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33 Fermented Vegetables

HISTORICAL PERSPECTIVE

The wide variety of fermented foods can be classified by the products of the fermentation, such as alcohol (beer, wine); organic acids, including lactic acid and acetic acid (vegetables, dairy); carbon dioxide (bread); and amino acids or peptides from protein (fish fermentations and others) (35, 56, 96, 97). Food fermentation is one of the earliest technologies developed by humans. Littoral foragers in Asia during the primitive pottery age (8000 to 3000 B.C.) are believed to have fermented vegetables prior to the development of crop-based agriculture (57). Dairy fermentations in the Middle East likely followed the domestication of cattle around this time. It is likely that the first product of fermentation to be discovered was alcohol from fermented fruits. More sophisticated fermentation skills using cereals to make alcohol were developed around 4000 B.C., with beer produced in Egypt and rice wine in northeast Asia (56). Early written references to fermentation technology in Asia are found in the historic Chinese book of poems *Shijing* (1100 to 600 B.C.), which celebrates "the thousand wines of Yao," a reference to a kingdom in China from 2300 B.C.

It is believed that cucumbers were first fermented around 2000 B.C. in the Middle East. Early written records of cucumber pickles come from paper fragment remains of a play (*The Taxiarchs*) by the Greek writer Eupolis (429–412 B.C.), and pickles are also mentioned several times in the Christian Bible. Korean-style fermented cabbage, kimchi, is thought to have originated in the primitive pottery age from the natural fermentation of withered vegetables stored in seawater (56). Early references to kimchi include the Korean poem "Gapoyugyeong in Donggugisanggukjip" by Yi Kyu-Bo (1168-1241 A.D.). European-style sauerkraut is thought to have originated in China, and the technology may have been brought to Europe during the Mongol invasion of central Europe in the 13th century. Today, industrial vegetable fermentation is carried out on a massive scale. In the United States, companies producing cucumber pickles can have at one location as many as 1,000 fermentation tanks of 40,000-liter capacity, totaling 40 million liters.

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VEGETABLE FERMENTATION OVERVIEW

The primary retail fermented vegetable products produced in the United States and Europe are cucumber pickles, olives, and sauerkraut. In Asia, a variety of fermented vegetable products are available, including pickles and fermented cabbage, notably kimchi in South Korea. The word "pickle" by itself usually refers to a pickled cucumber. The current market for pickled vegetables (fermented and acidified) in the United States is roughly $2 billion. The retail market for cucumber pickles, however, is dominated by acidified, pasteurized, and refrigerated products which are not fermented. In addition to cucumbers, a number of nonfermented pickled vegetable products, mainly acidified peppers, are also popular in the retail market (35). Commercial sale of hamburger dill pickle slices to the food service industry makes up most of the U.S. market for fermented cucumber pickles. Other cucumber pickles include deli-style, half-sour cucumber pickles, which are partially fermented prior to consumption (28). Fermented cabbage, i.e., sauerkraut, was introduced in the United States by immigrants from Germany and other European countries. Although the popularity of pickles and sauerkraut in Europe continues today, consumption has declined in the United States. Commercial production of kimchi in South Korea is increasing, due to the purchase of commercially prepared kimchi by people living in urban areas. Fermented olives are produced primarily in southern Spain, which has the world's largest export market for table olives (31). Examples of acid-fermented vegetables produced in different regions of the world are listed in Table 33.1 (55).

Reports on the microbiology and biochemistry of vegetable fermentations first appeared in the scientific literature in the early 1900s. Early research on the "lactic bacilli" present in fermenting vegetables was done by E. B. Fred at the University of Wisconsin (92, 93). Carl Pederson, at Cornell University, studied sauerkraut fermentation from the 1930s to early 1970s. He reported on various aspects of the subject, which culminated in a comprehensive review article (86). J. L. Etchells and R. N. Costilow published extensively in the field of pickled vegetables. Included in these studies were the development of pasteurization methods (30, 49), investigations of the yeasts that are responsible for spoilage of cucumber pickle products (26, 29), and a preservation-prediction chart to describe the storage stability of sweet pickles based on salt and sugar concentrations (2). H. P. Fleming and coworkers, along with Costilow, developed the purging technology that is now commonly used in commercial cucumber fermentations (17, 33). Other important developments include methods for controlled fermentations (6, 27), an understanding of the role of the malolactic enzyme of lactobacilli in the production of carbon dioxide during cucumber fermentation (75), the development of a *Lactobacillus plantarum* strain that lacks the ability to carry out the malolactic reaction (19), and the use of calcium to improve the texture of pickled vegetables (73, 74).

The fermentation process for vegetables can result in nutritious foods that may be stored for extended periods, 1 year or more, without refrigeration. Prior to fermentation, fresh fruits and vegetables harbor a variety of microorganisms, including aerobic spoilage microflora such as *Pseudomonas*, *Erwinia*, and *Enterobacter* species, as well as yeasts and molds (83). The cell populations for these bacteria, which spoil the vegetables if allowed to grow, range from 10^4 to 10^6 CFU/g. Brining vegetables for fermentation results in the production by lactic acid bacteria (LAB) of organic acids and a variety of antimicrobial compounds (11, 23). LAB are initially present on fresh vegetables in lower numbers, 10^2 to 10^3 CFU/g, compared with other mesophilic microorganisms. During fermentation, diffusion of organic acids into the brine, and the low pH that results, influences microbial growth across

