USE OF MICROBIAL CULTURES: VEGETABLE PRODUCTS

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□ THE PRESERVATION OF VEGETABLES by fermentation began before recorded history. Salting (brining) is a requisite for preservation of vegetables and certain fruits by fermentation. Salt tends to direct the course of the fermentation and helps prevent softening and other undesirable changes in the vegetables. Brining and fermentation were primary methods for preserving vegetables before the advent of canning and freezing. Today, they are still primary methods of vegetable preservation in much of the Orient and in underdeveloped areas of the world.

Although secondary to modern preservation methods in Western civilization, brining and fermentation remain important methods for preserving certain vegetables, even in highly developed countries, because they: (1) yield 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 the use of these age-old methods of preservation in our modern, energy-sensitive world.

THE FERMENTATION PROCESS

Cabbage, olives, cucumbers, and peppers account for the largest volume of vegetables and fruits commercially brined and fermented in the Western hemisphere. However, most vegetables probably have been preserved by various salting and fermentation methods, either commercially or in the home.

A generalized flow chart for brine fermentation of vegetables is given in Figure 1. Vegetables may be brined whole, diced, shredded, or pierced. Salt may be added in the dry form, as with cabbage, or as a brine solution, as with most other vegetables. The concentration of salt used varies widely among vegetables (Table 1), depending to a major extent upon the tendency of the vegetable to soften during brine storage. Pectinolytic and other enzymes of microbial origin or those native to the vegetable soften brined vegetables if the salt concentration is too low. Softening can be inhibited by adjusting the level of salt to inhibit the activity of these enzymes (Bell and Etchells, 1961). The level of salt also regulates the type and extent of microbial growth, and consequently the quality and safety of the fermented vegetable product. Extensive reviews are available on the brining and fermentation of sauerkraut (Pederson, 1960), olives (Vaughn, 1954; 1975) and cucumbers (Etchells et al., 1975).

Fresh vegetables, like most plant material, contain a numerous and varied epiphytic microflora, including many potential spoilage microorganisms, and an extremely small population of lactic acid bacteria (Etchells et al., 1961; Mundt and Hammer, 1968). When vegetables are properly brined in salt concentrations up to about 8%, growth or fermentation by a sequence of various types of microorganisms occurs. This sequence may be categorized into four stages: initiation, primary fermentation, secondary fermentation, and post-fermentation (Table 2).

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Lactic acid bacteria responsible for the natural fermentation of vegetables are within the genera *Streptococcus, Leuconostoc, Pediococcus,* and *Lactobacillus.* The lactics which reportedly predominate initiation and primary fermentation of brined vegetables include *Streptococcus faecalis, Leuconostoc mesenteroides, Lactobacillus brevis, Pediococcus cerevisiae* (probably *Pediococcus pentosaceus,* according to recent classification; Buchanan and Gibbons, 1974), and *Lactobacillus plantarum* (Pederson, 1960; Vaughn, 1954; 1975; Etchells et al., 1975).

Numerous chemical and physical factors influence the rate and extent of growth of various microorganisms, as well as their sequence of appearance, during the fermentation. Acidity, pH, and buffer capacity 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, the amount of fermentable carbohydrates in the vegetable, and availability of nutrients in the brine are other important factors that also affect the fermentation.

INOCULATION WITH LACTIC ACID BACTERIA

Early studies were made on the pure culture inoculation of sauerkraut by LeFevre (1919; 1920; 1928) and Pederson (1930). Pederson (1960) indicated that inoculation was impractical and unnecessary, since the organisms responsible for fermentation occur naturally in adequate numbers, and that proper fermentation will occur if temperature and salt concentration are suitable. Stamer (1968) indicated that a proper ratio of hetero- and homofermentative lactics is probably necessary to ensure superior kraut, and that this ratio is unknown.

