# Storage Stability of Vegetables Fermented with pH Control

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## ABSTRACT

Vegetables fermented by *Lactobacillus plantarum* with pH control were microbiologically stable during 12-months' storage in hermetically sealed jars at ca. 24°C provided all fermentable sugars were removed during fermentation, and the products were stored at pH 3.8 or below. Fermented green beans, cucumbers, red and green bell peppers, and green tomatoes were thus rendered microbiologically stable. Fermented red beets and carrots, which contained residual sucrose, underwent secondary fermentation. Calculated carbon recoveries of the fermented vegetables ranged from 74–146%.

## INTRODUCTION

BRINING, which may or may not be accompanied by fermentation, has long been used for commercial bulk storage of many fruits and vegetables. It is a low-energy means for temporary storage of perishable produce. Cucumbers, certain types of olives, and cabbage undergo a lactic acid fermentation during storage, while other products such as cherries and peppers may not. In nonfermented, brined products, preservatives such as sodium benzoate or sulfur dioxide, or high concentrations of NaCl must be added to prevent fermentation. Fermentation offers the advantages of acid formation and removal of fermentable sugars which serve to prevent growth of pathogenic microorganisms and to stabilize the products. Also, fermentation offers the potential for flavor enhancement in the products.

Before pasteurization was introduced into the United States pickle industry by Etchells (1938), commercial preservation of many pickle products relied upon conversion of fermentable carbohydrates to organic acids during bulk storage, and/or the addition of sufficient amounts of vinegar, sugar and other ingredients to fully cured and packed cucumbers to preclude microbial growth (Bell and Etchells, 1952; Dakin, 1962). The preservation of genuine dill pickles (Fabian and Switzer, 1941), which were not pasteurized, depended upon the added salt, the acid formed, and by being "fully cured." "Fully cured" refers to the complete removal of fermentable sugars and change in the flesh from an opaque to a translucent appearance. Process dill pickles and sour pickles were also prepared from fully cured brine stock, but final products were supplemented with acetic acid. Although such fully fermented, unpasteurized products were not subject to gaseous spoilage, the pickles were subject to softening during storage. Pasteurization of genuine dills was introduced as a means of preventing softening (Jones et al., 1941). Even today, however, some packers do not pasteurize genuine dills, while others do as added insurance against softening. The microbiological stability of Spanish-style green olives is dependent upon a completed fermentation; they are not pasteurized (Fernandez-Diez, 1971). Pimiento used for stuffing of fermented olives must also be fermented to prevent a secondary fermentation (Gonzalez-Cancho et al., 1972).

Successful fermentation of brined vegetables is influenced by numerous chemical and physical factors including the concentration and type(s) of fermentable carbohydrate of the raw product, and buffering capacity of the vegetables (Fleming, 1982). During primary fermentation, carbohydrates are converted to acids and other end products by lactic acid bacteria and yeasts. If sugars are incompletely fermented during primary fermentation, the product will be susceptible to secondary fermentation by yeasts. Fermentative yeasts can grow and cause gaseous spoilage even after growth of lactic acid bacteria has been inhibited by low pH. Etchells et al. (1973) added sodium acetate to brined cucumbers, which permitted complete fermentation of cucumber sugars by the added culture of *Lactobacillus plantarum*. Neutralization of brine acid by addition of NaOH has been suggested as an inexpensive means of assuring complete fermentation of cucumbers (Lingle, 1975).

Collective evidence indicates, therefore, that fermented vegetables may rendered microbiologically stable provided essentially all fermentable carbohydrates are removed during primary fermentation, sufficient acid is present to prevent growth of spore-forming spoilage bacteria, and oxygen is excluded from the products to prevent surface growth of yeasts, molds and spoilage bacteria. However, no systematic investigation has been made to establish general applicability of these principles to an array of vegetables.

The objective of this study was to determine problems associated with the microbiological and product stability during storage of seven vegetables fermented with pH control to facilitate complete removal of fermentable sugars. The vegetables included in this initial survey were green beans, red beets, carrots, cucumbers, green and red bell peppers, and green tomatoes. A procedure for pH control of fermentations by sodium hydroxide addition is described. Fermentable sugars and products of fermentation were determined for each vegetable.

## MATERIALS & METHODS

### Fresh vegetables

The vegetables used in this study were grown using standard production practices for North Carolina and were harvested at optimum quality. Cultivars were as follows: green beans, cv. Bush Blue Lake 274; red beets, cv. Ruby Queen; carrots, cv. Danvers 126; cucumbers, cv. Chipper; green and red bell peppers, cv. Keystone Resistant Giant no. 3; green tomatoes, cv. UC 82B. The vegetables were hand picked and stored at 10°C (50°F) at 90% relative humidity until use. Tap water and food-grade, granulated salt (noniodized without anticaking agents) were used in brine preparation. All other chemicals were reagent grade. Deionized water was used for all analytical work.

### Vegetable preparation

All vegetables were washed. Cucumbers (3.8–4.4 cm diameter) were cut into 5 mm slices. The stems and seeds were removed from bell peppers, and the hulls were cut into quarters or eights. Green tomatoes and red beets were quartered. The ends were removed from the green beans, and they were broken into two or

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three pieces. Carrots were cut longitudinally into four to six sections. All vegetables were heated in 82°C water until the internal temperature of the vegetable reached 76.7–79.4°C (170–175°F). They were held at that temperature for 3 min and rapidly cooled in 20°C water.

