Note Added in Proof

Since the completion of this chapter, several promising developments have occurred that have a bearing on genetic engineering (recombinant DNA) of starter bacteria used in fermented milks. Currently, we have experimental evidence for transduction, conjugation and transformation among lactic streptococci. Genetic recombination through protoplast fusion of lactic streptococci has also been reported (Gasson, 1980). For recent developments on genetics governing the metabolic activities of lactic streptococci, the reader should refer to Davies and Gasson (1981) and Kempler and McKay (1981). Genetic determinants for host–phage restriction modification systems in lactic streptococci have also been described (Sanders and Klaenhammer, 1981). At present, active research on the genetics of yogurt starter bacteria is being carried out in the U.S.A. (Somkuti *et al.*, 1981) and Italy (Vescovo *et al.*, 1982). These developments undoubtedly will have far-reaching effects on scientific control of dairy fermentations and in new-product development.

In this chapter, manufacturing aspects of fermented milks were discussed only briefly. For more detailed treatments of these aspects, the recent review by Chandan (1982) is recommended.

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7. Fermented Vegetables

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I. INTRODUCTION

Preservation of foods by fermentation is thought to have originated in the Orient before recorded history (Pederson, 1960). Salting (brining) is a requisite for preserving vegetables and certain fruits, such as olives, by fermentation because it helps to direct the course of the fermentation and prevent softening and other degradative changes in plant tissues. Brining probably preceded or occurred simultaneously with fermentation as a food preservation method. The type and extent of microbial action in salted vegetables is highly dependent on the concentration of salt. Microbial fermentation may be rapid at low concentrations of salt, but extremely slow or non-existent at high concentrations. Other chemical and physical factors are also important in regulating the rate and extent of fermentation.

Brining and fermentation were primary methods for preserving vegetables throughout the World prior to the advent of canning and freezing. Although secondary to modern preservation methods in Western civilization, brining and fermentation remain important methods for preserving certain vegetables in highly developed countries because they: (1) impart certain desired organoleptic qualities in the products; (2) provide a means for extending the processing season for fruits and vegetables; and (3) require comparatively little mechanical energy input, a fact that enhances the potential for these age-old methods of preservation in our modern energy-sensitive World.

Probably most vegetables have been preserved by brining and/or fermentation. Comprehensive reviews are available on the fermentation of sauerkraut (Pederson, 1960, 1979; Stamer, 1975), olives (Vaughn *et al.*, 1943; Vaughn, 1954, 1975) and cucumbers (Etchells *et al.*, 1951, 1975). Examples of other vegetables and fruits that have been brined for home and commercial purposes include carrots (Niketic-Aleksic *et al.*, 1973), celery (Bates, 1970), various vegetable blends (Orillo *et al.*, 1969), green beans, lima beans, green peas, corn, okra and green tomatoes (Etchells *et al.*, 1947), cauliflower (Dakin and Milton, 1964), and whole peppers, pepper mash, onions and citron. In addition, fermented beets, turnips, radishes, chard, Brussels sprouts, mustard leaves, lettuce, fresh peas and vegetable blends have been produced in considerable quantity in the Orient (Pederson, 1979). The purpose of this review is to summarize general principles governing brine fermentation of vegetables and certain fruits. Fermentations of cabbage, olives and cucumbers are emphasized to illustrate those principles.

II. BRINING OPERATIONS FOR NATURAL FERMENTATIONS

The general procedure for preserving vegetables by natural (i.e. by naturally occurring micro-organisms) fermentation is outlined in Figure 1. Specific treatments vary among vegetables, some of which will be indicated.

A. Prebrining Treatments

Vegetables may be treated in various ways before brining, depending on the nature of the fresh vegetable and the product desired. Examples of such prebrining treatments include grading, washing, cutting, piercing and exposing to alkali.

Cabbage for sauerkraut is wilted, cored, trimmed and shredded prior to placement in a fermentation tank. Olives destined for table use receive various prebrining treatments, depending on desired qualities of the end product (Vaughn, 1954; Fernandez-Diez, 1971). Olives contain oleuropein, an extremely bitter phenolic glucoside, and varieties rich in this compound must be debittered. In the preparation of Spanish-style green olives, the olives undergo a prebrining treatment with 1 to 2% sodium hydroxide to destroy the bitter principle. The alkali is allowed to penetrate about three-quarters of the distance to the pit, and air is

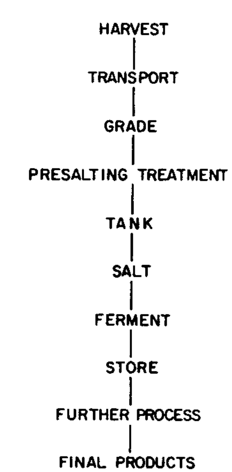

Fig. 1. Flow chart for preservation of vegetables by brining.

excluded during this operation to prevent darkening. The olives are then washed several times to remove alkali. During washing, some of the fermentable sugars and other nutrients are also removed from the olives. Sicilian-style green olives are not debittered before brining, since the varieties of olive used contain relatively low concentrations of the bitter principle. Greek-style ripe olives are prepared from naturally tree-ripened olives and are not debittered. The final product retains a level of bitterness desirable to some people.

Cucumbers are usually graded to size before brining because size influences the brining procedure. Cucumbers mostly are brined whole, although residual cut pieces from the manufacture of fresh-pack cucumbers (prepared by direct acidification and pasteurization of fresh cucumbers) are brined for use in relishes. Cucumbers may be pierced to prevent internal accumulation of gas (bloater formation) during fermentation, but this practice is limited primarily to specialty products such as overnight and genuine dills by a few briners. Cucumbers destined for natural fermentation are not normally washed before brining. It has been suggested, however, that the initial brine be drained from small cucumbers, which usually retain fungal-laden flowers, in order to lower the concentration of softening enzymes (Etchells *et al.*, 1955, 1958).

B. Brining Procedures

Vegetables may be brined at various salt concentrations by either the dry-salt method or the brine-solution method (Table 1). By the dry-salt method, salt is added with the vegetables, such as cabbage, as they are placed in the tanks; the tanks are then headed with timber. By the brine-solution method, as used with cucumbers and olives, a small amount of brine is added to the tanks to cushion the fall of the vegetables as they are loaded into the tanks. Then, the tanks are headed with timber and additional brine is added to cover the vegetables and heading timber. The added salt and water-soluble constituents of the vegetables interdiffuse until equilibrium of all constituents is attained. The equilibration process continues until some time after the fermentation is complete. With proper salting and under correct holding conditions, the fermented vegetables may be stored for several months or years without serious loss in texture or other quality factors.

Table 1
Brining procedures for vegetables

Method of salting	Concentration (%, w/v) of salt during: Fermentation	Storage	Vegetable
Dry salting	2–3	2–3	Cabbage
Brine solution	5–8	8–16	Cucumbers
	4–7	4–7	Green olives

The concentrations of salt indicated generally are used for commercial brining of cabbage, cucumbers and olives in the United States. Wide variations exist in the salt concentration used for peppers, onions and cauliflower.

Salt for brining purposes may include solar, rock and granulated types. Borg *et al.* (1972) observed no significant differences in microbial populations of these three types of salt and found that lactic-acid bacteria grew equally well in the presence of all types.

For sauerkraut fermentation, dry salt is distributed uniformly into the shredded cabbage as it is added to the tank. The rate of salt addition is such that the final concentration will be 2 to 3% (preferably 2.25%) sodium chloride. That concentration is maintained during fermentation and storage. The preferred temperature during fermentation is around 18°C (Pederson, 1960).

