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# Fermentation of Cucumbers in Anaerobic Tanks

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## ABSTRACT

A procedure was developed and tested for fermentation of cucumbers at low concentrations of NaCl in experimental, anaerobic tanks. The procedure included washing of the cucumbers, use of a buffered cover brine composed of 0.045M calcium acetate, sodium chloride to equilibrate at 2.7% or 4.6%, *Lactobacillus plantarum* culture, and $N_2$ purging to remove dissolved $CO_2$. The fermentations were predominantly homofermentative, lactic acid accounting for 95% of the cucumber sugars fermented. Firmness retention of the fermented cucumbers during storage for 1 year was improved by heating packaged products to 69°C before storage, but firmness retention was acceptable in unheated products.

## INTRODUCTION

APPROXIMATELY 700,000 TONS of pickling cucumbers are grown in the U.S. each year for processing (USDA, 1986). Of this production, about 40% is temporarily preserved by brine fermentation and storage in tanks typically ranging from 19,000 to 38,000L capacity. Brining in bulk tanks offers important economic advantages to the pickle industry, including rapid and relatively inexpensive temporary preservation of cucumbers and distribution of processing equipment and labor requirements throughout the year. Traditional tanks have open tops and are kept outdoors to permit exposure of the brine surface to sunlight, which retards growth of oxidative microorganisms. Unfortunately, exposure of the open tops allows dilution of the brine by rainwater and provides an avenue for entrance of foreign matter. Excess salt is added as insurance against spoilage of the brined cucumbers (Fleming et al., 1987).

The desirability of using covered cucumber brining tanks has been recognized for many years. Finley and Johnston (1956) proposed the use of post-fermentation covers to be mounted on the tanks. At that time tanks were constructed of wood, and proper mounting of the covers required that each tank be specially fitted. The covers could not be put into place until after fermentation because of $CO_2$ production during fermentation. The pickle industry tested an experimental, vinyl-coated, nylon tank cover (Etchells, 1964). Use of the cover was cumbersome, requiring special adaptation to each tank. Furthermore, any leaks through punctures in the cover or around the tank wall and cover juncture permitted the growth of oxidative yeasts and molds which could result in spoilage and sanitation problems. The covers were not adopted commercially.

Enclosed tanks have been used for many years for fermentation and storage of olives (Vaughn, 1975; Borbolla y Alcala et al., 1969). Plastic sheeting is commonly used to cover the surface of sauerkraut in concrete or wooden tanks (Pederson and Albury, 1969). The susceptibility of cucumbers to bloater damage caused by $CO_2$ produced during fermentation is at least partly responsible for delay in adoption of closed tanks by the pickle industry. The open top provides an avenue for escape of $CO_2$ produced during fermentation. Purging of $CO_2$ from fermenting brines has been shown in recent years to be an effective means of preventing bloater damage (Fleming et al., 1973, 1975; Costilow et al., 1977; Costilow and Uebersax, 1982) and is now in use throughout the industry. Thus, bloater damage is no longer a justification for not using enclosed tanks.

Reduced salt usage and disposal problems, improved and more uniform product quality, and improved sanitation are some of the potential benefits that the pickle industry could derive from the use of closed brining tanks. However, technological and economic factors must be considered before widespread use of closed tanks by the industry can be expected. Some of the technological factors have been addressed, including product handling (Fleming et al., 1983a) and tank design (Humphries and Fleming, 1986). The industry currently is testing experimental closed tanks with a nitrogen-blanketed headspace to maintain anaerobiosis and is assessing the economic and practical feasiblity for their use on a commercial scale.

Recent studies have shown that firmness of brined cucumbers can be maintained at substantially lower concentrations of NaCl than those traditionally used if calcium salts also are present (Fleming et al., 1978, 1987; Buescher et al., 1979, 1981; Buescher and Hudson, 1986). The use of closed tanks, in combination with addition of calcium salts, could greatly reduce NaCl requirements and the attendant disposal problems. The brining operation could be greatly facilitated, particularly with closed tanks, if addition of dry salt to the cucumbers after tanking was not required to maintain traditional concentrations of NaCl (5 to 8% for fermentation and 12 to 16% for storage; Fleming et al., 1987).

There is need for development of a suitable brining procedure for use in closed tanks. Etchells et al. (1973) developed a controlled fermentation procedure that involved washing of cucumbers to remove foreign material, chlorination followed by acidification of the cover brine to suppress growth of undesired microorganisms before inoculation, addition of sodium acetate buffer to assure complete sugar utilization by the added culture, inoculation with a culture of lactic acid bacteria, addition of dry salt as needed to maintain the desired NaCl concentration, and nitrogen purging to remove $CO_2$. While this procedure was effective for use in experimental open top tanks, it has not been widely adopted commercially, perhaps because of expense and complexity. Certain features of the procedure, however, are appropriate for use in closed tanks.

The objectives of this research were to develop and test a procedure for brining cucumbers in closed, anaerobic tanks and to determine the quality and stability of products produced by this procedure. Other objectives were to determine predominance of the added culture compared to naturally occurring lactic acid bacteria during the fermentation and to account for fermentation substrates and products.

## MATERIALS & METHODS

### Cucumbers

Pickling cucumbers used in this study were locally grown, size 2B (3.5–3.8 cm diameter), cv. Calypso. The fruit were free of serious mechanical damage and generally of good quality, except for evidence of slight mold infection in a small percentage of the fruit.

### Brining and fermentation procedures

The cucumbers were fermented in experimental, closed-top tanks according to the general procedure outlined in Fig. 1. After holding in the tanks for 2 months, during which time fermentation was completed, the cucumbers were processed as summarized in Fig. 2. In

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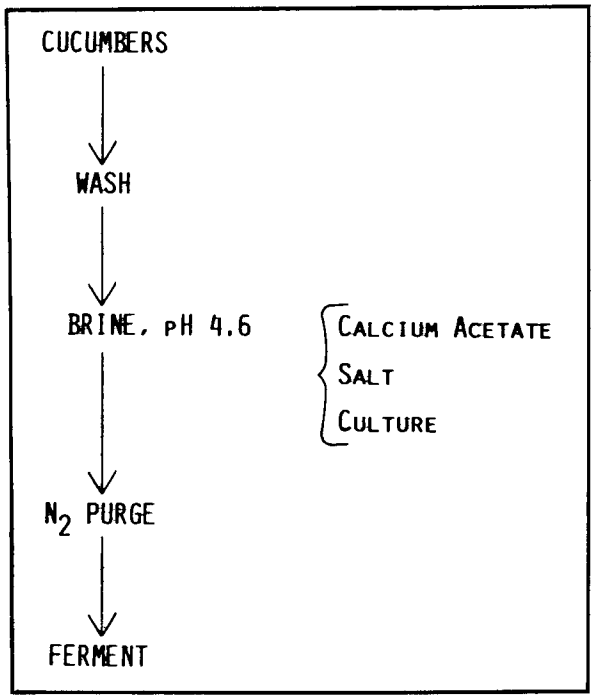