### Brining procedure

The fermentation vessel consisted of a 5-gal (18.9 liters) plastic pail fitted with a clear, plastic side-arm tube (Fig. 1). The side arm tube (4.7 cm i.d. x 38 cm tall) served as a mixing chamber for NaOH addition and for nitrogen purging, and contained 650 ml when filled to the pail-return-inlet (Fig. 1). The pail and side arm were completely filled with 200 ppm chlorine solution and then emptied just before addition of the vegetable. Immediately after cooling, 9.5 kg of each vegetable and an equal weight of brine were added to the pail. The brine contained 5% NaCl and 0.1% acetic acid adjusted to pH 4.5 with NaOH. The brines were inoculated with $10^6$ cells/ml, total volume, of *L. plantarum* WSO which had been grown in MRS broth with 2% NaCl for 8 hr prior to inoculation. A plastic mesh header was placed on top of the vegetables to hold them under the brine. The pail was covered with a tight-fitting lid that had a brine-filled gaslock fitted through a septum to allow escape of $N_2$ and $CO_2$ during fermentation. A needle for delivery of standardized, 10N NaOH was placed through a septum near the bottom of the side arm. Nitrogen at a flow rate of 25 ml/min was bubbled into the side arm in order to remove $CO_2$ produced in the fermentation and to maintain anaerobic conditions. The brine was continuously circulated with a Vibrostaltic pump (Chemical Rubber Co., Cleveland, OH) at a flow rate of ca. 650 ml/min. A combination pH electrode was fitted into the side of the pail for removal of brine samples with a sterile syringe.

The electrode was connected to a pH controller which was set to deliver 10N NaOH solution for 15 sec after a 30-min equilibration cycle, provided the pH of the brine was below 3.8. The equilibration period was programmed to prevent rapid multiple base additions before thorough mixing of the NaOH occurred. The pH of the brine was continuously recorded, the number of NaOH additions was counted, and the NaOH was delivered from a weighed volumetric flask so the amount of solution dispensed could be determined.

### Sampling

During fermentation, brine samples were taken with a sterile syringe through the septum placed in the middle of the side of the fermentation pail (Fig. 1). These samples were immediately plated with appropriate media for microbiological counts. Other samples were preserved by freezing for chemical analysis.

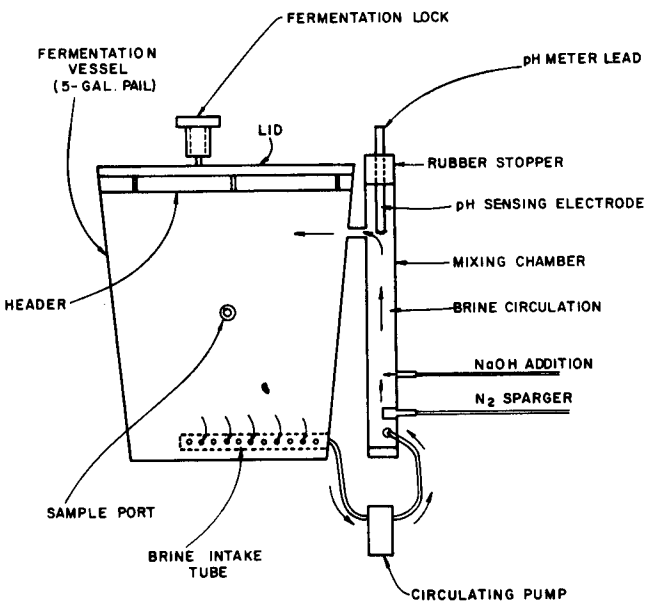


*Fig. 1—Fermentation pail used to maintain pH control during vegetable fermentations.*

Samples of the vegetables were taken for HPLC analysis before and after fermentations. The blanched vegetables (50g) were mixed with an equal weight of cover brine and frozen. After fermentation, equal weights of vegetable and brine were also frozen. For analysis the frozen samples were partially thawed and blended with a Tekmar tissue homogenizer. The particles were removed by brief centrifugation and the supernatant was filtered through a 0.22$\mu$ Millipore filter prior to chromatography.

### Microbiological analyses

Standard plating techniques were used to enumerate microorganisms in fermenting brines. The total aerobic population was enumerated with Standard Methods Agar (BBL, Cockeysville, MD); lactic acid bacteria with LBS Agar (BBL), containing 200 ppm cycloheximide (Calbiochem, San Diego, CA); and yeasts with Dextrose Agar (BBL) acidified with 5 ml of 10% sterile tartaric acid per 100 ml of medium immediately before plating. All plates were incubated at 30°C.

### Analysis of sugars, acids and ethanol

Fermentation substrates and products were analyzed by HPLC. The system consisted of a Waters 6000A pump, U6K injector and model 401 refractive index detector (Waters Associates, Milford, MA) and a Spectra-Physics 4100 integrator (Spectra-Physics, Santa Clara, CA). A Dupont Zorbax ODS $C_{18}$ column was used for the separation of sucrose, lactic acid, acetic acid, and ethanol. Separation conditions were similar to those described by Coppola et al. (1978). The mobile phase was pH 2.8, 0.05M phosphoric acid-ammonium phosphate buffer. Succinic acid was added to the samples as an internal standard. A 10–25 $\mu$l sample was injected.

