fruit products. Whiskeys, gin, vodka, rum, cordials, and liqueurs are examples of distilled spirits. Although the process for producing most products of these types is quite similar to that for beers, the content of alcohol in the final products is considerably higher than for beers. *Rye* and *bourbon* are examples of whiskeys. In the former, rye and rye malt, or rye and barley malt, are used in different ratios, but at least 51% rye is required by law. Bourbon is made from corn, barley malt, or wheat malt, and usually another grain in different proportions, but at least 51% corn is required by law. A sour wort is maintained to keep down undesirable organisms, the souring occurring naturally or by the addition of acid. The mash is generally soured by inoculating with a homolactic such as *L. delbrueckii* subsp. *delbrueckii*, which is capable of lowering the pH to around 3.8 in 6–10 hours.[58] The malt enzymes (diastases) convert the starches of the cooked grains to dextrins and sugars, and upon completion of diastatic action and lactic acid production, the mash is heated to destroy all microorganisms. It is then cooled to 75–80°F (24–27°C) and pitched (inoculated) with a suitable strain of *S. cerevisiae* for the production of ethanol. Upon completion of fermentation, the liquid is distilled to recover the alcohol and other volatiles, and these are handled and stored under special conditions relative to the type of product being made. *Scotch whisky* is made primarily from barley and is produced from barley malt dried in kilns over peat fires. *Rum* is produced from the distillate of fermented sugar cane or molasses. *Brandy* is a product prepared by distilling grape or other fruit wines.

Palm wine or Nigerian palm wine is an alcoholic beverage consumed throughout the tropics and is produced by a natural fermentation of palm sap. The sap is sweet and dirty brown in color, and it contains 10–12% sugar, mainly sucrose. The fermentation process results in the sap becoming milky-white in appearance due to the presence of large numbers of fermenting bacteria and yeasts. This product is unique in that the microorganisms are alive when the wine is consumed. The fermentation has been reviewed and studied by Faparusi and Bassir[26] and Okafor[49] who found the following genera of bacteria to be the most predominant in finished products: *Micrococcus*, *Leuconostoc*, "*Streptococcus*," *Lactobacillus*, and *Acetobacter*. The predominant yeasts found were *Saccharomyces* and *Candida* spp., with the former being the more common.[48] The fermentation occurs over a 36- to 48-hour period, during which the pH of sap falls from 7.0 or 7.2 to <4.5. Fermentation products consist of organic acids in addition to ethanol. During the early phases of fermentation, *Serratia* and *Enterobacter* spp. increase in numbers, followed by lactobacilli and leuconostocs. After a 48-hour fermentation, *Acetobacter* spp. begin to appear.[26,50]

Sake is an alcoholic beverage commonly produced in Japan. The substrate is the starch from steamed rice, and its hydrolysis to sugars is carried out by *A. oryzae* to yield the koji. Fermentation is carried out by *Saccharomyces sake* over periods of 30–40 days, resulting in a product containing 12–15% alcohol and around 0.3% lactic acid.[58] The latter is produced by hetero- and homolactic lactobacilli. Other fermented products of this type are further summarized in Table 8–1.