*Fig. 1—Flow diagram for brining of cucumbers in closed tanks.*

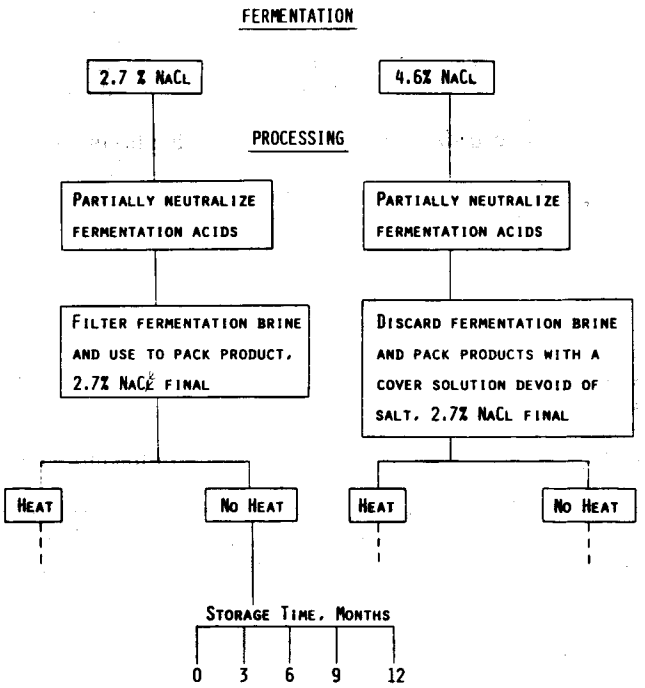


*Fig. 2—Experimental design for fermentation, processing and storage of whole cucumbers.*

addition, some cucumbers from the 4.6%, w/v, NaCl fermentation were sliced before the heating and storage treatments to determine storage stability of sliced as compared to whole products. All products equilibrated at approximately the same NaCl concentration, 2.7%.

The cucumbers were washed with an Osborn U-brush washer, model 810-273, and conveyed into the 4,428-L tanks. Approximately 2,600 kg of cucumbers were added per tank, thus occupying about 60% of the tank volume. The previously prepared cover brine (about 1,800L) was placed in the tank before entry of the cucumbers. The brine was composed of either 6.8% or 11.4% sodium chloride and calcium acetate buffer (0.133M acetic acid + 0.045M calcium hydroxide). The pH of this buffered brine was 4.6 ± 0.1. The brine was prepared by mixing vinegar (containing 20% acetic acid, v/v), food-grade lime [$Ca(OH)_2$] and food-grade sodium chloride to give the desired concentration of components cited above. The compounds added to the cover brine were intended to equilibrate with the cucumbers to concentrations of either 2.7 or 4.6% NaCl, 0.053M acetic acid and 0.018M calcium. The *Lactobacillus plantarum* culture, as later described, was mixed well with the cover brine immediately before being pumped into the brining tank. Cucumbers were then loaded into the inoculated brine. Brine temperatures were 26–30°C during fermentation, reflecting ambient conditions.

The brining tanks were cylindrically shaped and constructed of fiberglass (Warner Fiberglass Products, Belding, MI). The tanks were 152 cm in diameter and 244 cm high with a 20-cm domed top containing a 61-cm diameter, flanged, manway opening in its center. The washed, raw cucumbers were conveyed through this opening into the tanks containing the premixed, inoculated cover brine. The brine was circulated by pumping during the filling operation, thereby, providing brine uniformity throughout the tank. After the tanks were filled with cucumbers, a header device was placed below the neck of the manway opening to restrain the cucumbers. A capping device was mounted on the manway, and additional brine was added to rise above the cucumbers and into the cap. A sight glass was mounted adjacent to the cap to facilitate viewing of the brine level. The brined cucumbers were purged with nitrogen at a rate of 15 SCFH (424 L/hr). After fermentation, the cucumbers were removed through the opening by brine flotation. Details of the brining tank, heading and purging devices, and loading and unloading systems are more fully described by Fleming et al. (1983a).

### Product evaluation

Quality of the cucumbers was evaluated 2 months after brining. Samples of cucumbers from various depths of the tank were taken as the tanks were unloaded by brine flotation through the manway (Fleming et al., 1983a). Samples were taken to represent cucumbers from 1/4 (top), 2/4, 3/4 and 4/4 (bottom) depths of the tanks. Bloater damage and visual cure were determined on 25 cucumbers and firmness on 20 cucumbers from each sample. Bloater damage was expressed as bloater index, and visual cure was expressed as a percent of the flesh of longitudinally cut cucumbers that appeared translucent as described previously (Fleming et al., 1977). Firmness of whole cucumbers was determined with a USDA Fruit Pressure Tester (FPT) with a 5/16-inch (0.79 cm) tip and expressed as pounds force (Bell et al., 1955). Firmness of mesocarp and endocarp tissues was determined with an Instron Universal Testing Machine (UTM) according to Thompson et al. (1982).

### Processing and storage

Processing and storage treatments of products from the two fermentations are outlined in Fig. 2. The brine of the 2.7% NaCl fermentation was filtered of bacterial cells (see later) and used as the cover liquor for the products packed from this fermentation. The brine from the 4.6% NaCl fermentation was discarded and a cover liquor devoid of salt was used. Thus, for storage, products from both fermentations equilibrated at about 2.7% NaCl.

The titratable acidity in both fermentations was reduced from about 1.4 to 0.7% by neutralization with KOH. For neutralization, the brines were withdrawn from the tanks, either 13.6 kg (2.7% NaCl brine) or 15.0 kg (4.6% NaCl brine) of KOH pellets were dissolved by mixing, and then the brines were returned to their respective tanks. The cucumbers and brine were allowed to equilibrate for 4 days, during which time nitrogen purging at 15 SCFH assured circulation of the brine.