For glucose and fructose measurements a Waters $\mu$Bondapak carbohydrate column with an acetonitrile-$H_2O$ (84:16) mobile phase was used (Hurst and Martin, 1977). The presence of NaCl in the samples resulted in a peak that interferred with fructose. Rhamnose was added to the samples as an internal standard. Then the NaCl was removed from samples by addition of a mixed bed ion exchanger (Rexyn I-30, Fisher Scientific Co., Raleigh, NC) which had been washed with water and methanol to remove colored material. A 10–25 $\mu$l sample was injected onto the column.

### Calculation of conversion efficiency

Wood (1961) gave a detailed description for calculation of fermentation balances. A somewhat simplified version of this approach was used to calculate the efficiency of conversion of sugars to fermentation products. Nitrogen purging removed $CO_2$ during fermentation so that is was not practical to measure $CO_2$ production. It was assumed that a mole of $CO_2$ was produced for each mole of ethanol or acetic acid found during fermentation. Therefore,

$$\text{Conversion efficiency (\%)} = \frac{(L_f - L_i) + (A_f - A_i) + (E_f - E_i)}{2\,[(G_i - G_f) + (F_i - F_f) + 2(S_i - S_f)]} \times 100$$

where L, A and E represent molar concentrations of products formed (lactic acid, acetic acid, and ethanol, respectively) and G, F and S represent molar concentrations of carbohydrate substrates (glucose, fructose and sucrose, respectively). The initial and final concentrations of these compounds are shown by the subscripts i and f, respectively.

### Storage stability

Immediately after fermentation, a 300-g sample of each vegetable containing equal amounts of brine and fermented product at pH 3.8 was blended in a Waring Blendor. Portions (100g) of the slurry were titrated with NaOH or HCl to determine the amount of acid or base required to adjust the pH of portions of the products to 3.3 and 4.3 for storage studies. The appropriate amount of 6N HCl or NaOH was added to 1 liter of brine, and the brine was added to 1 kg of product in a gallon jar to attain the desired pH. The jars were kept at 10°C for 3 days for partial equilibration of acid/base before repacking into 8-oz jars for storage at room temperature.

The experimental design for storage studies is shown in Fig. 2. Jars (8 oz) were packed with equal weights of product and brine equilibrated at pH 3.3, 3.8 and 4.3. Eight jars were packed for each product at each pH, except for carrots (only six jars at each pH). Another set of eight jars (except carrots, six jars) containing product at pH 3.8 was capped and pasteurized by heating to an internal

temperature of 74°C and holding for 15 min. The jars were cooled in 20°C water and then stored inverted at room temperature (ca. 24°C) in cardboard boxes. Two jars from each treatment were evaluated 2 wk after packing (initial) and 3, 6 and 12 months thereafter. Brine pH, titratable acidity, brine turbidity, and vacuum were determined at each sampling period for each vegetable.

### Taste panel

For each session, the taste panel consisted of five to seven members of this laboratory. At the beginning of each session, a refrigerated control sample of the commodity to be evaluated was equilibrated to room temperature. The control was the fermented vegetable (pH 3.8), refrigerated immediately after fermentation. Consensus evaluations of firmness and off-flavor were established by tasting the control. The panelists then rated the samples from the four treatments for each of these factors using a one (no off-flavor, extremely soft) to ten (extreme off-flavor, extremely firm) rating scale. At anytime during the session a panelist could refresh his evaluation of the control sample. All panel sessions were duplicated, and samples were coded by nonparticipating personnel.

### Kramer shear analysis

The Instron Universal Testing Machine equipped with a Kramer shear cell, model CS-1, was used to test the firmness (peak height) of the fermented vegetable products. Twenty ±0.05g samples were uniformly layered over the bottom of a 500-kg compression shear cell for each test. Six replications per product per treatment were made using cross head and chart drive speeds of 100 mm/min.

The final preparation of the vegetable samples prior to testing was dependent upon the natural geometry and preparation of the vegetables prior to brining. The carrot samples were cut into eights, longitudinally, and then cut to length to fit the shear cell. The green beans were cut to length to fit the shear cell. The cucumber slices were cut in half, and only half of each slice was used to obtain the required sample weight. The quartered tomatoes (brined quartered) were sliced into approximately three to four crescent-shaped pieces. The red and green peppers were cut into squares of approximately 19–25 mm, with care being taken to avoid the "ridges" which occur on the inside of the pepper. The beets were cut into ca. 13-mm cubes.

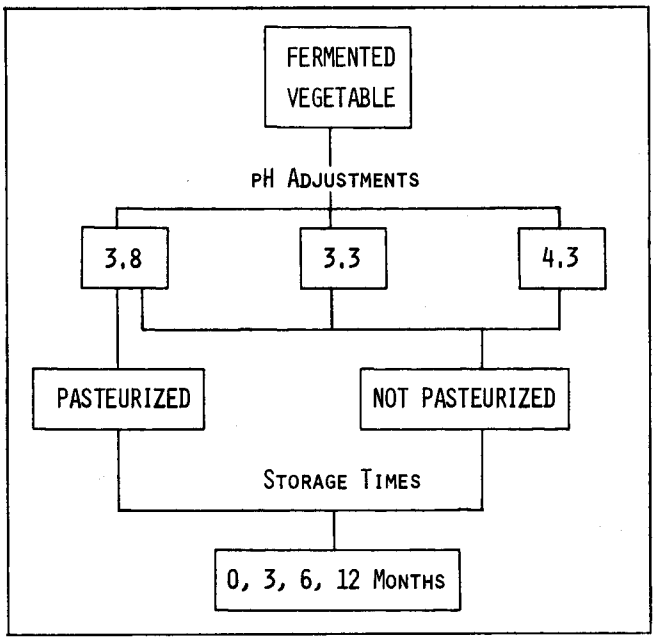


*Fig. 2—Experimental design for storage study for each of the seven fermented vegetables.*

