Chapter

4

Mixed Cultures in Vegetable Fermentations

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Introduction

Many vegetables have been preserved by salting, with various degrees of fermentation, depending upon the salt concentration. Cucumbers, cabbage (for sauerkraut) and olives account for the largest volume of fermented vegetables in the western hemisphere, but smaller quantities of peppers, carrots, cauliflower, and okra are preserved by brining.[2] The fermentation of vegetables involves a complexity of physical, chemical, and microbiological factors that have been well characterized over the past several decades. The reader is encouraged to consider previous reviews on this subject[3] in addition to the original research cited.

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[3] Andersson et al. 1988; Etchells et al. 1975; Fernandez Diez 1983; Fleming 1982; Pederson and Albury 1969; Stamer 1983, 1988; Vaughn 1954, 1982.

Microbial activities during the natural fermentation and storage of vegetables have been divided into four stages: initiation of fermentation, primary fermentation, secondary fermentation, and postfermentation (Table 4.1). This classification was based upon characterizations of the fermentations of cucumbers, cabbage, and olives by various researchers during this century. This review is organized according to this classification to emphasize similarities and dissimilarities in the fermentation of these commodities. Although some overlapping of activities among stages occurs, criteria for distinguishing the stages are sufficiently distinct to serve as a guide in developing controlled fermentation methods.

During the past 30 years, efforts have been made to develop pure-culture and controlled fermentation methods, many of which are discussed herein. Some novel control procedures have been employed commercially, such as the purging of CO_2 from fermenting cucumbers to prevent bloater formation. However, comprehensive controlled fermentation methods such as those used in the production of alcoholic beverages and fermented dairy products have not been used commercially on a large scale. Economic and technical reasons are responsible for this state of affairs. It is conceivable that technological advancements will mandate commercial acceptance of improved methods for fermentation control. One such advancement could be the development of microbial cultures with novel and valuable properties. The likelihood of developing such cultures during the next decade seems reasonable, considering the recent progress in genetic technology of microorganisms. To take advantage of such microbial technology, however, other technological advances also must occur (e.g., improved

TABLE 4.1 Stages of Microbial Activities During the Natural Fermentation of Vegetables

Stage	Prevalent microorganisms (conditions)
Initiation of fermentation	Various gram-positive and -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)
Postfermentation	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)

SOURCE: Modified from Fleming, 1982.

methods for sanitization and containment of the vegetables). It is the intent of this review to provide a better understanding of the principles that govern successful fermentation of vegetables, with the hope of encouraging the development of improved controlled fermentation methods that may eventually become commercially acceptable.

Initiation of Fermentation

Many vegetables, in the presence of appropriate concentrations of salt (NaCl) and under suitable environmental conditions, will undergo fermentation by lactic acid bacteria (LAB). Although the number of LAB is usually quite low compared to the total number of microorganisms (Table 4.2), the LAB eventually predominate due to the production of acids and other products which restrict growth and survival of other groups of microorganisms. The rate and consistency of LAB gaining predominance also is a reflection of many factors, including initial populations of all microorganisms, physical and chemical properties of the vegetables, and the environment (physical and chemical) in which the vegetables are held. Each vegetable may reflect unique responses during initiation of fermentation as exemplified with cucumbers, cabbage, and olives. Salt is known to exert two important effects in its preservative role: It directs the course of microbial activities and it prevents softening of the vegetable tissue. Although salt is commonly added in vegetable fermentations, the concentration used (Table 4.3) and its effects on the fermentation and product quality vary widely.

Cucumbers

Pickling varieties of cucumbers are used in various methods for commercial processing, including brine fermentation. Fermentation accounts for about 40 percent of commercially processed cucumbers, with pasteurization (40 percent) and refrigeration (20 percent) ac-

TABLE 4.2 Microorganisms on Raw Vegetables Used for Fermentation

	Number/g fresh weight		
	Cucumber [a]		
Microorganism	Fruit	Flower	Cabbage[b]
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.

TABLE 4.3 Salt Concentrations Used for the Fermentation and Storage of Vegetables

Method of salting	Salt concentration, %[a]		Vegetable
	Fermentation	Storage	
Dry salting	1.5–2.5	1.5–2.5	Cabbage
Brining	5–8	5–16	Cucumbers
Brining	4–8	4–8	Green olives

[a]The concentrations of salt indicated generally are used by commercial firms in the United States. Modified from Fleming, 1982.

counting for the remainder (Fleming and Moore, 1983). The primary purpose of fermentation is to serve as a means for temporary preservation of the cucumbers in bulk tanks until they are needed for processing into various types of sweet and sour pickles. The fermented cucumber products may or may not be pasteurized, depending upon the type of product and its intended use.

The cucumbers may be harvested by hand or mechanically, and usually are graded by size before being placed in bulk tanks containing a salt solution where they are allowed to ferment. The cucumbers usually are not intentionally washed before brining, so the initial microbial load reflects the soil type and weather conditions to which the fruit were exposed, as well as harvesting and handling methods. The fruit may be rinsed if they are received in a water-cushioned hopper or pit or conveyed in liquid. Most companies do not brush-wash the fruit before brining, although washing has been suggested for use in controlled fermentation procedures (Etchells et al. 1973, Fleming et al. 1988a). The microbial load on the raw fruit may vary widely, particularly if the flower remains attached, as is more common with smaller fruit (Fig. 4.1).

The flower is of particular significance also because it may harbor pectinolytic enzymes from mold growth which may result in softening of the cucumbers. In the southeastern United States where softening was a severe problem with small cucumbers for many years, pectinolytic enzymes resulting from mold growth within the flowers were found to be the causative agents of softening (Bell 1951, Etchells et al. 1958). Previously, aerobic, spore-forming bacilli had been thought to be responsible for softening (Vaughn et al. 1954). The softening problem with small fruit was greatly reduced by removal of the flowers before brining or by draining of the cover brines from the cucumbers after about 37 h (Etchells et al. 1955). In recent years the practice of draining brine from cucumbers to prevent softening has been curtailed due to environmental problems associated with salt disposal. Attempts now are made to remove the flowers from fruit be-

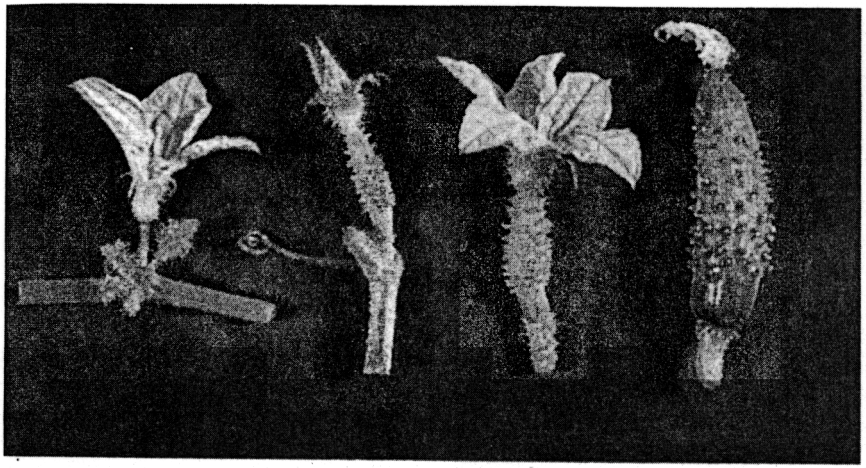

Figure 4.1 Flowers and immature fruit of the cucumber plant. (*From Etchells et al., 1953.*)

fore brining. Also, the addition of calcium salts to the cover brine has been shown to reduce softening of brined cucumbers (Fleming et al. 1978, 1987), even in the presence of mold polygalacturonase (Buescher et al. 1979, 1981).

The microflora are located mostly on or in the outer surface of raw cucumbers (Samish et al. 1963). For many years some scientists in the field considered the interior of healthy, fresh cucumbers to be sterile. However, it has been reported that the interior of fresh cucumbers may contain a latent population of bacteria (Samish and Dimant 1959, Meneley and Stanghellini 1974), probably of the family Enterobacteriaceae. Furthermore, it has been shown that bacteria may grow within the cucumbers during fermentation (Samish et al. 1963), and that the relative amount of fermentation by lactic acid bacteria within the cucumbers and in the brine surrounding the cucumbers can be manipulated by modifying the internal gas atmosphere of the fresh fruit before brining (Daeschel and Fleming 1981). Oxygen within the fresh cucumbers is quickly metabolized after the fruit are brined, and CO_2 is formed. The CO_2 is much more soluble than oxygen, thus a vacuum is formed within the fruit (Corey et al. 1983, Fleming et al. 1980). Bacteria may enter the fruit through stomata located in the skin (Fig. 4.2). Yeasts do not enter the fruit because of their larger size (Daeschel et al. 1985).