Table 33.1 Examples of acid-fermented vegetables produced in different regions of the world

Product name	Country	Major ingredients	Microorganisms	Usage
Sauerkraut	Germany	Cabbage, salt	*Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*	Salad, side dish
Kimchi	Korea	Korean cabbage, radish, various vegetables, salt	*L. mesenteroides*, *Lb. brevis*, *Lb. plantarum*	Salad, side dish
Dhamuoi	Vietnam	Cabbage, various vegetables	*L. mesenteroides*, *Lb. plantarum*	Salad, side dish
Dakguadong	Thailand	Mustard leaf, salt	*Lb. plantarum*	Salad, side dish
Burong mustasa	Philippines	Mustard	*Lb. brevis*, *Pediococcus cerevisiae*	Salad, side dish

the surface of the vegetable material. As sugars diffuse from the vegetables into the brine, the LAB grow rapidly. Because the LAB are more acid resistant than the spoilage microbiota, they dominate brined vegetable fermentations. In the absence of brine, spoilage microbiota are able to grow, unhindered by the metabolic end products of the LAB. The growth of spoilage bacteria results in deterioration of the vegetable material, due to the elaboration of degradative enzymes (proteases, lipases, amylases, nucleases, and others).

Leuconostoc mesenteroides and related LAB, including *Weissella* and other *Leuconostoc* species, are important in the initiation of the fermentation of many vegetables, i.e., cabbages, beets, turnips, cauliflower, green beans, sliced green tomatoes, olives, and sugar beet silages. *L. mesenteroides* grows more rapidly than most other LAB over a range of temperatures (5 to 35°C) and NaCl brine concentrations (0 to 5%). *L. mesenteroides* carries out a heterolactic fermentation of vegetable sugars, typically fructose and glucose, and produces carbon dioxide and acids (lactic and acetic). The production of acid quickly lowers the pH, thereby inhibiting the development of undesirable microorganisms and the activity of their enzymes. The carbon dioxide produced replaces air and provides anaerobic conditions favorable for the stabilization of ascorbic acid and the natural color of the vegetables. The high acidity produced by *L. mesenteroides* and other LAB subsequently inhibits the growth of these heterofermentative microbes in favor of more acid-tolerant homofermentative LAB. Homofermentative species, such as *Lb. plantarum*, produce exclusively lactic acid from the remaining sugars. In most vegetable fermentations, *Lb. plantarum* will eventually outcompete other LAB because of its superior acid tolerance (67). In brined vegetables with initial NaCl concentrations of 6 to 12%, such as pickled cucumbers and olives, *Lactobacillus* species, primarily *Lb. plantarum*, dominate the fermentation from the start, with little or no evidence of heterolactic species present. There are several excellent reviews of cucumber pickle, sauerkraut, kimchi, and olive fermentations (14, 31, 35, 37, 102). Here we present a summary of the commercial production practices, microbiology, and biochemistry of cucumber, cabbage, and olive fermentations.

CUCUMBER FERMENTATIONS

In the United States, commercial cucumber (*Cucumis sativus*) fermentations are commonly done in 30,000- to 40,000-liter, open-top, fiberglass tanks that are located out-of-doors so the brine surface is exposed to sunlight. The UV radiation in sunlight is relied upon to kill aerobic surface yeasts that can metabolize lactic acid produced by the fermentation. Cucumbers are covered with salt brine and held below the brine surface with wooden headboards. Fermentations are typically carried out in brine equilibrated at about 6% NaCl. Calcium chloride (0.1 to 0.4%, equilibrated) is added to the cover brine to maintain the firm, crisp texture of the fermented cucumbers during fermentation and storage (35). Cucumber fermentations typically undergo a homolactic acid fermentation, which does not result in production of carbon dioxide from sugars (glucose and fructose, about 1% each). However, carbon dioxide may be generated from the respiration of cucumbers and by the decarboxylation of malate during the initiation of fermentation (75). Some LAB have an inducible malolactic enzyme that converts malate to lactate and carbon dioxide. The malolactic enzyme reaction occurs intracellularly and results in the uptake of a proton, thereby increasing the internal cell pH. While it is a desirable reaction in winemaking (used for de-acidifying wines), malolactic fermentation in cucumbers may result in the creation of "bloaters," or fermented cucumbers with undesired internal gas pockets, decreasing the production yield. In an effort to prevent bloater formation, cucumber fermentations are purged with air to remove excess carbon dioxide from the tank (17). Potassium sorbate (~0.04%) or 0.16% acetic acid can be used as processing aids to limit the growth of aerobic microorganisms in air-purged cucumber fermentations, particularly molds and yeasts (42). Excessive growth of aerobic microorganisms may also be controlled by stopping air purging for several hours each day. After fermentation by *Lb. plantarum* and related LAB, cucumbers may be stored in the fermentation tanks for 1 year or more. In areas where the temperature decreases to below 0°C, the concentration of NaCl is often increased during storage to as high as 10 to 15% to minimize freezing damage and maintain the desirable texture of fermented cucumbers. Prior to sale, cucumbers are washed to remove excess salt and then packed in a variety of containers (plastic pails, pouches, jars) with an appropriate cover liquor. The cover liquor typically contains acetic acid and spices in addition to residual lactic acid. Fermented pickles may be pasteurized, but large containers are not heat treated. Further microbial growth is prevented by the organic acids, low pH, and lack of fermentable sugars.