After neutralization, about 20L of brine from the 2.7% NaCl fermentation were filtered with an Amicon, model DC 10L, filtration apparatus, using a model H5MP01-43, hollow fiber filter (1,000,000 molecular weight exclusion). This filtered brine was used as the cover liquor for the products packed from this fermentation. The cucumbers were rinsed before packing to remove bacterial cells. The brine was supplemented with dill and garlic flavors, yellow no. 5 colorant and 0.1% sodium benzoate and then added to 46-oz (1.3L) jars which contained the cucumbers (about 55% by volume).

Brine from the 4.6% NaCl fermentation was discarded. The cover liquor for products packed from this fermentation consisted only of water, flavor and color ingredients, 0.1% sodium benzoate and 0.2% $CaCl_2$. Since no salt was present in the cover liquor, the NaCl after

equilibration was about 2.7%. The $CaCl_2$ was added so that the $Ca^{++}$ concentration in products from both fermentations would be the same.

Cucumbers from both fermentations were packed whole or sliced (7 mm thick) into the jars. Some of the products were heated, others were not (Fig. 2). Products were heated to reach an internal temperature of 69°C (no holding time).

### Microbial culture and enumeration of microorganisms

The bacterial starter culture, *L. plantarum* WSO, was screened for naturally occurring streptomycin-resistant variants within the population. This was achieved by incorporating streptomycin sulfate (Sigma Chemical Co.) into MRS (Difco) agar plates at an initial concentration of 500 μg/mL. Colonies of cells naturally resistant to this concentration of streptomycin occurred at a frequency of less than 1 per 10 million cells. This procedure was repeated with a pure culture of cells resistant to 500 μg/mL streptomycin sulfate to obtain variants resistant to 1 mg/mL of drug. Such variants again were obtained at a frequency of less than 1 per 10 million cells. A culture resistant to 1 mg/mL streptomycin was used as the starter culture in order to facilitate selective enumeration of starter culture growth on LBS agar plates containing streptomycin sulfate. A starter culture of streptomycin-resistant *L. plantarum* WSO cultivated overnight at 30°C in MRS broth containing 4% NaCl was added to each tank of brined cucumbers to give a population of about 5 x $10^5$ cells per mL of brined material. The culture was added to the brine and mixed well immediately before addition of the cucumbers. The brine was continually circulated during filling of the tank. Brine samples were periodically taken during fermentation for microbial enumeration. LBS agar medium (BBL Microbiology Systems, Cockeysville, MD) was used to enumerate total lactic acid bacteria, which included endogenous as well as added lactic acid bacteria. This same medium supplemented with streptomycin sulfate (1 mg/mL) was used to selectively enumerate the starter culture. The difference in plate counts represented nonstreptomycin-resistant lactic acid bacteria, presumably those naturally present from the cucumbers.

Yeasts were enumerated with acidified dextrose agar (5 mL 10% w/v tartaric acid per 100 mL dextrose agar, BBL), and *Enterobacteriaceae* with violet red bile agar with 1% glucose added. An aerobic plate count was obtained with standard methods agar (BBL). All pour plates were duplicated and incubated at 30°C.

### Chemical analyses and fermentation balances

Duplicate samples of 16 cucumbers each were taken during filling of the fermentation tanks. The cucumbers were blended in a Waring Blendor, and a sample of the filtered juice was used for analysis of the initial fermentation substrate concentrations in the cucumbers. Brine samples were taken at the end of the fermentation period and analyzed for the concentrations of fermentation end products. Concentrations of fermentable sugars, organic acids and ethanol were determined by HPLC (McFeeters et al., 1984). Analysis of tritratable acidity, pH and salt during the storage study was done according to procedures described by Fleming et al. (1984).

Fermentation balances were calculated by general procedures described by Wood (1961). These balances were based on glucose and fructose as substrates, and lactic acid, acetic acid, ethanol and mannitol as products. Theoretical amounts of lactic acid from degradation of malic acid by the malolactic reaction were subtracted from product recoveries so that balances could be based on hexose fermentation. No attempt was made to account for $CO_2$ production since the brines were purged during fermentation to prevent bloater damage. Carbon and oxygen recoveries reported do not reflect any $CO_2$ that may have been produced from hexoses.

## RESULTS

### Microbiological changes during fermentation

Changes in microbial populations in the brines of cucumber fermentations at 2.7 and 4.6% NaCl are summarized in Fig. 3. The selective medium facilitated differential enumeration of the added and the endogenous lactic acid bacteria. The initial number of added *L. plantarum* WSO was 3 to 5 x $10^5$ cells/mL brine and approximated the total lactic acid bacteria enumerated. Within 1 to 3 days after brining, however, numbers of total lactic acid bacteria exceeded those of the added culture for the remaining period of most active fermentation (first 8 days).

Total aerobic bacteria approximated or exceeded the lactic acid bacteria in numbers throughout the fermentation. *Enterobacteriaceae* declined from initial numbers to less than $10^2$/