Cucumbers, graded by size, are conveyed into bulk tanks of 7500- to 75,000-L capacity containing brine (6 to 12% NaCl). The tanks are

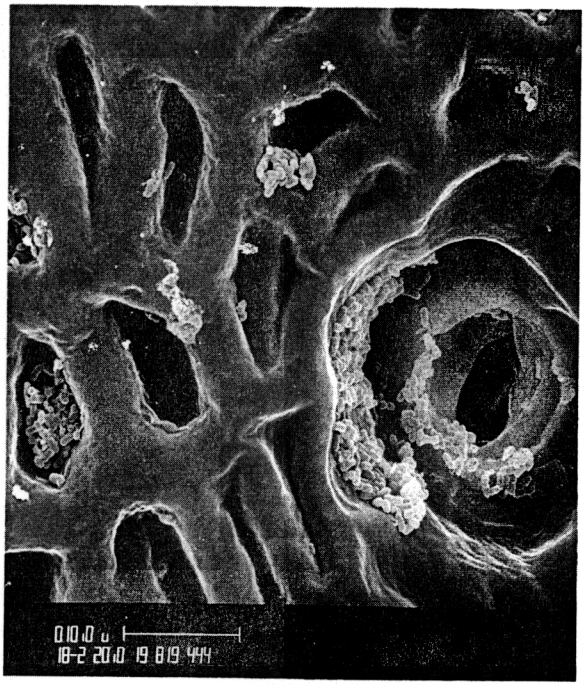

Figure 4.2 Bacteria associated with a stomate on the surface of a cucumber fermented by *L. plantarum*. Bar, 10 μm. (*From Daeschel and Fleming, 1981.*)

made of wood, fiberglass, or polyethylene. After the tanks are filled with cucumbers, wooden headboards are mounted over the cucumbers for their restraint after further brine addition. Additional brine is added to cover the cucumbers and to a level of 6 to 12 inches above the headboards, which are spaced or perforated to allow movement of liquid above and below the boards. The tanks (Fig. 4.3) are held outdoors to allow sunlight to strike the brine surface and thereby prevent growth of oxidative microorganisms (e.g., molds and film yeasts) at the brine surface. Dry salt is added onto the headboards as needed to attain the final equalized concentration for fermentation (5 to 8 percent), or to compensate for rainwater. Although open-top tanks have been used commercially for many years, recent efforts have been made to develop a closed-top (anaerobic) tanking system (Fleming et al. 1983a, 1988a), as is discussed later.

After brining, salt from the cover solution diffuses into the cucumbers and soluble substrates diffuse from the cucumber into the brine, the rate of diffusion being dependent upon the cucumber size and other variables (Potts et al. 1986). The cucumbers become flaccid to

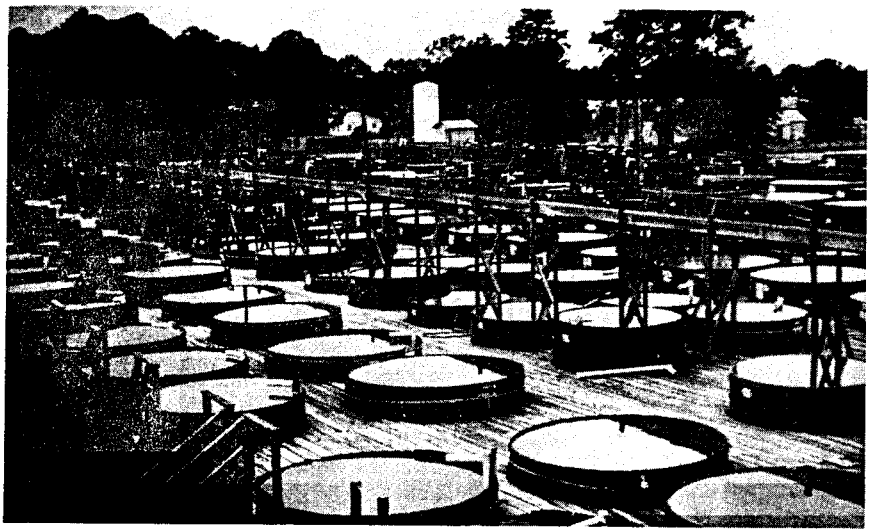

Figure 4.3 A tank yard for the fermentation and storage of pickling cucumbers. The tops of the tanks are open to the atmosphere, necessitating exposure of the brine surface to sunlight to prevent surface growth of yeasts and molds. Note the tall, white tank in the center background which is used for liquid nitrogen storage. Nitrogen gas is piped to all tanks for use in purging of CO_2 from fermenting brines to prevent bloater damage. (*From Fleming, 1984.*)

varying degrees, depending upon fruit size and concentration of salt, due to osmotic differential. Bacteria may be drawn into the fruit due to the vacuum created therein (Daeschel and Fleming 1981).

Cabbage

The microflora of cabbage are more numerous on outer leaves and diminish in number within the head and the relative number of LAB is higher within the head (Keipper et al. 1932). Cabbage heads are trimmed of green, outer leaves, which amounts to about 30 percent weight loss, before being further processed. The cabbage then may be washed, which reduces the total microflora in relation to the LAB (Keipper et al. 1932). The heads are then cored (the core is either removed or diced mechanically while within the head) and sliced (Stamer 1983). Efforts are made to avoid inclusion of discolored or diseased cabbage and foreign material before shredding, since it is practically impossible to do so later. Dry salt is sprinkled onto the shredded cabbage before it is conveyed into the fermentation vessel.

Fermentation vessels may be constructed of reinforced concrete, wood, or fiberglass and hold 20 to 180 tons of shredded cabbage (Stamer 1983). Brine is quickly generated due to osmotic conditions created by the added salt. The salt added typically constitutes 2 to

2.25 percent (w/w) of the shredded cabbage in the United States. Kandler (1981) reported use of 0.75 to 1.5 percent salt in German kraut manufacture. The amount of salt influences the amount of brine generated and the rate and type of microbial action. The concentration of salt necessary to ensure acceptable texture of sauerkraut is much lower than that of cucumbers, although softening has been attributed to low salt concentration (Pederson and Albury 1969). Neither enzymatic activity nor microorganisms responsible for such softening have been well characterized. The relatively low salt concentration and more immediate availability of nutrients from the sliced cabbage result in more rapid initial growth of microorganisms than in the case of cucumbers.

Olives

While differences in the fermentation of cucumbers and cabbage can be attributed largely to differences in salt concentration used and physical handling of the product, the fermentation of olives is unique partly because of the presence of the bitter phenolic glucoside, oleuropein (Fig. 4.4). In the Spanish-style process for production of fermented green olives, the olives are exposed to alkali to degrade the oleuropein (thereby eliminating bitterness) and then washed to remove excess alkali. Then the olives are placed in brine (to equalize with the olives at about 7% NaCl) where they are allowed to ferment. The initial microbial load is influenced by the alkali treatment, in addition to other variables.

The possible presence of a microbial inhibitor(s) in olives has been conjectured for many years, and oleuropein was suggested as an inhibitor of the LAB (Vaughn 1954). It has been shown, however, that oleuropein itself is not very inhibitory to the lactic acid bacteria, but certain of its hydrolysis products are (Fleming et al. 1973c). The aglycone of oleuropein and elenolic acid (Fig. 4.4) both have been shown to inhibit LAB. Further evidence that oleuropein is not appreciably inhibitory is the fact that it has been shown to serve as a sub-

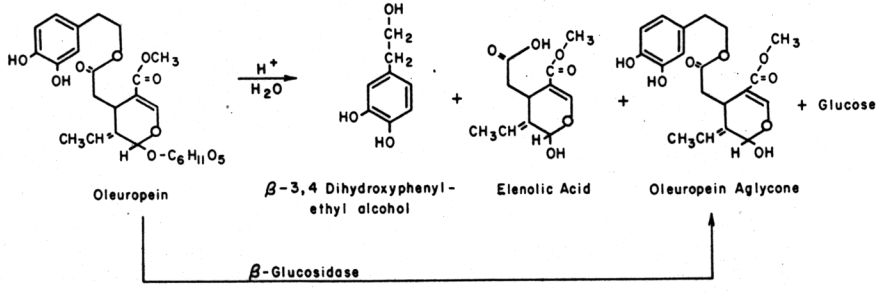

Figure 4.4 Oleuropein and its hydrolysis products. (*From Walter et al., 1973.*)

strate for the LAB (Garrido Fernandez and Vaughn 1978). If green olive varieties containing appreciable levels of oleuropein (e.g., Manzanillo, Mission) are brined without (or inadequate) lye treatment, they will undergo fermentation by yeasts and not LAB (Etchells et al. 1966). Neither the hydrolysis products of oleuropein nor oleuropein itself inhibit yeasts species tested (Fleming et al., 1973c).

It has been speculated that inhibitory hydrolysis products of oleuropein are formed when olives are brined (Fleming et al. 1973c). However, the mechanism by which these products are formed has not been established. It is thought that beta-glucosidase or a similar enzyme hydrolyzes oleuropein to the inhibitory compounds after the olives are brined. In fact, exposure of pure oleuropein to beta-glucosidase has been shown to produce the aglycone, which is inhibitory (Walter et al. 1973).

More recent studies have shown that phenolic compounds from olives previously shown to be noninhibitory, when tested in complex media, were inhibitory when *Lactobacillus plantarum* was exposed to the purified compounds in water (Ruiz Barba et al. 1990). These authors suggested that the presence of organic nitrogenous compounds in complex media can mask the bactericidal effects because of binding with the phenolic compounds. Thus, while the mechanism of inhibition of LAB in green olive fermentation has not been fully established, continuing interest in the subject promises to reveal new insights.

