

17 Vegetable Fermentations*

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1 Introduction

The preservation of vegetables by fermentation is thought to have originated before recorded history and the technology developed by trial and error. PEDERSON (1979) presumed early man to observe that when vegetables were flavored with salt or brine and packed tightly in a vessel, they changed in character but remained appetizing and nutritious. He concluded that the Chinese were the first to preserve vegetables in this manner and assumed that fermentation in salt brines occurred first and that dry salting came later. The Chinese have been credited with introducing fermented vegetables into Europe.

Many, if not all, vegetables have been preserved by fermentation throughout the world. This chapter is devoted to summarizing the current scientific principles and technology involved in the commercial preservation of cucumbers (for pickles) and cabbage (for sauerkraut) by fermentation. The preservation of kimchi, a Korean fermented vegetable mixture of radishes, Chinese cabbage, cucumbers, and other components, is also summarized.

2 Cucumbers for Pickles

2.1 Raw Product

Various authors have attributed the origin of the cucumber (*Cucumis sativus* L.) to Africa, China, India, or the Near East (MILLER and WEHNER, 1989). Later domestication occurred throughout Europe, and cucumbers are now grown throughout the world using field or greenhouse culture, but with various characteristics, depending upon region.

Cucumbers are bred either for fresh market or processing (pickling). The fresh market varieties possess a relatively tough skin, which serves to extend their storage life as fresh produce. Pickling varieties, however, possess thin, relatively tender skin. Pickling cucumbers are harvested in a relatively imma-

ture stage, before the seeds mature and before the seed area becomes soft and starts to liquefy. The fruit contains an endo-polygalacturonase in the area surrounding the seeds which causes pectin hydrolysis and, thus, liquefaction in the seed area as the fruit matures (MCFEETERS et al., 1980). The value of pickling cucumbers to the processor varies inversely with fruit size, and growers are paid accordingly. Size grades are determined by fruit diameter, and grading devices sort the fruit by diameter. There are limited efforts to also grade the fruit by length, but it is a more common practice to cut overly long fruit to match jar sizes preferred by the processor/consumer.

Pickling varieties of cucumbers have been carefully bred to resist diseases and environmental stresses, grow well in the specific region for which they were developed, produce high yields, and possess the desired physical and chemical attributes (MILLER and WEHNER, 1989). Among the physical attributes desired are relatively small seed area, thin and tender skin, straight and uniform shape, a length to diameter ratio of approximately 3.0, firm texture, typical green color, and absence of internal defects. Comparatively little has been done to manipulate the chemical composition of cucumbers, although research has indicated several possibilities for consideration.

Cucurbitacins (ENSLIN et al., 1967) responsible for bitterness; sugar content (MCCOMBS et al., 1976; MCCREIGHT et al., 1978), important in fermentation; malic acid (MCFEETERS et al., 1982b), important in bloater formation during fermentation; and polygalacturonase activity (MCFEETERS et al., 1980), involved in softening as the fruit ripens, are some of the chemical constituents that have been considered for possible manipulation. Although most improvements in cucumber varieties to date have been accomplished by traditional breeding programs (MILLER and WEHNER, 1989), the tools of modern molecular genetics are in the early stages of application (STAUB and BACKER, 1995).

2.2 Processing

Cucumbers are harvested by hand or mechanical means, depending upon availability of labor, land size and conformation, and other factors. Cucumbers are a seasonal crop that is grown in various geographical regions and shipped to the processor. Great changes have occurred in the United States over the past 20 years as to origin of the fruit which a processor receives. While once a mainly regional and seasonal enterprise, some large processors now receive fresh cucumbers nearly the entire year. Figures of the production of cucumbers for pickles in the U.S. are shown in Tab. 1. The fruit are grown from Mexico to Canada and shipped fresh to processors according to their demands. Cucumbers grown near the processor may be processed within 24 hours. Cucumbers are shipped under refrigeration if grown at distant locations from the processor. The demand for a year-round supply of fresh cucumbers varies according to the types of products that the processors manufacture. Brined cucumbers, being more stable, are transported inter-continentially.

Pickling cucumbers are preserved by three basic methods, fermentation (40% of overall

production), pasteurization (40%), and refrigeration (20%) (FLEMING and MOORE, 1983), as outlined in Fig. 1. Fermentation is the oldest method of preservation and was the only commercial method until about 1940. Pasteurization of fresh cucumber pickles was introduced into the United States industry in the 1940s and resulted in increased consumption of pickles because of their milder acid flavor and more uniform quality. The process involved heating properly acidified cucumbers to an internal temperature of 74°C and holding for 15 min (ETCHELLS and JONES, 1942; MONROE et al., 1969). Some processors deviate from this standard process, depending upon their products and experiences. Fermented cucumbers also may be pasteurized to increase shelf stability, but at lower temperatures and times (JONES et al., 1941). Refrigerated pickles were introduced on a national scale in the United States in the 1960s. Most of these products are preserved by addition of low concentrations of vinegar and a chemical preservative (e.g., sodium benzoate), in addition to refrigeration at 1–5°C. Microbial growth in these products is not desired. Non-acidified, refrigerated pickles, originally popular among certain ethnic groups, also are marketed in some metropolitan areas. These

Tab. 1. U.S. Production Statistics for Pickles and Sauerkraut for 1992

Crop/State	Harvested (1000 acres)	Yield per Acre (tons)	Total Production (1000 tons)	Total Value (\$1000)
Cabbage for Sauerkraut				
New York	1.4	25.0	35.0	1365
Wisconsin	2.6	38.8	100.8	3348
Other states	1.5	23.6	35.9	1799
Total U.S.	5.5	31.1	171.7	6512
Cucumbers for Pickles				
North Carolina	20.1	4.0	80.4	17125
Michigan	21.5	5.2	111.8	17776
Other states	61.6	—	397.4	85912
Total U.S.	103.2	5.7	589.6	120813

Source: ANONYMOUS (1993a)

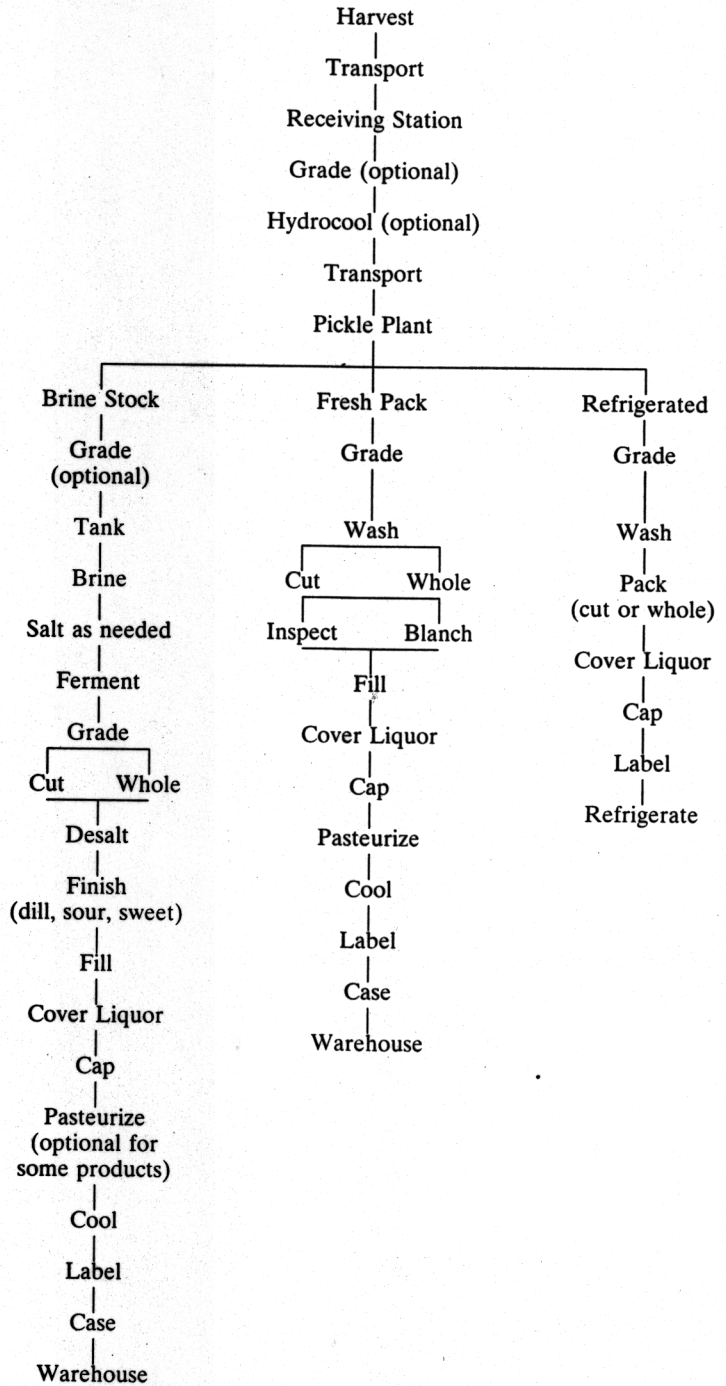


Fig. 1. Flow diagrams for three methods of cucumber processing. From FLEMING and MOORE (1983).

products may or may not be allowed to undergo fermentation before refrigeration. After packaging, these non-acidified pickles undergo a slow lactic acid fermentation while under refrigeration, the rate and extent of which dictates the storage life (up to about 3 months) and quality of the product.

Commercial firms may produce any or all of the three basic types of pickles, depending upon their size, area of distribution, and expertise. Waste generation, particularly excess salt generated by fermentation in brine, is becoming more significant because of environmental concerns about chloride in ground and surface waters of North America. In fact, problems with waste generation influence the types of products that some companies manufacture.

2.3 Fermentation Microbiology

Various groups of microorganisms associated with the cucumber fruit are indicated in Tab. 2. Some fruit, especially the smaller sizes, retain the withered flower from which they emanated. These flowers contain much higher numbers of microorganisms than the attached fruit (Tab. 2). Attempts are made to remove these flowers before brining to prevent enzymatic softening of the cucumbers due to polygalacturonases which may remain due to possible growth of fungi on the nectar within the flower during growth of the plant (BELL, 1951; ETCHELLS et al., 1958).

2.3.1 Natural Fermentations

Most commercial cucumber fermentations are the result of naturally occurring microorganisms and the environmental conditions that influence their growth. Fresh cucumbers are placed in brine contained in bulk tanks (Fig. 2). The microbial activities in the brine occur in various stages during fermentation (Tab. 3). The pH, temperature, and salt concentration of the brine greatly influence the types and rates of microbial activities. The brine typically is acidified (vinegar or acetic acid) to pH 4.5 or slightly below to facilitate CO₂ removal by purging (COSTILOW et al., 1977). This pH is well below the pK 6.1 of bicarbonate, thus resulting in a higher proportion of CO₂, which can be removed by purg-



Fig. 2. Fiberglass tanks used for the fermentation and storage of brined cucumbers. These tanks contain approximately 35000 L.