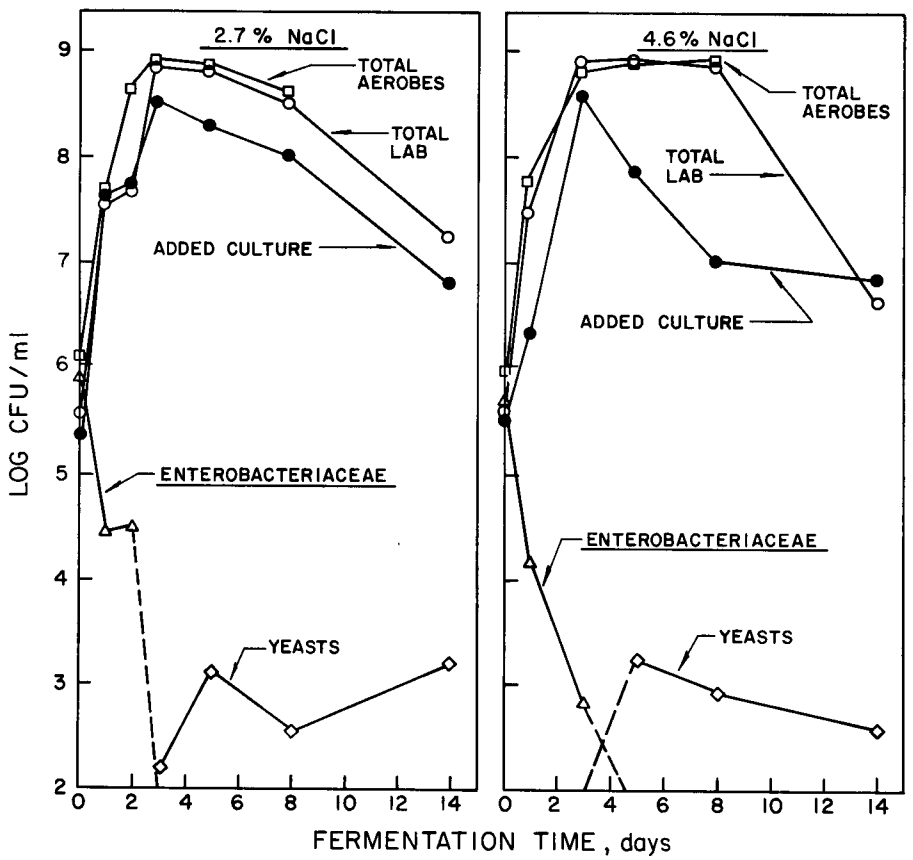


*Fig. 3— Microbiological changes in the brine of cucumbers fermented at either 2.7 or 4.6% NaCl in closed tanks. LAB, lactic acid bacteria.*

mL after 3 days in both fermentations. The brines contained less than $10^2$ yeasts/mL for the first 2 days, but approximated $10^3$/mL later during both fermentations.

### Chemical changes during fermentation

Chemical changes of fermentations at 2.7 and 4.6% NaCl were similar (Fig. 4), except that initiation of fermentation at 4.6% NaCl lagged about 1 day behind that at 2.7% NaCl. The brine pH was 4.5 initially, increased slightly during the first 1 or 2 days, and then declined as acid production began. The initial acidity was due to the acetic acid/acetate buffer and was expressed as lactic acid (Fig. 4). The acidity decreased slightly during the first day due to diffusion of added acid into the cucumbers, but then increased due to lactic acid production. The lactic acid fermentation was essentially complete at 4.6% NaCl after 13 days, as evidenced by no change in acidity after 30 days and the absence of sugar in the brine. Fermentation at 2.7% NaCl was about 95% complete after 13 days. The terminal pH was about 3.4 in both fermentations, being slightly lower at the higher salt concentration.

### Fermentation balances

Since the weight of cucumbers placed in the tanks and the volume of the tanks were known, it was possible to estimate substrate conversion to products. The raw cucumbers contained approximately equal molar concentrations of glucose (73 mM) and fructose (76 mM) and no other detectable fermentable sugars. These hexoses are expressed in Table 1 as concentrations after assumed equilibrium between the cucumbers and brine. Malic acid of the cucumbers is similarly expressed.

Fermentation products in Table 1 are expressed on the basis of their concentrations in the fermentation brine after 13 days, which was considered to be the endpoint of the primary lactic acid fermentation. No fermentable sugar was present in the brine after 13 days, and the lactic acid concentration did not change appreciably (<5%) over the next 2 months. Elemental recoveries were calculated based on conversion of hexoses to the products shown. It was assumed that malic acid was converted completely to lactic acid and $CO_2$, since malic acid was not detected in the fermentation brine. Thus, the concentration of malic acid in the equilibrated cucumbers was subtracted from the concentrations of lactic acid shown in Table 1 so that lactic acid and other products would be expressed relative to only hexoses as substrates. In so doing, recoveries of substrate elements exceeded 100%. Carbon recovery was 113.4% at 2.7% salt and 108.2% at 4.6% salt (Table 1). Apparently, we did not account for sufficient substrate for the products formed or inherent analytical errors were incorporated, as is later discussed.

The hydrogen-to-oxygen ratio (H/O) in the products exceeded the 2.0 ratio of the hexose substrate. The presence of ethanol and mannitol accounted for the slightly high H/O ratio. The origin of the ethanol may have been from yeast activity