Alkali-treated and washed green olives to be prepared into Spanish-style olives are brined and kept at 4 to 7% sodium chloride during fermentation and storage. Sicilian-style green olives are brined by the solution method, and dry salt added as needed to maintain the brine at 6 to 7% sodium chloride (Vaughn, 1954). Greek-style, naturally ripe olives may be dry-salted or placed in a brine solution, with salt concentrations ranging from 7 to 19% (Balatsouras, 1966). Olives for eventual processing as California-style, black-ripe olives are temporarily stored in brines of 5 to 7% sodium chloride (Vaughn, 1954). These olives undergo fermentation. Alternatively, olives for black-ripe processing may be held in a salt-free solution containing 0.67% lactic acid, 1% acetic acid, 0.3% sodium benzoate and 0.3% potassium sorbate (Vaughn *et al.*, 1969b) or in similar proprietary solutions. These olives do not ferment. Salt-free storage is now used primarily for Sevillano and Ascolano varieties which are more likely to shrivel in salt solutions;

brining is still an important method of storing smaller varieties (Manzanillo and Mission) of olives (J.R. Webster, personal communication).

Cucumbers are brined in a solution of 5 to 8% sodium chloride, and dry salt is added to maintain the brine at this concentration during fermentation. Etchells and Hontz (1972) suggested that the concentration of sodium chloride at equilibrium be adjusted according to brine temperature, i.e. lower salt concentrations at lower temperatures (e.g. about 5% sodium chloride at 20°C and below) and higher salt concentrations at higher temperatures (e.g. about 8% sodium chloride at 30°C). Brines containing large-size cucumbers are purged with nitrogen gas during the fermentation period to remove carbon dioxide from solution and thereby prevent bloater damage (Etchells *et al.*, 1973) as will be discussed later.

C. Brining Vessels

Vegetables are brined in vessels varying in size from a few litres for home and small-scale commercial use to over 75,000 litres for large-scale commercial operations. The size is dictated by the volume of fresh intake, physical properties of the vegetable and economic factors. Materials used for brining vessels include earthenware (as in stone crocks for home use), wood (e.g. cypress, fir, redwood), food-grade plastics, glazed tile, fibre-glass, and concrete. When fibre-glass or concrete is used, the interior of the vessel is coated with a food-grade material. Metal deteriorates rapidly in the saline and acidic environment and is not used within the brining vessels, but steel straps or rods may be placed externally for tank reinforcement.

Brine depth is the primary dimension that governs tank size for vegetable fermentations. Fresh vegetables contain air, many having a specific gravity of less than one, and are therefore buoyant when placed in brine solutions that have specific gravities above one. Headboards are mounted in the tops of brine tanks to keep freshly tanked vegetables submerged in the brine. The buoyancy of freshly brined cucumbers can cause physical damage to those cucumbers in the top section (Fleming *et al.*, 1977). Tank depths of about 2.5 metres are typical for brined vegetables, but may reach 4 metres for some cucumber tanks. The buoyancy problem is especially serious with peppers because they are

hollow, necessitating placement of baffles (false heads) at about 1.25-metre intervals in deep tanks to prevent the peppers at the top of the tank from being crushed.

The manner in which the vessel is headed and the environment in which it is held are extremely important relative to the attention that the brined vegetables must receive during fermentation and storage. Cucumbers, for example, are brined in cylindrical tanks with the brine surface exposed to the atmosphere (Fig. 2). Growth of film yeasts, moulds, and spoilage bacteria on the surface of the brine must be restricted. This growth is restricted if the brine surface is exposed to sunlight; but evaporation, rainwater and extraneous contamination from the atmosphere create problems. The tanks may be sheltered, but then the surface must be periodically skimmed to remove surface growth.

Sauerkraut tanks or vats are covered with plastic that seals the kraut surface from the atmosphere. The salted cut cabbage creates its own brine. When the plastic cover that is draped over the tank sides is properly weighted with water, the brine is kept level with the surface of the sauerkraut. Thus, the brine surface is anaerobic, and is not susceptible to growth by surface yeasts, moulds, and spoilage bacteria (Pederson and Albury, 1969).

Surface growth on olive brines exposed to air is similar to that on cucumber brines. No problem with surface growth occurs when the olives are fermented and stored in air-tight wooden or plastic drums. Bulk storage in large tanks has been of recent interest in Spain (Borbolla y Alcala *et al.*, 1969) and the United States (Vaughn, 1975). The use of 'anaerobic' bulk tanks in the olive brining industry is an important development which offers a means of eliminating the surface-growth problem (Vaughn, 1975). Some olive companies have designed their own special tanks to maintain anaerobiosis (Figs. 3 and 4). One company uses 45,000-litre capacity, fibre-glass tanks (Fig. 3) normally used for petroleum storage. The tanks are coated internally for food use and fitted with two manholes, each 0.91 m in diameter, for filling and emptying. These tanks are then buried with only the manholes protruding above ground. The tanks are filled with olives, brine is added until the manhole is partially filled, and a plastic disc is floated on the brine surface to further restrict exposure of the brine surface to the atmosphere. Underground storage of vegetables offers the advantage of uniform and moderate temperatures. This advantage has been put to

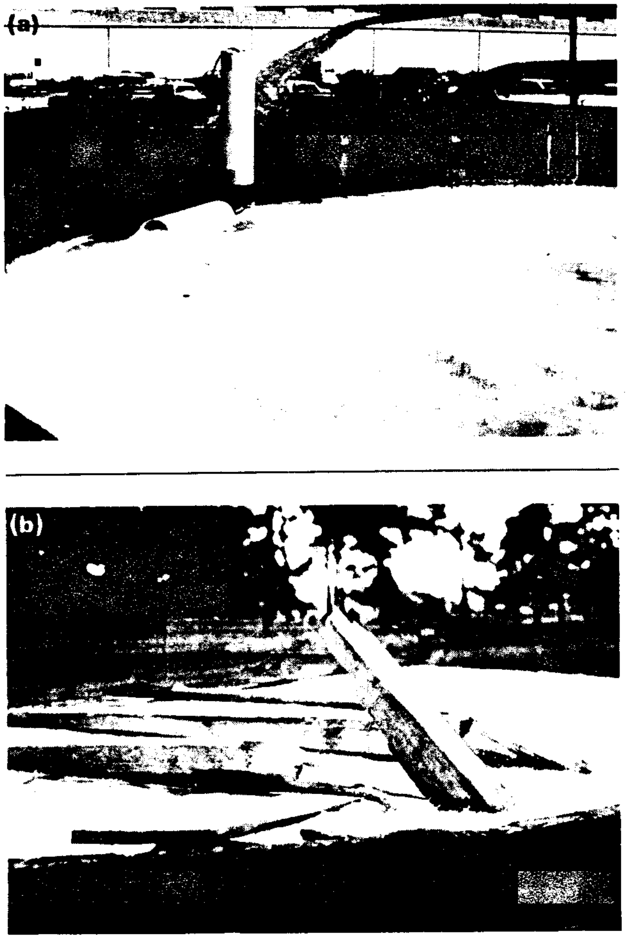

Fig. 2. Photograph of the surface of cucumber brining tanks during an active stage of fermentation. (a) Surface of a 23,000-litre, wooden tank that is being purged of carbon dioxide. Note that the headboards are not visible, giving no evidence of excessive buoyancy pressure. Dissolved carbon dioxide being removed from the brine by purging with nitrogen gas with the side-arm apparatus shown causes the frothy surface. The tank in the background is being flume-filled. (b) Surface of a 23,000-litre tank that is not being purged of carbon dioxide. Note the heavy timbers that are broken due to excessive buoyancy pressure created by bloated cucumbers. The tank must be reheaded to prevent air exposure and eventual spoilage of the cucumbers. The photographs are reproduced with compliments of Mount Olive Pickle Company, Inc., Mount Olive, N.C.