Tab. 2. Microorganisms on Raw Vegetables Used for Fermentation

Microorganisms	Number/g Fresh Weight Cucumber ^a		Cabbage ^b
	Fruit	Flower	
Total aerobes	1.6×10^4	1.8×10^7	1.3×10^5
Enterobacteriaceae	3.9×10^3	6.4×10^6	3.9×10^3
Lactic acid bacteria	5×10^0	2.6×10^4	4.2×10^1
Yeasts	1.6×10^0	3×10^3	<10

^a From ETCHELLS et al. (1975)

^b After trimming outer leaves

From FLEMING et al. (1988a)

Tab. 3. Stages of Microbial Activities During the Natural Fermentation of Vegetables

Stage	Prevalent Microorganisms (Conditions)
Initiation of fermentation	Various Gram-positive and Gram-negative bacteria
Primary fermentation	Lactic acid bacteria, yeasts (sufficient acid has been produced to inhibit most bacteria)
Secondary fermentation	Fermentative yeasts (when residual sugars remain and LAB have been inhibited by low pH) Spoilage bacteria (degradation of lactic acid when pH is too high and/or salt/acid concentration is too low, e.g., propionic acid bacteria, clostridia)
Post-fermentation	Open tanks: surface growth of oxidative yeasts, molds, and bacteria Anaerobic tanks: none (provided the pH is sufficiently low and salt or acid concentrations are sufficiently high)

Modified from FLEMING (1982)

ing. Acidification also influences the types of bacteria that grow during initiation of fermentation and suppresses growth of undesirable bacteria such as the Enterobacteriaceae (MCDONALD et al., 1991).

Fermentation by lactic acid bacteria (LAB) is preferred, and the rate and extent of growth by these bacteria is dictated by brine concentration (5–8% salt initially) and tem-

perature (15–32°C). Species of LAB involved in fermentation are summarized in Tab. 4. At about 5% NaCl and 21–27°C, fermentation by LAB is relatively rapid, and fermentable sugars are converted mostly to lactic acid, with relatively little gas formation (ETCHELLS and JONES, 1943; JONES and ETCHELLS, 1943). At 10 to 15% salt, however, the rate and extent of acid production by LAB is re-

Tab. 4. Lactic Acid-Producing Bacteria Involved in Vegetable Fermentations

Genus and Species	Fermentation Type ^a	Main Product (molar ratio)	Configuration of Lactate
<i>Streptococcus faecalis</i>	Homofermentative	Lactate	L(+)
<i>Streptococcus lactis</i>	Homofermentative	Lactate	L(+)
<i>Leuconostoc mesenteroides</i>	Heterofermentative	Lactate:acetate:CO ₂ (1:1:1)	D(-)
<i>Pediococcus pentosaceus</i>	Homofermentative	lactate	DL, L(+)
<i>Lactobacillus brevis</i>	Heterofermentative	Lactate:acetate:CO ₂ (1:1:1)	DL
<i>Lactobacillus plantarum</i>	Homofermentative	Lactate	D(-), L(+), DL
	Heterofermentative ^b	Lactate:acetate (1:1)	D(-), L(+), DL
<i>Lactobacillus bavaricus</i>	Homofermentative	Lactate	L(+)

Adapted from KANDLER (1983)

^a With respect to hexose fermentation

^b Heterofermentative with respect to pentoses (facultatively heterofermentative)

duced, and gas production by yeasts is increased.

Various species of yeasts have been isolated from cucumber fermentations. Fermentative species include *Hansenula anomala*, *Hansenula subpelliculosa*, *Saccharomyces baillii*, *Saccharomyces delbrueckii*, *Saccharomyces rosei*, *Torulopsis holmii*, *Torulopsis lactis-condensii* (*Torulopsis caroliana*), and *Torulopsis versatilis* (*Brettanomyces versatilis*) (ETCHELLS et al., 1961). Oxidative species include *Candida krusei*, *Debaromyces hansenii* (*Debaromyces membranaefaciens* var. Holl.), *Pichia ohmeri* (*Endomycopsis ohmeri*), *Rhodotorula* sp., *Saccharomyces rouxii* (*Zygosaccharomyces halomembranis*) (ETCHELLS and BELL, 1950b).

During fermentation, the brine is purged with either nitrogen or air to prevent bloater formation. Nitrogen purging presents fewer problems with yeast and fungal growth, and with off-flavors and colors (FLEMING, 1979). However, air purging is used more commercially because of its lower expense. To offset the potential growth and softening spoilage by fungi, potassium sorbate is added (0.035%) to the brine to prevent their growth (GATES and COSTILOW, 1981). Typically, the fermentation of all sugars to acids and other products is completed within about 3 weeks, depending upon temperature and salt concentration.

2.3.2 Controlled Fermentation

PEDERSON and ALBURY (1961) found that *Lactobacillus plantarum* terminated cucumber fermentations, regardless of the species of LAB used for inoculation. Other LAB tested included *Streptococcus faecalis*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, and *Pediococcus cerevisiae*. Apparently, the greater acid tolerance of naturally occurring *L. plantarum* permitted this bacterium to grow after the added cultures had become inhibited by high levels of acidity. Later, ETCHELLS et al. (1973) used an acid-tolerant strain of *L. plantarum* as inoculum in a controlled fermentation procedure (Fig. 3). This procedure included addition of sodium acetate buffer to assure complete fermentation of sugars to lac-

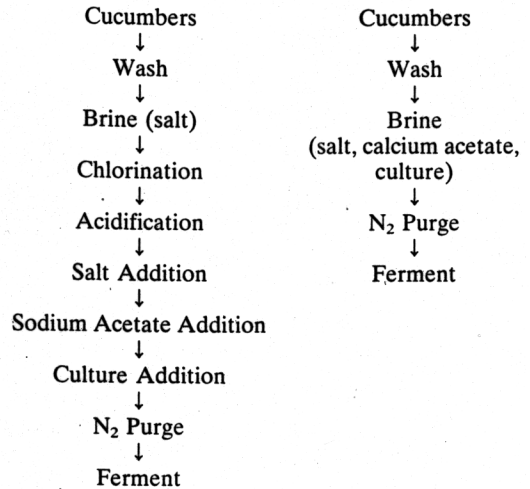


Fig. 3. Controlled fermentation procedures for brined cucumbers. From ETCHELLS et al. (1973), left, and FLEMING et al. (1988a), right.

tic acid and, thereby, prevent residual carbohydrate for fermentation and gas production by yeasts. The *L. plantarum* strain used, however, was found to result in CO₂ production (FLEMING et al., 1973b) and bloater formation (FLEMING et al., 1973a). Thus, the procedure also included purging of CO₂ from the brine to prevent bloater formation. At the time, it was unclear why *L. plantarum* caused bloater formation, since it does not produce CO₂ from hexoses. Later it was found that cucumbers contain malic acid (MCFEETERS et al., 1982a), which is degraded to lactic acid and CO₂ (Fig. 4) and resulted in bloater formation in unpurged fermentations (MCFEETERS et al., 1982b).

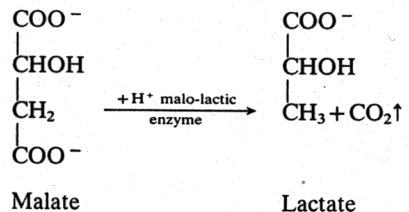


Fig. 4. Proposed malolactic reaction in cucumber fermentation (MCFEETERS et al., 1982b).

Most commercial firms now purge cucumber brines during fermentation (2–3 weeks), even for natural fermentations, as cited above. Nitrogen originally was recommended for purging because of its inertness (ETCHELLS et al., 1973; FLEMING, 1979). Air has been shown to encourage enzymatic softening by fungi and bleaching discoloration (FLEMING et al., 1975b). COSTILOW et al. (1980) confirmed that air results in softening of cucumbers by mold growth where aerated brine exits the side arm purger (Fig. 5). This is the location where dissolved oxygen in the brine contacting the cucumbers is presumed to be highest. GATES and COSTILOW (1981) determined, however, that addition of 0.035% potassium sorbate to the fermentation brine prevents mold and yeast growth and enzymatic softening of air-purged cucumbers. Also, it has been shown that addition of 0.16% acetic acid to the fermentation brine will prevent growth of fungi responsible for fruit softening (POTTS and FLEMING, 1982).

A procedure for fermentation of cucum-

bers in closed tanks has been proposed (FLEMING et al., 1983). Nitrogen is used for purging of the brine during fermentation, and an anaerobic headspace of nitrogen is maintained during storage. Calcium acetate addition to the fermentation brine was found to allow fermentation/storage of the cucumbers at a relatively low salt concentration (FLEMING et al., 1978). Addition of calcium acetate and *L. plantarum* to brines of cucumbers fermented in closed tanks resulted in a product of high quality (firm with desirable flavor and color) at relatively low salt concentrations (FLEMING et al., 1988a). See Fig. 3 for procedure. The closed tank concept has yet to be commercially adopted on a large scale probably because of greater expense of the tanks and the necessity for a different handling system. Removable, flexible covers have been proposed as a possible means of converting open to closed tanks, thereby achieving the advantages of open tanks for product handling and closed tanks for fermentation and storage (HUMPHRIES and FLEMING, 1991).

2.3.3 Pure Culture Fermentation

Pure culture fermentation of cucumbers requires the inactivation of naturally occurring microorganisms. ETCHELLS et al. (1964) used hot water blanching (66 to 80°C for 5 min) or gamma-radiation (0.83 to 1.0 Mrad) to accomplish this goal. They determined the rate and extent of fermentation of pasteurized cucumbers by *P. cerevisiae* (probably *Pediococcus pentosaceus*), *L. plantarum*, *L. brevis*, and six other LAB. The above three named species are common to cucumber fermentations, and their sequence of growth during fermentation of a three-species mixture as inoculum was similar to that thought to occur in natural fermentations. Although pure culture procedures can result in fermented cucumbers of high and consistent quality, such procedures have been considered impractical for commercial application. Cucumbers to be brined in bulk tanks during the hectic harvest season must be handled quickly and economically. Cucumbers for pasteurized or refrigerated products command first attention during this period.