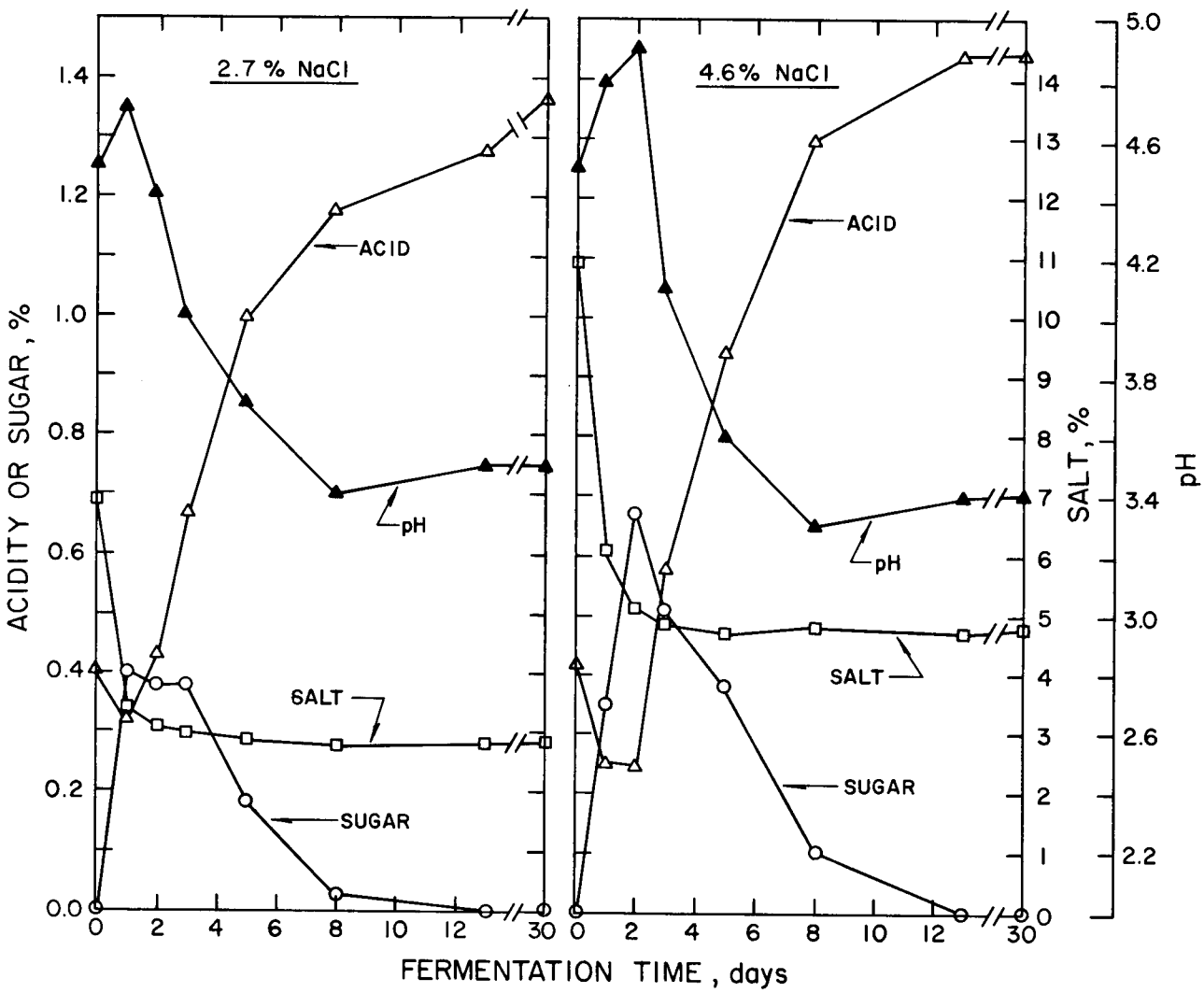


*Fig. 4— Chemical changes in the brine of cucumbers fermented at either 2.7 or 4.6% NaCl in closed tanks. LAB, lactic acid bacteria.*

since this compound did not appear until about the third day. A small number of yeasts appeared after several days (Fig. 3). Mannitol first appeared in the brine at 2 to 3 days in both fermentations. At 2.7% NaCl, mannitol concentration reached 8 mM after 5 days and then decreased to 3.3 mM after 13 days. No mannitol was detected after 13 days at 4.6% NaCl. The origin of the mannitol as well as ethanol and acetic acid could have been heterofermentative lactic acid bacteria (Pederson and Albury, 1969). In either case, however, the products formed indicated that the fermentation at both salt concentrations was primarily due to homofermentative lactic acid bacteria. At both concentrations of NaCl 1 mmol of hexose yielded 1.9 mmol lactic acid when corrected for malic acid conversion, which approaches the theoretical limit of 2 mmol lactic acid by the homofermentative pathway.

### Brine-stock quality

Evaluations of brine-stock quality are summarized in Table 2. Overall quality was excellent. The brine stock was firm as determined by the FPT at the four depths evaluated. However, slight stem end softening, as determined by hand, occurred in about 20% of the cucumbers brined at 2.7% NaCl. This softening problem has been reported elsewhere (Walter et al., 1985) and is later discussed herein. There was slight bloater damage in the top region of the tanks. Little or no damage was evident in the middle and bottom sections of the tanks. The cucumbers were visually cured (translucent) except for a slight uncured appearance at the top of the tanks. The brine stock yield was slightly lower in the 4.6 compared to the 2.7% NaCl fermentation.

*Table 1—Fermentation balances for brined cucumbers*

| | Salt, % | |
|---|---|---|
| | 2.7 | 4.6 |
| **Substrates, mM[a]** | | |
| Hexoses | 84.2 | 91.6 |
| Malic acid[b] | 12.4 | 11.6 |
| **Products, mM** | | |
| Lactic acid[b] | 172.5 | 186.4 |
| Acetic acid[c] | 13.6 | 18.3 |
| Ethanol | 22.7 | 16.9 |
| Mannitol | 3.3 | 0.0 |
| **Elemental recoveries from hexoses, %[b]** | | |
| Carbon | 113.4 | 108.2 |
| Hydrogen | 118.5 | 118.0 |
| Oxygen | 108.9 | 105.1 |
| Hydrogen/oxygen (H/O) | 2.18 | 2.12 |
| **Lactic acid formed per mM hexose fermented, mM[b]** | 1.90 | 1.91 |

[a] The concentrations of substrates are after assumed equilibrium between the cucumbers and the brine and are based on concentrations in the raw fruit.
[b] It was assumed that malic acid was converted to lactic acid and $CO_2$ since no malic acid was present after fermentation. The hypothetical concentration of lactic acid that would be produced from malic acid was subtracted from lactic acid shown as a product, which permitted elemental recoveries to be based solely on hexose fermentation.
[c] This represents the net increase in acetic acid over that added initially in the cover brine.

*Table 2—Quality of fermented cucumbers after 2 months*

| NaCl (%) | Tank position depth[a] | Firmness FPT, lb[b] | Bloater index | Visual cure, (%) | Brine-stock yield, (%)[c] |
|---|---|---|---|---|---|
| 2.7 | 1/4 (top) | 19.4 (1.9) | 4.8 | 98 | 95.6 |
| | 2/4 (upper middle) | 18.2 (2.4) | 1.4 | 99 | |
| | 3/4 (lower middle) | 19.0 (1.6) | 0 | 100 | |
| | 4/4 (bottom) | 19.4 (1.5) | 0 | 100 | |
| 4.6 | 1/4 (top) | 19.9 (2.6) | 5.6 | 85 | 92.3 |
| | 2/4 (upper middle) | 20.4 (1.5) | 0 | 100 | |
| | 3/4 (lower middle) | 19.7 (2.1) | 0 | 100 | |
| | 4/4 (bottom) | 20.4 (1.8) | 0 | 100 | |

[a] Cucumbers taken from the various depths from top of the tanks as indicated.
[b] Standard deviations in parentheses.
[c] Based on weights of fresh and fermented cucumbers for the entire tank.

### Storage stability of fermented cucumbers

Fermented cucumbers packed in 1.3L jars were evaluated for firmness and chemical and visual changes over a storage period of 12 months at room temperature (about 24°C). From a practical standpoint, firmness retention as measured by FPT or Instron UTM was not greatly influenced by any of the treatments (Tables 3 and 4). With the exception of stem end softening in a few pickles, overall firmness was highly acceptable. There were, however, several statistically significant differences that bear consideration. Whole cucumbers fermented at 4.6% NaCl were initially slightly firmer by FPT ($P \leq 0.01$) than those fermented at 2.7% NaCl. This firmness difference persisted throughout the storage period (Tables 3 and 4). There were no significant differences in firmness of mesocarp and endocarp tissues between the two tanks as determined by the Instron UTM ($P \geq 0.05$). Heating (69°C internal) of whole pickles after packing resulted in greater firmness retention than no heating as determined by FPT ($P \leq 0.05$) and Instron UTM ($P \leq 0.01$) tests (Table 3). Storage time resulted in reduced firmness ($P \leq 0.05$). This overall effect, as determined by ANOVA (Table 3), obviously was due to loss of firmness in unheated cucumbers since the firmness of heated cucumbers was not reduced. This conclusion is strengthened by the significant ($P \leq 0.05$) heat X time interaction (Table 3). The firmness stability of sliced cucumbers, heated and unheated, is summarized in Table 5. Again, improved firmness retention in whole cucumbers due to heating is indicated for mesocarp, but not endocarp tissue. Slicing of cucumbers before packing appeared to have no important effect on firmness retention, whether heated or unheated.