Most commercial cucumber fermentations rely upon growth of the LAB that are naturally present on the surface of cucumbers. However, some processors choose to use starter cultures to enhance product consistency. A commercial starter culture of *Lb. plantarum* that does

not decarboxylate malic acid (and hence does not contribute to the formation of bloaters) has been developed (19) and tested to determine the ability of the culture to grow in cucumber fermentations (6). A method for the preparation of a starter culture that meets kosher requirements is also available to processors (88).

The initial pH of brined cucumbers is about 6.5. In practice, commercial fermentations may use recycled brine, or acetic acid may be added to brine solutions. The addition of acid can help remove excess CO_2 and also help select for the growth of LAB, so the initial pH of commercial fermentations can vary substantially. In addition to lactic acid, the LAB produce a variety of metabolites, e.g., bacteriocins, peroxides, and peptides, that can be inhibitory to other bacteria (23). At the end of the fermentation, there may be 1.5% lactic acid, a pH of 3.1 to 3.5, and little or no residual sugar. In this anaerobic, acidic, high-salt environment lacking sugar, very few microorganisms are capable of growing or surviving, effectively preserving the cucumbers. Occasionally, fermented cucumbers undergo an undesired secondary fermentation during storage, which is characterized by an increase in pH, the disappearance of lactic acid, and the formation of propionic and butyric acids. The incidence of fermented cucumber spoilage tends to increase at the beginning of the spring season, when the ambient temperature increases. The increase in the concentration of propionic and butyric acids causes a malodorous spoilage (32). The microbial ecology of this type of spoilage is currently not well defined but may include the growth of spore-forming bacteria such as clostridia when the pH increases above 4.6.

Fermented cucumbers have salt concentrations (6% or greater) that are too high to be used in products for direct human consumption. Hence, prior to packing and distribution, the salt is reduced to about 2% by washing with water. This results in a waste stream with high concentrations of salt plus a high biological oxygen demand from the organic components that are present in the brine and that diffuse out of the cucumbers during the desalting process. To reduce the environmental impact, cucumber brine from the desalting process is usually recycled and may be used in subsequent fermentations (72). Prior to recycling, fermentation brines may be processed to remove "softening enzymes," primarily polygalacturonases (10), which can degrade pectic substances in the cucumber cell wall and soften the fruits.

CABBAGE FERMENTATIONS

Commercial production of fermented cabbage consists primarily of kimchi, made from the Chinese cabbage, *Brassica rapa*, in Korea, and sauerkraut, from *Brassica oleracea*, in the United States and Europe. Sauerkraut fermentations are done in bulk fermentation tanks that may contain 100 tons or more of shredded or chopped cabbage. The cabbage for these fermentations consists of large heads, typically 3.6 to 4.5 kg. The outer leaves and woody core of the cabbage are removed prior to shredding or chopping. This core of the cabbage contains sucrose, which can lead to dextran formation by *L. mesenteroides*, resulting in a slimy or stringy texture. The shredded cabbage is dry salted as it is conveyed to fermentation tanks. This process results in a brine forming in the fermentation tanks, with an NaCl concentration of about 2 to 3%. During the first 24 to 48 h, carbon dioxide gas and lactic and acetic acids are produced. Some of the excess brine formed from the salted cabbage may be removed from the tanks during the first week of fermentation. Cabbage typically contains 4 to 5% sugar, consisting of about 2.5% glucose and 2% fructose (38).

The initial heterolactic stage of the fermentation results in production of both lactic and acetic acids. The volatile acetic acid makes an important contribution to the flavor and aroma of the final product. Heterofermentative microorganisms also use fructose as an electron acceptor, converting it to mannitol (71). After about 1 week of fermentation, the heterofermentative LAB, which may grow to 9 log CFU/ml or greater, die off. They are replaced by the more acid-tolerant homofermentative microorganisms. This biphasic pattern of growth and death can be seen by plating total LAB using MRS agar, with anaerobic growth at 30°C (35, 36). High-quality sauerkraut can be produced without a starter culture if the equilibrated NaCl concentration is adjusted to 2% and the temperature is maintained at 18°C (85). The fermentation end products present after both stages of the fermentation can include mannitol and acetic acid (about 1% each) and lactic acid, which may exceed 2%, because sugar is in excess and does not limit the extent of fermentation. For most manufacturers in the United States, sauerkraut may be stored for up to 1 year in fermentation tanks until it is processed for food service or retail sale. While bulk storage is economical, the products may become very sour as lactic acid accumulates. European manufacturers typically package sauerkraut at the end of the heterolactic fermentation stage (about 1 week after the beginning of fermentation) to produce a product with mild acid flavor (35). Spices, wines, and other ingredients may be added to the sauerkraut to augment flavor.

The traditional composition of the microbiota present in sauerkraut fermentations was described by Pederson and Albury (86). The report, written at the

end of Pederson's career after 40 years of research, described two heterolactic species, *L. mesenteroides* and *Lactobacillus brevis*, and two homolactic species, *Pediococcus cerevisiae* and *Lb. plantarum*, as the primary bacteria present in the fermentation. In these early studies, microbial ecology data were obtained primarily by microscopic observation of stained cells and biochemical tests using isolated cultures. The advent of molecular techniques allowed for a more detailed examination. Organisms that were morphologically similar and possessed similar biochemical pathways were determined to be genetically different. LAB such as *Leuconostoc citreum*, *Leuconostoc argentinum*, *Leuconostoc fallax*, *Lactobacillus paraplantarum*, *Lactobacillus coryniformis*, and *Weissella* spp. were identified in commercial sauerkraut fermentations by analysis of the 16S rRNA sequences from hundreds of commercial isolates obtained from four fermentations (90). Surprisingly, only a few isolates of *Lb. brevis* and *Pediococcus* spp., which were considered to be two of the four principal microorganisms present in sauerkraut, were isolated in the study. Substantial variation between fermentations was observed. Heterolactic *Weissella* predominated in the early stage of one fermentation, not the *Leuconostoc* species (90). The biochemical changes, for these fermentations were very similar, and the differences in microbiota did not affect the resulting quality of the sauerkraut.