Table 8–1 Summary of a Variety of Fermented Products

Products	*Substrate*	*Fermenters*	*Where Found*
Nonbeverage plant products			
Bongkrek	Coconut presscake	*Rhizopus oligosporus*	Indonesia
Cocoa beans	Cacao fruit (pods)	*Candida krusei* (*Issatchenkia orientalis*), *Geotrichum* spp.	Africa, South America
Coffee beans	Coffee cherries	*Erwinia dissolvens*, *Saccharomyces* spp.	Brazil, Congo, Hawaii, India
Gari	Cassava	"*Corynebacterium manihot*," *Geotrichum* spp.	West Africa
Kenkey	Corn	*Aspergillus* spp., *Penicillium* spp., lactobacilli, yeasts	Ghana, Nigeria
Kimchi	Cabbage and other vegetables	Lactic acid bacteria	Korea
Miso	Soybeans	*Aspergillus oryzae*, *Zygosaccharomyces rouxii*	Japan
Ogi	Corn	*L. plantarum*, *L. lactis*, *Zygosaccharomyces rouxii*	Nigeria
Olives	Green olives	*L. mesenteroides*, *L. plantarum*	Worldwide
Ontjom*	Peanut presscake	*Neurospora sitophila*	Indonesia
Peujeum	Cassava	Molds	Indonesia
Pickles	Cucumbers	*P. cerevisiae*, *L. plantarum*	Worldwide
Poi	Taro roots	Lactics	Hawaii
Sauerkraut	Cabbage	*L. mesenteroides*, *L. plantarum*	Worldwide
Soy sauce (shoyu)	Soybeans	*A. oryzae*; or *A. soyae*; *Z. rouxii*, *L. delbrueckii*	Japan
Sufu	Soybeans	*Mucor* spp.	China and Taiwan
Tao-si	Soybeans	*A. oryzae*	Philippines
Tempeh	Soybeans	*Rhizopus oligosporus*; *R. oryzae*	Indonesia, New Guinea, Surinam
Beverages and related products			
Arrack	Rice	Yeasts, bacteria	Far East
Beer and ale	Cereal wort	*Saccharomyces cerevisiae*	Worldwide
Binuburan	Rice	Yeasts	Philippines
Bourbon whiskey	Corn, rye	*S. cerevisiae*	United States
Bouza beer	Wheat grains	Yeasts	Egypt
Cider	Apples; others	*Saccharomyces* spp.	Worldwide
Kaffir beer	Kaffircorn	Yeasts, molds, lactics	Nyasaland (Malawi)
Magon	Corn	*Lactobacillus* spp.	Bantus of South Africa
Mezcal	Century plant	Yeasts	Mexico
Oo	Rice	Yeasts	Thailand
Pulque†	Agave juice	Yeasts and lactics	Mexico, U.S. Southwest
Sake	Rice	*Saccharomyces sake* (*S. cerevisiae*)	Japan

continues

Table 8–1 continued

Products	*Substrate*	*Fermenters*	*Where Found*
Scotch whiskey	Barley	*S. cerevisiae*	Scotland
Teekwass	Tea leaves	*Acetobacter xylinum*, *Schizosaccharomyces pombe*	
Thumba	Millet	*Endomycopsis fibuliges*	West Bengal, India
Tibi	Dried figs; raisins	*Betabacterium vermiforme*, *Saccharomyces intermedium*	
Vodka	Potatoes	Yeasts	Russia, others
Wines	Grapes, other fruits	*Saccharomyces* "*ellipsoideus*" strains	Worldwide
Vinegar	Cider, wine	*Acetobacter* spp.	Worldwide
Palm wine	Palm sap	*Acetobacter* spp., lactics, yeasts	Nigeria
Breads			
Idli	Rice and bean flour	*Leuconostoc mesenteroides*	Southern India
Rolls, cakes, etc.	Wheat flours	*S. cerevisiae*	Worldwide
San Francisco sourdough bread	Wheat flour	*S. exiguus*, *L. sanfranciscensis*	Northern California
Sour pumpernickel	Wheat flour	*L. mesenteroides*	Switzerland, other areas
Vodka	Potatoes	Yeasts	Russia, Scandinavia

**N. sitophila* is used to make red ontjom; *R. oligosporus* for white ontjom.
†Distilled to produce tequila.

Kombucha is a home-prepared tea that is produced by fermenting sweetened black tea with a mixed culture of bacteria and yeasts. It is consumed mainly in China, Russia, and Germany. It has been consumed for over 2000 years, and is believed (at least by some) to provide a number of health benefits. The tea fermentation occurs at room temperature for 7 to 10 days, and the finished product contains organic acids, tea components, vitamins, minerals, and is slightly carbonated.[35] The most predominant organism in the fermentaion mat is *Acetobacter xylinum*. Among the large number of yeasts found are species of *Brettanomyces*, *Candida*, *Pichia*, *Saccharomyces*, and *Zygosaccharomyces*.