Some types of olives are not treated with alkali before fermentation either because they contain low levels of oleuropein and, thus, are low in bitterness (e.g., Sicilian type); or they are allowed to ripen on the tree before harvest, which apparently reduces the bitterness (Greek-type ripe olives); or they are allowed to ferment by yeasts (for processing into ripe olives). More detailed accounts on various types of olive fermentations are available (Fernandez Diez 1983; Fernandez Diez et al. 1985; Vaughn 1954, 1982). The present review will emphasize the Spanish-type process, since it accounts for the largest quantity of fermented olives and the yeast fermentation of non-alkali-treated olives. The salt-free storage of olives without fermentation for processing into "green-ripe" olives has become widely used in the United States in the last 20 years due to salt disposal problems, but will not be discussed herein (see Vaughn et al. 1969b, Vaughn 1982).

Primary Fermentation

Lactic acid bacteria

Four genera of lactic acid–producing bacteria have been reported in fermenting vegetables (Table 4.4). *Streptococcus* species occur on the fresh vegetables (Mundt et al. 1967) and may be present in the initi-

TABLE 4.4 Lactic Acid–Producing Bacteria Involved in Vegetable Fermentations

Genus and species	Fermentation type[a]	Main product (molar ratio)	Configuration of lactate
Streptococcus faecalis	Homofermentative	Lactate	L (+)
Streptococcus lactis	Homofermentative	Lactate	L (+)
Leuconostoc mesenteroides	Heterofermentative	Lactate:acetate: CO_2 (1:1:1)	D (−)
Pediococcus pentosaceus	Homofermentative	Lactate	DL, L (+)
Lactobacillus brevis	Heterofermentative	Lactate:acetate: CO_2 (1:1:1)	DL
Lactobacillus plantarum	Homofermentative	Lactate	D (−), L (+), DL
	Heterofermentative[b]	Lactate:acetate (1:1)	D (−), L (+), DL
Lactobacillus bavaricus	Homofermentative	Lactate	L (+)

[a]With respect to hexose fermentation.
[b]Heterofermentative with respect to pentoses (facultatively heterofermentative).
SOURCE: Adapted from Kandler, 1983.

ation stage of fermentation. Their significance in the primary fermentation and upon product quality has not been established. The other three genera (*Leuconostoc, Pediococcus*, and *Lactobacillus*) characteristically appear in vegetable fermentations. The relative numbers, time of occurrence, and significance of each vary with the vegetable commodity, salt concentration, and temperature, as is more fully discussed later in reference to specific vegetables.

Fructose and glucose are the principal fermentable carbohydrates in vegetables, although small quantities of sucrose and other sugars may be present. Fermentation of fructose and glucose yields mainly lactic acid by homofermentative and facultatively heterofermentative species, but obligately heterofermentative species produce CO_2, ethanol, acetic acid, and mannitol in addition to lactic acid. Organic acids of the vegetables also may be metabolized. Malic acid is degraded to lactic acid and CO_2 (McFeeters et al. 1982b). Metabolism of other organic acids such as citric (to acetic, pyruvate, and CO_2) by LAB has been reported (DuPlessis 1964). Similar reactions might be expected in vegetable fermentations, depending upon presence of the acids and appropriate species of bacteria. Amino acids may be decarboxylated to yield CO_2 and amines (Rodwell 1953).

Yeasts

Yeasts may or may not be involved in the primary fermentation of vegetables, depending upon their occurrence on the raw product and

the chemical and physical environments in which the product is fermented. Species of yeasts associated with the fermentation of cucumbers and olives are summarized in Table 4.5. Little information has been published on the involvement of yeasts in the fermentation of sauerkraut.

While the presence of oxygen has little influence on the fermentation products of LAB, it dictates metabolic end products of yeasts involved in vegetable fermentations. Under anaerobic conditions, yeasts produce mainly ethanol and CO_2 from hexoses. Aerobically, they produce mainly CO_2. Although anaerobic conditions normally prevail during vegetable fermentations, the use of air for purging of cucumber brines has been shown to encourage aerobic metabolism by yeasts present (Potts and Fleming 1979). Many species of yeasts can oxidize

TABLE 4.5 Yeasts Associated with the Fermentation of Cucumbers and Olives

Yeast[a]	Cucumbers[b]	Olives[c]
Candida diddensii		D
Candida krusei	B	C
Candida rugosa		C
Candida solani		C
Candida tenuis		C
Candida valida (C. mycoderma)		C
Debaromyces hansenii (D. membranaefaciens var. Holl.)	B	
Hansenula anomala	A	D
Hansenula subpelliculosa	A	C
Pichia membranaefaciens		C,D
Pichia ohmeri (Endomycopsis ohmeri)	B	
Rhodotorula sp.	B	
Rhodotorula glutinus var. *glutinus*		E
Rhodotorula minuta var. *minuta*		E
Rhodotorula rubra		E
Saccharomyces baillii (S. elegans)	A	
Saccharomyces delbrueckii	A	
Saccharomyces oleaginosus		D
Saccharomyces rosei	A	
Saccharomyces rouxii (Zygosaccharomyces halomembranis)	B	
Torulopsis candida		D
Torulopsis holmii	A	C
Torulopsis lactis-condensii (T. caroliniana)	A	
Torulopsis sphaerica (imperfect form of *Kluyveromyces lactis*)		C
Torulopsis versatilis (Brettanomyces versatilis)	A	

[a]Names consistent with Lodder (1970). Names in parentheses are synonyms used by the authors indicated.

[b]The letter "A" indicates Etchells et al. (1961) as the source, and they classified these as fermentative species. "B" indicates Etchells and Bell (1950) as the source of occurring in cucumber fermentations, and they classified these as oxidative species.

[c]The letter "C" indicates Mrak et al. (1956) as the source of occurring in green olive fermentations. The letter "D" indicates Duran-Quintana and Gonzalez-Cancho (1977) as the source of occurring in natural black olive fermentations. The letter "E" indicates Vaughn et al. (1969b) as the source occurring in green olive fermentations.

lactic and other organic acids aerobically, which can lead to spoilage if insufficient acids remain to inhibit growth of undesirable bacteria.

Until recently, yeasts have been considered to be incidental during primary fermentation of vegetables at best, and spoilage microorganisms at worst. Purging of brines to remove CO_2 and, thereby, preventing the formation of gas pockets within the vegetable has opened the possibility for use of yeasts and other gas-forming microorganisms in the fermentation, as is discussed below.

Cucumbers

Natural fermentation. Salt concentration can exert a great influence on the fermentation of cucumbers. In the early decades of this century, it was common commercial practice to use high concentrations of salt to prevent softening and other spoilage problems. Perhaps the best illustration of the effects of salt concentration on relative activities of LAB, yeasts, and Enterobacteriaceae during cucumber fermentation is in the work of Etchells and Jones (1943; Fig. 4.5). A companion paper summarizes the corresponding chemical changes at the three salt concentrations tested (Jones and Etchells, 1943; Fig. 4.6). These papers showed that a high salt concentration (15 percent) resulted in slow and limited growth by LAB and low acid production with vigorous yeast growth after about 14 days and high concentrations of CO_2. A low salt concentration (5 percent) resulted in rapid growth by LAB and high acid production, with limited yeast growth and low concentrations of CO_2.

Maintenance of structural integrity is highly important in the fermentation of whole vegetables such as cucumbers, which adds complications not encountered in the fermentation of liquid products such as wine and beer. Bloater damage (Fig. 4.7) results from gas accumulation inside the cucumbers during fermentation. Tissue softening may be caused by pectinolytic enzymes of either microbial (primarily fungal) origin (Etchells et al. 1958) or of the cucumber fruit itself (McFeeters et al. 1980). Off-flavors and off-colors also may result from improper fermentation. Bloater damage has been attributed to CO_2 production by yeasts (Etchells et al. 1953), Enterobacteriaceae (Etchells et al. 1945), heterofermentative LAB (Etchells et al. 1968b), and even homofermentative LAB (Fleming et al. 1973a). Sorbic acid has been used to reduce the yeast growth and bloater damage resulting therefrom (Costilow et al. 1957).