Kimchi fermentation is microbiologically similar to sauerkraut fermentation, although the ingredients, flavor, and preparation methods differ. Chinese cabbage and Asian radishes are popular primary ingredients in kimchi fermentations. For cabbage kimchi (known as baechu kimchi), the fresh cabbage is cut in half lengthwise or quartered and initially soaked in brine of 5 to 10% NaCl to wilt the cabbage. The cabbage is then washed and drained. An aqueous paste of ground red pepper is prepared and mixed in with the cabbage leaves. Small amounts of additional ingredients, such as garlic, ginger, and jeotgal, a highly salted (20% NaCl) anchovy product (63), are usually included along with additional vegetable material, such as green onion. After the cabbage and other ingredients are packed into containers (jars, pouches), the final salt concentration is between 3 and 6%. In rural areas, kimchi was traditionally packed into earthen jars and buried in the soil to allow constant temperatures for fermentation and a supply of vegetables through the winter. In urban South Korea today, kimchi is prepared commercially or by individuals using household "kimchi refrigerators." These are small, programmable-temperature refrigerators that provide an initial 18°C fermentation period of a few days, followed by very cold refrigeration (1 to 2°C). This procedure allows the initial heterolactic stage of fermentation to occur but delays the onset of the homolactic stage of fermentation, keeping kimchi from becoming too sour. Optimum taste is attained when the pH and acidity reach approximately 4.0 to 4.5 and 0.5 to 0.6%, respectively.

The vitamin B content increases during sauerkraut and kimchi fermentations, and vitamin C and A are preserved (35, 56). Many Koreans prefer a lightly fermented, carbonated kimchi, characteristic of the initial, heterolactic stage of fermentation. In addition to cabbage or baechu kimchi, there are many other types of kimchi, depending on the type of vegetables used in the fermentation and the ingredients added. These include white kimchi, which contains no red pepper. Commercially prepared refrigerated kimchi, either factory packaged in pouches (containing a CO_2 adsorbent) or freshly made in grocery stores, represents a rapidly growing market in South Korea.

As described above for sauerkraut, the traditional kimchi fermentation has a biphasic heterofermentative and then homofermentative pattern of LAB succession, with a few prominent species (*L. mesenteroides*, *P. cerevisiae*, *Lb. brevis*, and *Lb. plantarum*) (56, 79). More recent studies of kimchi fermentation, including DNA-based, culture-independent methods, have revealed a complex microbiota as described above. A variety of *Weissella*, *Leuconostoc*, *Pediococcus*, and *Lactobacillus* species, as well as yeasts and *Archaea*, have been identified. The *Archaea* are likely found in kimchi due to the presence of extreme halophiles present in jeotgal. Molecular methods used in these studies include microarrays (82), denaturing gradient gel electrophoresis (12), and 16S sequencing (15, 84).

OLIVE FERMENTATIONS

Like cucumber and cabbage fermentations, olive fermentation practices are based on traditional methods that have been modified for large-scale commercial production. There are several methods used for processing olives (31). The principal types of products include green table olives, natural black olives in brine, and canned ripe black olives. Green table olives are treated with lye (NaOH) and then washed prior to being brined and fermented. Following fermentation, they may be pitted and stuffed before sale. Natural black olives are prepared by a slow fermentation without lye or further treatment. Ripe black olives are prepared by darkening olives through oxidation in an alkali followed by washing and canning.

The preparation of green table olives, commonly sold stuffed with pimento or a variety of other materials, involves treating olives with 1 to 3% NaOH prior

to fermentation. The addition of a strong base serves multiple purposes. The NaOH treatment helps reduce the natural bitterness of the fruit, due to the degradation of oleuropein (40), and reduces the antimicrobial activity of the phenolic components of olives (76). The NaOH treatment also makes the skin of the olive more permeable, aiding sugar diffusion during fermentation. After NaOH treatment, the olives are washed to remove excess alkali and then brined in 10% NaCl, so the salt equilibrates with the fruit and results in a final concentration of around 5 to 6% for fermentation. The initial pH of the fermentation can be above 7 depending on how much washing was done after the NaOH treatment. As a consequence, the initial microflora during fermentation can include a variety of gram-positive bacilli (*Bacillus* species) and gram-negative enteric bacteria (*Enterobacter*, *Citrobacter*, *Klebsiella*, and *Escherichia*). As organic acids accumulate and the pH decreases below 6, the LAB, principally *Lb. plantarum*, dominate the fermentation to the exclusion of the other gram-positive and gram-negative microbes. Yeast species may also be present (*Candida*, *Pichia*, *Saccharomyces*, and others) (31, 43) and contribute desirable flavor characteristics to the brined olives. As with cucumber fermentation, some purging with air may be done to remove excess CO_2 and prevent gas pockets that may form in the fruit. However, this can lead to growth of oxidative yeasts, which consume lactic acid and result in elevated pH and spoilage problems.