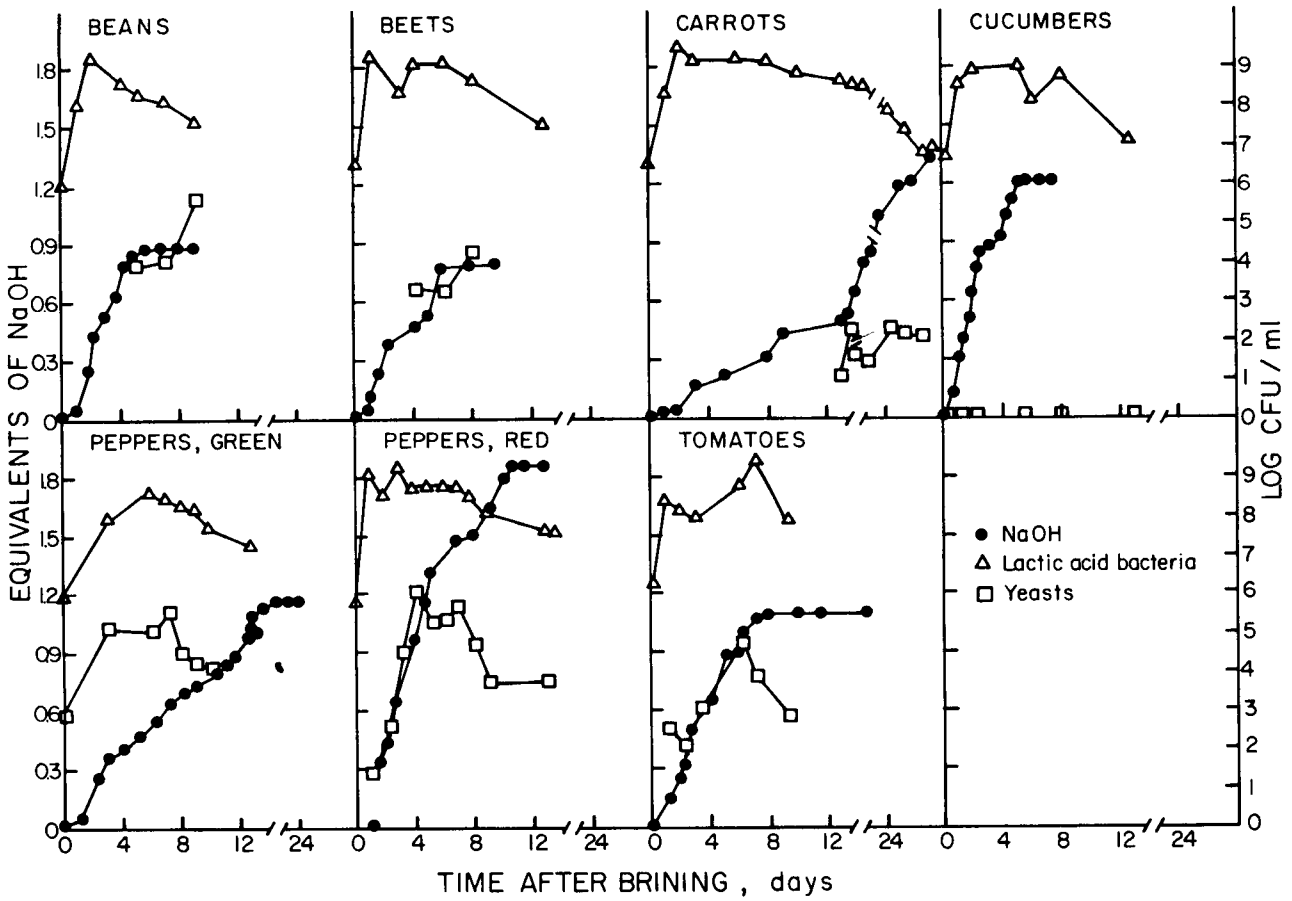


*Fig. 3—NaOH addition, lactic acid bacteria, and yeast counts obtained during pH-controlled fermentation of seven vegetables.*

## RESULTS

### Fermentation

Fermentation progress of the seven vegetables as indicated by sodium hydroxide addition is shown in Fig. 3. There was an initial lag in NaOH addition due to the lag in growth of the bacterial inoculum and to a drop in pH from 4.5 in the initial cover brine to the set point of 3.8 for the pH controller. The subsequent rate of NaOH addition varied among the seven vegetables, being fastest for cucumbers and slowest for carrots. Total equivalents of NaOH added also varied from a low of 0.9 for beets to a high of 1.9 for red peppers. The end point of fermentation was indicated by the time when NaOH addition ceased. By this indicator, fermentation of all products, except carrots, appeared to be complete within 14 days or less. Fermentation of carrots was not complete after 30 days.

Growth of lactic acid bacteria was rapid in all products and reached maximum cell numbers of $10^9$/ml or greater for all products except green peppers. The rate of bacterial growth in green peppers was slower than in other products, and reached a maximum of $4 \times 10^8$ cells/ml. The total aerobic plate counts (data not shown) were nearly identical to lactic acid bacteria counts in all products. Although not intended, yeasts also grew in all products, except cucumbers, but in much lower numbers ($10^2$ to $10^6$ cells/ml maximum) than lactic acid bacteria (Fig. 3).