Pure culture fermentation. To ensure only growth of the added LAB culture, the natural flora must be inactivated or prevented from growing. Etchells et al. (1964) accomplished this by use of ionizing irradi-

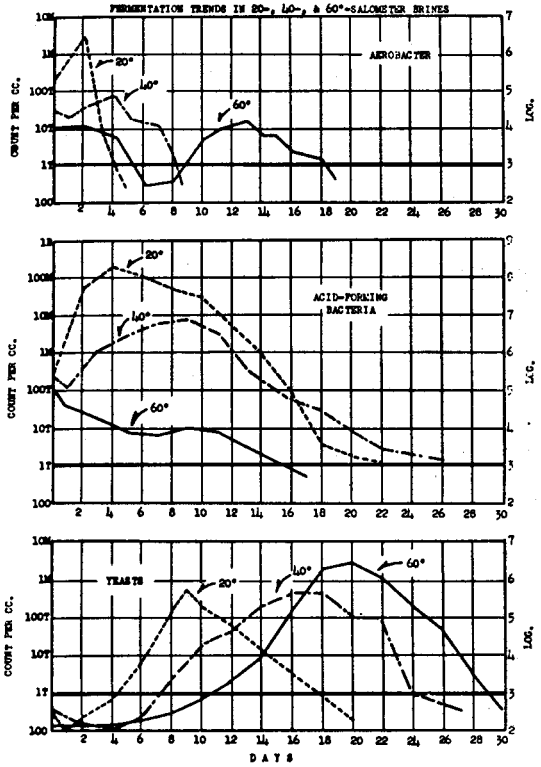

Figure 4.5 Growth of microorganisms in cucumber fermentations at initial brine concentrations of 20, 40, and 60°S (percent saturation with respect to salt = 5.3, 10.6, and 15.8%, w/w, respectively). (*From Etchells and Jones, 1943.*)

ation or heat shocking of the raw fruit and mild acidification of the cover brine. Fermentable sugars were not reported in this study, but, based on the author's experience, sugars undoubtedly remained in the fermentations with homofermentative LAB. Although the pure-culture procedure has been suggested for commercial production in small containers (Etchells et al. 1968*a*), it has not been considered practical for bulk fermentation due to energy requirements to inactivate the natural microflora and the aseptic conditions required for its success.

Controlled fermentation. Various efforts have been made to influence the fermentation of cucumbers by addition of cultures to the unpasteurized raw product. Such procedures are considered to be controlled rather than pure-culture fermentations, since the natural

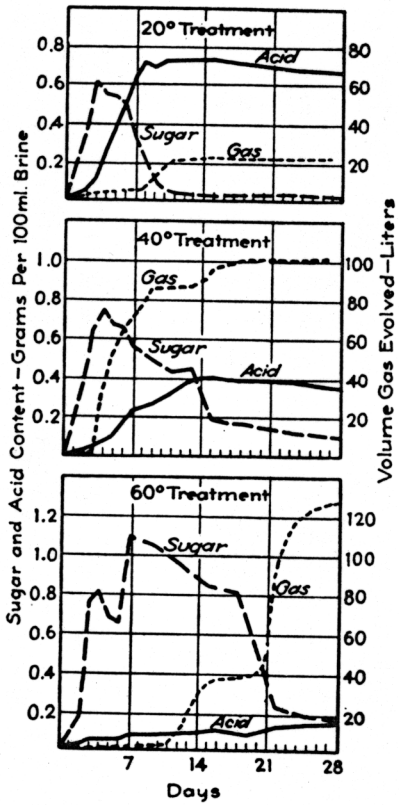

Figure 4.6 Chemical changes in cucumber fermentations at initial brine concentrations of 20, 40, and 60°S. (*From Jones and Etchells, 1943.*)

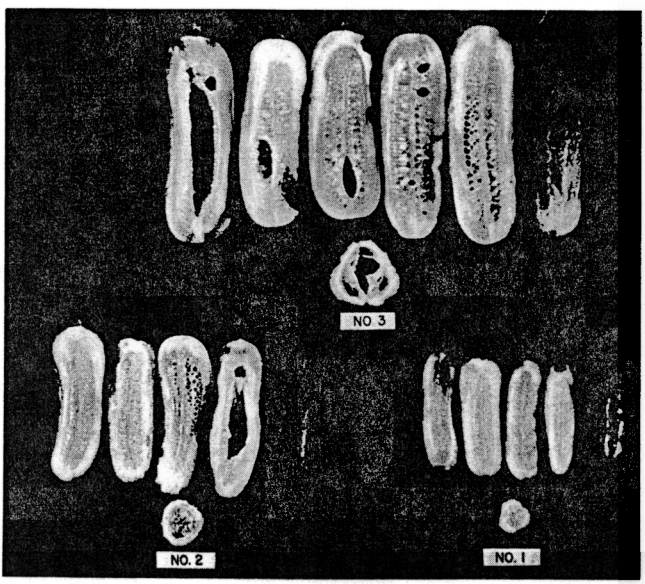

Figure 4.7 Bloater damage in fermented cucumbers. Note the more serious damage in larger fruit.

microflora may play a significant role. For example, Pederson and Albury (1961) found that *Lactobacillus* plantarum eventually predominated fermentations regardless of the species of LAB added initially. Apparently, the greater acid tolerance of *L. plantarum* permitted its growth after less-acid-tolerant species were inhibited by acid produced during fermentation.

The controlled fermentation procedure of Etchells et al. (1973) included the use of an acid-tolerant culture of *L. plantarum*. In addition, other control measures helped to assure success of the procedure. These measures included washing of the fruit before brining, chlorination and mild acidification of the cover brine, addition of sodium acetate buffer to assure complete sugar utilization, and nitrogen purging of CO_2 from the fermenting brine to prevent bloater formation. Certain features of this procedure have been successfully applied on a commercial scale, but the overall procedure has not been accepted commercially.

Purging of CO_2 from brines to prevent bloater formation represents an evolving story that continues today. Further research is needed and a historical perspective is warranted. The need for purging was recognized when cucumbers fermented by *L. plantarum* were observed to have bloater damage, even though there was no evidence of heterofermentative LAB or yeasts (Etchells et al. 1969, unpublished observation). This was surprising since *L. plantarum* does not produce CO_2 from glucose or fructose, the fermentable sugars of cucumbers. A subsequent study revealed that *L. plantarum* produced sufficient CO_2 from cucumber fermentation, which when added to that produced by the cucumber tissue, was sufficient to cause bloater damage (Fleming et al. 1973a). A nondestructive method for monitoring bloater formation, based on brine volume increase due to gas formation within the tissue, was used. Nitrogen purging was shown to relieve the expansion volume after bloater formation had occurred, or to prevent bloater formation altogether if CO_2 was continuously purged from the brine. The CO_2 produced during fermentation was shown to be derived about equally from the cucumber tissue and the *L. plantarum* culture (Fleming et al. 1973b). Respiratory CO_2 from the cucumbers was expected, but *L. plantarum* was not expected to produce CO_2. Later, malic acid was found to be the principal organic acid of cucumbers (McFeeters et al. 1982a) and was shown to be the major source of CO_2 produced by the *L. plantarum* starter culture (McFeeters et al. 1982b). A procedure was developed for mutation and selection of *L. plantarum* strains that do not produce CO_2 from malic acid (Daeschel et al. 1984; Fig. 4.8).

The pickle industry has adopted purging as a general practice, particularly for larger sizes of fruit that are more susceptible to bloater

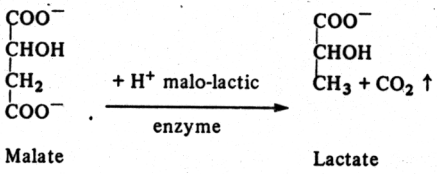

Figure 4.8 Proposed malolactic reaction in cucumber fermentation. (*From McFeeters et al., 1982b.*)

damage. Both nitrogen and air have been shown to effectively prevent bloater formation. Nitrogen has been recommended to avoid growth of undesired aerobic microorganisms and potential oxidative changes in flavor and color components (Etchells et al. 1973). However, air is used by many companies using a side-arm purging device described by Costilow et al. (1977) or modifications because it is less expensive.

Cucumbers fermented solely by *L. plantarum* may be too acidic in flavor without removal of part of the acid before final processing. Thus, use of microorganisms that produce neutral end products may be desirable. Heterofermentative LAB have been shown to ferment all of the fermentable carbohydrates in green beans with the production of a mild flavor (Chen et al. 1983a). *Lactobacillus cellobiosus* was found to be particularly efficacious in this regard and has shown promise for cucumber fermentations. Fermentative yeasts, historically considered to be spoilage agents because of bloater damage (Etchells et al. 1953), also have been considered as a means of producing more mildly acidic cucumber products. Fermentation of sterile cucumber juice could be manipulated to produce varying amounts of lactic acid and ethanol by mixed cultures of *L. plantarum* and the yeast *Saccharomyces cerevisiae* (Daeschel et al. 1988).

Before any procedure is accepted for commercial fermentation and storage of cucumbers, it must be economically justifiable. Furthermore, in order for controlled fermentation to occur, the environment in which the product is held must be controlled. Since the pickle industry has become accustomed to use of open tanks (see Fig. 4.3) for various reasons, the problem of control is exacerbated. However, the pickle industry has supported research to develop a closed-top tanking system for the anaerobic fermentation and storage of cucumbers. Pilot tanks and the overall handling system have been described (Fleming et al. 1983a). A simplified brining procedure was developed for use in closed tanks, which included addition of calcium acetate buffer to assure complete utilization of fermentable sugars (Fleming et al. 1988a). The calcium served to improve firmness retention in the cucumbers. Using the procedure in pilot tanks, approximately 95 percent of the fermentable sugars were converted to lactic acid, which is consistent

with the *L. plantarum* starter culture that was added. However, the starter culture did not predominate the entire fermentation, as is indicated by recovery of the marked culture in Fig. 4.9. Apparently, naturally occurring homofermentative LAB overcame the added culture after a day or so.