Natural black olives are also prepared by fermentation but do not receive an NaOH treatment prior to brining. They are picked in a ripened state and have a black color as well as a softer texture than green table olives. Fermentation is a much slower process in black olives because of the lack of NaOH treatment. Antimicrobial phenolic compounds diffuse into the brine, which slows fermentation, and diffusion of sugars is also reduced compared with the NaOH-treated green olives. As a consequence, the fermentation may take months to complete. Commercial "ripe black olives" are also prepared from green or semiripened olives that have been brined without an initial NaOH treatment. Following storage in brine for up to 1 year, the olives are subjected to one or more vigorous oxidation treatments with pressurized air in the presence of 1 to 2% NaOH. This treatment blackens the olives, which are then washed with water to remove NaOH and bring the pH down to around 7 or less. The olives are then canned in a 1 to 3% NaCl brine and processed in a retort to sterilize the fruit. Sterilization is needed for these black olives to prevent botulism, because the pH is significantly above 4.6. Because the color of olives blackened by oxidation is not stable, iron-containing compounds, such as iron gluconate or iron lactate, can be added to help stabilize the black color (31).

Problems associated with the commercial fermentation and processing of olives include disposal of waste NaCl brines and NaOH solutions, as well as malodorous spoilage fermentations. As with other vegetable fermentations, waste NaCl brine disposal can cause environmental problems if the scale of the brining operation is large. As a consequence, fermentation brines can be recycled, but this may result in the accumulation of off-flavors and contribute to spoilage problems. For olive processing, sodium hydroxide solutions are also reused, although they must eventually be disposed of. To reduce disposal problems with NaOH, green table olives may have limited washing, resulting in an initial pH that may be high enough to result in excessive growth of enteric bacteria during the early stage of fermentation. Vaughn and coworkers (89, 102) characterized *Bacillus* and *Clostridium* species that cause excess gas production and malodorous (known as zapatera) fermentation. Propionic acid bacteria may also be involved in spoilage fermentations (43).

BACTERIOPHAGES IN VEGETABLE FERMENTATIONS

Bacteriophages that infect LAB were first identified in 1935 (103) in dairy fermentations. Bacteriophages were not investigated in vegetable fermentations (kimchi and sauerkraut) until recently (105–107). Dairy fermentations are typically carried out with pasteurized milk and a single starter culture or cocktail of a few cultures. If the dairy starter culture(s) fails to ferment milk due to bacteriophage infection, the result may be a costly spoilage problem. Vegetable fermentations, however, do not typically use starter cultures, but if phages are present and inhibit one strain of bacteria, other (resistant) strains of indigenous LAB will grow instead. It is possible, however, that bacteriophages have an impact on microbial succession. Since the initial reports mentioned above, over 100 bacteriophages have been isolated and characterized from cucumber and cabbage fermentations (1, 60, 61, 81, 104). In one large study of commercial sauerkraut fermentations (61), more than 40 phages and LAB were characterized to determine host range. Isolates included phages from both the early (heterolactic) and late (homolactic) stages. Lytic phages active against *Lb. plantarum* were isolated for up to 60 days after the start of fermentation, when the pH was below 4.0. Interestingly, the host-range data revealed that some phages were capable of attacking more than one

species. Genome sequence analyses have been done for phages from both cucumber and sauerkraut fermentations (58, 59). Genomic analysis of a sauerkraut phage active against *L. mesenteroides* has revealed a similar pattern of genome organization to sequenced dairy phages, but phage protein sequences had little similarity to dairy phages (58). The impact of phages on fermentation ecology remains unclear, and this is an area ripe for further research.

BIOCHEMISTRY OF VEGETABLE FERMENTATIONS

Fermentation is by definition an anaerobic process. During the fermentation of cucumbers, cabbage, and olives, glucose and fructose are converted to lactic acid, acetic acid, ethanol, and CO_2 by LAB and yeasts. The primary pathway for homofermentative LAB involves the breakdown of one six-carbon sugar (glucose) to give two three-carbon lactic acid molecules. Heterofermentative organisms use a more complex metabolism. Glucose is initially converted to CO_2 and a five-carbon sugar phosphate, which is further degraded to lactic acid and a two-carbon compound, ethanol or acetic acid. The details of these metabolic pathways have been previously reported (44). Here we shall focus on the biochemical aspects of vegetable fermentation that relate to product quality.

There is continuing research interest in fermentation and storage of vegetables, particularly cucumbers, with reduced salt. Chloride waste from vegetable fermentations could be greatly reduced if the salt required for fermentation and storage could be reduced sufficiently to eliminate the need for a desalting step prior to conversion into final products. The relationship between salt type and concentration has been investigated. Lu et al. (62) studied the effects of replacement of NaCl with different anions and cations on the sugar fermentation in cucumber juice. Interestingly, fructose was determined to be the preferred sugar for *Lb. plantarum*, as more fructose than glucose was fermented in almost every experiment. Sugar utilization decreased as cation or anion concentrations increased with the addition of different salts (62).