The packed products initially had a clear, light, golden-yellow color. The color remained desirable and constant throughout storage. It was noted, however, that the product packed with the filtered fermentation brine was slightly turbid in the heated but not in the unheated jars (Table 4). Upon storage, the filtered brine in all of the unheated jars also became turbid. The turbidity was not due to microbial growth, as determined by microscopic examination. The turbidity apparently was due to coagulation or precipitation of organic or inorganic matter from the filtered fermentation brine. The brine remained clear in products packed from cucumbers brined at 4.6% NaCl, using fresh brine rather than the fermentation brine.

There was no evidence of microbial spoilage during storage of the products based on pressure due to headspace gases. All jars were under vacuum at the time of sampling. Titratable acidity increased slightly (< 0.2%) in the unheated but not in the heated jars (Table 4). The pH increased slightly (< 0.2 unit) in heated and unheated products (Table 4).

## DISCUSSION

THE BRINING PROCEDURE developed for use in fermentation of cucumbers in anaerobic tanks (Fig. 1) appeared to be satisfactory and applicable for commercial testing. The procedure incorporated important features of the controlled fer-

*Table 3—Analysis of Variance on effects of salt concentration, heating and storage time on firmness of cucumbers fermented and stored whole*

| Source of variation | Degrees of freedom | F ratios for firmness measurements[b] | | |
|---|---|---|---|---|
| | | FPT, lb | Instron UTM, N | |
| | | Whole | Mesocarp | Endocarp |
| Salt conc (S) | 1 | 14.09** | 3.78 | 3.60 |
| Heat (H) | 1 | 6.72* | 12.55** | 13.68** |
| Time (T) | 4 | 3.30* | 2.54 | 2.85* |
| S × H | 1 | 0.52 | 0.10 | 1.53 |
| T × S | 4 | 0.42 | 0.12 | 2.32 |
| T × H | 4 | 2.34* | 1.14 | 1.78 |
| Error[a] | 24 | | | |

[a] Error constructed by pooling the sum of squares of the third order interaction, S × H × T, with the original error term.
* = $P \leq 0.05$; ** = $P \leq 0.01$.

*Table 4—Storage stability of whole cucumbers fermented at 2.7 and 4.6% NaCl*

| Treatments | | | | | | | Firmness measurements | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | FPT, lb | Instron UTM, N | |
| NaCl, % | | | | | | | Whole | Mesocarp | Endocarp |
| Fermentation | Storage | Cover brine | Storage time (months) | pH | Acid, % | Brine turbidity[a] | Mean (SD)[b] | Mean (SD) | Mean (SD) |
| **Heated** | | | | | | | | | |
| 2.7 | 2.7 | Filtered fermentation brine | 0 | 4.07 | 0.74 | + | 17.9 (3.0) | 11.31 (2.8) | 2.01 (0.4) |
| | | | 3 | 4.08 | 0.75 | + | 16.2 (2.2) | 12.43 (2.0) | 2.12 (0.6) |
| | | | 6 | 4.13 | 0.73 | + | 16.7 (2.3) | 13.23 (3.4) | 2.49 (1.0) |
| | | | 9 | 4.24 | 0.76 | + | 18.2 (2.0) | 11.38 (3.9) | 2.44 (1.0) |
| | | | 12 | 4.26 | 0.74 | + | 18.0 (2.6) | 11.49 (2.4) | 2.44 (0.4) |
| **Unheated** | | | | | | | | | |
| 2.7 | 2.7 | Filtered fermentation brine | 0 | 4.08 | 0.74 | – | 17.5 (3.2) | 11.53 (2.8) | 1.85 (0.6) |
| | | | 3 | 4.04 | 0.79 | + | 16.3 (2.8) | 11.82 (1.3) | 2.04 (0.3) |
| | | | 6 | 4.04 | 0.81 | + | 15.8 (2.0) | 10.91 (2.7) | 2.36 (0.9) |
| | | | 9 | 4.14 | 0.88 | + | 17.1 (1.9) | 10.21 (2.9) | 2.10 (0.7) |
| | | | 12 | 4.18 | 0.88 | + | 14.4 (3.2) | 10.43 (2.1) | 2.09 (0.6) |
| **Heated** | | | | | | | | | |
| 4.6 | 2.7 | Fresh brine | 0 | 3.90 | 0.65 | – | 18.7 (2.6) | 12.12 (2.8) | 2.41 (0.7) |
| | | | 3 | 3.90 | 0.68 | – | 17.8 (2.8) | 13.10 (2.8) | 2.39 (0.7) |
| | | | 6 | 3.93 | 0.58 | – | 17.0 (1.6) | 12.26 (2.4) | 2.56 (0.8) |
| | | | 9 | 4.08 | 0.59 | – | 19.6 (2.8) | 12.94 (1.9) | 2.81 (0.8) |
| | | | 12 | 4.08 | 0.58 | – | 19.1 (2.4) | 11.63 (2.3) | 2.55 (0.6) |
| **Unheated** | | | | | | | | | |
| 4.6 | 2.7 | Fresh brine | 0 | 3.90 | 0.58 | – | 19.4 (2.5) | 11.75 (2.2) | 2.25 (0.6) |
| | | | 3 | 3.90 | 0.62 | – | 17.8 (3.3) | 11.36 (2.0) | 2.22 (0.5) |
| | | | 6 | 3.90 | 0.70 | – | 17.1 (1.9) | 11.57 (3.0) | 2.42 (0.8) |
| | | | 9 | 4.06 | 0.66 | – | 17.3 (1.8) | 11.72 (2.8) | 2.52 (1.0) |
| | | | 12 | 4.06 | 0.68 | – | 17.4 (2.0) | 10.72 (2.6) | 1.85 (0.6) |

[a] Brine turbidity was determined by visual observation.
[b] Standard deviation (SD).