MISCELLANEOUS PRODUCTS

Coffee beans, which develop as berries or cherries in their natural state, have an outer pulpy and mucilaginous envelope that must be removed before the beans can be dried and roasted. The wet method of removal of this layer seems to produce the most desirable product, and it consists of depulping and demucilaging followed by drying. Whereas depulping is done mechanically, demucilaging is accomplished by natural fermentation. The mucilage layer is composed largely of pectic substances,[29] and pectinolytic microorganisms are important in their removal. *Erwinia dissolvens* has been found to be the most important bacterium during the demucilaging fermentation in Hawaiian[30]

and Congo coffee cherries,[83] although Pederson and Breed[59] indicated that the fermentation of coffee berries from Mexico and Colombia was carried out by typical lactic acid bacteria (leuconostocs and lactobacilli). Agate and Bhat[3] in their study of coffee cherries from the Mysore state of India found that the following pectinolytic yeasts predominated and played important roles in the loosening and removal of the mucilaginous layers: *Saccharomyces marxianus*, *S. bayanus, S.* "*ellipsoideus*," and S*chizosaccharomyces* spp. Molds are common on green coffee beans, and in one study, 99.1% of products from 31 countries contained these organisms, generally on the surface.[45] Seven species of aspergilli dominated the biota, with *A. ochraceus* being the most frequently recovered from beans before surface disinfection, followed by *A. niger* and species of the *A. glaucus* group. The toxigenic molds, *A. flavus* and *A. versicolor*, were found, as were *P. cyclopium*, *P. citrinum*, and *P. expansum*, but the penicillia were less frequently found than the aspergilli.[45] Microorganisms do not contribute to the development of flavor and aroma in coffee beans as they do in cocoa beans.

Cocoa beans (actually cacao beans–cocoa is the powder and chocolate is the manufactured product), from which chocolate is derived, are obtained from the fruits or pods of the cacao plant in parts of Africa, Asia, and South America. The beans are extracted from the fruits and fermented in piles, boxes, or tanks for 2–12 days, depending on the type and size of beans. During the fermentation, high temperatures (45–50°C) and large quantities of liquid develop. Following sun or air drying, during which the water content is reduced to less than 7.5%, the beans are roasted to develop the characteristic flavor and aroma of chocolate. The fermentation occurs in two phases. In the first, sugars from the acidic pulp (about pH 3.6) are converted to alcohol. The second phase consists of the alcohol being oxidized to acetic acid. In a study of Brazilian cocoa beans by Camargo et al.,[16] the biota on the first day of fermentation at 21°C consisted of yeasts. On the third day, the temperature had risen to 49°C, and the yeast count had decreased to no more than 10% of the total biota. Over the seven-day fermentation, the pH increased from 3.9 to 7.1. The cessation of yeast and bacterial activity around the third day is due in part to the unfavorable temperature, lack of fermentable sugars, and increase in alcohol. Although some decrease in acetic acid bacteria occurs because of high temperature, not all of these organisms are destroyed. The importance of lactic acid in the overall process was shown earlier.[54,66]

In one study, the cocoa fermentation was carried out with a defined microbial cocktail consisting of only five organisms rather than the 50 or so that have been isolated from natural fermentations.[74] The five consisted of *Saccharomyces cerevisiae* var. *chevalieri*, *Lactobacillus plantarum*, *L. lactis*, *Acetobacter aceti*, and *Gluconobacter oxydans* subsp. *suboxydans*. The defined inoculum led to a product highly similar to that produced by natural fermentation. The key roles for the yeasts involved elevating pH from about 3.5 to 4.2, breaking down citric acid in pulp, producing ethanol, producing organic acids (oxalic, succinic, malic, etc.) that destroy bean cotyledons, producing volatile substances that may play a role in chocolate flavor, and reducing viscosity of pulp. *S. cerevisiae* was the most important organism in the above activities.

Although yeasts play important roles in producing alcohol in cocoa bean fermentation, their presence appears even more essential to the development of the final, desirable, chocolate flavor of roasted beans. Levanon and Rossetini[42] found that the endoenzymes released by autolyzing yeasts are responsible for the development of chocolate precursor compounds. The acetic acid apparently makes the bean tegument permeable to the yeast enzymes. It has been shown that chocolate aroma occurs only after cocoa beans are roasted and that the roasting of unfermented beans does not produce the characteristic aroma. Reducing sugars and free amino acids are in some way involved in the final chocolate aroma development.[67] For an extensive review, see reference 81.