Sauerkraut

Natural fermentation. The natural fermentation of sauerkraut is characterized by a progression in the growth of LAB species, typically starting with the acid-sensitive *Leuconostoc mesenteroides* and terminating with the acid-tolerant *L. plantarum* (Pederson and Albury 1969). *Pediococcus cerevisiae* and *Lactobacillus brevis* species have been indicated to grow between these two extremes. Temperature and salt concentration are extremely important factors which regulate the type of fermentation. Optimum conditions to produce high-quality sauerkraut are temperatures of 13 to 24°C and salt concentrations of 1.8 to 2.25 percent (Pederson and Albury 1969). Various spoilage problems have been reported for sauerkraut including softening, discoloration, and off-flavors, some of which occur during primary fermentation due to low or uneven salt concentrations. The aggravating

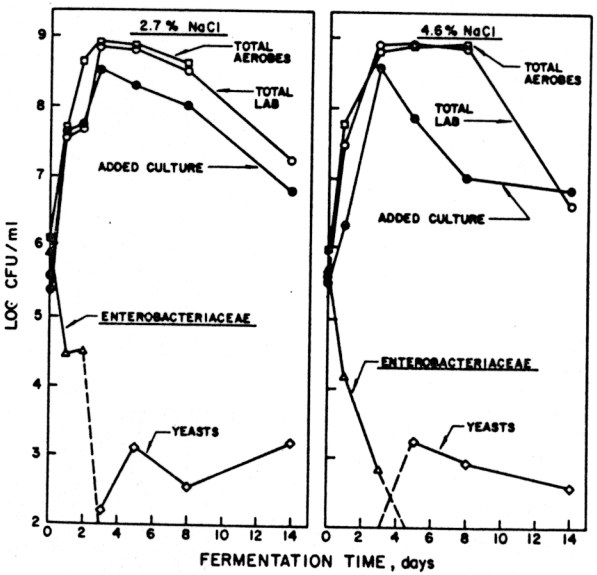

Figure 4.9 Microbial growth in brined cucumbers inoculated with an *L. plantarum* starter culture. Note that the added culture (marked with streptomycin resistance for detection) did not predominate the lactic acid fermentation at either 2.7 or 4.6% NaCl. (*From Fleming et al., 1988a.*)

problem of heaving occurs during the first day or so after shredded cabbage and salt are placed into the fermentation tank. This problem is due to the high concentration of CO_2 produced by the cabbage and by *L. mesenteroides* and other gas-forming bacteria early in the fermentation. After heaving, the tank must be reheaded. Tanks today typically are headed with a plastic cover weighted down by water, but the primitive method of weighting down the fermenting cabbage with stones is still practiced to a limited extent (Fig. 4.10).

Considerable differences exist between methods for sauerkraut production in the United States and certain other parts of the world. The above conditions cited by Pederson and Albury (1969) refer principally to sauerkraut produced in the United States. The sauerkraut is fermented and held in bulk tanks until it is needed for further processing. Thus, the acidity varies greatly, depending upon the initial concentration of fermentable sugars in the raw cabbage. In Europe, however, the sauerkraut is held only until the desired acidity is reached (e.g., 1 percent as lactic acid). The product is then canned, heat processing being used to stabilize the product to further fermentation. Thus, American processors retain the economic advantage of bulk storage but compromise on product uniformity in comparison to their European counterparts. There are additional economic reasons for the differences in methods of sauerkraut production beyond the scope of this review.

Controlled fermentation. Various attempts have been made to influence the fermentation of sauerkraut by addition of bacterial cultures

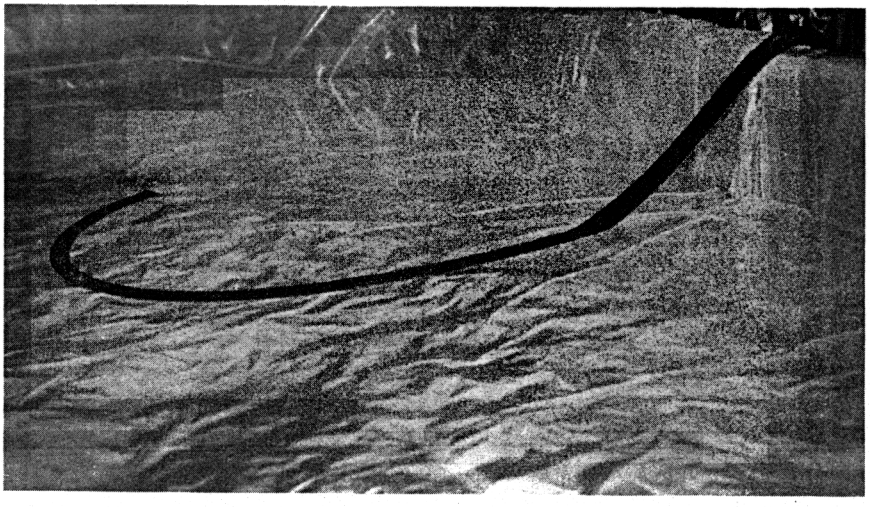

Figure 4.10a Heading of sauerkraut tanks with either water (*a*) or stones (*b*).

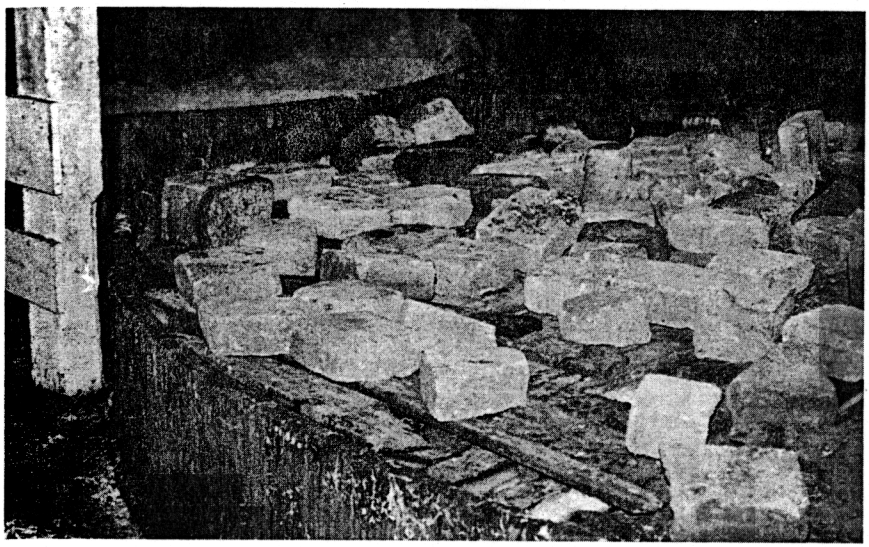

Figure 4.10b (*Continued*)

(Pederson and Albury 1969). However, some have concluded that culture additions are not needed to yield desirable sauerkraut, provided optimum temperature and salt concentrations are maintained (Pederson and Albury 1969, Stamer 1983). Stetter and Stetter (1980) isolated *Lactobacillus bavaricus* from sauerkraut, which produces exclusively L(+) lactic acid. A German patent has been granted for the production of sauerkraut with this culture (Eden-Waren 1976). There has been some health concern by the presence of high concentration of D(−) lactic acid (FAO/WHO 1966), thus the above culture may provide a significant advantage in such products. More recent indications are that the presence of D(−) lactic acid in the diet may only be important in infants (WHO 1974).

A significant factor influencing the uniformity of quality of sauerkraut, or lack thereof, is the distribution of salt within the product. Noel et al. (1979) have suggested a brine circulation system to ensure uniformity of salt and acid throughout the product. A fermenter has been designed which permits creation of a vacuum to prevent the heaving problem (Christ et al. 1981).

The sauerkraut fermentation is highly complex and involves physical, chemical, and microbiological factors which influence quality of the product. The fermentation has been broadly categorized into two stages, gaseous and nongaseous, based on studies using a specially designed laboratory fermentor (Fleming et al. 1988b). The physical, chemical, and microbiological changes that occurred during fermentation used to characterize these two stages are given in Figs. 4.11 to

4.13. During the first 2 days, CO_2 rapidly increased, and the brine level increased due to gaseous expansion within the shreds of cabbage (Fig. 4.11). Purging of CO_2 from the brine relieved the gas pressure, as evidenced by a decrease in the brine level (Fig. 4.11*a*). Heterofermentative LAB, presumably *L. mesenteroides*, predominated for approximately 8 days, after which homofermentative LAB predominated and continued to ferment until all sugar was fermented or fermentation ceased due to low pH. This conclusion is based on the evidence of rapid fructose depletion and rapid formation of mannitol, acetic acid, and ethanol, which are products of heterofermentative metabolism (Fig. 4.12). Also, the microbial growth profile indicated two stages of LAB growth, which included a rapid increase in numbers of LAB during the first 4 days (which were determined to be all gas-formers), followed by a decrease thereafter and a subsequent slight increase in numbers (all non-gas-formers) after 8 days (Fig. 4.13).