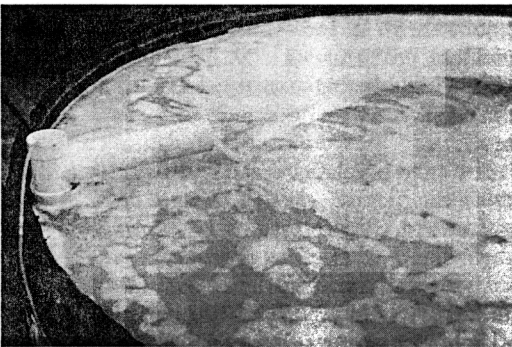


Fig. 5. A tank of fermenting cucumbers being purged to prevent bloater formation. Note the brine and foam that are exiting the side arm purger. The side arm is constructed of a polyethylene tube (10 cm diam.) that extends nearly to the bottom of the tank. The purging gas (air or nitrogen) is introduced through a gas diffuser into the brine as fine bubbles near the bottom of the tank and within the polyethylene tube. This causes the brine to rise in the tube and exit the side arm. Thus, the purging action serves to remove CO₂ from the brine and to circulate the brine within the tank. This tank is constructed of wood and contains approximately 23000 L.

2.4 Fermentation Chemistry

Glucose and fructose, the primary fermentable sugars of cucumbers, are converted to lactic and acetic acids, ethanol, mannitol, and CO₂ (see Tab. 4), depending upon the species that grow. *L. plantarum*, homofermentative to hexoses, normally predominates cucumber fermentations and converts hexoses primarily to lactic acid. In cucumber juice, 95% or more of the sugars are converted to lactic acid by *L. plantarum* (PASSOS et al., 1994). Malic acid, a natural constituent of cucumbers (MCFEETERS et al., 1982a), is degraded to lactic acid and CO₂ (MCFEETERS et al., 1982b) (see Fig. 4). Heterofermentative LAB, when active, produce mannitol, ethanol, acetic acid, and CO₂ from hexoses, in addition to lactic acid. Fermentative yeasts produce ethanol and CO₂.

Cucumber fruits typically constitute about 60% of the volume in bulk tanks, the remaining 40% being occupied by the cover brine. For cucumbers containing 2% fermentable sugars, about 1.2% sugar theoretically would be present if allowed to come to equilibrium (this does not occur because of fermentation). Thus, about 1.1% lactic acid is present after exclusive fermentation of hexoses by *L. plantarum*. Since malic acid (0.2–0.3% of cucumbers) is degraded to lactic acid, this additional amount must be considered in predicting total lactic acid formation. When less than 1.1% lactic acid is present after fermentation, growth by microorganisms other than homofermentative LAB or a leaking tank is implicated.

2.5 Sensory Properties

Textural properties are highly important in fermented, as well as pasteurized and refrigerated cucumber pickles. A firm, crisp texture is desired. In fermented products such as hamburger dill chips and sweet pickles, the tissue is translucent (referred to as a "cured" appearance) due to expulsion of gas during brine storage. This is normal for these products and, therefore, expected. This translucent appearance is not desired in pasteurized or refrigerated pickles since white, opaque

appearance in these products is associated with freshness. Internal tissue voids are objectionable in all products. Cucumber products with small, immature seeds and a small seed area are preferred.

The flavor of cucumber pickles varies widely, depending upon the spices and flavorings that are added to the numerous products. Lactic acid is a natural component of fermented pickles, but its flavor is too tart and objectionable to some in certain products such as hamburger dill chips, where custom has resulted in a preference for an acetic acid (vinegar) flavor. Lactic acid is removed from the brined cucumbers by the desalting operation and is replaced with vinegar to acidify the final product. In other products such as "genuine" dill pickles, the lactic acid flavor is preferred, and these products can be sold in the brine in which they were fermented.

A clean flavor which does not detract from the added spices and flavorings is desired. Proper levels of acid and salt in the finished product are essential and vary among the many products that are made. However, pasteurized pickles typically contain 0.5 to 0.6% acetic acid and possess a pH of about 3.7. The salt concentration varies from 0% (in dietetic pickles) up to about 3%. Fermented pickles vary widely in types of products that are produced. In relish and salad cube products the acetic acid and sugar concentrations are varied to give products that are characterized by their degrees of sweetness and acidity. The ratios and concentrations of acetic acid and sugar can be varied to preserve sweet pickles without the need for pasteurization (BELL and ETCHELLS, 1952). Although the preservation of some mildly acidic and sweet, fermented pickles is assured by pasteurization today, before about 1940 pasteurization was not used.

2.6 Spoilage Problems

Gaseous spoilage (bloat formation) is due to the growth of gas-forming microorganisms such as yeasts (ETCHELLS and BELL, 1950a), heterofermentative LAB (ETCHELLS et al., 1968), and Enterobacteriaceae (ETCHELLS et al., 1945). *L. plantarum* also contri-

butes CO₂ by decarboxylation of malic acid (MCFEETERS et al., 1984), as mentioned earlier. CO₂ from the fermenting brine diffuses into the cucumber tissue faster than entrapped nitrogen can diffuse out, thus gas pressure greater than 1 atmosphere results within the tissue, which can result in bloater formation, depending upon resistance of the fruit to internal gas pressure, brine depth, and other factors (FLEMING and PHARR, 1980). Although bloated cucumbers can be used to make relish, the product is of lower value. As mentioned above, bloater formation can be prevented by purging CO₂ from solution during fermentation.

Softening spoilage of fermented cucumbers can arise from various sources. Polygalacturonase enzymes of fungal origin on the surface of the fruit, and especially within the flowers retained on small fruit, can cause softening of the fruit during brine storage (ETCHELLS et al., 1958). This type of softening is rather uniform throughout the tissue of individual fruit, and can occur within the entire tank. Softening of individual fruit in spots normally is due to fungal or bacterial growth on the fruit before brining. Softening of the interior of the fruit is normally associated with ripening of the fruit and the natural endo-polygalacturonase that develops in the fruit as it matures (MCFEETERS et al., 1980). Softening can be reduced by removing flowers from the fruit before brining (ETCHELLS et al., 1958), by brining only disease-free fruit as soon after harvest as possible, and by avoiding over-mature fruit. High concentrations of salt (NaCl) will prevent softening by polygalacturonases (BELL and ETCHELLS, 1961), but can present a waste disposal problem. The addition of CaCl₂ to the brine (0.2–0.4%) has been shown to reduce the concentration of NaCl needed to prevent softening (BUESCHER et al., 1979, 1981; FLEMING et al., 1987; MCFEETERS and FLEMING, 1990).

Bleaching of the green color from brined cucumbers can be due to exposure of the fruit to sunlight. Excessive concentrations of potassium sorbate (>0.035%) have been reported to cause bleached or gray-colored fruit.

Off-flavors and odors in fermented cucumbers result from the growth of undesirable

microorganisms. Oxidative yeasts can grow on the surface of cucumber brines that are not exposed to sunlight, utilize lactic acid which causes the pH to rise, and allow spoilage bacteria to grow. Butyric and propionic acids have been shown to be produced, resulting in offensive odors, when the cucumbers are brined at very low (e.g., 2.3%) concentrations of NaCl (FLEMING et al., 1989).

3 Cabbage for Sauerkraut

3.1 Raw Product

The modern, hard-head cultivars of cabbage (*Brassica oleracea*) are reported to have descended from wild, non-heading brassicas originating in the eastern Mediterranean and in Asia Minor (DICKSON and WALLACE, 1986). Cabbage is grown for the fresh market and for the production of sauerkraut. For use in the production of sauerkraut it is desired that the cabbage heads be large (typically 8–12 lbs), compact (i.e., dense), have a minimum of green outer leaves, and possess desirable flavor, color, and textural properties when converted into sauerkraut. Cabbage varieties are bred for yield, disease and insect resistance, storage stability, and dry matter content (DICKSON, 1987; DICKSON and WALLACE, 1986). Although varieties have been developed for fresh market, as well as sauerkraut, when demand exceeds supply, varieties can serve multiple purposes.

Cabbage for sauerkraut is grown in cooler climates in the U.S., and primarily in the states of New York and Wisconsin (see Tab. 1), with production in lesser quantities in Ohio, Oregon and other states. Processing plants for sauerkraut production also are located mostly in these states.

3.2 Processing

Fresh cabbage for sauerkraut is harvested mechanically or by hand mostly in the months of August to November. The cabbage is transported to the processor, where it is

graded, cored, trimmed, shredded, and salted. The waste from the coring and trimming operations typically is returned to the field, where it is plowed into the soil. This waste constitutes about 30% of the weight of the fresh cabbage.

After shredding (ca. 1 mm thick), the cabbage is conveyed by belt, where salt is added (Fig. 6A), to the fermentation tanks (Fig. 6B). The tanks typically hold 20–180 tons of shredded cabbage (STAMER, 1983). Most tanks today are constructed of reinforced concrete,

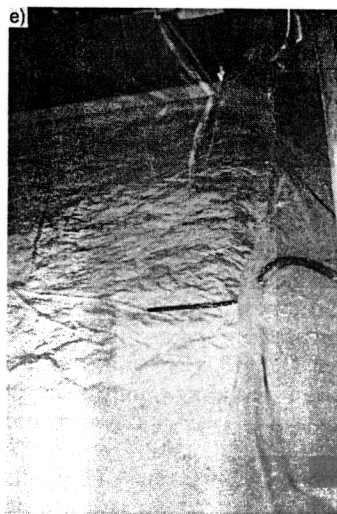
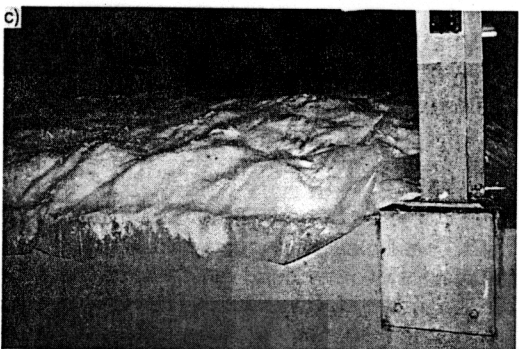
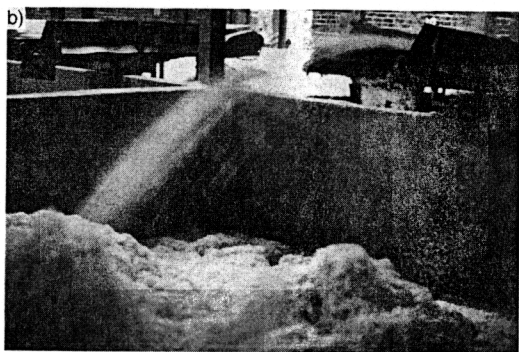
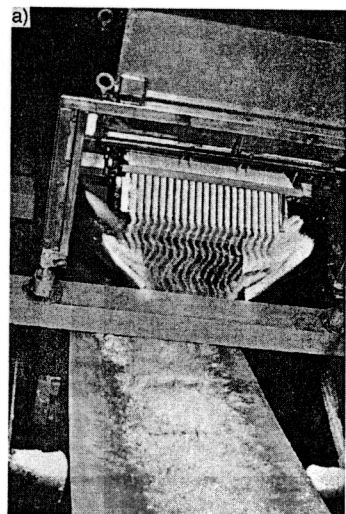


Fig. 6. Salting, conveying, tank filling, and covering sliced cabbage for fermentation into sauerkraut. (A) The sliced cabbage is conveyed by a belt, where it is salted. (B) The salted, sliced cabbage is conveyed into the tank. (C) The cabbage is heaped above the tank and loosely covered with plastic sheeting. (D) After about 24 h, the cabbage is allowed to be lowered by removal of brine from the tank bottom and is then leveled. (E) The cabbage is covered with one or more plastic sheets. A plastic tube may be inserted between the cabbage and the plastic sheet to allow for escape of gas during fermentation. The reinforced concrete tank shown contains approximately 80 000 kg of cabbage.

but some wooden tanks remain. The tank is uniformly filled, heaped to extend slightly above the top of the tank, and loosely covered with plastic sheeting (Fig. 6C). After about 24 h, brine generated and located at the bottom of the tank is allowed to drain from the tank to allow the top of the cabbage to settle below the top of the tank. Then, the cabbage is manually distributed to create a slightly concave surface (Fig. 6D). The plastic sheeting is then placed on the surface and water is added on top to weight it down and to provide an anaerobic seal (Fig. 6E). Gas generated during fermentation escapes by forcing its way between the tank wall and the cabbage, or through a plastic tube placed between the cabbage and the cover (Fig. 6E). In cabbage with a low moisture content, excess brine may be insufficient to allow for settling of the salted cabbage from above the top of the tank. In this case the cabbage is not heaped above the tank at filling.