Fig. 3. Photographs of 45,000-litre, fibre-glass tanks for underground storage of Spanish-style green olives. The tanks will be placed underground, leaving only the two manholes protruding above ground. The manholes will be used for filling and unloading, and are fitted with plastic floats to further restrict air exposure of the brine surface. (a) Shows tanks aligned in their burial site. (b) Shows site of the buried tanks. The photographs are reproduced with the compliments of J.R. Webster, Lindsay Olive Growers, Lindsay, California, U.S.A.

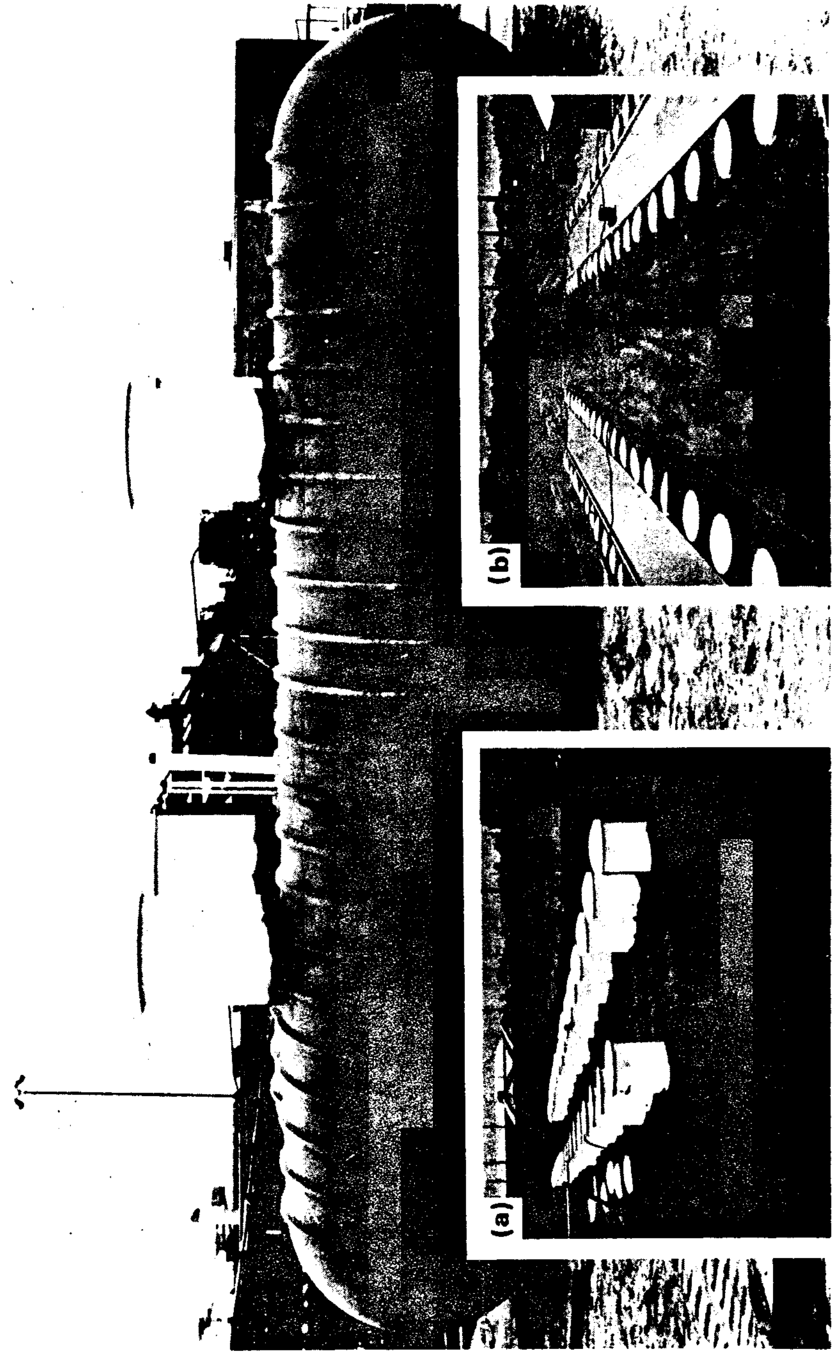

Fig. 3. (For legend see facing page.)

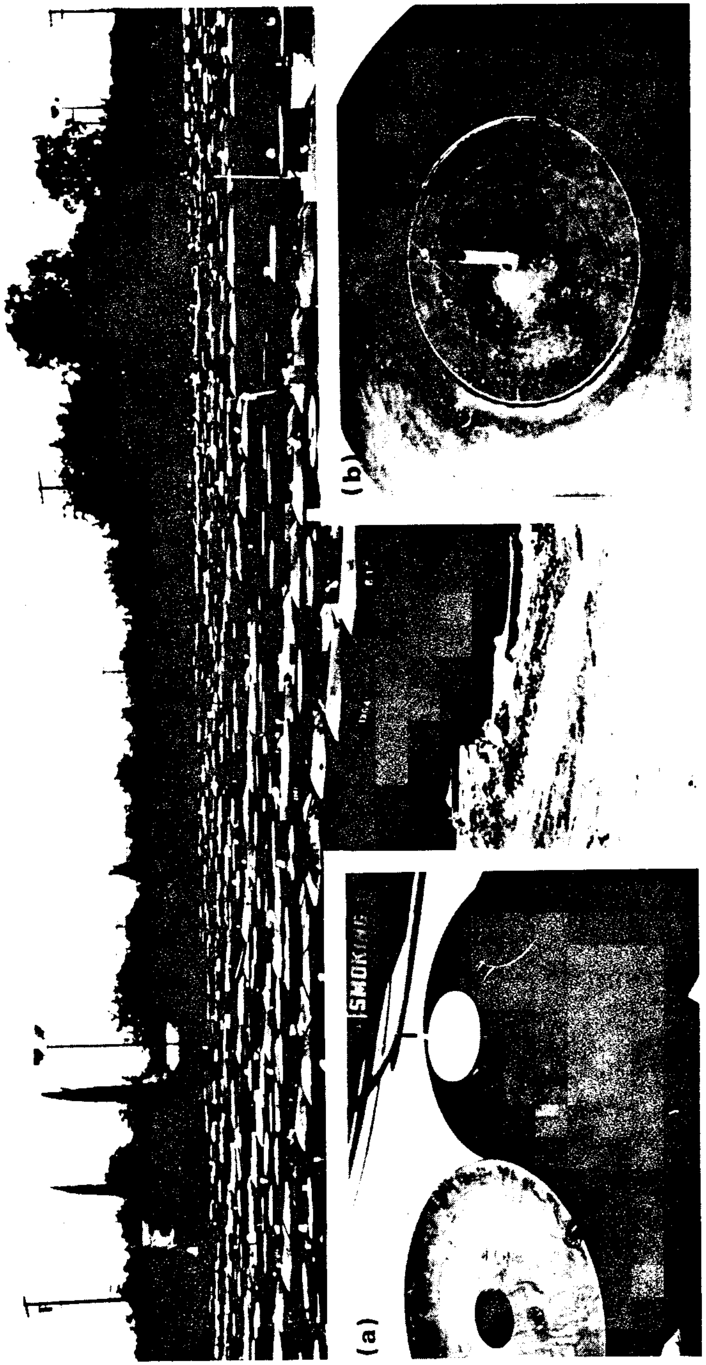

Fig. 4. (For legend see facing page.)

use by storage of olives underground in southern California to avoid high brine temperatures that occur during summer months and which result in softening of the olives.

Important changes in design, size and materials of brining vessels have occurred in recent years. Further changes should be expected because of the trend toward larger tanks, the quest for higher quality and more economical products, and greater regulatory restrictions on disposal of salt and other wastes.