Kimchi

The early phase of *kimchi* fermentation is similar to sauerkraut fermentation. However, *kimchi* typically includes several vegetables (e.g., Korean cabbage, garlic, green onion, red pepper powder) that are allowed to ferment to only a slightly acidic product. In this regard, *kimchi* is highly perishable and must be consumed within a few days, depending upon temperature, before excessive acidity results. Like

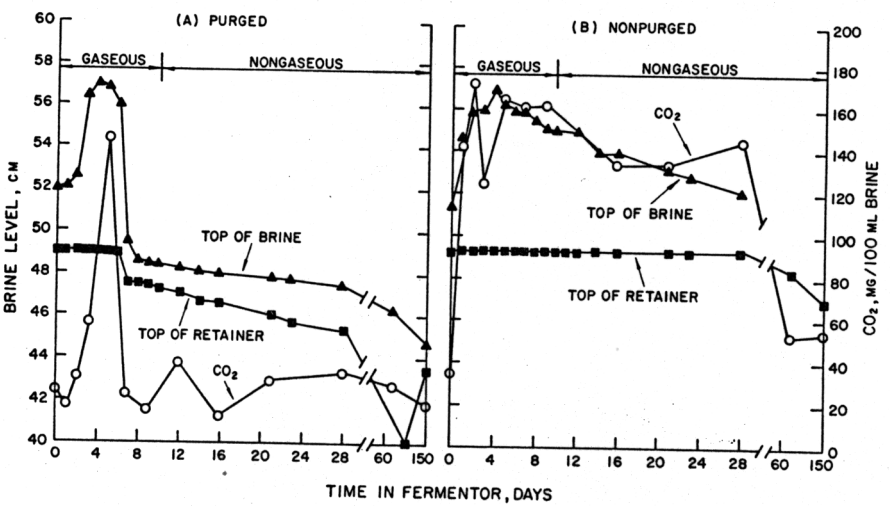

Figure 4.11 Changes in liquid and kraut bed volumes and in CO_2 concentration of purged and nonpurged sauerkraut fermentations. Note that purging of CO_2 from the brine (*a*) resulted in CO_2 reduction and reduced internal gas pressure, as evidenced by lower brine levels. (*From Fleming et al., 1988b.*)

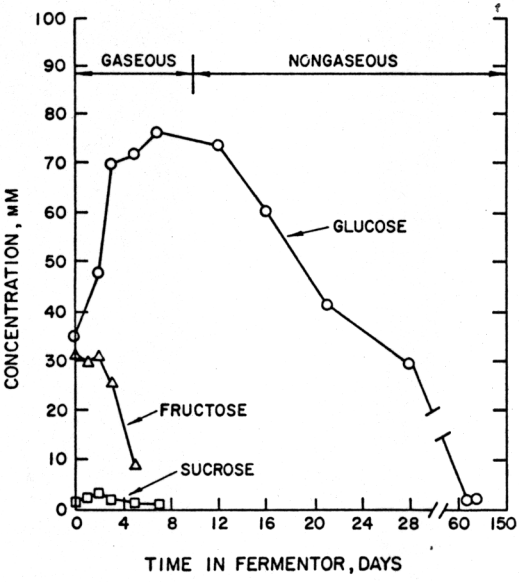

Figure 4.12a Substrate depletion in sauerkraut fermentation. (*From Fleming et al., 1988b.*)

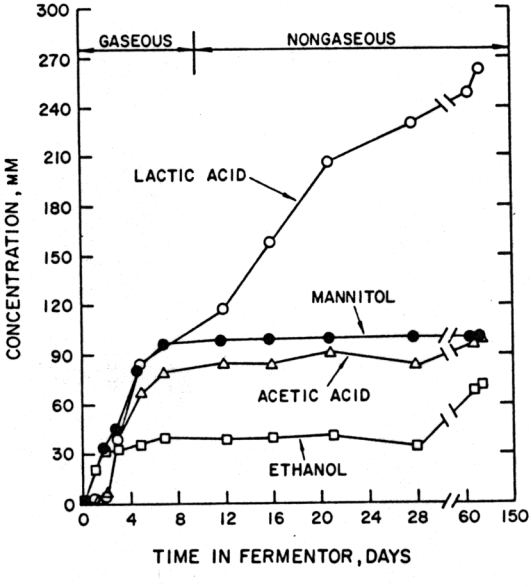

Figure 4.12b Product formation in sauerkraut fermentation. (*From Fleming et al., 1988b.*)

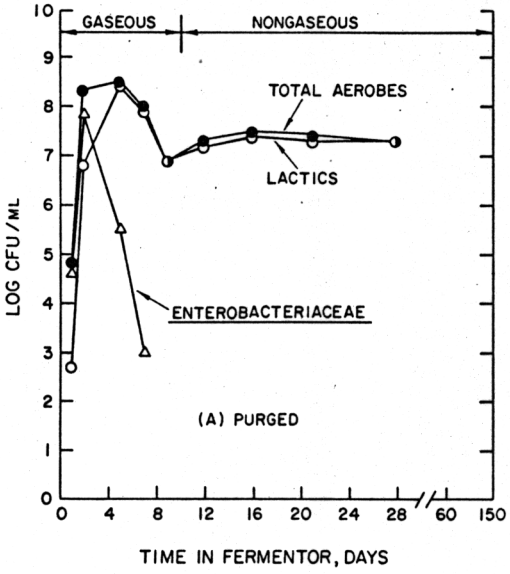

Figure 4.13 Microbial growth in sauerkraut fermentation. (*From Fleming et al., 1988b.*)

sauerkraut, *L. mesenteroides* is involved in the early phase of fermentation. *L. plantarum*, which grows later, is considered to be a spoilage microbe when it produces excessive amounts of acid (Mheen and Kwon 1984). The optimum pH, acidity (as lactic acid), and salt of *kimchi* was found to be 4.2, 0.6 to 0.8 percent, and 3.0 percent, respectively, and the temperature for achieving the best flavor to be 5 to 14°C (Mheen and Kwon 1984).

Olives

Natural fermentation. After alkali treatment and washing to remove excess alkali according to the Spanish-type process previously mentioned, the olives undergo fermentation by naturally occurring LAB. The species of LAB involved in the fermentation vary with the salt concentration used. Some olive varieties such as Sevillano (which are more susceptible to shrivel) are brined at 4 to 5% NaCl, while most varieties today are brined at 7 to 8% NaCl (Vaughn 1982). *L. plantarum* is consistently found in all fermentations, while other LAB may occur, depending on olive variety and salt concentration used (Vaughn 1982). *L. mesenteroides* and *Streptococcus faecalis* have been reported in olive fermentations at low salt concentrations, while *L. brevis* and *P. cerevisiae* may or may not occur at various periods during the fermentation (Vaughn et al. 1943, Vaughn 1982).

L. plantarum has been shown to be primarily responsible for the occurrence of "yeast spots" on the surface of fermented olives (Vaughn et

al. 1953). This blemish, which appears as raised white spots on the olive surface, may be considered by some to be unsightly, but the olives are not otherwise affected adversely. Gas pocket formation within the flesh of fermenting olives, however, is a serious defect. This problem is analagous to bloater formation in cucumbers and has been shown to be associated with the growth of gas-forming bacteria and yeasts (Vaughn 1982). Some of the gas-forming bacteria such as Enterobacteriaceae (Vaughn et al. 1943, West et al. 1941) or butyric acid bacteria (Gilliland and Vaughn, 1943) may grow during the initiation stage of fermentation, before primary fermentation by lactic acid bacteria becomes established. These problems likely are associated with the improper removal of alkali and resultant initial high pH. Most processors add sufficient acid to the brine today to lower the pH to 4.5 to 5.0 (Vaughn 1982), which should reduce these problems. Tissue softening and malodorous fermentations also result from growth of these same types of bacteria (Vaughn 1982).

Yeasts also may be active during fermentation of Spanish-type olives (Table 4.5). Mrak et al. (1956) reported predominantly fermentative yeasts during the first 7 weeks of olive fermentation and a more aerobic flora during weeks 9 to 16. Some yeasts have been associated with the softening and gas-pocket formation of brined olives (Vaughn et al. 1969a, 1972). In non-alkali-treated olives, yeasts are primarily responsible for the fermentation (Duran Quintana and Gonzalez Cancho 1977; Table 4.5).

Spanish researchers have begun to explore ways to take advantage of yeasts for the fermentation of non-alkali-treated olives for the production of ripe olives. Two relatively recent areas of study involve naturally ripe black olives (i.e., olives that are allowed to ripen on the tree before harvest) and post-harvest-ripened black olives (i.e., olives that are harvested green and subsequently debittered and blackened by the use of NaOH and air).

The fermentation of naturally ripe black olives of four varieties was shown to be due principally to yeasts (Duran Quintana et al. 1971). Naturally occurring yeasts from the fermentation have been identified (Duran Quintana and Gonzalez Cancho 1977, Gonzalez Cancho et al. 1975; Table 4.5). A serious problem with this type of fermented olive is the formation of gas pockets within the flesh, referred to as *alambrado* by Spanish researchers. The problem is analogous to bloater formation in brined cucumbers. The problem is more severe in tree-ripened olives than in green olives, due apparently to the softer flesh of the tree-ripened fruit. Purging of CO_2 from the brine with air or nitrogen prevented *alambrado* (Garcia Garcia et al. 1982). Procedures for air purging of brines to prevent the problem have been described (Duran Quintana et al. 1986, Garcia Garcia et al. 1985). More

recently, air purging has been shown to be useful in the fermentation of green olives for postharvest ripening (Brenes Balbuena et al. 1986a,b).