Soy sauce or shoyu is produced in a two-stage manner. The first stage, the koji (analogous to malting in the brewing industry), consists of inoculating either soybeans or a mixture of beans and wheat flour

with *A. oryzae* or *A. soyae* and allowing them to stand for 3 days. This results in the production of large amounts of fermentable sugars, peptides, and amino acids. The second stage, the moromi, consists of adding the fungal-covered product to around 18% NaCl and incubating at room temperatures for at least a year. The liquid obtained at this time is soy sauce. During the incubation of the moromi, lactic acid bacteria, *L. delbrueckii* subsp. *delbruckeii* in particular, and yeasts such as *Zygosaccharomyces rouxii* carry out an anaerobic fermentation of the koji hydrolysate. Pure cultures of *A. oryzae* for the koji and *L. delbrueckii* subsp. *delbrueckii* and *Z. rouxii* for the moromi stages have been shown to produce good quality soy sauce.[93]

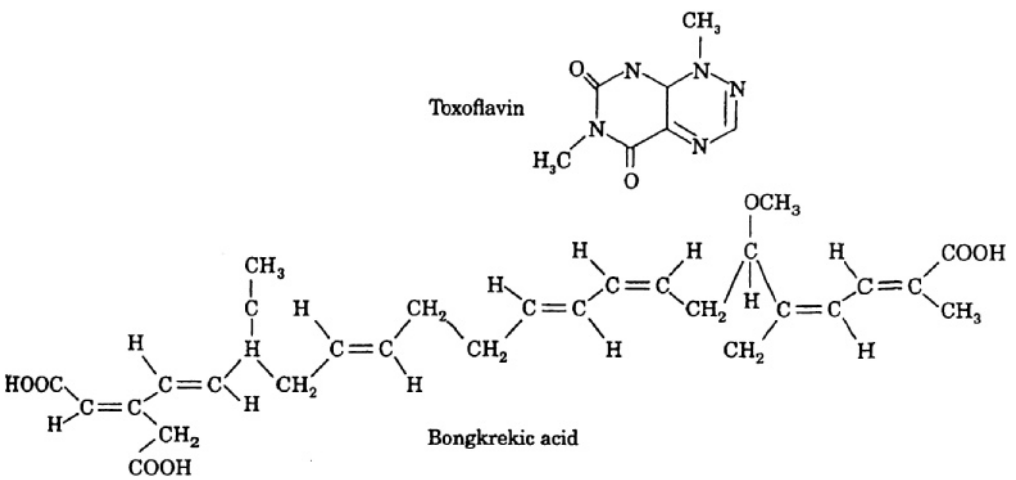
Tempeh is a fermented soybean product. Although there are many variations in its production, the general principle of the Indonesian method for tempeh consists of soaking soybeans overnight in order to remove the seed coats or hulls. Once seed coats are removed, the beans are cooked in boiling water for about 30 minutes and spread on a bamboo tray to cool and surface dry. Small pieces of tempeh from a previous fermentation are incorporated as starter, followed by wrapping with banana leaves. The wrapped packages are kept at room temperature for 1 or 2 days during which mold growth occurs and binds the beans together as a cake—the tempeh. An excellent product can be made by storing in perforated plastic bags and tubes with fermentations completed in 24 hours at 31°C.[27] The desirable organism in the fermentation is *Rhizopus oligosporus*, especially for wheat tempeh. Good soybean tempeh can be made with *R. oryzae* or *R. arrhizus*. During the fermentation, the pH of soybeans rises from around 5.0 to values as high as 7.5.

Miso, a fermented soybean product common in Japan, is prepared by mixing or grinding steamed or cooked soybeans with koji and salt and allowing fermentation to take place usually over a 4- to 12-month period. White or sweet miso may be fermented for only a week, whereas the higher-quality dark brown product (*mame*) may ferment for 2 years. In Israel, Ilany-Feigenbaum et al.[38] prepared miso-type products by using defatted soybean flakes instead of whole soybeans and fermenting for around 3 months. The koji for these products was made by growing *A. oryzae* on corn, wheat, barley, millet or oats, potatoes, sugar beets, or bananas, and the investigators found that the miso-type products compared favorably to Japanese-prepared miso. Because of the possibility that *A. oryzae* may produce toxic substances, koji was prepared by fermenting rice with *Rhizopus oligosporus* at 25°C for 90 days; the product was found to be an acceptable alternative to *A. oryzae* as a koji fungus.[76]