III. MICRO-ORGANISMS DURING NATURAL FERMENTATION

A. Growth Sequence of Microbial Groups

Fresh vegetables contain a numerous and varied epiphytic microflora, including many potential spoilage micro-organisms, and an extremely small population of lactic-acid bacteria. Fresh pickling cucumbers, for example, may contain over $1 \cdot 10^7$ total aerobic micro-organisms per g but only $5 \cdot 10^3$ acid-forming bacteria per g of cucumber (Table 2; Etchells *et al.*, 1961, 1975). Less than 10 lactobacilli per g of plant material were enumerated in the majority of various plant materials analysed by Mundt and Hammer (1968). Streptococci and *Leuconostoc mesenteroides* occurred about 14 times as frequently as lactobacilli in a survey of 109 fresh plant samples, which included vegetables, grasses, fodders, legumes and cereals (Mundt and Hammer, 1968). Stirling and Whittenbury (1963) found that fresh grasses and legumes contained extremely low numbers of lactic-acid bacteria, but that the numbers were much higher on the harvesting equipment. They suggested that nutrients made available through cutting of the plant material provided a medium for growth of the few lactic-acid bacteria, which apparently were latent on the intact plant.

When vegetables are properly brined at salt concentrations of up to about 8% and allowed to undergo natural fermentation, the brine

Fig. 4. Photograph of a brine yard with 1500-litre plastic storage containers for Spanish-style green olives. (a) Shows the cap and plastic float assembly used to restrict the surface of the brine to air. (b) Shows the top of a storage container with a plastic float assembly in place. After olives are placed in the container, brine is added until it reaches a specified level in the reservoir of the cap. The plastic float is then installed and the brine level is monitored by the indicator stick extending from the float through the cover plate. The photographs are reproduced with the compliments of J.R. Webster, Lindsay Olive Growers, Lindsay, California, U.S.A.

Table 2
Microbial populations on cucumber fruit and blossoms

	Colony counts (thousands)			
	Cucumber fruit		Cucumber blossoms	
Microbial group	Per g	Per unit	Per g	Per unit
Bacteria				
Aerobes				
Total	16,000.0	182,320.0	18,200,000	476,000
Spores	17.0	218.0	67,800	1,940
Anaerobes				
Total	1,830.0	19,800.0	3,092,000	78,760
Spores	0.8	9.8	2,100	191
Coliforms	3,940.0	49,125.0	6,400,000	167,530
Acid-formers	5.0	60.0	26,000	765
Yeasts	1.6	18.0	3,030	82
Moulds	3.4	44.0	11,300	295

Data shown are based on results of Etchells *et al.* (1961) as recapitulated by Etchells *et al.* (1975).

solution supports growth or fermentation by a sequence of various types of micro-organisms. This sequence may be categorized into four stages, namely *initiation*, *primary fermentation*, *secondary fermentation*, and *post-fermentation* (Table 3). These four stages are based on changes in the chemical and physical environments during fermentation and storage of cabbage, olives, cucumbers and other vegetables. These environments

Table 3
Sequence of microbial types during natural fermentation of brined vegetables

Stage	Prevalent micro-organisms
Initiation	Various Gram-positive and -negative bacteria
Primary fermentation	Lactic-acid bacteria, yeasts
Secondary fermentation	Yeasts
Post-fermentation	Open tanks[a], surface growth of oxidative yeasts, moulds and bacteria
	Anaerobic tanks, none

[a] This refers to tanks with the brine surface exposed to the atmosphere. Exposure of the brine surface to sufficient sunlight will restrict surface growth, but surface growth may be great if the brine surface is shaded.

dictate the type and extent of microbial growth. The rapidity of transition between stages varies among vegetables due to properties of the vegetables as well as the chemical and physical conditions under which they are held.

The *initiation* stage may include growth by many of the faculative and strictly anaerobic micro-organisms originally present on the fresh material, but as lactic-acid bacteria become established, the pH value is lowered and growth of undesirable micro-organisms such as Gram-negative and spore-forming bacteria is inhibited. The quality of the final product depends largely on the rapidity with which the lactic-acid bacteria are established and the undesirable bacteria are excluded.

During the *primary fermentation* stage, lactic-acid bacteria and fermentative yeasts are the predominant active microflora. They grow in the brine until the fermentable carbohydrates are exhausted or until the lactic-acid bacteria are inhibited by low pH values, resulting from production of lactic and acetic acids. Buffering capacity and the fermentable carbohydrate content of the plant material are important factors which govern the extent of fermentation by lactic-acid bacteria and the extent of subsequent fermentation by yeasts.

Secondary fermentation is essentially due to fermentative yeasts. These yeasts may become established during the primary fermentation, are acid tolerant and, if fermentable sugars remain after the lactic-acid bacteria are inhibited by low pH values, continue to grow until the fermentable carbohydrates are exhausted.

During the *post-fermentation* stage, when fermentable carbohydrates are exhausted, microbial growth is restricted to the surface of brines exposed to air. When the surface of brines is so exposed, oxidative yeasts, moulds, and ultimately spoilage bacteria may become established on the surface of improperly managed tanks. No surface growth occurs in anaerobic tanks.

Numerous chemical and physical factors influence the rate and extent of growth of various micro-organisms, as well as their sequence of appearance during fermentation. Acidity and pH value greatly influence establishment and extent of growth of lactic-acid bacteria. Salt concentration, temperature, natural inhibitory compounds of plant origin, chemical additives, exposure of the brine surface to air and sunlight, fermentable carbohydrate content of the vegetable and availability of nutrients in the brine are other important factors that affect fermentation. Most of these factors are dealt with to some extent in this review.

B. Lactic-Acid Bacteria

The lactic acid bacteria that predominate initiation and primary fermentation of brined vegetables include *Streptococcus faecalis*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Pediococcus cerevisiae* (probably *Pediococcus pentosaeceus*, according to recent classification; Buchanan and Gibbons, 1974), and *Lactobacillus plantarum* (Pederson, 1960; Vaughn, 1954, 1975; Etchells *et al.*, 1975). These species are listed in order of increasing total acid production and in the order of their appearance during the sauerkraut fermentation (Pederson, 1960). Only the last three species appear to grow to any appreciable extent in olives or cucumbers, however, apparently due to the higher brine strengths used for these products than for sauerkraut. All factors that govern sequential growth of lactic-acid bacteria are not known, but the size of the initial populations, rapidity of growth in the brine solution, and limiting pH values are highly important. Limiting pH value, or the lowest pH value at which the organism will grow, and corresponding acidities for these bacteria in pure culture fermentations are given in Table 4. *Streptococcus faecalis* and *L. mesenteroides*, not being as salt and acid tolerant as the other species, are probably not of major significance in vegetables brined above about 5% sodium chloride (Pederson, 1960; Etchells *et al.*, 1964).

Table 4
Terminal pH values and acidities tolerated by lactic-acid bacteria involved in pure-culture vegetable fermentations

Organism	Final pH values and acidities[a]			
	Cabbage juice[b]		Cucumbers[c]	
	pH Value	Acid (%)	pH Value	Acid (%)
Leuconostoc mesenteroides	3.9	1.04	—	0.23
Lactobacillus brevis	3.9	1.06	3.7	0.54
Pediococcus cerevisiae	3.5	0.90	3.4	0.63
Lactobacillus plantarum	3.5	1.40	3.2	0.91

[a] Acidity expressed as lactic acid.
[b] From Stamer *et al.* (1971) for fermentation of filter-sterilized cabbage juice.
[c] From Etchells *et al.* (1964). The salt concentration was 5.5% for all fermentations, except *L. mesenteroides* (4.2% salt; these data are from Etchells *et al.*, 1975).