### Fermentation substrates and products

Fermentation substrates and products of the seven vegetables are summarized in Table 1. Fructose and glucose constituted the known fermentable sugars in beans, cucumbers and peppers. Sucrose, in addition to glucose and fructose was present in carrots and peppers (green and red). Beets contained only sucrose. These fermentable sugars were removed from all vegetables during the 8- to 35-day fermentation period, except for beets and carrots which contained residual sucrose.

Lactic acid was the major constituent of the three end products determined from all fermentations (Table 1). Acetic acid was produced in small amounts in all products except carrots. Fermented carrots contained a comparatively high amount of acetic acid, 0.59%, for which explanation is not readily apparent. Ethanol was detected only in beans and green and red peppers. The conversion efficiency of fermentable sugars to the above three end products varied from 74.3% in green peppers to 146.2% in carrots.

### Storage of fermented vegetables

**Microbiological stability.** Changes in brine acidity and gas pressure within the jar were used to indicate microbiological stability of the fermented and stored vegetables (Table 2). Based on these criteria, green beans, cucumbers, and tomatoes were microbiologically stable at all pH values tested. Acidity changes and gas pressure were negligible in these products. They did not vary appreciably from changes that occurred in the pasteurized products stored at pH 3.8. None of these products contained fermentable sugars after fermentation (Table 1).

Beets underwent secondary yeast fermentation in the jar at all pH values as evidenced by gas pressure and microscopic observation in all jars except the pasteurized lot (Table 2). Brine seeped from all jars under gas pressure. The acidity increased in unpasteurized beets held at pH 3.8 and 4.3, but not at 3.3. Secondary fermentation of the unpasteurized beets was not unexpected, since they contained 2.8% sucrose after fermentation ceased.

Carrots underwent secondary yeast fermentation at pH 3.3 and 3.8 as evidenced by gas pressure, but not at pH 4.3. Titratable acidity increased in the pH 4.3 carrots, however, indicating bacterial growth (Table 2). The carrots contained 0.95% sucrose after fermentation (Table 1).

Bell peppers, both green and red, underwent secondary fermentation when stored at pH 4.3 as evidenced by gas pressure in some jars and by a slight increase in acidity (Table 2). The products appeared to be microbiologically stable at pH 3.3 and 3.8. The peppers did not contain measurable fermentable sugars after fermentation (Table 1).

A noticeable brine turbidity and/or microbial sediment was observed in the beets (all pH values) and carrots (pH 4.3) that underwent secondary fermentation (data not shown). No microbial growth was evidenced in any of the other stored products.

**Firmness changes.** The change in firmness of the seven commodities over 12 months of storage is shown in Fig. 4. Firmness of green beans increased noticeably during the first 3 months of storage and then declined. There was a gradual but variable decline in firmness of the other six

*Table 1—Sugar, acid and ethanol concentrations in products before and after fermentation*[a]

| Vegetable | Before or after fermentation | Fermentable sugars | | | | Lactic acid | Acetic acid | Ethanol | Conversion efficiency[d] (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | Fructose | Glucose | Sucrose | Total | | | | |
| Beans | Before | 0.51 | 0.47 | —[c] | 0.98 | — | 0.05 | — | |
| | After (13)[b] | — | — | — | — | 1.23 | 0.12 | 0.03 | 142.2 |
| Beets | Before | — | — | 3.88 | 3.88 | — | 0.04 | — | |
| | After (12) | — | — | 2.80 | 2.80 | 0.91 | 0.07 | — | 84.0 |
| Carrots | Before | 0.37 | 0.49 | 1.96 | 2.82 | — | ND[e] | — | |
| | After (35) | 0.04 | — | 0.91 | 0.95 | 1.93 | 0.59 | — | 146.2 |
| Cucumbers | Before | 0.38 | 0.40 | — | 0.78 | — | 0.04 | — | |
| | After (8) | — | — | — | — | 0.98 | 0.08 | — | 133.0 |
| Peppers, green | Before | 0.25 | 0.35 | 0.48 | 1.08 | — | 0.03 | — | |
| | After (25) | — | — | — | — | 0.62 | 0.06 | 0.08 | 74.3 |
| Peppers, red | Before | 0.84 | 0.87 | 0.04 | 1.75 | — | 0.04 | — | |
| | After (14) | — | — | — | — | 1.53 | 0.14 | 0.23 | 121.6 |
| Tomatoes | Before | 0.56 | 0.51 | — | 1.07 | — | 0.04 | — | |
| | After (16) | — | — | — | — | 1.34 | 0.11 | — | 135.0 |

[a] Results are expressed as percent (w/w) of the vegetable/cover brine mixture.
[b] Time in days after brining when analyses were made.
[c] —, none detected.
[d] Conversion of sugars to lactic acid, acetic acid and ethanol. See Materials & Methods for method of calculation.
[e] Not determined.

products. Firmness retention for all products as affected by pH adjustments, pasteurization treatments and storage times is shown relative to initial firmness of the unpasteurized product adjusted to pH 3.8. Initial firmness of the seven fermented products varied significantly ($P \leq 0.05$, Table 3). Likewise, initial firmness loss as affected by pasteurization varied; there was a significant loss in firmness of beets, peppers and tomatoes upon pasteurization, but not for green beans, carrots and cucumbers (Table 3).