*Table 5—Storage stability of whole and sliced cucumbers fermented at 4.6% NaCl*

| | Instron UTM firmness, N[a] | |
|---|---|---|
| Treatment before storage | Mesocarp | Endocarp |
| Whole, heated | 12.41[A] | 2.54[A] |
| Whole, not heated | 11.42[B] | 2.25[A] |
| Sliced, heated | 11.83[A,B] | 2.66[A] |
| Sliced, not heated | 12.08[A,B] | 2.54[A] |

[a] Treatment means within columns with the same superscript are not significantly different ($P \geq 0.05$). Means are averages of samples taken at 0, 3, 6, 9, and 12 months of storage at room temperature.

mentation procedure of Etchells et al. (1973) but was simplified to increase the potential for commercial use in anaerobic tanks. By incorporation of all components needed to complete the fermentation in the initial cover brine, brine handling was greatly simplified. Problems associated with dry salt additions also were eliminated. The addition of calcium to permit use of lower concentrations of sodium chloride is a key factor that allows all salt needed for cucumber texture retention and fermentation control to be incorporated in the cover brine without excessive shriveling of the cucumbers. The addition of calcium acetate to the cover brine served the dual functions of a buffer to help assure complete fermentation of sugars and a source of calcium to help assure cucumber firmness retention. Calcium acetate has been shown to be effective for these dual functions in experimental fermentations of cucumbers (Fleming et al., 1978) and green tomatoes (Laleye and Simard, 1982). In addition, acetate serves to direct the course of the fermentation by suppressing growth of *Enterobacteriaceae* (McDonald et al., 1986). The cucumber briner can easily prepare calcium acetate from vinegar and lime, both of which are readily available and relatively inexpensive.

Washing of the cucumbers before tanking, as in the Etchells et al. (1973) procedure, is deemed an important feature of the brining procedure developed for closed tanks. By washing the cucumbers before tanking, dirt and other foreign materials are removed that could influence flavor of the final product. Also, cucumber flowers and other harbors for pectinolytic enzymes are removed, which improves the likelihood of firmness retention of the cucumbers at the relatively low concentration of NaCl used. Purging of $CO_2$ from the brine is essential to prevent bloater damage to the cucumbers.

## Fermentation

The buffered brine effectively suppressed growth of *Enterobacteriaceae.* Lactic acid bacteria predominated fermentations at 2.7 and 4.6% NaCl. The added culture of *L. plantarum* grew, but did not predominate the total lactic acid bacteria population. The fermentation balance indicated, however, that the fermentation was essentially homofermentative. We have isolated the predominating lactic acid bacterium, which was identified as a strain of *L. plantarum.* This strain has a significantly faster growth rate than *L. plantarum* WSO under the brine conditions from which it was isolated. This strain is being modified and evaluated for possible use as a commercial starter culture for fermented vegetables. Slight yeast activity later in the fermentation did not appear to present significant problems in terms of $CO_2$ production or substrate utilization.

It is not clear why carbon recoveries in fermentation products exceeded 100% (108 and 113%) of theoretical from hexoses present in the cucumbers. Our fermentation balances were based on analyses of brine surrounding the cucumbers and the assumption of uniform distribution of compounds between brine and cucumbers. We have found this assumption to be reasonably accurate when the fermentation is complete, but a small error should be expected in this assumption. Entrapped gas and cell wall and other solid structures within the fruit influence the distribution of solutes. However, these inherent analytical errors may not account entirely for the high carbon recoveries noted. Also, our recoveries were based on the concentration of hexoses measured. Sucrose or starch was not detected in the cucumbers used, although small amounts of both have been reported to be present in cucumbers (Handley et al., 1983). Certainly, sucrose was not present in sufficient concentration to account for the 108% and 113% carbon recoveries noted (Table 1). Perhaps, cell wall material was converted to some of the products measured. De Man (1957) reported evidence that indicated that lactic acid bacteria fer-

mented cell wall substances of potato pulp. Further studies are needed to develop simple, accurate analytical techniques for determining fermentation balances in nonhomogeneous materials.

### Product quality and storage stability

The excellent firmness of the cucumbers fermented at 2.7 and 4.6% NaCl indicated that it might be possible to reduce or eliminate salt disposal problems as discussed above. The stem end softening in some of the cucumbers brined at 2.7% NaCl has been attributed to mold infection of the fruit before brining (Walter et al., 1985). The fact that stem end softening was much less at 4.6% NaCl suggested that salt concentration might influence firmness retention in fruit that were mold infected before brining. Further studies are needed to elucidate the importance of mold infection of raw cucumbers on firmness retention at the relatively low concentrations of NaCl that would be desirable to eliminate salt disposal.

Bloater damage in the top or neck region of the tanks, although representing a small portion of the tank contents, is undesirable. If the damaged cucumbers are removed before unloading the remainder of the tank, grading problems can be minimized. Several factors probably contribute to the problem of more bloater damage at the top of brined cucumbers. Buoyancy pressure is greater and hydrostatic pressure is lower near the top of the tank, and both conditions have been shown to be conducive to bloater formation (Fleming et al., 1977). The extent of the problem has been variable among numerous fermentations during the past 2 years, ranging from little or no damage to serious damage to depths of 2 to 3 feet. Thus, biological as well as engineering factors are being considered in an attempt to solve this minor but aggravating problem.

Some means of reducing acidity seems essential in the new brining procedure, since all of the acid formed in the fermentation is present in the final product. Partial neutralization of the acidity produced during fermentation resulted in a desirable acid/salt balance and contributed to the highly acceptable flavor of the products. Such a procedure may be necessary if the fermentation liquid is clarified and used in final packing. In the present case, neutralization to about 0.7% acid (calculated as lactic) provided the desired flavor response. The pH after neutralization was about 4.1, and increased slightly during storage. Although we observed no evidence of microbial spoilage in the stored products, the stability of such products warrants further study. Spoilage due to softening has been observed in fermented vegetables stored at pH 4.3 (Fleming et al., 1983b).

Also, we recently observed microbial instability during storage of small cucumbers fermented at 2.3% NaCl (Fleming et al., 1986). The cucumbers fermented normally to 1.1% lactic acid, pH 3.7. However, upon storage the lactic acid was metabolized with a resultant pH of 4.8 and appearance of butyric and propanoic acids. *Clostridium tertium* was identified as the probable producer of the butyric and propanoic acids, but an intermediate fermentation by unidentified bacteria was indicated. The aberrant fermentation was attributed to the low concentration of NaCl, and the use of small (ca. 2.5 cm diameter) cucumbers which were prone to retain relatively more soil per unit weight than larger fruit.