Lactobacillus plantarum is the most acid-tolerant species, and terminates the lactic fermentation of vegetables. Even when other species were introduced initially into otherwise natural fermentations of cucumbers, *L. plantarum* was the final organism to grow (Pederson and Albury, 1961).

Heterofermentative lactic-acid bacteria produce sizeable quantities of acetic acid, ethanol, carbon dioxide, mannitol, dextrans, and traces of other compounds in addition to lactic acid; homofermentative lactic-acid bacteria produce mainly lactic acid (Table 5). Heterofermentative lactic-acid bacteria are desirable in sauerkraut fermentation because the volatile acids and other compounds they produce impart desired flavours to the final product (Pederson, 1960; Stamer, 1975). In cucumbers, heterofermentative lactic-acid bacteria have been implicated in bloater damage due to their large production of carbon dioxide (Etchells *et al.*, 1968b). However, even the relatively small amount of carbon dioxide produced by the homofermentative *L. plantarum*, when combined with that produced by the cucumber tissue, was found to induce bloater damage (Fleming *et al.*, 1973a).

Although the contributions of lactic-acid bacteria and other organisms to the flavour of many fermented foods are well known, comparatively little is known about their importance to the flavour of fermented vegetables. The concentration of volatile acid is known to be

Table 5
Products of lactic-acid bacteria during vegetable fermentations[a]

Fermentation type or species	Major products from fermentation of vegetable sugars[b]	Lactic acid configuration
Heterofermentative	Lactic acid, acetic acid, ethanol, carbon dioxide, mannitol, dextran	
Leuconostoc mesenteroides		D(−)
Lactobacillus brevis		DL
Homofermentative	Lactic acid	
Pediococcus cerevisiae		DL
Lactobacillus plantarum		DL

[a] Primary catabolism of hexoses is by the phosphoketolase pathway for heterofermentative and the Embden-Meyerhof-Parnas pathway for homofermentative lactic-acid bacteria (Doelle, 1975).
[b] The major fermentable sugars in vegetables are glucose, fructose and sucrose.

important to the flavour of sauerkraut (Pederson, 1960). Acetaldehyde and diacetyl may be present early in the fermentation (Hrdlicka *et al.*, 1967). During the pure-culture fermentations of cucumbers, changes that occur in the concentrations of ethanol and various aldehydes may influence flavour (Aurand *et al.*, 1965). Fleming *et al.* (1969a) concluded that the most important contribution of *L. plantarum* to the flavour of olives in pure-culture fermentation studies was to produce preservative concentrations of acid and exclude undesirable micro-organisms which produce off-flavours.

C. Yeasts

Yeasts are probably active in most vegetable fermentations. Surface or film (oxidative) and subsurface (fermentative) yeasts may populate vegetable brines, depending on various chemical and physical conditions of the brines during fermentation and storage. Yeasts may be present in relatively low numbers on fresh vegetables such as cucumbers (Table 2, p. 238). Usually, they are present in very low numbers in brines of cucumbers (Etchells and Jones, 1943) and olives (Mrak *et al.*, 1956) during the first few days after brining. Fermentative yeasts grow throughout the fermentation of cucumbers and olives, including the primary and secondary stages, until fermentable carbohydrates are exhausted. Fermentative yeasts apparently are not found in high numbers during sauerkraut fermentations. A partial explanation is that the low concentrations of salt used favour a primary fermentation predominated by lactic-acid bacteria.

J. L. Etchells and his coworkers have made extensive studies on the yeasts of cucumber brines, including their identification and factors affecting their growth (Etchells and Bell, 1950a,b; Etchells *et al.*, 1952, 1953, 1961). The principal species of fermentative yeasts (the recent classification of yeasts by Lodder, 1970, is given in parentheses), listed in the approximate order of their occurrence in commercial cucumber brines, were: *Brettanomyces versatilis* (now *Torulopsis versatilis*); *Hansenula subpelliculosa*; *Torulopsis caroliniana* (now *T. lactis-condensi*); *Torulopsis holmii*; *Saccharomyces rosei*; *Saccharomyces elegans* (now *S. baillii*); *Saccharomyces delbrueckii*; *Brettanomyces sphaericus* (now *Torulopsis etchellsii*); and *Hansenula anomala* (Etchells *et al.*, 1961). The principal oxidative yeasts were: *Debaryomyces membranaefaciens* var. *Holl.*; *Endomycopsis ohmeri* (now *Pichia ohmeri*); *Zygosaccharomyces halomembranis* (now *Saccharomyces rouxii*); and *Candida krusei* (Etchells and Bell, 1950b). Several other fermentative and oxidative yeasts were present in smaller numbers. *Rhodotorula* species, for example, occurred infrequently, but were recognizable by the pink film they formed on brine surfaces.

Mrak *et al.* (1956) found predominantly fermentative yeasts in commercial Spanish-style green olives during the first seven weeks after brining, which approximated the period of lactic acid fermentation. The principal species were: *Candida krusei*; *C. tenuis*; *C. solani*; *Torulopsis sphaerica*; *T. holmii*; and *Hansenula subpelliculosa*. During the next several weeks they found, in addition to the fermentative yeasts, the following principal species of oxidative yeasts: *Pichia membranaefaciens*; *C. mycoderma* (now *C. valida*); and *C. rugosa*.

In the fermentation of cucumbers, high concentrations (e.g. 10 to 16%) of sodium chloride favour growth of yeasts, whereas low concentrations (e.g. 5 to 8%) favour growth of lactic-acid bacteria (Etchells and Jones, 1943). The concentration of sodium chloride also influences species of yeasts in cucumber (Etchells and Bell, 1950a; Etchells *et al.*, 1952) and olive brines (Mrak *et al.*, 1956). Of the oxidative yeasts, species of *Debaryomyces* and *Zygosaccharomyces* (now *Saccharomyces*) grew in brines at sodium chloride concentrations of up to 20%, *Pichia* species up to 15%, and *Candida* species up to 10%, as shown by luxuriant film formation (Etchells and Bell, 1950a). Brine acidity also influences the species of yeasts. For example, *T. lactis-condensii* occurs early in cucumber fermentation when the acidity is low, but apparently is eliminated as the acidity increases (Etchells *et al.*, 1952). On the other hand, *Torulopsis* species may be active throughout brine storage (Etchells *et al.*, 1952). Yeasts generally have been considered as spoilage organisms, or at best have been viewed with indifference, in regard to cabbage, cucumber, and olive fermentations. Fermentative yeasts produce large amounts of carbon dioxide which can cause gaseous deterioration in brined cucumbers (Jones and Etchells, 1943; Etchells *et al.*, 1952). Pink discolouration in sauerkraut is caused by *Rhodotorula* species which may grow if the cabbage contains over 3% salt (Pederson, 1960).

Oxidative yeasts can utilize lactic acid and lower the brine acidity to allow other spoilage micro-organisms to grow (Etchells *et al.*, 1975; Mrak *et al.*, 1956). Utilization of brine acidity by these yeasts is limited to aerobic conditions, however, as they are unable to utilize organic acids under anaerobic conditions (Mrak *et al.*, 1956).

Yeasts have not been implicated in the softening of brined cucumbers. Although *Saccharomyces fragilis* and four strains of *Sacch. cerevisiae* have been found to hydrolyse pectin, none of these yeasts has been isolated from cucumber brines (Bell and Etchells, 1956). However, species of *Debaryomyces*, *Pichia* and *Candida* isolated from films on cucumber brines were able to de-esterify pectin (Bell and Etchells, 1956). *Rhodotorula glutinis* var. *glutinis*, *R. minuta* var. *minuta* and *R. rubra* have been found to cause softening in brined olives (Vaughn *et al.*, 1969a).