Many of the volatile components in cucumbers fermented with *Lb. plantarum* in 2% NaCl were identified by Zhou and McFeeters (108). Thirty-seven volatile compounds were identified, although for most there was little change as a result of fermentation. The most notable effect of fermentation on cucumber volatiles was the inhibition of production of (E, Z)-2,6-nonadienal and 2-nonenal, the two most important odor impact compounds in fresh cucumbers. Marsili and Miller (65) identified *trans*- and *cis*-4-hexenoic acid as the most potent odorants that define the characteristic brine aroma of cucumbers fermented commercially in about 6% NaCl. Zhou et al. (109) exposed fermented cucumber slurries with 2% NaCl to oxygen and observed nonenzymatic formation of hexanal plus a series of *trans* unsaturated aldehydes with five to eight carbon atoms that correlated with the development of oxidized odor intensity of the fermented cucumber tissue. Calcium disodium EDTA at a concentration of at least 100 μg/ml protects nonfermented pickles against lipid oxidation and bleaching of pigments in the presence of light (10). However, there was some reduction in firmness retention in pickles when this compound was used.

Retention of firmness is a key quality issue in the fermentation and storage of cucumbers and peppers. It has not been possible to assure the retention, in cucumbers fermented in reduced salt, of firmness equivalent to that which can be achieved by fermenting in 6% NaCl and storage in 6% or greater NaCl concentrations (34). However, in the past several years there has been increased understanding of cucumber tissue softening. Fleming et al. (39) determined that calcium is beneficial in maintaining the firmness of fermented cucumbers. Nonenzymatic softening of blanched, acidified cucumber tissue was found to follow first-order kinetics (73). This kinetic behavior made it possible to determine the entropy and enthalpy of activation for nonenzymatic softening of cucumbers, even though the chemical reactions responsible for softening were not known. Both the enthalpy and entropy of activation were high at pH 3.0 in the presence of 1.5 M NaCl. Calcium inhibited cucumber softening because it reduced the entropy of activation so much that the overall free energy of activation was reduced (74). This thermodynamic behavior is more like that which occurs when polymers change conformation, such as occurs in protein denaturation. It is very different from the characteristics observed for acid hydrolysis of pectin (54). Krall and McFeeters (54) found that the rate of acid hydrolysis of pectin was too slow to be the cause of nonenzymatic cucumber tissue softening. McFeeters et al. (69) determined the combined effects of temperature and salt and calcium concentrations on the rate of softening of fermented cucumber tissue. The kinetics of softening for fermented cucumbers did not follow a simple first-order reaction.

As with many other plant tissues, cucumbers contain enzymes that can degrade components of the plant cell walls, which may result in changes in texture. Pectinesterase, exopolygalacturonase, and endopolygalacturonase activities have been found in cucumbers

(4, 70, 91). Pectinesterase removes methyl groups from pectin when cucumbers are fermented or acidified (47, 68, 99). However, it has not been determined if enzymatic hydrolysis of pectin by cucumber polygalacturonases is a significant factor in the softening of fermented cucumbers. Commercially important enzymatic softening of fermented cucumbers has been associated with the introduction of fungal polygalacturonases into fermentation tanks, particularly on flowers attached to small cucumbers (3). Buescher and Burgin (9) developed a sensitive diffusion plate assay to measure polygalacturonase activity in fermentation brines and determined that an alumino-silicate clay can adsorb and remove polygalacturonase activity from fermentation brines that are recycled.

In addition to enzymes that degrade pectin, enzymes that may degrade other cell wall polysaccharides in cucumbers have been investigated to a very limited extent. Meurer and Gierschner (78) reported endo-β-1,4-gluconase activity in the cucumber that is inactivated below pH 4.8 and an endoglucomannan-splitting enzyme that retains activity down to pH 4.0 but is inactivated during fermentation. They detected six enzymes in fresh cucumbers that hydrolyze *p*-nitrophenylglycosides of α-D-galactose, β-D-galactose, β-D-glucose, β-D-xylose, α-D-mannose, and α-L-arabinose, which were inactivated during fermentation. Enzymes capable of hydrolyzing these synthetic substrates are common in plants; e.g., most of the same enzymatic activities have been found in pears (25), olives (45), and Semillon grapes (98). Maruvada (66) detected the same *p*-nitrophenylglycosidases observed by Meurer and Gierschner (78) in cucumbers. She determined that the activity of all of these enzymes declined to nondetectable levels during the first week of fermentation in 2% NaCl brines. Fleming et al. (34) combined blanching of fresh cucumbers to partially inactivate enzymes, calcium addition, and rapid fermentation with a malolactic-negative *Lb. plantarum* strain in order to ferment cucumbers and maintain desirable texture with the NaCl concentration reduced to 4%.

Olives are fermented in 5 to 6% salt concentrations, similar to cucumbers (77). As noted above, before fermentation olives are treated with NaOH to remove components that inhibit growth of fermentative bacteria but not yeasts. Fleming et al. (40) and Ruiz Barba et al. (94) suggested that oleuropein and degradation products of oleuropein are the primary components responsible for inhibition of bacteria when olives are not treated with NaOH. However, Medina et al. (76) determined that the dialdehydic form of decarboxymethyl elenolic acid and one isomer of oleoside 11-methyl ester were inhibitory to bacteria instead of oleuropein. Gordal olives, which have low levels of these phenolic compounds, could be fermented without NaOH treatment, whereas Manzanilla olives, which have high levels of phenolic compounds, could not (77).