The 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, although they have not been used extensively since then (Vaughn, 1975). The addition of cultures increased the rate of acid production during the first 2 months after brining, which resulted in less gas-pocket formation and other types of spoilage (Vaughn et al., 1943). More recently, lactic acid has been added to the brines of lye-treated and washed olives to reduce the pH to 7.0 or below; when the need for inoculation is indicated, normal brine is used to reseed abnormal fermentations (Vaughn, 1975).

Pederson and Albury (1956) altered the course of natural cucumber fermentations by addition of pure cultures of hetero- and homofermentative lactic acid bacteria. They subsequently found, however, that *L. plantarum* completed all fermentations, regardless of species used for inoculation, apparently because of its greater acid tolerance (Pederson and Albury, 1961). Etchells et al. (1964; 1968) attained pure culture fermentation by hot-water blanching or by gamma irradiation of cucumbers prior to inoculation with lactic acid bacteria. *L. plantarum* produced the highest level of acid and grew at the lowest pH at 8% NaCl. *P. cerevisiae* and *L. brevis* also grew well at 8% salt, but several thermophilic lactics were limited in salt tolerance to about 2.5-4% NaCl. More recently, a controlled fermentation

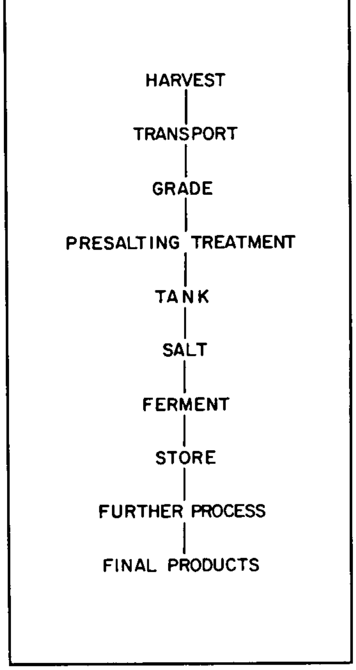

Fig. 1—**PRESERVATION** *of vegetables by brining: generalized flow chart*

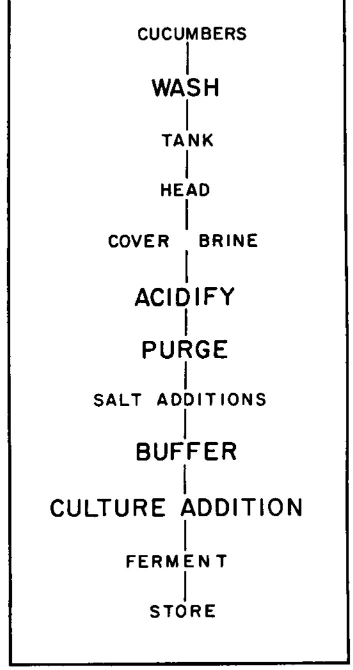

Fig. 2—**BRINE FERMENTATION** *of cucumbers: flow chart. Steps that have been added to the overall conventional procedure (natural fermentation) to render "controlled fermentation" are indicated in boldface. See Etchells and Hontz (1972) and Etchells et al. (1973) for details of procedures for natural and controlled fermentation of cucumbers*

procedure for brined cucumbers (Fig. 2) was developed by Etchells et al. (1973; 1976) and has been in commercial use for several years.

In addition to certain steps taken previously with commercial, natural fermentations of brined cucumbers, the additional steps outlined in Figure 2 foster a "controlled" fermentation. The brines are inoculated with *L. plantarum* with or without *P. cerevisiae.* The brine undergoes rapid fermentation by the added culture at 25-30°C and is complete 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, relishes, etc.

The foregoing procedure does not result in a pure culture fermentation. Rather, the key features of the procedure which are emphasized in Figure 2 serve to set the conditions to favor growth by the added culture over that by the residual, natural microorganisms.