Relationships between firmness as determined by taste panel and by Kramer shear tests were significant for all products ($P \leq 0.05$). Correlation coefficients between the two methods were: beans, 0.71; beets, 0.84; carrots, 0.92; cucumbers, 0.91; green peppers, 0.75; red peppers, 0.83; and tomatoes, 0.63.

**Flavor of products.** The flavor of all products was acceptable immediately after fermentation. Each product had distinctive characteristics. Flavor changes during storage depended upon the product, pH and whether the product was pasteurized. Off-flavor ratings and predominant comments from the panelists are summarized in Table 4. The greatest increase in off-flavor development during storage occurred in green and red peppers and green tomatoes. Green and red peppers were characterized by bitterness, which intensified during storage. In addition, red peppers acquired a chemical off-flavor. Tomatoes acquired a phenolic off-flavor at pH 4.3. Beets had an alcoholic flavor and carrots had a yeasty flavor, apparently due to yeast growth. Off-flavors in green beans and cucumbers were characterized mainly as haylike and musty.

## DISCUSSION

COMPLETE FERMENTATION of green beans, cucumbers, red and green bell peppers, and green tomatoes rendered these products microbiologically stable when they were hermetically stored at pH 3.3 or 3.8 at room temperature (ca. 24°C). These products did not undergo secondary fermentation, as evidenced by the absence of gas pressure in the storage jars, the lack of significant change in brine acidity when compared to pasteurized lots, and absence of brine sediment or turbidity. Complete removal of fermentable carbohydrates, and perhaps other essential nutrients, apparently precluded growth by fermentative bacteria and yeasts in these products. Fleming et al. (1983) showed that brines from these fermented vegetables did not ferment when inoculated with yeasts or lactic acid bacteria. When the brines were supplemented with glucose, however, all of the brines supported growth of yeasts and the culture of *L. plantarum* with which the products were originally inoculated. Yeasts and lactic acid bacteria are the only microorganisms capable of growing anaerobically that have been implicated in spoilage of pickle products acidified below pH 4 (Etchells and Bell, 1976). Thus, preclusion of growth of these microorganisms by absence of fermentable carbohydrates was not unexpected.

When the above products were stored at pH 4.3, however, red and green bell peppers produced gas pressure, and the brine acidity increased. Although we found no residual sugars in these fermented products, levels of sugar below the sensitivity of the HPLC method employed could account for at least part of the acid formed. However, this does not explain why the products stored at pH 3.8 did not also ferment. Another plausible explanation could be that fermentable sugars were released from these products during storage. Growth of the flat sour bacterium, *Bacillus coagulans*, could account for the acid produced in the peppers stored at pH 4.3, if sugars were released during storage. This organism has long been known to cause spoilage in tomato and other fruit products stored at above pH 4 (Becker and Pederson, 1950). *Clostridium thermosaccharolyticum* has been reported to grow and produce spoilage in canned vegetables down to pH 4.1 and at temperatures as low as 37°C (Ashton, 1976); and this bacterium hydrolyzes starch (Buchanan and Gibbons, 1974). But the optimum temperature for growth of this thermophilic bacterium is 55°C; they seldom grow below 32°C (Ashton, 1976). The products in this study were stored at room temperature (24°C).

*Table 2—Storage stability of fermented vegetables as indicated by brine acidity and gas pressure*

| Fermented vegetable | Measurement[a] | pH Adjustment for storage[b] Not pasteurized 3.3 | 3.8 | 4.3 | Pasteurized 3.8 |
|---|---|---|---|---|---|
| Beans | Initial acid, % | 0.88 | 0.64 | 0.44 | 0.58 |
| | Change in acid, % | 0.11 | 0.08 | 0.08 | 0.08 |
| | Final pH | 3.2 | 3.6 | 4.0 | 3.7 |
| | Gas pressure | 0/6 | 0/6 | 0/6 | 0/6 |
| Beets | Initial acid, % | 0.88 | 0.64 | 0.40 | 0.56 |
| | Change in acid, % | 0.11 | 0.17 | 0.70 | 0.10 |
| | Final pH | 3.4 | 3.7 | 3.7 | 3.8 |
| | Gas pressure | 6/6 | 6/6 | 6/6 | 0/6 |
| Carrots | Initial acid, % | 1.92 | 1.45 | 1.23 | 1.46 |
| | Change in acid, % | −0.01 | 0.08 | 0.58 | 0.09 |
| | Final pH | 3.4 | 3.8 | 3.8 | 3.8 |
| | Gas pressure | 1/4 | 2/4 | 0/4 | 0/4 |
| Cucumbers | Initial acid, % | 0.77 | 0.44 | 0.28 | 0.41 |
| | Change in acid, % | 0.04 | 0.04 | 0.03 | 0.06 |
| | Final pH | 3.2 | 3.8 | 4.1 | 3.8 |
| | Gas pressure | 0/6 | 0/6 | 0/6 | 0/6 |
| Peppers, green | Initial acid, % | 0.75 | 0.54 | 0.29 | 0.52 |
| | Change in acid, % | 0.03 | 0.05 | 0.13 | 0.02 |
| | Final pH | 3.3 | 3.7 | 4.1 | 3.8 |
| | Gas pressure | 0/6 | 0/6 | 2/6 | 0/6 |
| Peppers, red | Initial acid, % | 1.19 | 0.72 | 0.42 | 0.72 |
| | Change in acid, % | −0.07 | 0.04 | 0.13 | −0.01 |
| | Final pH | 3.2 | 3.7 | 4.1 | 3.7 |
| | Gas pressure | 0/6 | 0/6 | 4/6 | 0/6 |
| Tomatoes | Initial acid, % | 1.17 | 0.84 | 0.45 | 0.86 |
| | Change in acid, % | 0.05 | 0.02 | 0.05 | 0.00 |
| | Final pH | 3.3 | 3.7 | 4.1 | 3.6 |
| | Gas pressure | 0/6 | 0/6 | 0/6 | 0/6 |

[a] Titratable acidity is expressed as lactic acid. Changes in acidity are relative to initial acidity, which was measured after fermentation and before storage. Gas pressure is indicated as the number of jars under pressure out of the total number of jars tested over the 12-month period.