Controlled fermentation. Inoculation of alkali-treated green olives has been done with pure cultures of LAB (Borbolla y Alcala et al. 1952, 1964; Cruess 1937; Etchells et al. 1966) and with actively fermenting brine (Cruess 1930). Commercially, however, inoculation has not been practiced extensively. Although various spoilage problems occur, apparently none has justified the use of cultures. Stuck fermentations (failure to develop acidity) is one such problem that occurs occasionally. The causes of stuck fermentations are not always understood. Lack of sufficient sugar and the presence of compounds inhibitory to LAB have been suggested as possible reasons (Vaughn 1954). The inhibitory compound(s) of olives is inactivated by alkali (Fleming and Etchells 1967, Juven et al. 1968), which is coincidental to the use of alkali to remove bitterness. The presence of such inhibitors complicates the use of LAB cultures. When cultures were added to brined olives that had been neither alkali-treated nor heated, only yeasts grew (Etchells et al. 1966). The LAB culture was inhibited. When heat-shocked (74°C, 3 min), however, the olives underwent rapid fermentation (Fig. 4.14). *L. mesenteroides* was found to be more inhibited than

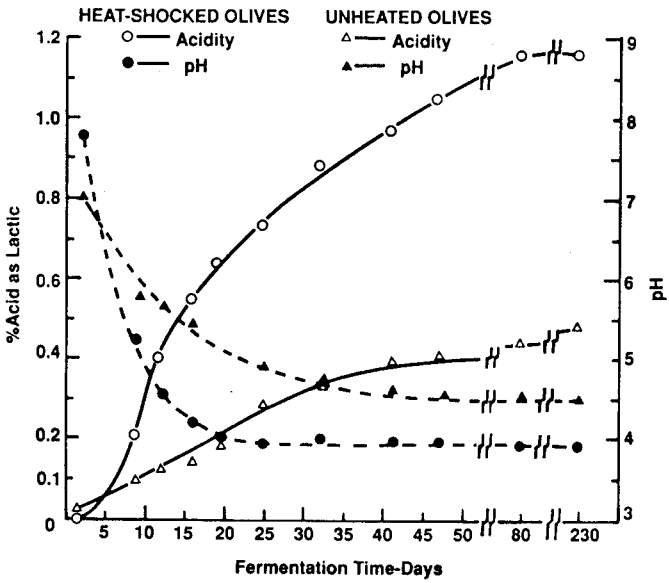

Figure 4.14 Effect of heat shocking Manzanillo olives on their subsequent acid fermentation by *L. plantarum*. Lye-treated, washed olives were either heated (74°C, 3 min) or unheated before inoculation. (*From Etchells et al., 1966.*)

either *L. plantarum* or *P. cerevisiae* (Fleming and Etchells 1967).

Although heating renders green olives fermentable by LAB even if the olives are not treated with alkali, this phenomenon has not been exploited on a large commercial basis. If a practical method for debittering olives other than alkali treatment could be developed, heat-shocking and inoculation with LAB could prove advantageous.

Secondary Fermentation

Yeasts

The problem of secondary fermentation by fermentative yeasts is particularly important with cucumbers in bulk vessels because of the bloater problem. Sorbic acid or its potassium salt may be added to suppress the growth of fermentative yeasts during primary fermentation (Costilow et al. 1957). However, the presence of residual fermentable sugars may allow eventual growth by yeasts if the sorbic acid is degraded or diluted. Thus, it is preferable for all fermentable sugars to be utilized before purging is discontinued. A buffering agent such as sodium or calcium acetate may be added to the cover brine (Etchells et al. 1973, Fleming et al. 1988a), or the acid may be partially neutralized with base during fermentation to ensure complete sugar utilization. Secondary yeast fermentation also is important in various fermented vegetables that are not heat-processed after packing for consumer use. Gas pressure buildup in the jar and cloudy brine in packaged products is undesirable.

Cucumbers, green beans, tomatoes, and peppers that were fermented with pH control to remove all fermentable carbohydrates were microbiologically stable (Fleming et al. 1983b). Carrots and red beets, however, could not be fermented by LAB to complete sugar utilization; these products underwent fermentation by yeasts after growth of LAB ceased. These observations led to the conclusion that the fermented vegetables that were microbiologically stable achieved stability due to: (1) the absence of fermentable carbohydrates, (2) the pH being 3.8 or lower, and (3) exclusion of oxygen. The maximum pH for assuring inhibition of spoilage bacteria is not known. Although pH 3.8 resulted in stability of several vegetables (Fleming et al. 1983b), in another instance butyric acid spoilage of cucumbers resulted when the lactic fermentation terminated at pH 3.7 (Fleming et al. 1989), as is discussed below.

Bacteria

Spoilage of Spanish-style green olives may result from two types of clostridial action, both of which result in malodorous products. In one type, *Clostridium butyricum* and closely related species produced

butyric acid from sugars during the primary fermentation stage (Gilliland and Vaughn 1943). A concentration of 7 to 8 percent salt in brines was reported sufficient to prevent this type of spoilage. In a second type of malodorous fermentation, *zapatera* spoilage resulted from decomposition of lactic acid when little or no sugar was present and before the pH had decreased below 4.5 (Kawatomari and Vaughn 1956). These workers stated that *zapatera* spoilage is prevented if the brine is pH 3.8 or below.

Plastourgos and Vaughn (1957) isolated propionic acid bacteria from commercial olives with *zapatera* spoilage. All isolates grew at 7 percent salt concentration in lactate medium between pH 4.8 and 8.0. They conjectured that propionic acid bacteria grew first in the olives and caused a rise in pH which permitted *Clostridium* species to grow. Borbolla y Alcala et al. (1975) and Rejano Navarro et al. (1978) reported the formation of propionic acid in Sevillian olives and suggested that acidity, salt concentration, and pH can be controlled to prevent its formation. These workers observed that the degradation of lactic acid to acetic and propionic acids occurs in the late stages of fermentation and concluded that this action could be prevented by sufficiently high salt concentration and sufficiently low pH.

Instability of fully fermented (no residual sugars) cucumbers due to bacterial action has only recently been documented. Fleming et al. (1989) observed butyric acid spoilage of cucumbers that had undergone a normal primary fermentation which resulted in > 1 percent titratable acidity (as lactic) and pH 3.7. The cucumbers were fermented and stored anaerobically. Products formed during secondary fermentation in relative order of concentration were acetic acid > butyric acid > *n*-propanol > propionic acid. Fermentation balances after primary fermentation and after spoilage are shown in Table 4.6. No botulinal toxin was detected. *Clostridium tertium* was identified as contributing to the spoilage but did not produce propionic acid or *n*-propanol under test conditions. Similarities exist between instability problems identified above for olives and the spoiled cucumbers. It was hypothesized that lactic acid was degraded to propionic and acetic acids by unidentified bacteria (possibly propionic acid bacteria), with resultant rise in pH. This rise in pH eventually allowed growth of *C. tertium* and perhaps other clostridia. The fact that the fermented cucumbers were pH 3.7 prior to subsequent microbial spoilage is an important distinction from the problem noted above with olives (where pH values in the 4.8 to 8.0 range were found necessary for growth of propionic acid bacteria). Bacterial instability of fermented cucumbers under anaerobic conditions at pH 3.7 was not expected, especially since *zapatera* spoilage in brined olives does not occur at pH 3.8 or below (Kawatomari and Vaughn 1956). However, the cucumbers were

TABLE 4.6 Substrates and Products of a Cucumber Fermentation that Underwent a Secondary Butyric Acid Fermentation[a]

Compound	Before fermentation	After primary fermentation	After spoilage
	Concentration of compounds, mM		
Glucose	27.1	ND	ND
Fructose	34.3	ND	ND
Malic acid	12.6	ND	ND
Acetic acid	53.2 (0.0)[b]	63.7 (12.5)[b]	105.3 (39.6)[b]
Lactic acid	ND[c]	140.1	ND
Ethanol	ND	7.3	1.7
Propionic acid	ND	ND	8.1
Propanol	ND	ND	34.5
Butyric acid	ND	ND	38.7
	Elemental recoveries, %		
Carbon		109.8	79.4
Hydrogen (H)		115.0	85.9
Oxygen (O)		104.9	46.2
	Hydrogen/oxygen ratio of compounds, mM		
	1.88	2.06	3.84

[a]After primary fermentation (30 days), the brine (2.3% NaCl) contained 1.09% acidity (as lactic) and was pH 3.7. After spoilage, the brine contained 0.59% acidity and was pH 4.8. From Fleming et al., 1989.

[b]Values in parentheses are those used in calculation of elemental recoveries and reflect the assumption that acetic acid was not a substrate for products measured.

[c]ND = none detected.

stored at only 2.3% NaCl, which is much lower than typical concentrations used commercially (5 to 12 percent).

Mannitol is formed from fructose during the heterolactic fermentation of vegetables, which may or may not be susceptible to metabolism, depending upon pH. Chen et al. (1983b) found that mannitol was not metabolized by *L. plantarum* at pH 3.5 and below, but was when the pH was raised to 3.9.