After filling and heading, the cabbage may present a heaving problem during the first few days. This is due to gas pockets being formed in the shredded cabbage from tissue respiration and microbial fermentation. In severe cases the cabbage may be lifted so high as to empty the water seal. In these instances more brine must be removed from the bottom of the tank to allow for settling of the cabbage, and then the tank reheaded with a plastic cover-water seal.

In the U.S. the cabbage is allowed to remain in the tanks until at least 1% lactic acid is formed (about 30 days minimum, depending upon the temperature), and is stored beyond this time and until such time as needed for processing. Thus, the sauerkraut tanks are used for fermentation as well as storage. Although extended periods of holding in the tanks can result in excess acid formation and waste generation due to the need to wash excess acid from the product before canning, this disadvantage is offset by the economic advantages of bulk storage. Also, most U.S. sauerkraut companies specialize in this product only, and desire the option of further processing of sauerkraut throughout the year so as to distribute labor and equipment needs. This U.S. procedure differs significantly from that of many European manu-

facturers who process the sauerkraut into finished products when it reaches the desired level of titratable acidity (calculated as lactic acid, typically 1%). Thus, European manufacturers have greater control over product uniformity, but lose the economic advantages of bulk storage. Also, the European method results in less waste generation since less excess acid is produced.

The sauerkraut is removed pneumatically, by mechanical fork, or by hand from the tanks as needed for processing during the year. The sauerkraut may be packaged in cans, glass, or plastic containers. When packaged in glass or plastic, the product is not heated. Rather, sodium benzoate (0.1%, w/w) and potassium metabisulfite are added as preservatives, and the product is held under refrigeration (5°C) (STAMER and STOYLA, 1978).

Canned sauerkraut is preserved by pasteurization without the addition of preservatives. Heating is performed either by steam injection into a thermal screw, or by a sauerkraut juice immersion-type cooker. The product is heated to 74–82°C for about 3 min and hot-filled into the cans (STAMER, 1983). After closure, the cans are immediately cooled to less than 32°C. The shelf life of enamel-lined canned products is estimated at 18–30 months, and that of glass- and plastic-packaged products is 8–12 months (STAMER, 1983).

3.3 Fermentation Microbiology

3.3.1 Natural Fermentation

Cabbage, like many fresh vegetables, contains numerous species of microorganisms and relatively high numbers of total aerobic bacteria. LAB constitute a relatively small proportion of the total bacteria (see e.g., Tab. 2). When properly shredded and salted, the cabbage (at a proper temperature) undergoes fermentation by a sequence of LAB that results in the distinctive flavor of sauerkraut. The most comprehensive review of the sauerkraut fermentation is that by PEDERSON and ALBURY (1969), which should be consulted by serious students of the subject. These re-

searchers listed five species of LAB as important in the sauerkraut fermentation in order of increasing total acid production: *Streptococcus faecalis* < *Leuconostoc mesenteroides* < *Lactobacillus brevis* < *Pediococcus cerevisiae* (*P. pentosaceus*) < *Lactobacillus plantarum*. Although the names of some of these species have been changed, it is believed that all of these, and perhaps others, are involved. However, it seems clear that *L. mesenteroides* is a major species in the early, heterofermentative stage of fermentation, and that *L. plantarum* is a major species involved in the late, homofermentative stage of fermentation. Salt concentration and temperature influence the relative extent of growth by these two species of LAB, and thus the quality of the sauerkraut. Low salt concentration and low temperature (e.g., 1%, 18°C) favor growth of heterofermentative LAB, while high salt concentration and high temperature (e.g., 3.5%, 32°C) favor growth of homofermentative LAB (PEDERSON and ALBURY, 1954).

The fact that sauerkraut first undergoes fermentation by heterofermentative and then homofermentative species is illustrated in Fig. 7. During the first 8 days of fermentation at 2% salt and 18.3°C, heterofermentative species were predominant. This stage of the fermentation resulted in much gas production. After this period, homofermentative species predominated and relatively little gas was formed.

3.3.2 Controlled Fermentation

Proper salt concentration and temperature have been concluded to be primary means for control of the sauerkraut fermentation (PEDERSON and ALBURY, 1954). Some have concluded that addition of cultures is not needed for desirable sauerkraut if these conditions are appropriate (PEDERSON and ALBURY, 1969; STAMER, 1983). However, cultures could be desirable if they have sufficiently unique and valuable properties, or if the environmental conditions (salt, temperature) are undesirable for traditional sauerkraut manufacture. Culture development for vegetable fermentations is discussed later in this chapter.

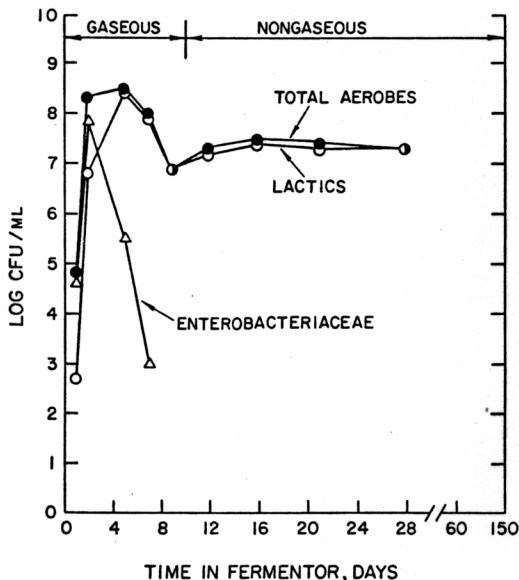


Fig. 7. Microbial growth in sauerkraut fermentation. From FLEMING et al. (1988b). Heterofermentative LAB predominated during the gaseous stage and homofermentative during the non-gaseous stage.

Some European manufacturers inoculate sliced cabbage with brine from a previous fermentation, and this seems to result in a desirable heterolactic fermentation. The mild acidity (1%), relatively low salt concentration (e.g., 1%), rapid fermentation (e.g., 1 week), and immediate canning of German sauerkraut probably account for its high quality and uniformity. Since American sauerkraut may be held in bulk storage for several months, it is even more important that heterofermentation by *L. mesenteroides* attain its maximum potential. If temperature or salt is not ideal, homofermentation by *L. plantarum* can result in a harshly acidic product. Thus, culture selection and predominance could have a significant impact on quality and uniformity of American sauerkraut.

3.4 Fermentation Chemistry

The fermentable sugars of cabbage are primarily glucose and fructose, with a smaller

Tab. 5. Composition of Raw Cabbage Used in Fermentations

Compound	Concentration ^a in Leaves mM	SD ^b	Concentration ^a in Core mM	SD ^b
Sucrose	7.0	4.6	53.1	16.4
Glucose	132.5	9.9	75.7	15.9
Fructose	114.2	3.2	60.3	7.3
Malic acid	12.2	1.6	7.1	3.0

Source: FLEMING et al. (1988b)

^a Averages of four replicates^b SD=standard deviation

concentration of sucrose (see, e.g., Tab. 5). In the cabbage used for the following discussion, the core accounted for 23% and the leaves for 77% of the cabbage weight. The core region contained a relatively high concentration of sucrose (53.1 mM) compared to the leaves (7.0 mM). Fermentation of this cabbage at 18.3°C in laboratory fermentors resulted in sugar depletion and product formation, as illustrated in Figs. 8 and 9 (FLEMING et al., 1988b). These chemical changes are consistent with the microbiological changes noted in Fig. 7, where the fermentation was characterized into gaseous and non-gaseous stages.

After coring, shredding, salting, and packing the cabbage into the fermentors, the brine generated was analyzed periodically, as indicated in Figs. 8 and 9. Although fructose and glucose were in similar concentrations in the raw cabbage, the fructose concentration was 30 mM in the initial brine and declined thereafter. However, glucose concentration continued to increase in the brine up to 75 mM after 7 days, and then declined to near 0 after 60 days. Mannitol, acetic acid, and ethanol increased rapidly until about 7 days and plateaued. Lactic acid production continued until all of the fermentable sugars were de-

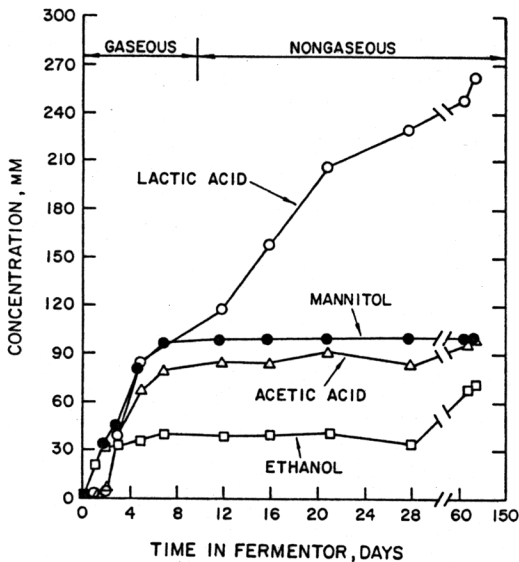


Fig. 8. Product formation in sauerkraut fermentation. From FLEMING et al. (1988b).