Concentrated cultures, commercially prepared for the specific purpose of brine fermentation of cucumbers and other vegetables, are available from several commercial sources. The optimum method of freezing liquid starter culture concentrates is by immersion of seamless, extruded-aluminum cans of culture in liquid nitrogen or its vapor. The cultures are shipped to the briner in frozen form with dry ice and may be held for several weeks on dry ice or in a —40°C freezer by the briner (Porubcan and Sellars, 1979). Success has also been claimed with the use of improved lyophilized cultures, which may be shipped at ambient temperature and then held under refrigeration (Porubcan and Sellars, 1975).

SHELF LIFE OF FERMENTED PRODUCTS

Brine fermentation requires little mechanical energy input for the preservation of vegetables, compared to heat processing and freezing. Some vegetables can be stored for years in high-concentration NaCl solutions without serious loss in texture and other organoleptic properties, although economic considerations usually favor storage for no more than about 1 year. Brine storage offers a rapid method of preserving large amounts of vegetables during the harvest season. The brine-fermented product can then be further processed during the remainder of the year; this serves to distribute labor and equipment usage. The product may or may not require mild heat processing subsequent to packaging. This depends on several factors, including the type and extent of microbial activity during fermentation. Even when heat processing is used, the energy requirements are considerably lower than those required for retorting of low-acid foods.

It is interesting to note that cucumber pickles were not preserved by heat processing in the United States prior to the pasteurization studies of Etchells (1938). Preservation was accomplished by proper salting during bulk storage, followed by the incorporation of preservative levels of

Table 1—**SALTING PROCEDURES FOR VEGETABLES**[a]

	Level of salt(%)		
Method of salting	During fermentation	During storage	Vegetable
Dry salting	2-3	2-3	Cabbage
Brine solution	5-8	8-16	Cucumbers
	4-7	4-7	Green olives

[a]The levels of salt indicated generally are used for the commercial brining of cabbage, cucumbers, and olives in the U.S. Wide variations exist in the salt concentration used for peppers, onions, cauliflower, etc.

Table 2—**SEQUENCE OF MICROBIAL TYPES** *during natural fermentation of brined vegetables*

Stage	Prevalent microorganisms
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, molds, 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

vinegar and sugar into the appropriately desalted and packaged product. Principles underlying the use of vinegar, sugar, and chemical preservatives for pickles were established long ago (see, for example, Bell and Etchells, 1952; Dakin, 1962). Alternatively, fully fermented cucumbers were manufactured into such products as sours and dills by ensuring that fermentable sugars were absent. Both of these methods are used today, but mild heat processing may be used to ensure against fermentation and enzymatic softening in the finished product.

Microbial action during fermentation in bulk containers influences shelf stability of the packaged product in several ways. The Etchells et al. (1973) controlled-fermentation procedure for brined cucumbers incorporates several basic factors, discussed earlier and emphasized in Figure 2, that are relevant to maximizing shelf stability of pickles. The extent of fermentation in bulk containers is important, in that complete conversion of fermentable carbohydrates to lactic and acetic acids and other end-products can render the packaged product stable to subsequent fermentation. If the packaged product contains sufficient acid from the fermentation or from supplementation and is hermetically sealed, further microbial fermentation is normally precluded. Thus, fully fermented, brine-stock cucumbers may be processed into hamburger dill chips, whole and sliced sours, etc., and packaged without the need for heat processing to prevent fermentation. Fully fermented green olives likewise do not require heat processing (Vaughn, 1954; Fernandez Diez, 1971). The presence of residual sugar in such fermented vegetables can lead to gas pressure and unsightly brine turbidity in the final package as a result of yeast and lactic acid bacterial growth.

High levels of acidity produced during fermentation may adversely influence texture retention in cucumbers (Thompson et al., 1979) and olives (Etchells et al., 1966). Lactic acid at levels of 0.8-1% in pickles has been implicated in softening (Bell et al., 1972).