## REFERENCES


Bell, T.A., Etchells, J.L., and Jones, I.D. 1955. A method for testing cucumber salt-stock brine for softening activity. U.S. Dept. of Agriculture, ARS-72- 5.

Borbolla y Alcala, J.M.R. de la, Fernandez Diez, M.J., Gonzalez Cancho, F., and Cordon Casanueva, J.L. 1969. Studies on the pickling of green olives. XXIII. Bulk fermentations. Grasas y Aceites 20: 55.

Buescher, R.W. and Hudson, J.M. 1986. Bound cations in cucumber pickle mesocarp tissue as affected by brining and $CaCl_2$. J. Food Sci. 51: 135.

Buescher, R.W., Hudson, J.M., and Adams, J.R. 1979. Inhibition of polygalacturonase softening of cucumber pickles by calcium chloride. J. Food Sci. 44: 1786.

Buescher, R.W., Hudson, J.M., and Adams, J.R. 1981. Utilization of calcium to reduce pectinolytic softening of cucumber pickles in low salt conditions. Lebensm. Wiss. Technol. 14: 65.

Costilow, R.N., Bedford, C.L., Mingus, D., and Black, D. 1977. Purging of natural salt-stock pickle fermentations to reduce bloater damage. J. Food Sci. 42: 234.

Costilow, R.N. and Uebersax, M. 1982. Effects of various treatments on the quality of salt-stock pickles from commercial fermentations purged with air. J. Food Sci. 47: 1866.

De Man, J.C. 1957. The fermentation of cell wall substances in grass silage and in potato pulp. Antonie van Leeuwenhoek 23: 87.

Etchells, J.L. 1964. Personal communication. U.S. Dept. of Agriculture, N.C. State Univ., Raleigh, NC.

Etchells, J.L., Bell, T.A., Fleming, H.P., Kelling, R.E., and Thompson, R.L. 1973. Suggested procedure for the controlled fermentation of commercially brined pickling cucumbers—the use of starter cultures and reduction of carbon dioxide accumulation. Pickle Pak Sci. 3: 4.

Finley, N. and Johnston, M.R. 1956. Influence of plastic vat cover on the fermentation of cucumbers. Food Technol. 11: 437.

Fleming, H.P., Etchells, J.L., Thompson, R.L., and Bell, T.A. 1975. Purging of $CO_2$ from cucumber brines to reduce bloater damage. J. Food Sci. 40: 1304.

Fleming, H.P., Humphries, E.G., and Macon, J.A. 1983a. Progress on development of an anaerobic tank for brining of cucumbers. Pickle Pak Sci. VII: 3.

Fleming, H.P., McFeeters, R.F., Daeschel, M.A., and Pierson, M.D. 1986. Aberrant, secondary fermentation of brined cucumbers. Food Technol. 40: 159.

Fleming, H.P., McFeeters, R.F., Etchells, J.L., and Bell, T.A. 1984. Pickled vegetables. In "Compendium of Methods for the Microbiological Examination of Foods," 2nd ed., p. 663. American Public Health Association, Washington, DC.

Fleming, H.P., McFeeters, R.F., and Thompson, R.L. 1987. Effects of sodium chloride concentration on firmness retention of cucumbers fermented and stored with calcium chloride. J. Food Sci. 52: 653.

Fleming, H.P., McFeeters, R.F., Thompson, R.L., and Sanders. D.C. 1983b. Storage stability of vegetables fermented with pH control. J. Food Sci. 48: 975.

Fleming, H.P., Thompson, R.L., Bell, T.A., and Hontz, L.H. 1978. Controlled fermentation of sliced cucumbers. J. Food Sci. 43: 888.

Fleming, H.P., Thompson, R.L., Bell, T.A., and Monroe, R.J. 1977. Effect of brine depth on physical properties of brine-stock cucumbers. J. Food Sci. 42: 1464.

Fleming, H.P., Thompson, R.L., Etchells, J.L., Kelling, R.E., and Bell, T.A. 1973. Bloater formation in brined cucumbers fermented by *Lactobacillus plantarum*. J. Food Sci. 38: 499.

Handley, L.W., Pharr, D.M., and McFeeters, R.F. 1983. Carbohydrate changes during maturation of cucumber fruit. Implications for sugar metabolism and transport. Plant Physiol. 72: 498.

Humphries, E.G. and Fleming, H.P. 1986. Closed-top fermentation tanks for cucumbers. Trans. ASAE 2: 45.

Laleye, L.C. and Simard, R.E. 1982. Lactic acid fermentation of sliced green tomatoes. In "Developments in Industrial Microbiology," L.A. Underkofler (Ed.), Vol. 23, p. 601. Society for Industrial Microbiology, Arlington, VA.

McDonald, L.C., Fleming, H.P., and Daeschel, M.A. 1986. Effect of acidification on microbiological changes during initiation of cucumber fermentation. Food Technol. 40: 156.

McFeeters, R.F., Thompson, R.L., and Fleming, H.P. 1984. Liquid chromatographic analysis of sugars, acids and ethanol in lactic acid vegetable fermentations. J. Assoc. Off. Anal. Chem. 67: 710.

Pederson, C.S. and Albury, M.N. 1969. The sauerkraut fermentation. N. Y. State Agr. Expt. Sta. Bull. No. 824.

Thompson, R.L., Fleming, H.P., Hamann, D.D., and Monroe, R.J. 1982. Method for determination of firmness in cucumber slices. J. Text. Stud. 13: 311.

USDA. 1986. "Agricultural Statistics," U.S. Dept. of Agriculture, U.S. Government Printing Office, Washington, DC.

Vaughn, R.H. 1975. Lactic acid fermentation of olives with special reference to California conditions. In "Lactic Acid Bacteria in Beverages and Food," J.G. Carr, C.V. Cutting, and G.C. Whiting (Ed.), p. 307. Academic Press, New York.

Walter, W.M. Jr., Fleming, H.P., and Trigiano, R.N. 1985. Comparison of the microstructure of firm and stem-end softened cucumber pickles preserved by brine fermentation. Food Microstructure 4: 165.

Wood, W.A. 1961. Fermentation of carbohydrates and related compounds. In "The Bacteria," Vol. 2, p. 49. Academic Press, New York.



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