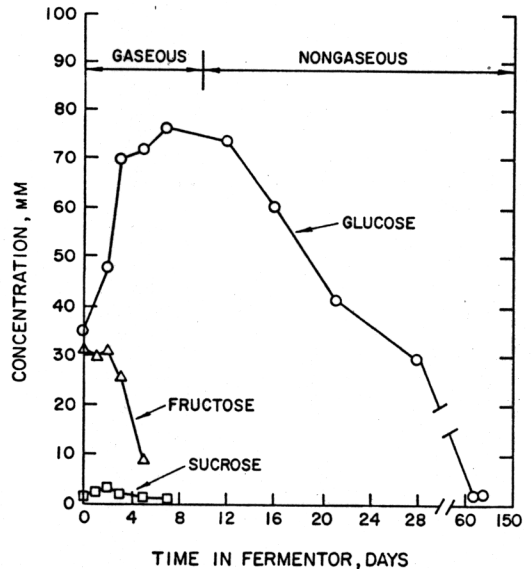


Fig. 9. Substrate depletion during sauerkraut fermentation. From FLEMING et al. (1988b).

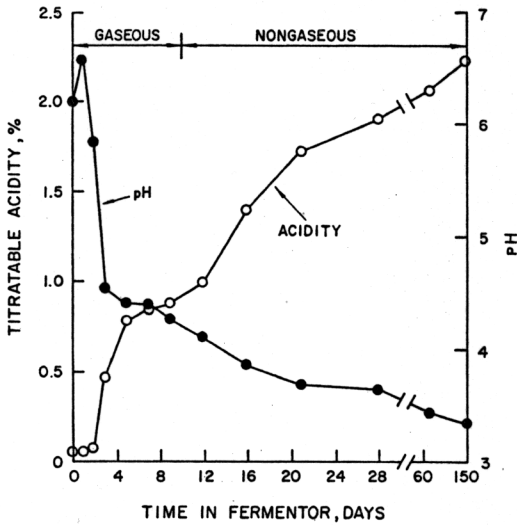


Fig. 10. Titratable acidity and pH changes during sauerkraut fermentation. From FLEMING et al. (1988b).

pleted. During fermentation, the pH was reduced from about 6 to about 3.4 and the titratable acidity, calculated as lactic acid, increased to 2.2% (Fig. 10).

3.5 Sensory Properties

U.S. Department of Agriculture grade standards for canned sauerkraut involve a 100-point scale with color (30 points), cut (10), absence of defects (20), character (10), and flavor (30) being individually evaluated and these factor scores totaled (ANONYMOUS, 1993b). It is preferred that the product be light to straw color. "Cut" refers to uniformity, thickness, and length of shreds. "Defects" refers to the relative absence of large and coarse pieces of leaves and core material, blemished pieces, spotted or discolored pieces, etc. "Character" refers to firmness and crispness. "Flavor" refers to the characteristic flavor of clean sauerkraut with the absence of off-flavors and off-odors.

Ideal ranges for percent titratable acidity (1.1–1.5), percent salt (1.7–2.4), salt/acid ratio (1.0–1.7), and lactic/acetic acid concentrations (3.0–5.0) were reported by PEDERSON (1940)

for canned sauerkraut. More recently, a survey of U.S. commercial sauerkraut revealed a slightly lower range in salt concentration (1.4–2.0%) and a lower lactic/acetic ratio (1.4–4.0) (CORBET et al., 1995). This may be due to the industry's trend to use lower salt concentrations for fermentation than the 1.8–2.25% recommended earlier by PEDERSON and ALBURY (1969).

3.6 Spoilage Problems

Although sauerkraut is simple to make, the commercial manufacture of a product of consistently high quality is not simple. American manufacturers of sauerkraut are confronted with the problem of maintaining product quality during storage of the sauerkraut for many months. In contrast, many European manufacturers can their product when it reaches the desired level of acidity (about 1 week) and, thus, are not confronted with quality maintenance during bulk storage.

Inferior or off-flavor can be a serious defect, depending upon the extent, and can result from an improper fermentation or growth of spoilage microorganisms during bulk storage. An inferior flavor can result if the cabbage is fermented at too high a temperature, resulting in predominant fermentation by homofermentative LAB and an improper ratio of lactic:acetic acids (PEDERSON and ALBURY, 1954). The production of butyric, propionic, and other short-chained fatty acids can result in a serious flavor defect (VORBECK et al., 1961). Growth by clostridia or other spoilage bacteria during the early stages of fermentation, before growth and acid production by the LAB, may be responsible for this problem. Improper salting procedures and use of excessively soiled cabbage are likely contributing factors. After fermentation and during storage, oxidative yeasts and molds may grow at the surface of sauerkraut that is exposed to air (PEDERSON and ALBURY, 1969), which can result in off-flavors. These microorganisms may metabolize the lactic acid, thereby allowing spoilage bacteria to grow. This problem can be reduced by assuring a proper seal of the plastic covers of the tanks.

Discoloration of sauerkraut in the fermentation tank can result from growth of pigmented (pink) yeast species (perhaps *Rhodotorula* sp.) due to improper salting (PEDERSON and KELLY, 1938). A red color has been shown to be produced by the non-pigmented bacterium, *L. brevis* (STAMER et al., 1973). In this case, the color formation was catalyzed by aeration and inhibited by cysteine and ascorbic acid, natural reductants of cabbage. Darkening of sauerkraut during fermentation or bulk storage, or after canning seems to be due to oxidative changes, perhaps influenced by the content of natural constituents of the cabbage and exposure to air. It is considered important that the ascorbic acid concentration in the sauerkraut be sufficiently high, e.g., 20 mg/100 g sauerkraut or higher, to serve as an antioxidant and color preservative. Exclusion of air during fermentation, bulk storage, and in consumer containers is important for color retention.

Soft sauerkraut can result from cabbage stored at less than 1.8% salt, whereas greater than 2.5% salt results in a tough texture (PEDERSON, 1946). Mold growth can result in enzymatic softening, perhaps by polygalacturonases and other enzymes, thus reinforcing the need to exclude air from the sauerkraut.

4 Kimchi

Kimchi is a general term applied to a Korean product made by the lactic acid fermentation of salted vegetables (dry salted or brined) with or without secondary ingredients. Unless otherwise specified, however, kimchi generally means a product made from Chinese cabbage as the primary vegetable with secondary ingredients. Kimchis made from vegetables other than Chinese cabbage as the primary vegetable are specified with a qualifying word, e.g., radish kimchi, cucumber kimchi, and green onion kimchi. Both solids and liquids of kimchis are consumed.

Kimchi has been a major table condiment in Korea for about 200 years (LEE, 1975). Kimchi provided a spicy and flavorful adjunct to a rather narrow choice of foods for Ko-

reans in difficult times, complementing the bland taste of cooked rice. Although kimchi still is a major dish in Korea today, its consumption has declined from 200–300 g per person per day in the 1950s (LEE et al., 1960) to 100 g today (CHO, 1993). Consumption is greater during the winter months (150–200 g/day) than in the summer months (50–150 g/day) (ANONYMOUS, 1974). Koreans now enjoy a more diversified diet thanks to industrialization of the country. Interestingly, however, demands for the product may be increasing in the United States and certain other countries where ethnic and regional foods seem to be gaining in popularity.

4.1 Raw Product

Raw materials used for kimchi preparation are divided into two basic groups, primary vegetables and secondary ingredients (Tab. 6). The division is not absolute and relative amounts used vary. Radishes (roots or greens), green onions, or Indian mustard greens, for example, can be primary vegetables in some kimchis but secondary ingredients in other kimchis, depending on the percentages comprising the kimchis. The two most frequently used primary vegetables are Chinese cabbage (*Brassica campestris* subsp. *pekinensis*) and radishes (*Raphanus sativus* L.). Other primary vegetables such as cucumbers, green onions, Chinese leeks, Indian mustard greens, turnips, sowthistle, and green peppers are used less frequently. Spinach, pumpkins, cabbages, and eggplants are used much less frequently.

More than 38 different varieties of kimchi are made and consumed in Korea, depending on vegetables and other ingredients used (SOHN, 1992). More kimchis are made in individual households than commercially, resulting in varying formulations to appeal to the preferences and traditions of each household.

4.2 Kimchi Processing

Winter kimchi has been most traditional and is made between mid-November and ear-

Tab. 6. Raw Materials Used for Kimchi Preparation

Raw Materials	
Primary vegetables	Chinese cabbage, radish (roots with or without leaves), cucumber, green onion, Chinese leek, Indian mustard greens, turnip, green pepper, sowthistle, spinach, pumpkin, cabbage, eggplant
Secondary ingredients	<p><i>Vegetables:</i> radish (root and/or greens), Indian mustard greens, water celery, carrot</p> <p><i>Seafoods:</i> shrimp, oyster, Alaskan pollack, squid, flounder, yellow corvenia</p> <p><i>Spices:</i> red pepper powder, green onion, garlic, ginger, Chinese leek, onion</p> <p><i>Fruit:</i> pine nut, ginkgo nut, pear, apple, chestnut, jujube</p> <p><i>Seasoning:</i> salt, salted fermented fish sauce (anchovy, shrimp, yellow corvenia), sugar, MSG</p> <p><i>Cereals:</i> cooked rice, cooked rice flour, cooked wheat flour</p>

Adapted from LEE and CHO (1990)

ly December, depending on the climate of the particular year. It is consumed until the following spring. The fresh ingredients are tightly filled into large earthen jars with earthen lids. The jars are partially buried (80–90% of container depth) under ground (Fig. 11) and are covered with bundles of rice straw for protection from direct sunlight and the climate. This procedure is still practiced in much of the rural areas and some households in urban areas. Households living in urban areas usually do not have a place to bury their kimchi jars in the close vicinity of their house and instead keep the jars covered with clothing and other insulating materials in the shaded places of apartments or in home cellars. The sub-surface temperature of the earth during

the winter season is fit for slow but excellent fermentation of kimchi and also for the subsequent storage. Over-acidification and development of yeasty flavor are the two most important problems in the long-term storage of kimchi. However, as the temperature goes up late in the following spring, the quality of winter kimchi is reduced.

Today, lesser amounts of winter kimchi are made due to more availability of food items, including meats, fish, and fresh vegetables, and due to year-round availability of vegetables for kimchi preparation. Koreans now enjoy freshly fermented kimchi anytime of the year due to both the year-round availability of fresh vegetables and the general availability of household refrigerators. It is common for Korean families to make small batches of kimchi as needed and to store it under refrigeration for short periods to extend the fresh quality.

Kimchis can be divided into two types simply depending on the way the primary vegetable is salted. Primary vegetables are salted (either by dry salting or brining) and then the liquid is drained off completely before blending with secondary ingredients in the preparation of general kimchis. However, sufficient brine is poured over the packed ingredients to cover whole cucumber fruits or radish roots. Pickles are usually prepared without secondary ingredients.

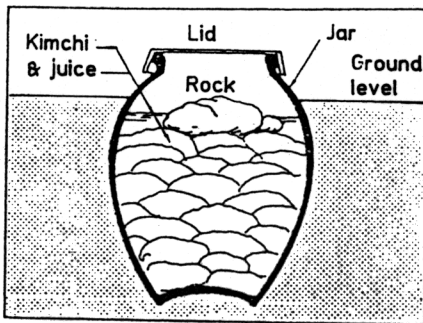


Fig. 11. Traditional storage of kimchi. From CHUN (1981).