[b] Acidity and final pH measurements are for duplicate, 8-oz jars stored for 12 months. Data for gas pressure and brine turbidity are compiled from analyses of duplicate jars for 3, 6, and 12 months of storage.

*Table 3—Effect of pasteurization on initial firmness loss in fermented vegetables*

| Fermented vegetable | Kramer shear before pasteurization, N/g[a] | Firmness loss due to pasteurization, %[b] |
|---|---|---|
| Beans | 63.7c | 4.3 |
| Beets | 48.0d | 34.4** |
| Carrots | 81.4e | 3.7 |
| Cucumbers | 60.8c | 8.2 |
| Peppers, green | 52.5d | 46.2** |
| Peppers, red | 30.4f | 56.8** |
| Tomatoes | 34.3f | 38.6** |

[a] Values were determined immediately after fermentation. Values with a common postscript are not significantly different ($P \leq 0.05$) by Duncan's New Multiple Range Test (Duncan, 1955).

[b] **Indicates that firmness loss due to pasteurization was significant ($P \leq 0.01$) for the product.

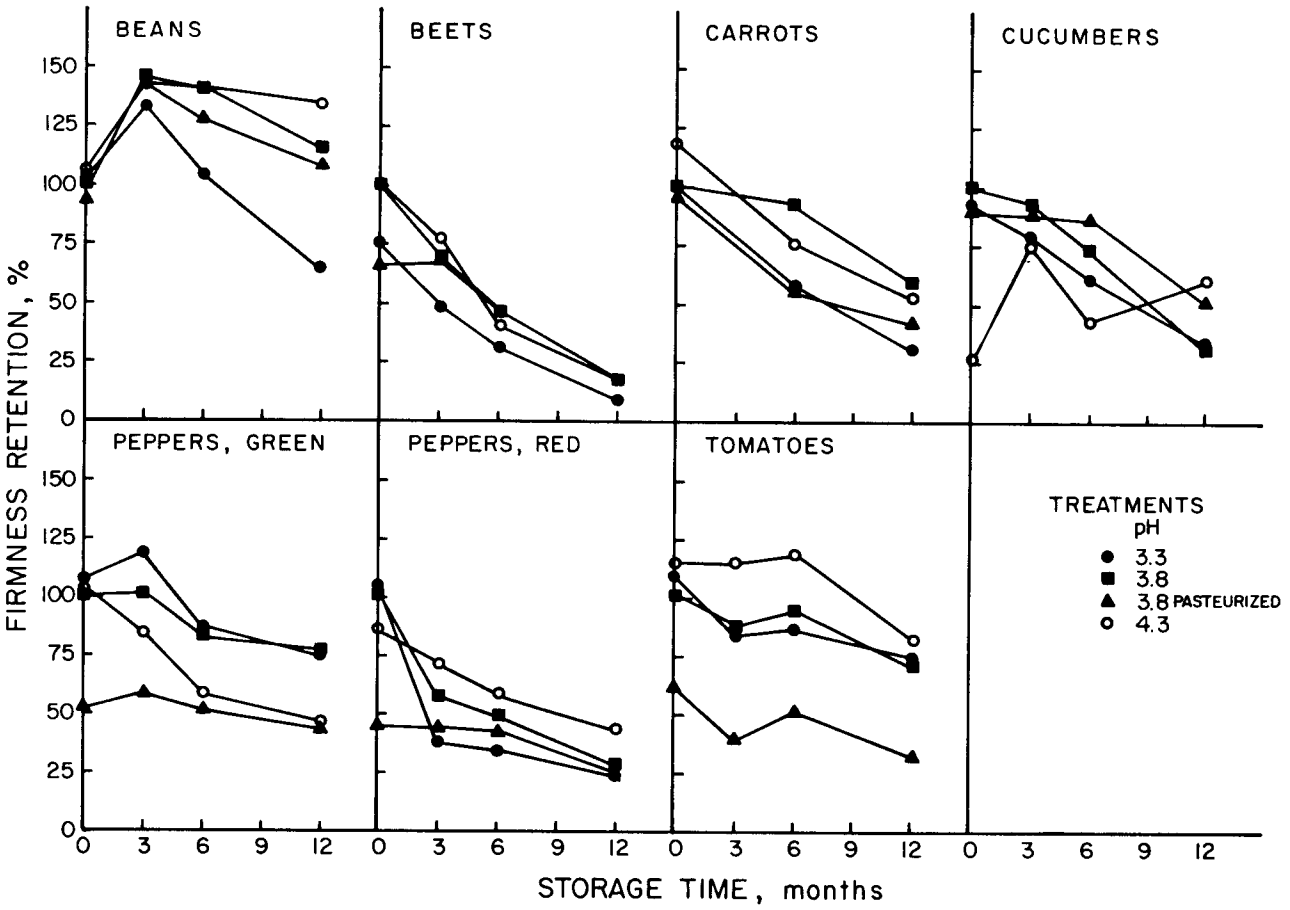


*Fig. 4—Retention of firmness in fermented vegetables during storage as determined by Kramer shear analysis.*