The exclusion of microorganisms that produce softening enzymes is important in prolonging the storage stability of brined vegetables. Mold polygalacturonases are particularly troublesome, as they are active at pH values at which the packaged products are held. These enzymes may be present on the raw vegetable (e.g., cucumber fruit and flower; Etchells et al., 1958) or may be formed on the brine surface of improperly attended cucumbers (Etchells et al., 1975), olives (Vaughn, 1975), and sauerkraut (Pederson, 1960). Storage of brined vegetables under anaerobic conditions is an important means for precluding such surface growth on olives (Vaughn, 1975) and sauerkraut (Pederson, 1960).

Certain chemical preservatives may be added to fermented vegetables as adjuncts to preservation. For example, sodium benzoate may be added to fully fermented vegetables to preclude or delay growth of film yeasts on the surface of the cover liquor when the container is opened by the consumer. Such a preservative is particularly useful when the product is stored in large containers such as 5-gal glass jars for dispensing over a period of days or weeks. Benzoic acid, sorbic acid, and potassium bisulfite may be used to increase the shelf stability of bagged sauerkraut (Stamer and Stoyla, 1978).

SAFETY OF FERMENTED VEGETABLES

Fermented vegetables have not been a significant source of microbial food poisoning. The Commissioner of the Food and Drug Administration stated that "No instances of illness as the result of contamination of commercially processed fermented foods with *Clostridium botulinum* have been reported in the United States" (FDA, 1979). He further stated that no cases of botulism caused by commercially processed, fermented foods were reported from his inquiry of health authorities in Canada, Japan, the United Kingdom, and other members of the European Economic Community. Botulism outbreaks have occurred in Japan, however, caused by home-prepared izushi, a fermented food containing vegetables, rice, and fish (Dolman, 1964; Iida, 1970). The food poisoning from izushi does not occur when fish is omitted from the recipe or when fermentation is not allowed to occur; clostridial toxin is produced during the course of fermentation (Nakano and Kodama, 1970).

Instances of botulism from improperly canned, especially home-canned, vegetables are well documented (e.g., Tompkin and Christiansen, 1976). The apparent low incidence of food poisoning from fermented vegetables has been attributed to rapid growth and acid production by lactic bacteria and the relatively high tolerance of these bacteria to acid and salt (Stamer, 1976). The lactic acid bacteria quickly predominate when unheated fresh vegetables are brined at about 2-8% NaCl. In addition, lactic acid bacteria may produce compounds that are antagonistic to spoilage bacteria (Hurst, 1972; 1973). Oxygen is rapidly depleted in freshly brined cucumbers, as a result of respiration of the plant tissue and bacterial growth (Potts and Fleming, 1979); this creates an anaerobic environment. From that point, it appears that competition between growth of food-poisoning bacteria and growth and acid production by lactic acid bacteria is consistently won by the lactics. It is generally accepted that growth of *C. botulinum* in foods is prevented at about 10% NaCl (Segner et al., 1966), and at pH 4.6 or below (Townsend et al., 1954). Interactive forces of low pH and high salt combine to produce an even less favorable environment for growth of *C. botulinum* (Ohye and Christian, 1967; Hauschild and Hilsheimer, 1979).

The apparently low incidence of food poisoning from fermented vegetables should not be viewed with complacency, however. Nature's mechanism for preserving vegetables by fermentation could be hindered, with potentially hazardous results. The lactic acid bacteria may be present in very low numbers and are more sensitive than bacterial spores to heat and some chemical treatments. Thus, heat-shocked cucumbers and olives are inoculated with pure cultures of lactic acid bacteria (Etchells et al., 1964; 1966). Washed cucumbers are also inoculated (Etchells et al., 1973). Green olives are treated with alkali to remove bitterness, and in the process, lactic acid bacteria are inactivated, necessitating subsequent inoculation or direct acidification to prevent growth by spoilage bacteria (Vaughn, 1975).

Natural antibiotic compounds in vegetables may act favorably or unfavorably to growth of lactic acid bacteria. Unidentified substances in cabbages inhibit growth or destroy certain undesirable gram-negative bacteria but not the natural lactic acid bacteria (Pederson and Albury, 1969). On the other hand, green olives, unless properly lye-treated or heat-shocked prior to brining, may yield compounds inhibitory to the lactic acid bacteria (Etchells et al., 1966; Fleming et al., 1973).