Sorbic acid has been used to discourage growth of fermentative and oxidative yeasts (Phillips and Mundt, 1950; Costilow *et al.*, 1957; Bell *et al.*, 1959). Although sorbic acid lowers the size of the yeast population, some concern exists that surviving yeasts may develop sufficient tolerance to the compound to yield a subsequent problem in the brine yard (Etchells *et al.*, 1961). Furthermore, fermentable sugars must be removed sooner or later if full advantage of fermentation as a method of preservation is to be obtained.

Yeasts also have a beneficial role in brined vegetables during bulk fermentation by utilizing fermentable sugars, since lactic-acid bacteria may be inhibited by low pH before the fermentation is complete. This is particularly true of fermentations involving vegetables containing a high sugar content such as carrots. Vaughn *et al.* (1976) reported that certain yeasts may be responsible for acid production in brined olives. Also, certain yeasts may impart a desirable flavour, although this possibility has not been fully explored in regard to vegetable fermentations.

D. Moulds

Moulds, especially highly pectinolytic species, can cause serious spoilage problems due to enzymic softening of vegetable tissue and should be avoided on the fresh vegetable and during brine storage and further processing. Pectinolytic enzymes may be present on the vegetable at the time of brining or may be produced by moulds that are permitted to grow on the brine surface. Raymond *et al.* (1960) found 73 species in 34 genera of moulds to be present on the blossoms, ovaries and fruit of cucumbers. Seven species in five of the genera accounted for most of the

isolates and included, in decreasing order of occurrence, *Penicillium oxalicum*, *Ascochyta cucumis*, *Fusarium roseum*, *Cladosporium cladosporioides*, *Alternaria tenuis*, *Fusarium oxysporum* and *Fusarium solani*.

IV. SPOILAGE PROBLEMS

Preservation of vegetables by brine fermentation is highly dependent on the brining treatment and the resulting type and extent of microbial action during and after fermentation in bulk containers. Complete conversion of fermentable carbohydrates into acids and other end products renders the vegetables stable to subsequent fermentation. Fully fermented cucumbers (Etchells *et al.*, 1951) and olives (Fernandez-Diez, 1971), for example, may be made into finished products without the need for heat processing. The presence of residual fermentable sugar in such products can lead to gas pressure and unsightly brine turbidity in the final package from growth of yeasts and lactic-acid bacteria. Fermentation, if properly done, offers advantages as a method for preserving vegetables, as indicated in the Introduction to this chapter, but improperly brined vegetables can result in serious spoilage.

A. Softening

Softening can be a very serious problem with brined vegetables and may be caused by enzymes of plant or microbial origin. The concentration of salt needed to prevent softening varies widely among vegetables and is a major reason for differences in salt concentrations used for the vegetables listed in Table 1 (p. 231). For example, 2% sodium chloride is sufficient to prevent softening in properly attended sauerkraut, but bell peppers must be saturated with salt (about 26%) to maintain firm texture. A low concentration of salt favours a rapid fermentation in sauerkraut, but a high concentration of salt precludes microbial fermentation in bell peppers. This difference in tendency to soften among vegetables apparently is related to the activity of natural softening enzymes in the plant tissue, or possibly to differences in resistance of the tissue to attack by microbial softening enzymes.

Soft kraut is caused by insufficient salt and by yeast and mould growth at the surface, where air has been allowed to contact the kraut. These problems can be largely eliminated by insuring that the proper concentration of salt is distributed uniformly throughout the kraut at the time of tanking, and by preventing air from contacting the kraut surface by proper heading such as with the use of an air-impermeable plastic cover over the kraut surface (Pederson and Albury, 1969).

Softening of olives also may result from pectinolytic enzymes produced by moulds (Vaughn, 1975) and yeasts (Vaughn *et al.*, 1969a) which are allowed to grow on brine surfaces (Vaughn, 1954, 1975). Methods for controlling surface growth of moulds and yeasts have already been discussed. Softening can also result from growth of undesirable bacteria in the early stages of brining, especially in Spanish-style olives, before the pH value is lowered by lactic-acid bacteria. Softening can be especially serious if the salt concentration is too low. Fortifying brines with additional salt and with acid (to lower the pH value below that at which spoilage bacteria can grow) will prevent bacterial softening.

Softening of cucumbers may occur due to the action of pectinolytic enzymes which originate in the cucumber fruit itself and from microbial sources. The pectin methylesterase content of cucumbers is approximately constant regardless of fruit size (Bell *et al.*, 1951), but polygalacturonase activity increases dramatically in the larger-size fruit as they ripen. The increase in polygalacturonase activity is thought to give rise to the problem of tissue softening in the seed area, a problem commonly termed 'soft centres' (McFeeters *et al.*, 1980). Softening may occur due to bacterial growth on the fruit prior to brining, but the major microbial source of polygalacturonase softening in brined cucumbers is fungi that populate the fruit and especially the fungal-laden flowers, which are frequently retained on small fruit (Etchells *et al.*, 1958). Bacterial softening is not a serious problem if the freshly harvested fruit is brined promptly after harvest because most bacterial polygalacturonases are not active in the pH range encountered in properly fermenting cucumbers (Etchells *et al.*, 1952). Mould enzymes present more of a problem, however, due to their activity at low pH values, and they should be removed. Draining the brine from tanks of small cucumbers about 36 hours after brining or washing the fruit prior to brining as in the controlled fermentation process (Etchells *et al.*, 1973) will effectively lower the level of these enzymes. Softening enzymes can be inhibited by tannin-like substances extracted from grape leaves (Bell and Etchells, 1958) and other plants (Bell *et al.*, 1962, 1965a,b), but such compounds have not been approved for use in brined vegetables.

Although polygalacturonase activity is suppressed by high concentrations of sodium chloride in the brine (Bell and Etchells, 1961), it is preferable to exclude polygalacturonase enzymes from the tank as much as possible. Current and projected restrictions on salt disposal demand concerted efforts to minimize salt usage in brining. Buescher *et al.* (1979) found that addition of calcium chloride to cucumber brines resulted in firm brine-stock pickles, even in the presence of added polygalacturonase enzymes from a mould. They suggested that the use of calcium may offer a practical means of lowering the sodium chloride concentration required for cucumber brining. Salt-free storage of olives (Vaughn *et al.*, 1969b) and cucumbers (Shoup *et al.*, 1975) has been proposed as a means of storing these products. The storage solution is acidified but does not contain salt; the product does not undergo fermentation.

B. Gaseous Deterioration

Fisheye spoilage in Spanish-style green olives is characterized by formation of gas blisters under the skin. The disorder is due to growth of gas-forming bacteria, especially coliforms, which grow when the brine contains less than 5% salt or has a high pH value (4.8–8.5). Brines may be fortified with the proper concentration of salt and acid to prevent this type of spoilage (Vaughn, 1954).

Bloater damage in brined cucumbers results from an increase in gas pressure inside the cucumbers during fermentation. This gas pressure is due to the combined effects of nitrogen, which is trapped inside the cucumbers when they are brined, and carbon dioxide (Fleming and Pharr, 1980). The carbon dioxide originates from the cucumber tissue (Fleming *et al.*, 1973b) and from gas-forming micro-organisms active in the brine such as yeasts (Etchells *et al.*, 1952), heterofermentative lactic-acid bacteria (Etchells *et al.*, 1968b), and even the homofermentative *L. plantarum* (Fleming *et al.*, 1973a). Bloater damage in brined cucumbers can be greatly decreased if carbon dioxide is purged from the brine, as is later discussed (p. 252).