4.3 Fermentation Microbiology

The total aerobic population in raw vegetables used to make the kimchi mixture typically is in the range of 10^3 – 10^7 cells/g. Total LAB, including *Lactobacillus* and *Leuconostoc* species, are in the range of 10^4 – 10^6 cells/mL (KIM and CHUN, 1966; MHEEN and KWON, 1984; KIM and LEE, 1988; LEE et al., 1992; KIM et al., 1989; SHIM et al., 1990a, b). SHIM et al. (1990b) reported the population of *Lactobacillus plantarum* on individual kimchi ingredients (Tab. 7) as determined by the most probable number (MPN) method to be in the range of 0.36 to 240/g (mean 7.3) for mixed ingredients and 0.0 and 0.36/g (mean 0.0) for Chinese cabbage.

LAB found in raw kimchi mixture include *Leuconostoc mesenteroides*, *L. dextranicum*, *Lactobacillus leichmannii*, and *L. sake*. Other LAB found during the course of the kimchi fermentation are *Lactobacillus fermentum*, *Pediococcus pentosaceus*, *Streptococcus faecalis*, *Lactobacillus plantarum*, and *L. brevis* (SHIM et al., 1990b). When LAB isolated from raw kimchi mixture and fermenting kimchi were inoculated into filter-sterilized fresh cabbage juice, *L. mesenteroides*, *L. leichmannii*, and *L. fermentum* produced 0.5–1.0% acid as lactic. *P. pentosaceus* and *L. plantarum* produced more acid than other species

and kept producing acid after cell growth ceased.

The types and numbers of initial microorganisms in kimchi mixture are heavily dependent on the quality of ingredients and the washing procedure. Red pepper powder, which is not washed before use, is a particular source of variation in microorganisms. Kimchi fermentation typically is initiated by *L. mesenteroides* and terminated by *L. plantarum* and/or *L. brevis*.

Good quality primary vegetables and proper combination of secondary ingredients are believed to be important prerequisites for the production of good-quality kimchi. Salt concentration and fermentation temperature are the two most important variables influencing bacterial growth and metabolism. The maximum number of microorganisms attainable and the time required to reach maximum numbers vary depending on the temperature and salt concentration (Fig. 12; MHEEN and KWON, 1984). As the salt concentration is increased, maximum microbial populations attained are lower and the time required to reach maximum numbers is longer. At all temperatures tested, *L. mesenteroides* was shown to reach the maximum population before any other LAB. *L. mesenteroides* declines fast after reaching the maximum populations and disappears from fermenting kim-

Tab. 7. Estimated Number of *Lactobacillus plantarum* in Individual Ingredients (cells/g) and Kimchi Mixture (cells/mL)

	Number of <i>Lactobacillus plantarum</i>	
	Mean	Range
Kimchi	7.3	0.36–240
Chinese cabbage	0.0	0.0–0.36
Radish	2.3	0.0–9.3
Ginger	4.6	0.0–30.0
Green onion	1.82	0.0–4.6
Garlic (unpeeled)	0.0	0.0–37.2
Red pepper powder	0.0	0
Red pepper (wet)	4.3	0.0–240

From SHIM et al. (1990b)

These estimates were based on the most probable number (MPN) technique and the fact that only *L. plantarum* and *L. brevis*, of the LAB isolates from kimchi, were able to grow in MRS broth containing 7% ethanol. It was possible to distinguish between these two species by the fact that *L. brevis* produces CO_2 from hexoses, but *L. plantarum* does not.

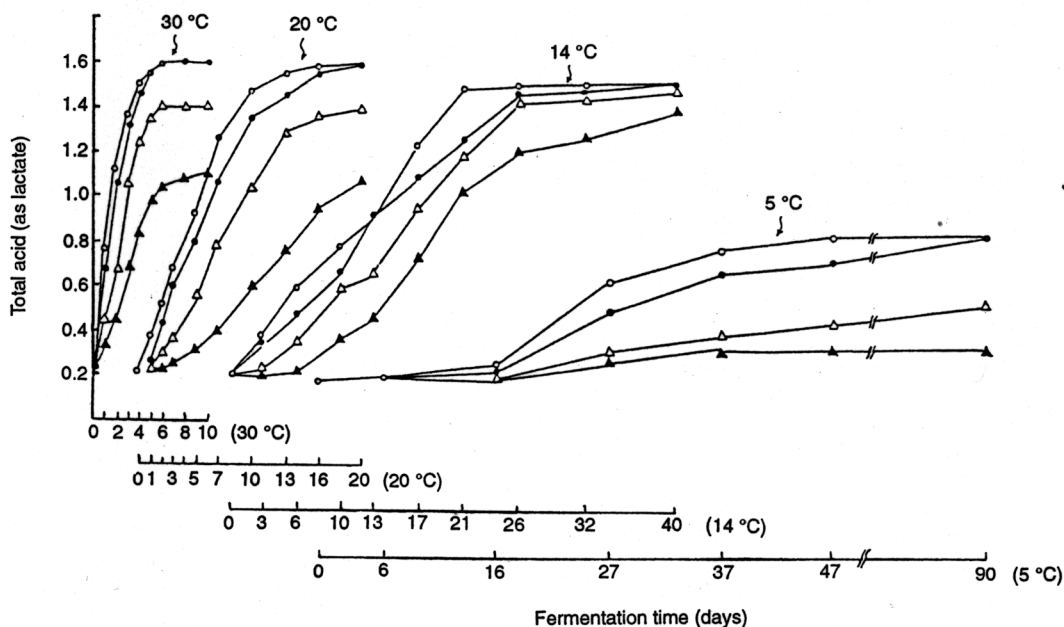


Fig. 12. Changes of total acid during kimchi fermentation at different temperatures and salt concentrations. NaCl: —○—, 2.25%; —●—, 3.5%; —△—, 5.0%; —▲—, 7.0%. From MHEEN and KWON (1984).

chis when other LAB, including *L. plantarum*, become predominant when the temperature is 14°C or above. At 5°C, however, *L. mesenteroides* is the dominant bacterium during the early phase of fermentation and does not decline as fast as at higher temperatures. High populations are maintained throughout fermentation. *L. plantarum* and *L. brevis*, which are blamed for over-acidification, do not appear in kimchi fermented at 5°C.

Various scientists (KIM and CHUN, 1966; MHEEN and KWON, 1984; SHIM et al., 1990a; LEE and YANG, 1975) agree that *L. mesenteroides* appears at the early stage of kimchi fermentation, followed by *L. plantarum*, as in the case with sauerkraut (PEDERSON and ALBURY, 1969).

4.4 Fermentation Chemistry

The pH and acidity of optimally fermented kimchis are 4.2–4.5 and 0.4–0.8% (as lactic acid), respectively (SONG et al., 1966; MHEEN and KWON, 1984; CHUN, 1981). Acid production, as well as microbial growth, is greatly in-

fluenced by salt concentration and fermentation temperature. More total acid is produced at lower salt concentrations and higher temperatures (Fig. 12). At the lower concentration of salt, maximum acidity is reached in a shorter period of time. Optimum acidity (0.6%) of kimchi is reached within 1 day at 30°C and at 2.25–3.5% salt, and the same level of acidity is reached in 2 and 4 days at 5.0 and 7.0% salt, respectively.

More volatile acid is produced when low concentrations of salt are used. The ratios of volatile to non-volatile acids are highest after only 2 days of fermentation, decline quickly, and are stabilized thereafter. Also, the ratios were higher at lower fermentation temperatures. Kimchis fermented at lower temperature typically are judged to be superior in quality than those fermented at higher temperatures. The time when the ratio of volatile to non-volatile acids reaches the maximum is considered to be the time when kimchi tastes best (MHEEN and KWON, 1984).

As the salt concentration is increased, the volatile to non-volatile acid ratio decreases, producing a less palatable product. *L. planta-*

rum is not as sensitive to salt as *L. mesenteroides*, which produces more volatile end products.

Organic acids other than lactic and acetic are found in kimchi and kimchi ingredients. These include citric, fumaric, oxalic, malonic, malic, and succinic acids as non-volatile acids and formic, propionic, valeric, butyric, caproic, and heptanoic acids as volatile organic acids. All these organic acids, except caproic and heptanoic, have been reported in small amounts in fresh raw ingredients such as Chinese cabbage and radishes (KIM and RHEE, 1975; RYU et al., 1984; TSUYUKI and ABE, 1979). Malic acid is in highest concentration among the natural organic acids found in fresh Chinese cabbage. Butyric, caproic, and heptanoic acids have been reported to contribute to the off-flavor of sauerkraut (VORBECK et al., 1961) and may be important in kimchi flavor.

The kinds of organic acids found in kimchi depend on the secondary ingredients used, as well as the fermentation. Concentrations of certain organic acids produced seem to be influenced by individual secondary ingredients when secondary ingredients are tested separately. More lactic acid is produced in kimchi made with red pepper powder, garlic, and green onion, and more acetic acid is produced in kimchi with garlic (RYU et al., 1984).

Mannitol has been shown to be produced during the fermentation of kimchi (HA et al., 1989). Fructose is said to be reduced by *L. mesenteroides* to mannitol, which is consumed by *L. plantarum* in the following stage of fermentation (FRAZIER and WESTHOFF, 1978).

4.5 Sensory Properties

Factors influencing kimchi flavor are sugar, amino acids, organic acids, salt, and volatile sulfur compounds. The sugar content of vegetables influences the taste of final products due to acidity formed or residual sweetness. Sweetness in the final product is desired. The soluble solids content ($^{\circ}$ Brix) varies for Chinese cabbage (1.2–6.6 $^{\circ}$) and radishes (2.6–5.1 $^{\circ}$) (KIM et al., 1989; SHIM et al., 1990a). The sugar composition of Chinese cabbage has been reported to be about 70% glucose,

17% mannose, and 10% fructose. No sucrose was reported (HA et al., 1989).

Free amino acids also influence the flavor of kimchi. Eighteen amino acids have been identified in raw and fermented kimchi, and reports on the amino acid changes during the fermentation are conflicting. CHO and RHEE (1979) reported that the content of free amino acids decreased as fermentation proceeded, while TAKAMA et al. (1986) and HAWER et al. (1988) reported that it doubled. TAKAMA et al. (1986) maintained that the content of free amino acids increased in kimchi because of the liberation of amino acids from plant proteins. Kimchi made with fermented fish (anchovy) sauce has a higher content of free amino acids and a richer flavor.