Cabbage contains a group of glucosinolates that have received considerable attention in recent years due to potential health benefits of some of the degradation products formed during the processing of cabbage. A recent report indicates that a high intake of sauerkraut correlates with a reduced incidence of breast cancer in women (95), although others have been concerned about potentially toxic compounds derived from glucosinolates (20). Tolonen et al. (100) determined isothiocyanates and allyl cyanide to be the predominant degradation products of glucosinolates in sauerkraut fermented with and without a starter culture. Only minor amounts of goitrin, a toxic compound, and the beneficial phytochemical sulforaphane nitrile were found in sauerkraut. Tolonen et al. (101) found greater amounts of glucosinolate degradation products in sauerkraut fermented with *Lactobacillus sakei* than in sauerkraut made with starter cultures consisting of other LAB. Ciska and Pathak (16) reported that ascorbigen, a compound formed from the reaction of a degradation product of indole glucosinolate (glucobrassicin) and ascorbic acid, is the dominant glucosinolate degradation product in sauerkraut. Glucoraphinin present in fresh cabbage was converted to sulforphorane during fermentation, although sulforphorane was a relatively minor glucosinolate degradation product in fermented cabbage.

There has been some concern about the formation of biogenic amines in sauerkraut. Kalač et al. (50) reported that tyramine was formed in sauerkraut stored for up to 12 months. Only trace levels of histamine, tryptamine, and spermine were detected. These results were confirmed in a survey of vegetable products (80) in which the concentration of tyramine was found to be 4.9 mg/100 g in canned sauerkraut, virtually the same concentration reported by Kalač et al. (50). These biogenic amine levels would not represent a health risk, with the possible exception of individuals taking medications containing monamine oxidase inhibitors.

GENOMICS OF LAB IN VEGETABLE FERMENTATIONS

With the advent of whole-genome sequencing, it has become apparent that the LAB present in vegetable fermentations have relatively small genomes compared with many other mesophilic organisms. It is known that LAB are nutritionally fastidious, and rich media (MRS agar) is used for cultivation (36). Since the sequencing of

the first LAB, *Lactococcus lactis* (5), genome data have rapidly accumulated (51, 64). Phylogenetic analysis of sequenced genomes indicates that LAB evolved from ancestral anaerobic organisms with a much more extensive metabolic capacity by a process of gene loss. A phylogenetic tree for LAB has been constructed using several methods, including concatenated protein sequences predicted from the sequenced genomes (64). Because LAB grow in a nutritionally rich environment, there may be a limited need to manufacture the complex biomolecules needed for cell growth. From the available genome data it is evident that the process of gene loss may be ongoing, and hundreds of apparently inactive "pseudogenes" are present in some of the sequenced genomes (64). It is also clear that LAB have acquired a number of genes, including genes used for transport of nutrients, by horizontal gene transfer. Multilocus sequence typing for a number of *Lb. plantarum* strains has indicated that recombination events have had a prominent role in generating genetic heterogeneity in these highly commercialized bacteria (22).

About 50% of the open reading frames (ORFs) identified in the 2- to 3-Mb genomes of LAB have been assigned putative functions related to the metabolism or transport of amino acids, carbohydrates, and inorganic ions (Table 33.2). About 7% of these ORFs are dedicated to energy production by fermentation of sugars. The *Lb. plantarum* sequence contains a large

Table 33.2 Distribution of ORFs among Clusters of Orthologous Groups (COG) functional categories[a]

COG categories	*Lactobacillus brevis* ATCC 367	*Leuconostoc mesenteroides* ATCC 8293	*Pediococcus pentosaceus* ATCC 25745	*Lactobacillus plantarum* WCFS1
Energy production and conversion	6.8	6.0	6.7	8.8
Cell cycle control, cell division, chromosome partitioning	2.4	2.2	1.8	2.2
Amino acid transport and metabolism	13.6	17.3	10.4	16.1
Nucleotide transport and metabolism	7.2	8.3	8.8	7.5
Carbohydrate transport and metabolism	14.4	12.8	17.0	21.9
Coenzyme transport and metabolism	5.5	7.1	4.9	7.8
Lipid transport and metabolism	3.8	3.9	3.9	4.3
Translation, ribosomal structure, and biogenesis	17.4	15.9	17.5	15.4
Transcription	15.7	11.3	14.0	20.2
Replication, recombination, and repair	16.2	11.7	13.2	12.8
Cell wall/membrane/envelope biogenesis	10.8	9.4	11.8	13.2
Cell motility	0.2	0.1	0.3	0.3
Posttranslational modification, protein turnover, chaperones	5.4	5.5	5.2	4.9
Inorganic ion transport and metabolism	10.6	9.0	8.5	11.2
Secondary metabolite biosynthesis, transport, and catabolism	3.1	2.3	1.4	2.6
General function prediction only	32.2	26.9	28.4	38.6
Function unknown	20.5	16.4	18.1	20.0
Signal transduction mechanisms	7.8	6.2	6.5	8.4
Intracellular trafficking, secretion, and vesicular transport	2.4	2.2	2.9	2.1
Defense mechanisms	4.1	3.0	4.1	5.4
Extracellular structures	0.0	0.1	0.1	0.3
Other ORF without a category	0.0	0.0	0.0	0.0

[a]Values are in percentages.

number of putative phosphotransferase system (PTS) genes, encoding up to 25 complete transport complexes and several incomplete complexes (53). *Pediococcus pentosaceus* has a reduced number of putative PTS genes compared with *Lb. plantarum*. *L. mesenteroides* apparently contains five functional PTSs and several incomplete systems.