C. Other Spoilage Problems

Discolouration is an important concern with some brined vegetables. Pink kraut may be caused by pink yeasts, which can grow when excess salt is used (Pederson and Albury, 1969). This problem can be avoided by proper concentrations and distribution of the salt. *Lactobacillus brevis* has been implicated in causing pink to brown off-colours (Stamer, 1975). Laboratory tests showed that increased quantities of an unidentified pink compound were formed when *L. brevis*-inoculated cabbage juice was buffered with calcium carbonate (Stamer, 1975). Bleaching may occur when brined vegetables are exposed to sunlight. Thus, it is important that the product not be excessively exposed to sunlight during brine storage or further processing. Spanish-style green olives may darken during the alkali prebrining treatment if air is allowed to contact the olives.

Undesirable flavours and odours may result from growth of undesirable micro-organisms during fermentation and storage of brined vegetables. Malodorous fermentations of olives occur when undesirable bacteria grow before the lactic-acid bacteria become established. Certain rancid odours are caused by butyric-acid bacteria, and 'zapatera' (characterized by a sagey offensive odour) is caused by unidentified bacteria (Vaughn, 1954). These problems can be prevented by proper lye and washing treatments and by use of brine concentrations that favour early growth of lactic-acid bacteria.

Off-flavours and odours in brined cucumbers originate from growth of coliform and other undesirable bacteria before lactic-acid bacteria become established, from oxidative yeasts, fungi and bacteria which grow on the surface of improperly maintained brines, and from oxidative changes brought on by exposure of the cucumbers to sunlight. Most of these problems occur because of negligence and can be avoided by proper brining, avoidance of surface growth, and by not allowing cucumbers to be exposed to sunlight for extended periods after headboards have been removed from the tank.

V. USE OF PURE CULTURES OF LACTIC-ACID BACTERIA

A. Pure-Culture Inoculation

Numerous studies have been made on pure-culture inoculation of sauerkraut, including those of Gruber (1909), LeFevre (1919, 1920, 1928), Pederson (1930) and Engelland (1962). Pederson (1960) concluded that inoculation was impractical and unnecessary, since the organisms responsible for the fermentation occur naturally in adequate numbers, and proper fermentation will occur if temperature and salt concentrations are suitable. Stamer (1968) indicated that a proper ratio of heterofermentative to homofermentative lactic-acid bacteria is probably necessary to ensure superior kraut, and that this ratio is unknown.

Use of lactic starter cultures for Spanish-style green olives was tested by Cruess (1937). Subsequently, starters of *L. plantarum* were used commercially in the California olive industry from 1937 to 1955; they have not been used extensively since (Vaughn, 1975). Addition of cultures increased the rate of acid production during the first two months after brining and resulted in less spoilage (Vaughn *et al.*, 1943). More recently, lactic acid has been added to the brines of lye-treated and washed olives, decreasing the pH value to 7.0 or below. When the need is indicated, normal brine is used to inoculate abnormal fermentations (Vaughn, 1975).

Etchells *et al.* (1966) found that heat shocking of lye-treated, green Manzanillo olives greatly increased their brine fermentation by pure cultures of lactic-acid bacteria. When the olives were neither lye-treated nor heated, added lactic cultures caused essentially no fermentation; only yeasts grew. They suggested that the heat treatment destroyed a naturally occurring inhibitor of lactic-acid bacteria, and that the inhibitor may account for the occurrence of stuck fermentations, which are characterized by the absence of lactic-acid bacteria and the presence of yeasts in the brine. Subsequent studies revealed that hydrolysis products of oleuropein, including its aglycone and elenolic acid (Fig. 5), are inhibitory to lactic-acid bacteria but not to yeasts (Fleming and Etchells, 1967; Fleming *et al.*, 1969b, 1973c). Fleming *et al.* (1973c) found that oleuropein was not greatly inhibitory to lactic-acid bacteria, contrary to earlier suggestions by Vaughn (1954) and Juven *et al.* (1968). Indeed, lactic-acid bacteria were found to utilize oleuropein (Garrido-Fernandez and Vaughn, 1978). Fleming *et al.* (1973c) suggested that oleuropein is degraded to its aglycone when unheated olives are brined, perhaps by the natural β-glucosidase which Cruess and Alsberg (1934) reported, and by further degradation to elenolic acid. Juven and Henis (1970) found that oleuropein became more inhibitory when it was treated with β-glucosidase. Perhaps heat inactivates β-glucosidase in the olives, preventing breakdown of oleuropein to yield the inhibitory aglycone when the olives are brined.

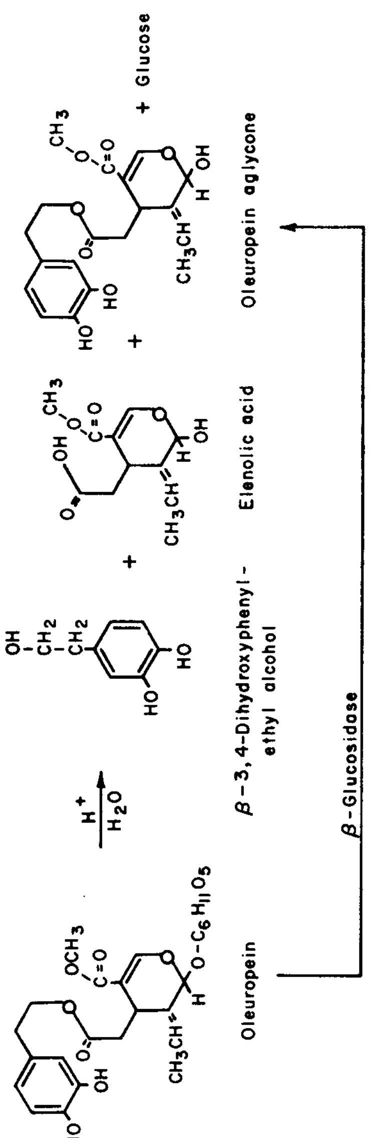

Fig. 5. Structures of oleuropein and its hydrolysis products. From Walter *et al.* (1973) and based on the work of Panizzi *et al.* (1960).

Pederson and Albury (1956) altered the course of natural cucumber fermentations by the addition of pure cultures of heterofermentative and homofermentative lactic-acid bacteria. No attempt was made to remove or inactivate the natural microflora. They found that *L. plantarum* completed all fermentations, regardless of the species of bacteria used for the inoculum, apparently because of its greater acid tolerance and presence among the natural microflora of cucumber (Pederson and Albury, 1961). Etchells *et al.* (1964, 1968a) obtained pure-culture fermentation by hot-water blanching or by gamma radiation of cucumbers prior to inoculation with lactic-acid bacteria. *Lactobacillus plantarum* produced the highest concentration of acid and grew at the lowest pH value at 8% sodium chloride. *Pediococcus cerevisiae* and *L. brevis* also grew well at 8% salt, but several thermophilic lactic-acid bacteria were limited in salt tolerance to about 2.5 to 4% sodium chloride.

B. Controlled Fermentation of Cucumbers

A controlled fermentation procedure for brined whole cucumbers was

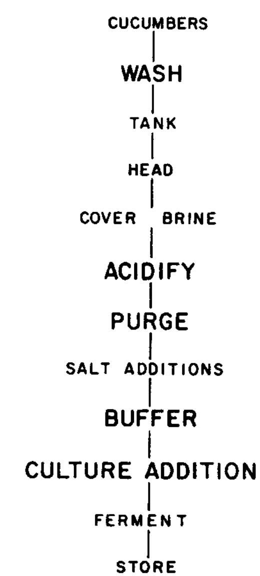

Fig. 6. Flow chart for brine fermentation of cucumbers. Steps that have been added to the overall conventional procedure (natural fermentation) to render 'controlled fermentation' are indicated in bold face. See Etchells and Hontz (1972) and Etchells *et al.* (1973) for details of procedures for natural and controlled fermentation of cucumbers.