Ogi is a staple cereal of the Yorubas of Nigeria, and it is often the first food given to babies at weaning. It is produced generally by soaking corn grains in warm water for 2–3 days followed by wet-milling and sieving through a screen mesh. The sieved material is allowed to sediment and ferment and is marketed as wet cakes wrapped in leaves. Various food dishes are made from the fermented cakes or the ogi.[10] During the steeping of corn, *Corynebacterium* spp. become prominent and appear to be responsible for the diastatic action necessary for the growth of yeasts and lactic acid bacteria.[4] Along with the corynebacteria, *S. cerevisiae* and *L. plantarum* have been found to be prominent in the traditional ogi fermentation, as are *Cephalosporium*, *Fusarium*, *Aspergillus*, and *Penicillium*. Most of the acid produced is lactic, which depresses the pH of desirable products to around 3.8. The corynebacteria develop early, and their activities cease after the first day; those of the lactobacilli and yeasts continue beyond the first day of fermentation. A newer process for making ogi has been developed, tested, and found to produce a product of better quality than the traditional process.[9] By this newer method, corn is dry-milled into whole corn and dehulled corn flour. Upon the addition of water, the mixture is cooked, cooled, and then inoculated with a mixed culture (starter) of *L. plantarum*, *L. lactis*, and *Z. rouxii*. The inoculated preparation is incubated at 32°C for 28 hours, during which time the pH of the corn drops from 6.1 to 3.8. This process eliminates the need for starch-hydrolyzing bacteria. In addition to the shorter fermentation time, there is also less chance for faulty fermentations.

Gari is a staple food of West Africa prepared from the root of the cassava plant. Cassava roots contain cyanogenic glucosides, *linamarin* and *lotaustralin*, which make them poisonous if eaten fresh or raw. The roots can be detoxified by the addition of linamarase, which acts on both.[13] In practice the roots are rendered safe by a fermentation during which the toxic glucoside decomposes with the liberation of gaseous hydrocyanic acid. In the home preparation of gari, the outer peel and the thick cortex of the cassava roots are removed, followed by grinding or grating the remainder. The pulp is pressed to remove the remaining juice and placed in bags for 3 or 4 days to allow fermentation to occur.[17] The organisms most responsible for the product include *L. plantarum*, *E. faecium*, and *Leuconostoc mesenteroides.*[13] The fermented product is cooked by frying.

Bongkrek is an example of a fermented food product that in the past has led to a large number of deaths. Bongkrek or semaji is a coconut presscake product of central Indonesia, and it is the homemade product that may become toxic. The safe products fermented by *R. oligosporus* are finished cakes covered with and penetrated by the white fungus. In order to obtain the desirable fungal growth, it appears to be essential that conditions permit good growth within the first 1 or 2 days of incubation. If, however, bacterial growth is favored during this time and if the bacterium *Burkholderia cocovenenans* is present, it grows and produces two toxic substances–toxoflavin and bongkrekic acid.[84,85,86,94] Both of these compounds show antifungal and antibacterial activity, are toxic for humans and animals, and are heat stable. Production of both is favored by growth of the organisms on coconut (toxoflavin can be produced in complex culture media). The structural formulas of the two antibiotics–toxoflavin, which acts as an electron carrier, and bongkrekic acid, which inhibits oxidative phosphorylation in mitochondria–follow:



Bongkrekic acid has been shown to be cidal to all 17 molds studied by Subik and Behun[79] by preventing spore germination and mycelial outgrowth. The growth of *B. cocovenenans* in the preparation of bongkrek is not favored if the acidity of starting materials is kept at or below pH 5.5.[84] It has been shown that 2% NaCl in combination with acetic acid to produce a pH of 4.5 will prevent the formation of the bongkrek toxin in tempeh.[14]

A fermented cornmeal product that is prepared in parts of China has been the cause of food poisoning by strains *B. cocovenenans*. The product is prepared by soaking corn in water at room temperature for 2–4 weeks, washing in water, and grinding the wet corn into flour for various uses. The toxic organisms apparently grow in the moist product during its storage at room temperature. The responsible

organism produced both bongkrekic acid and toxoflavin, as do the strains of *B. cocovenenans* in bongkrek.

Ontjom (oncom) is a somewhat similar but more popular fermented product of Indonesia made from peanut presscake, the material that remains after oil has been extracted from peanuts. The presscake is soaked in water for about 24 hours, steamed, and pressed into molds. The molds are covered with banana leaves and inoculated with *Neurospora sitophila* or *R. oligosporus*. The product is ready for consumption 1 or 2 days later. A more detailed description of ontjom fermentation and the nutritive value of this product has been provided by Beuchat.[12]

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