Predicted gene products related to pyruvic acid catabolism in *L. mesenteroides*, *Lb. plantarum*, and *P. pentosaceus* are listed in Table 33.3. Genes for lactate dehydrogenases, a malolactic enzyme, and an incomplete citric acid cycle are in all three bacterial genome sequences. The sequence data reveal that *Lb. plantarum* and *L. mesenteroides* contain more pyruvate-dissipating enzymes than *P. pentosaceus*, which may provide a metabolic advantage. This may contribute to the predominant role of *Lb. plantarum* and *L. mesenteroides* over *P. pentosaceus* in a variety of vegetable fermentations. The genome sequences of *L. mesenteroides*, *Lb. plantarum*, and *P. pentosaceus* contain multiple copies of the rRNA clusters, which display minimal or no polymorphism within a given genome. The 16S, 5S, and 23S rRNA sequence copies within each bacterium have 99 or 100% identity over their entire length. With the exception of the cysteine tRNA, most tRNAs are present in these genomes in multiple copies. The *L. mesenteroides* genome sequence contains four putative rRNA clusters, which are located close to the origin of replication. The *Lb. plantarum* and *P. pentosaceus* genome sequences contain five putative rRNA clusters distributed around the genome. The number and location of the rRNA operons may influence reproductive fitness (52), with more operons indicating greater fitness; however, the significance of the genome arrangement of rRNA operons remains to be determined.

LAB isolated from vegetable fermentations frequently contain plasmids. *Lb. plantarum* WCFS1 contains two small plasmids of approximately 2,000 bp and a larger plasmid of about 36,000 bp (53). Putative functions assigned to the proteins encoded by ORFs in these plasmids include conjugal plasmid transfer. Similarly, *L. mesenteroides* ATCC 8293 contains a plasmid of approximately 37,000 bp, which apparently encodes several mobile genetic elements. Plasmid-borne genes encoding proteins involved in bacteriocin production, lactose utilization, and citric acid utilization have been isolated from several *Leuconostoc* species; however, none of these functions appears to be present in the ATCC 8293 plasmid. The overall G+C content of these plasmids tends to be lower than the 40% chromosomal G+C content, suggesting that these plasmids were acquired from nonhomologous sources. Most of the ORFs identified in the sequenced plasmids have no assigned functions. Further research remains to be done to exploit the potential for genetic manipulation by conjugative transfer of genes in LAB from vegetable fermentations, although this technology has been extensively exploited with *Lactococcus* spp. from dairy fermentations (41).

CONCLUDING REMARKS

As we advance into the second century of research on fermented and acidified foods, researchers are building on the solid foundation laid by those noted above, as well as others. Recent research includes mathematical modeling of bacterial growth and competition (24), the

Table 33.3 Pyruvic acid-dissipating enzymes present in the predominant LAB in fermented vegetables

Pyruvate catabolism-related enzyme(s)	*Lactobacillus brevis* ATCC 367	*Leuconostoc mesenteroides* ATCC 8293	*Pediococcus pentosaceus* ATCC 25745	*Lactobacillus plantarum* WCFS1
Pyruvate oxidase	X[a]	X	X	X
Pyruvate dehydrogenase	X	X	X	X
Pyruvate formate lyase	–[b]	X	X	X
Acetolactate synthase	X	X	–	X
Lactate dehydrogenases	X	X	X	X
Hydroxyisocaproate dehydrogenase	–	X	–	X
Malic enzyme (EC 1.1.1.38)	–	–	–	X
Malate dehydrogenase (EC 1.1.1.40)	X	X	X	–
Pyruvate kinase	X	X	X	X
Oxaloacetate decarboxylate	–	–	–	–
Pyruvate carboxylase	–	X	X	X

[a]X, enzyme is present.
[b]–, enzyme is absent.

molecular ecology of vegetable fermentations (12, 15, 82, 84, 90), closed-tank fermentation technology to reduce salt waste (34), the use of clays to remove softening enzymes from recycled brines (10), studies on the sensory perception of pickled vegetable products (48), and studies on the safety of acidified foods (7, 8). Preservation of vegetables by fermentation or by the addition of acids without fermentation (21, 46, 87, 88) is based upon achieving acid concentrations to decrease pH values low enough (pH 3 to 4) to prevent growth of most microorganisms. Hence, sour flavor is important and a characteristic component of the flavor profile of the vegetable products produced by these processes. An understanding of the biological mechanisms of sour taste perception has lagged behind progress on the other four basic tastes. However, there has been recent progress regarding both the physiology (13) and chemistry of sour taste (18).

The recent advances in genomics, molecular microbial ecology, analytical biochemistry, plant breeding, and fermentation technology reveal a bright future for vegetable fermentation science and applications, enhancements of existing products, and new processing techniques. Principal areas for the application of this technology may include the development of commercial low-salt fermentations and nonfermentation preservation methods that will reduce or eliminate salt wastes. Current industrial fermentation practices are in large part based on traditional practices that have been adapted to a larger scale. The development of low-salt fermentations and the storage of fermented vegetables for commercial use present significant technological hurdles, including the potential need for starter cultures (and the impact of bacteriophages on starter cultures) and for new product-handling equipment. Future products may have nutritional properties superior to those of current products and incorporate nontraditional vegetables. The reasons for developing these products will be the same as they have been for centuries. Fermented vegetable products are microbiologically safe, nutritious, and flavorful; have appealing sensory characteristics; and can be conveniently stored for extended periods without refrigeration.

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