Acetic and lactic acids, generated by LAB activity, and several other acids contributed by the vegetable material, are important to kimchi flavor. Excess acidity is considered a spoilage problem, as will be discussed later. The optimum pH and total acidity for kimchi are 4.0–4.5 and 0.4–0.8%, respectively. Kimchis stored for an extended period of time may contain propionic, butyric, caproic, and heptanoic acids, which are believed to contribute to off-flavor (MHEEN and KWON, 1984).

The quality of kimchi fermented with 3% salt was reported to be superior to that with higher concentrations (MHEEN and KWON, 1984), and is the concentration used in commercial kimchi (Tab. 8). KIM and KIM (1990), who evaluated the sensory properties of low sodium kimchi, reported that they could replace more than 50% salt with potassium chloride without sacrificing kimchi flavor.

A firm, crisp texture is highly desired in the vegetable components of kimchi. Pectinmethylesterase exclusion (CHEONG et al., 1993) and heat treatments (55 $^{\circ}$ C, YOON et al., 1985) in the presence of 0.05 M CaCl₂ helped retain kimchi firmness. However, pre-heated kimchi was inferior in sensory quality to unheated controls (SONG et al., 1967). Fermentation temperature and time influence the firmness of kimchi (LEE and RHEE, 1986; JUNG and RHEE, 1986). Kimchi fermented at lower temperatures (6–10 $^{\circ}$ C) was firmer than that fermented at higher temperatures (22–24 $^{\circ}$ C). A

Tab. 8. Ingredient Composition for Commercially Produced Chinese Cabbage Kimchi

Ingredients	Composition (%) of Four Different Companies			
	A	B	C	D
Cabbage	73	85	76	85
Radish (shredded)	18	0	8	0
Salt	3	3	3	3
Red pepper powder	2	4	3	2
Garlic	0.7	2	0.8	1.7
Ginger	0.2	0	0.4	0.7
Green onion	0.4	0	2	3.8
Fermented fish sauce	0	5	2.5	0
MSG	0	<0.1	<0.1	0
Sugar	0	0.3	0.9	0.9

Adapted from LEE and CHO (1990)

similar result was reported by THOMPSON et al. (1979) that brined cucumbers retained firmness, provided the cucumbers are washed to remove softening enzymes and the storage temperature is 15.5°C or lower.

Freshly fermented, good quality kimchi should have rather distinct red and green colors contrasted against the white color of the cabbage stem. Kimchi made with red pepper powder of inferior quality may have a dull red color with a dark brownish tint, which is not desired. Kimchi loses its bright color and develops dullness in appearance when it is exposed to air after a long storage period, especially in the case of winter kimchi.

4.6 Spoilage Problems

Quality deterioration of kimchi during storage is mainly due to over-acidification, softening of vegetable tissues, the development of yeasty flavor, and a darkened appearance.

Over-acidification has been the problem most studied by Korean workers without much success. The growth and acid production by *L. plantarum* and *L. brevis* following the initial growth and activity of *L. mesenteroides* are regarded as undesirable in kimchi because they impart a harsh, strong acidic taste. Various methods have been tested to extend the storage period for good quality kimchi and to maintain pH 4.0–4.5 and acidity

of 0.4–0.8%. These include pasteurization after canning (85°C/25.2 min; LEE et al., 1968; GIL et al., 1984; LEE and CHUN, 1982) or in retort pouch (80–90°C/>10 or 95°C/>7 min; PYUN et al., 1983), gamma irradiation (KIM, 1962; CHA et al., 1989; LEE and LEE, 1965), addition of buffering agents (KIM and LEE, 1988; KIM, 1985), addition of antimicrobial agents (KWON and CHOI, 1967; SONG et al., 1966; CHOI et al., 1990), and others (KIM et al., 1991a; LIM et al., 1989; LEE et al., 1993; HONG and YOON, 1989; UM and KIM, 1990). Even though many workers claim they were successful, the effects were only marginal in most cases. Pasteurization seems to be more successful in preventing over-acidification, but the flavor defect caused by heating is undesirable.

Only a method which allows the growth and activity of *L. mesenteroides* but restricts *L. plantarum* and *L. brevis* will successfully prevent over-acidification of kimchi. Any method or chemicals used have to be microbially selective to be successful.

BREIDT et al. (1994a) inoculated nisin-resistant *L. mesenteroides* into a kimchi mixture with appropriate amounts of nisin. Nisin inhibited the growth of Gram-positive bacteria except for the nisin-resistant inoculum, which successfully initiated fermentation. Further research is needed to evaluate the addition of nisin to control kimchi fermentation, as was earlier proposed by CHOI et al. (1990).

Softening is another problem of stored kimchi but is not as serious a problem as over-acidification. It is caused by autolytic pectic enzymes of the vegetables. Pectinolytic enzymes of Chinese cabbage have been isolated and studied (BAEK et al., 1989). These scientists recommended that preheating cabbage at 50°C for 1.5 h in 0.05 M CaCl₂ solution will ensure crispness and firmness of kimchi. YOOK et al. (1985) made kimchi with preheated radish root and obtained a maximum firmness at 55°C for 2 h in 0.05 M CaCl₂ solution. This condition was mentioned to be optimal for pectinesterase activity, but inhibitory for polygalacturonase, a softening enzyme. Free carboxyl groups exposed due to pectinesterase activity are believed to form cross-linkages with Ca²⁺ to make tissues firmer. There are reports that firmness is increased in kimchi by adding sodium acetate (UM and KIM, 1990) and calcium acetate (KIM et al., 1991b).

Yeasty off-flavor occurs in winter kimchi due to air exposure after being stored for an extended period of time. Winter kimchi loses its bright red, green, and white colors and gains a dull dark color under these conditions. Very little research has been done to deal with these problems, but air exclusion seems important for their prevention.

5 Culture Development for Vegetable Fermentations

Currently, most vegetable fermentations rely on the natural microflora, although use of starter cultures has been suggested by various authors (ETCHELLS et al., 1973; DAESCHEL and FLEMING, 1984; HAMMES, 1990; LUCKE et al., 1990; BUCKENHUSKES, 1993). Various reasons have been proposed to explain the lack of commercial use of cultures, including economics, lack of sufficiently unique and valuable properties to justify their use, and the fact that vegetables will undergo a natural lactic fermentation under proper en-

vironmental conditions (FLEMING et al., 1985). However, recent research with cucumbers, sauerkraut, and olives indicates that use of special cultures for vegetable fermentations may find application in the near future. This section is focused upon methods that have been used for characterization and development of LAB strains for use in vegetable fermentations. Such methods include mutation and/or selection of unique cultures, marking of cultures for differential enumeration, and determination of growth kinetics.

5.1 Mutation and/or Selection of Cultures

In selecting cultures for food use, the first concern must be for impact of the culture on safety of the food for human consumption. Health concerns have been raised about the production of D(-)-lactic acid and biogenic amines by LAB during vegetable fermentations. Most LAB involved in vegetable fermentations produce DL-lactic acid, with the exception of *L. mesenteroides*, which produces D(-)-lactic acid (reviewed in FLEMING et al., 1985). Due to the difficulty of metabolism of D(-)-lactic acid, the WHO (1974) has suggested that infants should not consume D(-)- or DL-lactic acid. However, no limitation was placed on adult consumption of DL-products. Consumer demand for L(+)-lactate containing foods in Europe has led to the development of LAB starter cultures producing only this isomer for the fermentation of vegetables and vegetable juices (STETTER and STETTER, 1980; HAMMES, 1990; BUCKENHUSKES et al., 1990; BUCKENHUSKES, 1993). The production of biogenic amines (including histamine, putrescine, spermidine, and others) during the fermentation of vegetables has also been investigated (RICE et al., 1976; BRINK et al., 1990; HUIS IN 'T VELD et al., 1990; BUCKENHUSKES, 1993). Biogenic amines are formed by the decarboxylation of amino acids and are potentially hazardous to human health. Many LAB species are capable of decarboxylating one or more amino acids, producing biogenic amines. KUNSCH et al. (1989) demonstrated that a natural fer-

mentation of sauerkraut produced an excess of 200 $\mu\text{g/mL}$ of putrescine, along with other biogenic amines. They also showed that the production of biogenic amines could be substantially reduced using an *L. plantarum* starter culture.

Other consequences of LAB metabolism, such as the production of carbon dioxide by malolactic fermentation, can be considered in the development of bacterial starter cultures. McFEETERS et al. (1982b) found that the malolactic fermentation by LAB was a source of carbon dioxide in the fermentation of cucumbers. Prior to the introduction of purging technology for the pickle industry (ETCHELLS et al., 1973), product losses due to bloater damage were a major concern. While carbon dioxide is formed in cucumber fermentations from the respiration of cucumber tissue (FLEMING et al., 1973a), McFEETERS et al. (1984) showed that carbon dioxide formed from the malolactic reaction of *L. plantarum* was a direct cause of bloater damage. Toward this end, DAESCHEL et al. (1984) mutagenized *L. plantarum* and selected strains, using a differential medium, that do not produce CO_2 from malate. One mutant, MOP3-M6, has been further characterized and tested for use in vegetable fermentations (MCDONALD et al., 1993; BREIDT and FLEMING, 1992). BREIDT and FLEMING (1992) developed a selective medium (MS agar medium) that only allows LAB strains that carry out a malolactic fermentation (MDC^+) to grow. Using MS medium, they found that the LAB population in cucumber fermentations is predominantly composed of MDC^+ strains. They found that the rapid degradation of malate in cucumber fermentations could be prevented by inoculation of the fermentations with 10^6 CFU/mL of the MOP3-M6 starter culture. The conditions that would ensure the predominance of the starter culture, however, and therefore prevent the fermentation of malate, remain unclear (MCDONALD et al., 1993).

Many LAB strains have been shown to produce bacteriocins; these peptide antibiotics have been investigated for use in a wide variety of food products (RAY, 1992a, b; DAESCHEL, 1992). Intensive research has focused on the use of the bacteriocin, nisin (produced by *L. lactis*), and pediocins (pro-

duced by *Pediococcus* strains as food preservatives (reviewed by DE VUYST and VANDAMME, 1994; RAY, 1992a, respectively). Nisin has a broad spectrum of bacteriocidal activity against Gram-positive bacteria. Genes encoding nisin production, and immunity have been cloned and sequenced (reviewed in RAUCH et al., 1994), and the mechanism of action of nisin has been investigated (DE VUYST and VANDAMME, 1994). Bacteriocin-producing LAB have been investigated for use in vegetable fermentations. FLEMING et al. (1975a) reported that a *P. pentosaceus* strain, isolated from cucumber fermentation brines, produced a pediocin that was inhibitory to a variety of LAB species. HARRIS et al. (1992a) isolated a nisin-producing *Lactococcus* strain from fermenting cabbage and proposed using this bacteriocin-producing strain to control the fermentation of cabbage in the production of sauerkraut. In mixed culture broth fermentations, HARRIS et al. (1992b) demonstrated the production of nisin and the inhibition of *L. plantarum* (Fig. 13). BREIDT et al. (1994a), using this nisin-producing *Lactococcus*, demonstrated the effect of the nisin produced on the indigenous microflora in cabbage fermentations and isolated a nisin-resistant mutant of *L. mesenteroides* for use in cabbage fermentations (BREIDT et al., 1993). JIMENEZ-DIAZ et al. (1993) isolated a bacteriocin-producing *L. plantarum* strain from green olive fermentations. RUIZ-BARBA et al. (1994) demonstrated the use of this strain in controlling the microflora of olive fermentations. These experiments have shown that bacteriocin-producing LAB starter cultures can be effective in controlling the microflora in vegetable fermentations and will likely inspire the development of commercial starter cultures in the future.