Postfermentation

Normally it is desirable for microbial activity of brined vegetables to cease after primary fermentation. Secondary fermentation can be prevented by suitable pH, acidity, and salt concentration; the absence of fermentable carbohydrates; and the exclusion of oxygen. The exclusion of oxygen is dictated by design of the fermentation and storage vessel, while the other parameters can be controlled by brine additions.

The open-top tanks used for brined cucumber storage (refer to Fig. 4.3)

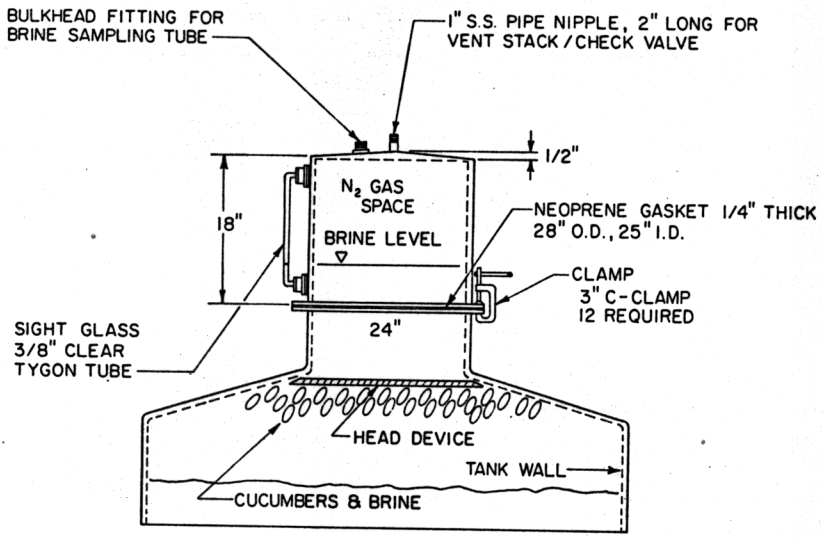

Figure 4.15 Cap assembly for anaerobic storage of brined cucumbers. (*From Fleming et al., 1983a.*)

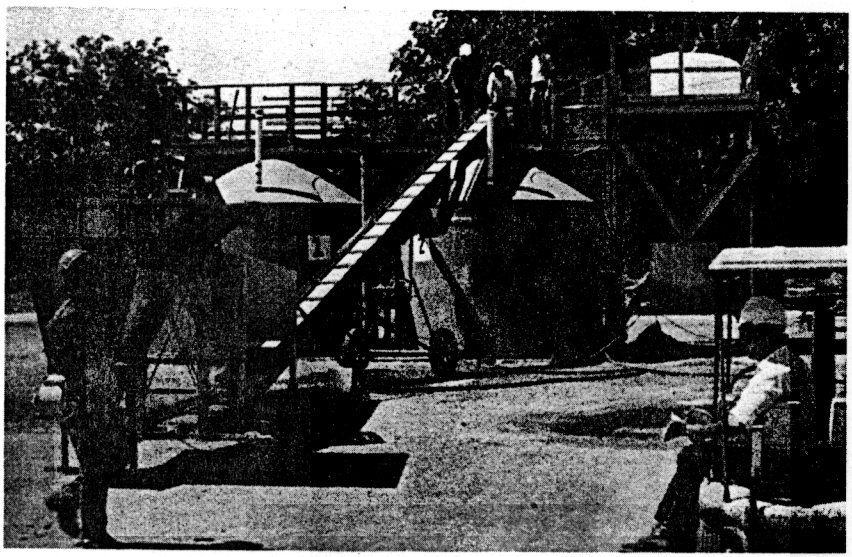

Figure 4.16 Experimental anaerobic tanks for the fermentation and storage of brined cucumbers. The tanks are made of reinforced fiberglass, coated on the interior with a food-grade resin, and contain about 30,000 L.

create multiple problems. As noted earlier, open tanks must be left outdoors to allow exposure of the brine surface to sunlight and resultant inhibition of aerobic microorganisms. If allowed to grow, film yeasts and molds will oxidize lactic acid, resulting in a rise in pH and growth of other spoilage microorganisms. Growth of molds and production of pectinolytic enzymes may result in softening of the cucumbers. Extra salt must be added to compensate for rainwater and to prevent freezing damage to the cucumbers in colder regions during winter. An anaerobic tanking system has been designed and is being tested commercially. An important feature of the tank design is the anaerobic headspace which is maintained with nitrogen (Fig. 4.15). This principle has been applied to commercial size tanks (Fig. 4.16).

Sauerkraut is covered with plastic sheeting, weighted down with water (Fig. 4.10). After the heaving problem that occurs in initiation and primary fermentation, the cover provides an excellent anaerobic seal. Aerobic spoilage microorganisms may grow, however, if the cover becomes dislodged. Also, if the cover is ruptured, the salt and acid of the sauerkraut will be diluted, which can result in spoilage.

Anaerobic tanks for olive fermentation and storage have been in use for over 20 years. An example of such tanks in southern California is shown in Fig. 4.17*a,b*. These tanks are being buried to provide a cooler temperature and better texture retention of the olives. The Spanish commonly use spherically shaped tanks constructed of fiberglass, which are buried, for olive fermentation (Fernandez Diez et al. 1985).

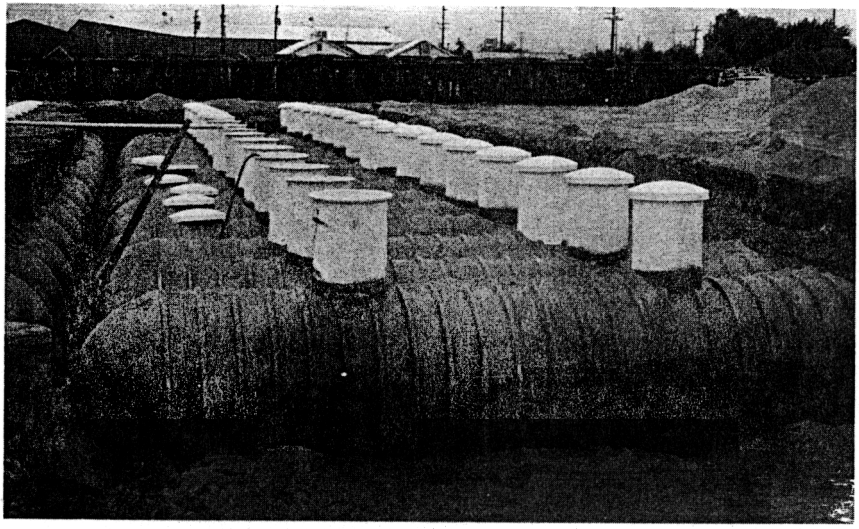

Figure 4.17a Anaerobic tanks for fermentation of Spanish-type green olives.

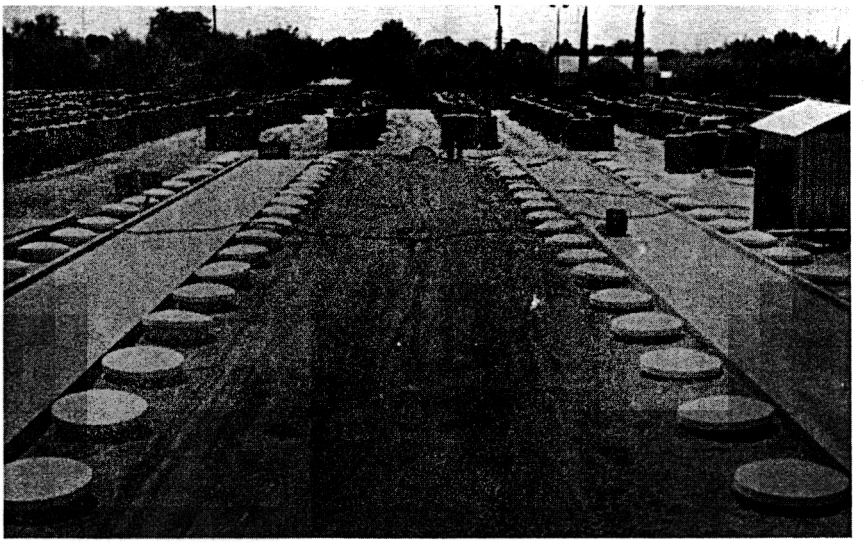

Figure 4.17b (*Continued*)

Only the manway for filling and emptying extends above a concrete pad. The tanks have a typical capacity of 16,000 L. The Spanish claim that burying provides moderate temperatures that are suitable for fermentation and storage, thus avoiding extremes due to seasonal temperature changes.

Summary

General principles involved in the fermentations of cucumbers, cabbage, and olives are summarized in this review. Traditionally, vegetables have been allowed to undergo spontaneous fermentation with salt concentration and temperature largely dictating the fermentation type. Recent studies have suggested additional controls which may enhance the potential of fermentation as an economical means for preservation and bulk storage of vegetables. These controls include purging of CO_2 from fermenting brine, use of anaerobic vessels, pH control, Ca^{2+} addition, and use of selected and genetically modified microbial cultures.

Acknowledgment

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