*Table 4—Off flavor ratings and comments for fermented and stored vegetables*

| Fermented vegetable | Storage time, months | Off flavor ratings and comments[a] Not pasteurized pH 3.3 | pH 3.8 | pH 4.3 | Pasteurized pH 3.8 |
|---|---|---|---|---|---|
| Beans | 0 | 2.5 | 1.2 | 2.2 | 4.7 E |
| | 12 | 1.5 | 3.2 C | 3.2 C, M | 2.6 M |
| Beets | 0 | 2.0 | 2.2 | 2.2 | 1.2 |
| | 12 | 2.7 A | 3.0 A | 3.0 A, Y | 2.0 E |
| Carrots | 0 | 2.4 | 1.1 | 1.6 | 2.0 E |
| | 12 | 1.9 Y | 2.2 Y | 1.9 S, M | 2.5 E |
| Cucumbers | 0 | 1.5 | 3.0 H | Spoiled[b] | 2.8 H |
| | 12 | 1.3 H | 1.4 H | 2.4 H, M | 3.7 H |
| Peppers, green | 0 | 1.9 B | 1.5 H | 2.2 B | 3.6 B |
| | 12 | 2.8 B | 3.9 B | 5.8 B, C | 6.5 B, S |
| Peppers, red | 0 | 1.5 B | 1.4 B | 1.8 B | 2.0 |
| | 12 | 6.4 B | 6.1 B, C | 5.4 B, C | 7.9 B, C |
| Tomatoes | 0 | 1.0 | 1.0 | 1.8 | 2.2 H, S |
| | 12 | 1.6 | 4.3 S, B | 4.6 P | 3.6 H |

[a] Numbers refer to taste panel scores with 1 being no off flavor and 10 being extreme off flavor. Letters adjacent to numbers refer to predominant comments by panelists with A = alcoholic, B = bitter, C = chemical, H = haylike, E = heated, M = musty, P = phenolic, S = stale, and Y = yeasty.
[b] The tissue was soft and partially liquified.

Secondary fermentation of the beets and carrots by yeasts was not unexpected, since both products contained residual sucrose. The *L. plantarum* culture used has been found to grow in test media with sucrose as the sole fermentable sugar. It has not been established why the lactic acid fermentation was so prolonged in the case of carrots, or apparently ceased in the case of beets before complete sugar removal, or how rapid complete fermentation can be assured. Further studies are needed to answer these questions.

Firmness retention of the fermented green beans during storage at pH 3.8 was exceptionally high among the seven products tested, which makes this vegetable particularly suitable for bulk fermentation and storage under the low-salt conditions used. Firmness of beets, carrots and cucumbers gradually declined over the 12-mo storage, which would make these products less suitable. However, addition of calcium to these products could make them also suitable for low-salt storage. Firmness retention has been shown to be improved in whole and sliced cucumbers held at 1.3% NaCl with addition of calcium acetate (Fleming et al., 1978). Further studies by Thompson et al. (1979) and Buescher et al. (1979, 1981) also indicate the value of calcium for firmness retention of cucumbers during low-salt brine storage. Off-flavor problems were greater for green and red bell peppers and green tomatoes during storage than for the other products. Also, tissue sloughing was particularly noticeable in these products. Thus, these products may not be suitable for storage under the conditions used in this study. However, other blanching and storage conditions, or addition of suitable chemicals could improve the organoleptic stability of these products.

It should be recalled that the vegetables used in this study were blanched to 77–80°C internally before fermentation. This treatment was done to inactivate undesirable microorganisms before inoculation with *L. plantarum*, and to inactivate softening enzymes. The blanching treatment was considered desirable as a standard treatment for all vegetables, especially to prevent softening. The vegetables were fermented and stored at only 2.5% NaCl. Softening of brined cucumbers (Bell and Etchells, 1961) and olives (Vaughn, 1954) has been reported to be more serious at

lower concentrations of NaCl. Spanish-style green olives and genuine dill pickles are not blanched before fermentation, but the NaCl content in these products is normally two to three times more than used in this study. The effects of blanching were not determined in this study. Pasteurization of the fermented products prevented microbial growth in the products, but did not prevent firmness loss and off-flavor changes. In fact, pasteurization caused a significant and immediate decrease in the firmness of beets, pepper and tomatoes. Possible advantages and disadvantages of blanching and pasteurization in stabilizing organoleptic properties of fermented vegetables is a subject for further study. Effects of these treatments on individual vegetables should be assessed, as indicated by the variability in response to pasteurization among fermented vegetables in this study.

Though many fermentation balances have been done for lactic acid bacteria in microbiological media with single carbon sources (Wood, 1961; DuPlessis, 1963), we were not aware of any published balances done in fermented vegetables. Since both the major fermentable sugars and fermentation products were determined, the conversion efficiencies of sugars to fermentation products were calculated. Five of the products had conversion efficiencies well above 100%. This suggests that other substrates which were not measured were converted to the measured product. In two instances the conversion efficiency was less than 100%, which indicates that some of the sugars were converted to products that were not measured.

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