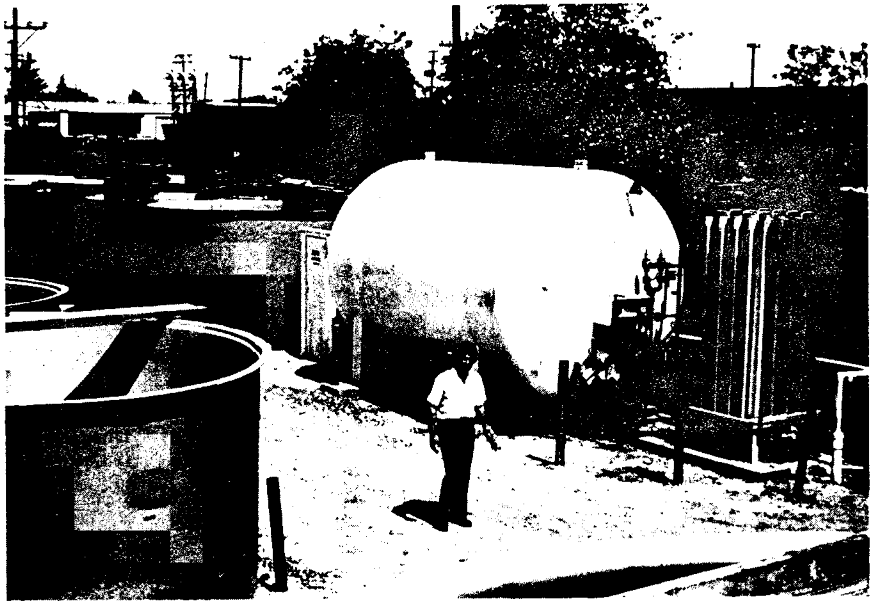

Fig. 7. Photograph of a modern cucumber brine yard with fibre-glass brining tanks and a 19,000-litre, liquid nitrogen storage tank for purging of carbon dioxide from fermenting brines. Nitrogen gas is piped to each of the brining tanks. The photograph is reproduced with the compliments of Straub Food Products, Stockton, California, U.S.A.

developed by Etchells *et al.* (1973, 1976), and has been in commercial use for several years (Fig. 6). In addition to certain steps taken previously with natural fermentations of brined cucumbers, the steps as outlined in Figure 6 foster a 'controlled fermentation'. The cucumbers are *washed* to lower the concentrations of softening enzymes, dirt and undesirable micro-organisms. After heading and addition of the cover brine (about 6.6% sodium chloride, w/w), the brine is immediately *acidified* with glacial acetic acid or vinegar (about 0.16% acetic acid, concentration at equilibration) to an initial pH value of around 2.8, which prevents growth of the natural microflora prior to addition of the culture. During the next 18 to 24 hours, nutrients diffuse from the cucumbers into the brine, and salt and acid diffuse into the cucumbers. The brine is *purged* continuously with nitrogen gas (Figs 2 and 7), which sweeps carbon dioxide from the brine until the fermentation is completed (Fleming *et al.*, 1973a, 1975; Etchells *et al.*, 1973). Salt is added in the dry form onto the top of the tank in two to three increments over the next few days to compensate for water in the cucumber (about 95%). This additional salt maintains the equilibrated brine strength at

5 to 8% sodium chloride. About 18–24 hours after brining, the brine pH value is altered from below 4.0 to around 4.6 by addition of sodium acetate, a *buffer* (Etchells *et al.*, 1973). Alternatively, commercial firms more commonly raise the pH value of the acidified brine to around 4.6 by carefully adding a predetermined amount of sodium hydroxide, hence, forming a buffer (Lingle, 1975). The sodium hydroxide is added, preferably, as pellets contained in 2.3 kg plastic bags. The bags are punctured for gradual dissolution and release of the alkali into the stream of acidified brine, which continuously exits from the side-arm purger. The lactic *culture* (*L. plantarum* alone or with *P. cerevisiae*) is then added to an initial population of around one billion cells per 5 litres of brined cucumbers. Fermentation by the added culture at 25°C to 30°C is completed within 7–12 days (Etchells *et al.*, 1973). The brine-stock cucumbers are then stored under ambient conditions until needed for further processing into dills, sweets, sours and relishes.

The foregoing procedure does not result in a pure culture fermentation. Rather, the key features of the procedure, as emphasized in Figure 6, serve to establish the conditions that favour growth of the added culture rather than the naturally occurring micro-organisms. Although the entire controlled fermentation procedure is in limited use, purging brines of carbon dioxide to prevent bloater formation has been widely adopted and has had a significant impact on the pickling cucumber industry through increased yields and improved quality of brine-stock pickles (Fleming *et al.*, 1975; Fleming, 1979; Costilow *et al.*, 1977). The effects of purging are further obvious in less buoyancy pressures on headboards of tanks, since the cucumbers do not become bloated by buildup of internal gas pockets (Fig. 2, p. 234).

A controlled fermentation procedure also has been described for sliced cucumbers (Fleming *et al.*, 1978). The cucumbers are sliced, heated, cooled and brined in a solution of sodium chloride and calcium acetate and inoculated with *L. plantarum*. Calcium acetate serves as a buffer and to firm the tissue.

Concentrated cultures specifically prepared for brine fermentations of cucumbers and other vegetables are available commercially. The cultures are shipped to the briner in frozen form with dry ice and may be kept for several weeks on dry ice or in a −40°C freezer (Porubcan and Sellars, 1979). Success has been claimed in the use of improved lyophilized cultures, which may be shipped at ambient temperature and then held under refrigeration (Porubcan and Sellars, 1975).

Although nitrogen was originally recommended as the purging gas, inert gas (obtained by combustion of the oxygen in air) and air have been used (Fleming, 1979). Air will sweep carbon dioxide from brines and thereby effectively prevent bloater formation, but may encourage growth of undesirable aerobic micro-organisms such as moulds, which may induce enzymic softening, or oxidative yeasts, which may utilize excessive amounts of lactic acid (Fleming *et al.*, 1975; Potts and Fleming, 1979 and unpublished work; Fleming, 1979).

VI. SUMMARY

Most vegetables can be preserved by brine fermentation. Major principles involved in successful vegetable fermentations include the presence of the proper amount of salt, conversion of fermentable carbohydrates into acids and other end products by certain lactic-acid bacteria and yeasts, and the preclusion of spoilage micro-organisms. Properties of the individual vegetable, particularly its susceptibility to softening, dictate the concentration of salt needed for preservation. In turn, the salt concentration greatly influences the type and extent of microbial action. Other factors that affect microbial growth include temperature, degree of exposure to air, and properties of the vegetable and the brine in which it is held (e.g. fermentable carbohydrate level, buffer capacity, pH value, acidity, natural inhibitory compounds, and availability of nutrients in the brine).

Considerable knowledge on vegetable fermentations has been accumulated during the past several decades and is available for further technological application. Anaerobic post-fermentation storage, controlled fermentation methods, purging of carbon dioxide from brines, and use of calcium chloride to lower salt requirements are recent developments which may be only the beginning of a new era in vegetable fermentations. Further research will undoubtedly yield new insights which may advance vegetable fermentations into the modern technological age that has been attained by many other fermentation industries.

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8. Coffee

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I. INTRODUCTION

Coffee is a general term which refers to the fruits, seeds and products of plants of the genus *Coffea*. Although there are over 40 species of this genus, those cultivated commercially are *Coffea arabica*, *Coffea canephora* (robusta), *Coffea arabusta*, *Coffea liberica* and *Coffea excelsa*. The coffee plant belongs to the family Rubiaceae. A mature coffee fruit is a fleshy spheroidal berry about 15–20 mm in diameter. The term berry is not botanically accurate because the coffee fruit is a drupe. It changes from a green to a cherry-red colour while ripening. The fruit normally contains two beans surrounded by a thin membrane known as the silver skin. The beans and the silver skin are protected by a hard, horny endocarp

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