The potential for bacteriophage problems with starter culture systems for vegetable fermentations should be considered (BUCKENHUSKES, 1993). The presence of bacteriophages in dairy fermentations has been recognized since 1935 (for review see LUNDSTED, 1983). It seems possible that any pure culture system may fall prey to bacteriophages. One reason that bacteriophages have not been observed in natural vegetable fermentations may be due to variation in the microflora and

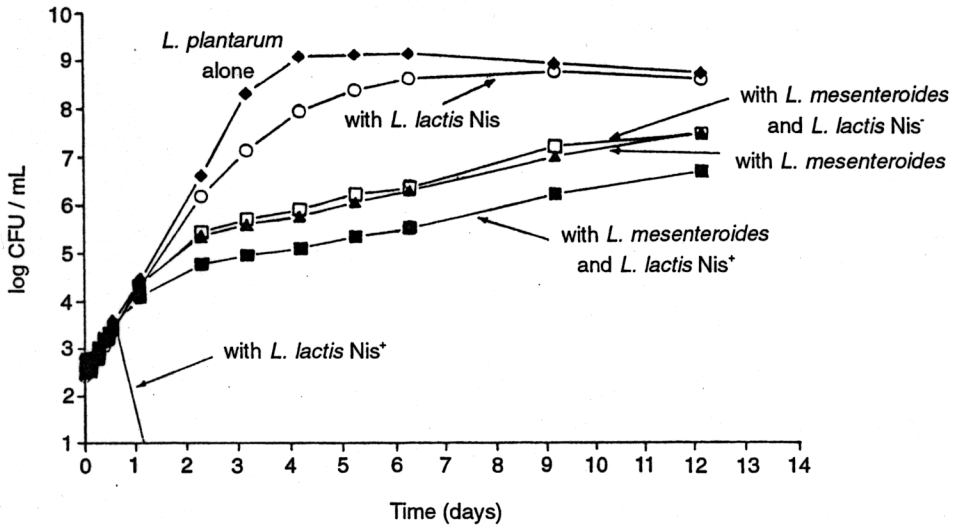


Fig. 13. Growth of *Lactobacillus plantarum* in a model sauerkraut fermentation. Growth of Nis⁺ *L. plantarum* ATCC 14917 in pure culture and mixed culture with Nis⁺ *Leuconostoc mesenteroides* NCK293, Nip⁺ *Lactococcus lactis* subsp. *lactis* NCK401, or Nip⁻ *L. lactis* subsp. *lactis* NCK402 in a model sauerkraut fermentation. Initial inoculum levels were 4×10^5 CFU/mL (*L. lactis* subsp. *lactis*), 2×10^3 CFU/mL (*L. mesenteroides*), and 3×10^2 CFU/mL (*L. plantarum*). These studies were done in cabbage juice broth. From HARRIS et al. (1992b).

a succession of LAB species during the course of the fermentation. RUIZ-BARBA et al. (1994) showed the rise and fall of naturally present *Lactobacillus* species between 20 and 30 days into an olive fermentation. They found 14 different *Lactobacillus* strains by following plasmid profiles. Little is known about the numbers of different strains of any given species in vegetable fermentations.

5.2 Enumeration of Starter Cultures in Vegetable Fermentations

Genetically marked cultures can be used to investigate the growth of starter cultures in food fermentations (RUIZ-BARBA et al., 1994; FOEGEDING et al., 1992; BREIDT and FLEMING, 1992; WINKOWSKI and MONTVILLE, 1992; FLEMING et al., 1988a). The availability of plasmid vectors for LAB with antibiotic resistance genes has greatly simplified the task of marking LAB cultures. A

marked culture can be selectively enumerated from a mixed population by taking advantage of the antibiotic resistance phenotype. It should be noted that these genetically marked strains are restricted to laboratory studies and are not meant for human consumption. The technology for isolation of plasmids from LAB (ANDERSON and MCKAY, 1983; KLAENHAMMER, 1984), transformation of LAB by electroporation (LUCHANSKY et al., 1988; SUVOROV et al., 1988; DAVID et al., 1989), and rapid analysis of LAB strains for the presence of plasmids (ANDERSON and MCKAY, 1983; ORBERG and SANDINE, 1985) has opened LAB to the tools of molecular biology. Plasmid cloning vectors, such as pGK12 (KOK et al., 1984), have been generated by marking cryptic plasmids from various strains of LAB with antibiotic resistance genes. The chloramphenicol acetyltransferase gene and the *ermC* methyltransferase gene (encoding resistance to erythromycin and other macrolide antibiotics) originally from the staphylococcal plasmids pC194 (IORDANESCU and SURDEANU, 1980) and pE194

(IORDANESCU et al., 1987), respectively, have been widely used for this purpose. pGK12 has been used to enumerate LAB starter culture strains in a variety of foods (FOEGEDING et al., 1992; BREIDT and FLEMING, 1992; WINKOWSKI and MONTVILLE, 1992). An alternative strategy has been to develop streptomycin or rifampin-resistant LAB starter culture strains by sequential selection for these phenotypes on increasing concentrations of the antibiotics (FLEMING et al., 1988a; RUIZ-BARBA et al., 1994). The use of either method must be validated by determining the stability of the antibiotic resistance marker. Experiments using genetically marked LAB starter cultures can be limited by the interference of bacterial populations in the indigenous microflora that are resistant to the antibiotic(s) used for selection of the marked strain. Antibiotic-resistant lactic acid bacterial isolates, from a variety of sources, including meats, dairy, and other products, have been reported (ORBERG and SANDINE, 1985; VESCOVO et al., 1982; VIDAL and COLLINS-THOMPSON, 1987), as well as the spontaneous appearance of antibiotic-resistant LAB mutants (CURRAGH and COLLINS, 1992).

Experiments with marked starter cultures to investigate the change in the microflora have been carried out. The dominance of a *L. plantarum* starter culture inoculated at 10^6 CFU/mL and the effect of the starter culture on the indigenous microflora of a laboratory cucumber fermentation were demonstrated by BREIDT and FLEMING (1992). In these experiments the starter culture achieved a cell concentration of 1×10^9 CFU/mL, while the concentration of the natural microflora was shown to reach a maximum of 5×10^5 CFU/mL. The indigenous microflora were enumerated with the use of a selective medium that prevented the growth of the starter culture but permitted the growth of the naturally present LAB. However, similar experiments with commercial-scale fermentations showed a starter culture inoculated at 10^6 CFU/mL did not predominate in an experimental, anaerobic tank, cucumber fermentation (FLEMING et al., 1988a). The controlled olive fermentations carried out by RUIZ-BARBA et al. (1994) showed that a bacteriocin-producing *L. plantarum* would dominate a brined

live fermentation with an inoculum of 10^5 , but a non-bacteriocin-producing derivative strain added to brined olives at the same concentration would not predominate, and was lost from the fermentation. Clearly, further experiments with marked starter cultures and methods to determine the effect of the starter cultures on the indigenous microflora will be needed to understand the factors affecting the ecology of controlled vegetable fermentations.

5.3 Growth Kinetics

Determining the growth kinetics of the starter culture strain can be an important test of the fitness of the strain for use in controlling vegetable fermentations. For laboratory analysis of growth kinetics, it is convenient to use broth cultures for optical density readings. Automated microtiter plate methods have been developed that allow convenient and rapid determination of bacterial growth kinetics (THOMAS et al., 1985; BREIDT et al., 1994b). Large amounts of data can be generated using these automated methods (Fig. 14). Multi-well microtiter plates can allow replicates of batch fermentations with a variety of growth media, salt concentrations, organic acids, pH, and other conditions to be tested simultaneously. A limitation of these methods is the need for the optical clarity of the growth medium. Vegetable juices prepared as a growth medium for kinetic tests can have inhibitory compounds or stimulatory compounds present. For example, inhibitors in cabbage juice have been identified, which are present in fresh cabbage juice, and may affect the growth of LAB species (KYUNG and FLEMING, 1994). The effect of temperature on the growth kinetics of LAB species can be investigated with a temperature gradient or Arrhenius block. To determine the "temperature character" of a bacterial strain, an Arrhenius block can be used to grow batch cultures over a range of temperatures (DROST-HANSEN, 1977; ROMICK, 1994). Temperature character is a measurement of the range of temperatures over which a culture can grow. Models for determining the temperature character of bacterial strains have been devel-

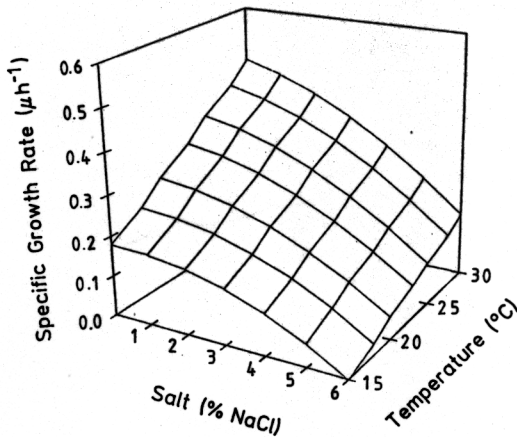


Fig. 14. The effects of temperature and NaCl on the growth kinetics of *Leuconostoc mesenteroides*. Response surface graph showing the surface determined from the predicted values for specific growth rates. From BREIDT et al. (1994b).

oped and characterized by a number of workers (RATKOWSKY et al., 1982; ADAIR et al., 1989; HANUS and MORITA, 1986).

Knowledge of the kinetics of growth, product formation, and substrate utilization by the fermenting bacterium is essential for modeling of vegetable fermentations. PASSOS et al. (1993) assessed the effects of key variables involved in cucumber fermentations and developed a model for predicting the growth of *L. plantarum*. Limiting conditions for growth in cucumber juice were pH 3.37 (lower limit), 69 mM undissociated lactic acid, 150 mM undissociated acetic acid, or 11.8% NaCl. A predictive equation, based on data collected, was developed and found to predict growth of *L. plantarum* reasonably well in batch cultures in cucumber juice. Later, a model was developed to describe substrate utilization and lactic acid production, in addition to growth by *L. plantarum* in cucumber juice (PASSOS et al., 1994).

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