The use of nitrates and nitrites in meat products as curing and preservative agents is a serious public health concern because nitrites react with nitrogenous compounds to form carcinogenic nitrosamines (Bacus and Brown, 1981). Raw vegetables may contain appreciable levels of nitrates (Tate and Alexander, 1975). The significance and fate of nitrates and nitrites in fermented vegetables is not well established. Certain lactic acid bacteria, including some strains of *L. plantarum,* have been shown to reduce nitrates to nitrites (Costilow and Humphreys, 1955). Hata and Ogata (1979) reported that nitrite concentration in salt-pickled cabbage increased from nondetectable levels initially to a maximum of 108 ppm after 5 days (apparently as a result of microbial action), then rapidly declined to initial levels.

Tate and Alexander (1975) reported that N-nitrosamine did not occur in sauerkraut and silage fermentations, even when supplemented with nitrate. Addition of dimethylamine to the fermentation did result in formation of nitrosamine. They concluded, however, that nitrosamine formation probably will not occur at appreciable levels even in products prepared from nitrate-rich plants, because of the absence of appreciable levels of natural requisite precursors.

Low pH in bacon inoculated with lactic acid bacteria has been shown to result in dissipation of residual nitrite, thus providing a natural control mechanism for this product (Bacus and Brown, 1981). Perhaps low pH accounted for dissipation of nitrite in the fermented cabbage of Hata and Ogata (1979). Because of the well-established effects of nitrite on germination and outgrowth of anaerobic spores (Duncan and Foster, 1968), it is interesting to speculate that the natural nitrate level of vegetables and its reduction to nitrite during fermentation may play an important role in inhibiting anaerobic, spore-forming bacteria in fermenting vegetables. Thus, it may be desirable to use nitrate-reducing lactics for pure culture fermentation of vegetables. Further research in this area appears warranted.

Mayer et al. (1973; 1974) and Mayer (1976) reported that histamine and tyramine are formed by certain lactic acid bacteria in sauerkraut and wine fermentations. *P. cerevisiae* was implicated in amine formation. Taylor et al. (1978) surveyed 50 sauerkraut samples, representing 9 commercial brands, for histamine content. The mean level was 5 mg/100 g, with a range of 0.9-13.0 mg/100 g. Mayer and Pause (1972) found 20 mg/100 g in a sample of sauerkraut. Based upon the available data, it does not appear likely that the level of biogenic amines in sauerkraut is sufficient to cause illness (Rice et al., 1976; Taylor et al., 1978). It would be preferable, however, to select non-biogenic-amine-producing lactic acid bacteria for pure culture fermentations. Apparently only a minor portion of *Lactobacillus* and *Pediococcus* strains produce histamine (Taylor et al., 1978).

NUTRITIVE VALUE OF FERMENTED VEGETABLES

The chief nutritive value of vegetables is in their content of vitamins, which may include important amounts of β-carotene, ascorbic acid, and folic acid, and less important amounts of riboflavin and some of the other B vitamins (Davidson et al., 1979). Vitamin B_{12} is not present in vegetables. Vegetables used for fermentation are an important source of fiber, but contain low levels of protein and energy.

The effect of brining and fermentation on the nutritive value of vegetables has been reviewed (Fellers, 1960; Jones, 1975); however, relatively little information is available on the subject. Lactic acid bacteria conventionally important in vegetable fermentations are nutritionally fastidious and would not be expected to increase essential nutrients during fermentation. Costilow and Fabian (1953) found that fermentation of cucumber juice with *Lactobacillus plantarum* resulted in 10-30% losses in pantothenic acid, leucine, isoleucine, valine, tryptophan, and cysteine. On the other hand, judicious selection of microbial cultures for the fermentation may offer a means for the enhancement of important nutrients. For example, Ro et al. (1979) significantly increased the vitamin B_{12} content of kimchi by inoculation with *Propionibacterium freudenreichii* ss. *shermanii*. Production of vitamin B_{12} in tempeh fermentation is attributed to bacterial action (Liem et al., 1977).

Although microbial action may alter the nutrient content of vegetables during fermentation, other factors may significantly influence the retention of nutrients during storage and further processing. If the vegetables are brined at high levels of salt, large losses in nutrients will result when the vegetables are subsequently desalted before use (Jones and Etchells, 1944). From a nutritive standpoint, therefore, it would be preferable to brine vegetables at a low level of salt. Unfortunately, softening and other spoilage problems dictate the use of higher levels of salt in some vegetables than would otherwise be desirable. There is nearly complete loss of ascorbic acid from salt-stock cucumbers, which are stored at 8-16% salt, when the cucumbers are desalted to 2-4% salt for use in finished products (Jones, 1975). Fellers (1960) reported 86, 82, and 28% losses for vitamin C, thiamin, and carotene, respectively, in desalted salt-stock cucumbers; but, in genuine dills, which are not desalted, 33-60% vitamin C retention was found. Sauerkraut requires only 2-3% salt for preservation and therefore is not desalted before use.

Ascorbic acid (Khan and Martell, 1967; Huelin et al., 1971; Kurata and Sakurai, 1967) and thiamin (Farrer, 1955) are stabilized by acid and exclusion of oxygen. Since fermented vegetables are normally held under such conditions, good retention of these vitamins would be expected. Ro et al. (1979) observed a 50% loss of vitamin C in kimchi during the first 5 weeks of fermentation, but no further loss occurred from 5 to 10 weeks. Pederson et al. (1956) found a range of 1-35 mg/100 g of ascorbic acid in commercially canned sauerkraut. This wide range was probably a result of variations in the handling and processing procedures and in vitamin content of the fresh product. Current nutritional labels on commercial sauerkraut show a usual ascorbic acid content of 50% of the U.S. RDA per 100-g serving.

Information is needed as to whether and to what extent *cis*-isomers of carotenoids form as a result of fermentation and storage of vegetables under acid conditions. The *cis*-isomers of carotenoids possess less pro-vitamin A activity than the natural *trans*-isomers. Sweeney and Marsh (1971) found that significant amounts of *cis*-isomers of carotenoids are formed during canning or cooking of vegetables. Zechmeister (1962) has shown that carotenoids in organic solvents isomerize when treated with dilute hydrochloric acid.

FURTHER RESEARCH MAY ENHANCE VALUE

Vegetables have been safely preserved by the low-energy method of fermentation for centuries. As discussed above, the presence of salt helps to prevent softening of fermented vegetables and to direct the course of the fermentation, and therefore is a requisite for preservation of vegetables by the lactic acid fermentation. Fermented vegetables have not been reported to be a significant source of food poisoning. Certain vegetables, when properly and fully fermented, are shelf-stable without the need for heat processing. Although the lactic fermentation apparently does not greatly enhance and may even deplete some nutrients, the acid and anaerobic conditions resulting from the fermentation may increase the storage stability of some vitamins. Recent studies have indicated that certain bacteria may be used to produce vitamin B_{12} in fermented vegetables, a vitamin that is absent in fresh vegetables. Other essential nutrients may be produced by selection, adaptation, or engineering of microorganisms for use in vegetable fermentations. Further scientific and technological inputs will help to retain and perhaps enhance the value of fermentation as a way of preserving vegetables in our modern, energy-sensitive society.

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Based on a paper presented during the IFT Food Microbiology Division program, "Use of Microbial Cultures to Increase the Safety, Shelf Life, and Nutritive Value of Food Products," at the 40th Annual Meeting of the Institute of Food Technologists, New Orleans, La., June 8-11, 1980.
Paper No. 6456 of the journal series of the North Carolina Agricultural Research Service, Raleigh, N.C.