

# Fermentation of Cucumbers Brined with Calcium Chloride Instead of Sodium Chloride

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**ABSTRACT:** Waste water containing high levels of NaCl from cucumber fermentation tank yards is a continuing problem for the pickled vegetable industry. A major reduction in waste salt could be achieved if NaCl were eliminated from the cucumber fermentation process. The objectives of this project were to ferment cucumbers in brine containing CaCl<sub>2</sub> as the only salt, to determine the course of fermentation metabolism in the absence of NaCl, and to compare firmness retention of cucumbers fermented in CaCl<sub>2</sub> brine during subsequent storage compared to cucumbers fermented in brines containing both NaCl and CaCl<sub>2</sub> at concentrations typically used in commercial fermentations. The major metabolite changes during fermentation without NaCl were conversion of sugars in the fresh cucumbers primarily to lactic acid which caused pH to decrease to less than 3.5. This is the same pattern that occurs when cucumbers are fermented with NaCl as the major brining salt. Lactic acid concentration and pH were stable during storage and there was no detectable production of propionic acid or butyric acid that would indicate growth of spoilage bacteria. Firmness retention in cucumbers fermented with 100 to 300 mM CaCl<sub>2</sub> during storage at a high temperature (45 °C) was not significantly different from that obtained in fermented cucumbers with 1.03 M NaCl and 40 mM CaCl<sub>2</sub>. In closed jars, cucumber fermentations with and without NaCl in the fermentation brine were similar both in the chemical changes caused by the fermentative microorganisms and in the retention of firmness in the fermented cucumbers.

**Keywords:** *Cucumis sativus*, lactic acid fermentation, *Lactobacillus plantarum*, pickles

## Introduction

Cucumbers are commercially fermented in a brine solution with 1.03 M (6%) NaCl in open top tanks that are typically about 40000 L in volume (Breidt and others 2007). Salt serves multiple functions in the fermentation process. It selects for salt tolerant homofermentative lactic acid bacteria, such as *Lactobacillus plantarum*, that are required for normal fermentations, it helps maintain the firm texture of fermented cucumbers while they are stored for up to a year in fermentation tanks, and it provides salty flavor in the products made from the fermented cucumber fruit (Fleming 1982). In cold climates additional salt, up to 12%, is added after fermentation to minimize freezing of the tanks in winter. About 30 y ago, calcium chloride (CaCl<sub>2</sub>) equilibrated at 20 to 40 mM began to be added to cucumber fermentation brines because it helped maintain the firmness of fermented cucumbers better than NaCl alone (Thompson and others 1979; Hudson and Buescher 1980; Tang and McFeeters 1983).

A continuing issue for the pickle industry is to meet discharge limits due to the high salt waste generated by this process. Recycling of the fermentation brines (Geisman and Henne 1973; Palnitkar and McFeeters 1975) is now widely practiced by the industry. However, the salt concentration in fermented cucumbers is above

levels normally consumed in foods so it must be partially washed out before the cucumbers are made into consumer products. This process results in a large volume of brine with high biological oxygen demand (BOD) and salt concentrations too low to be used for recycling. Fleming and others (1995) were not successful in brining cucumbers without NaCl. However they did demonstrate fermentation of cucumbers with reduced NaCl (4%) in pilot scale fermentations (Fleming and others 2002), but found it necessary to blanch cucumbers prior to fermentation to obtain adequate firmness retention of the cucumbers.

The fact that calcium ions at low concentration have a major effect on preserving the firmness of cucumber fruits (McFeeters and Fleming 1990) and the problem of disposal of waste salt has led to the concept of doing cucumber fermentations with higher calcium concentrations, but no NaCl in the fermentation brine (Maruvada and McFeeters 2009). CaCl<sub>2</sub> concentrations required to maintain cucumber firmness would be expected to be much lower than the current concentration of NaCl that is used. Since calcium is not toxic to plants, disposal of treated waste with CaCl<sub>2</sub> as the major salt would be less of an issue for processors.

The objectives of this project were to carry out laboratory scale cucumber fermentations in brine containing CaCl<sub>2</sub> as the only salt, to determine the course of fermentation metabolism in the absence of NaCl, and to compare firmness retention of cucumbers fermented in CaCl<sub>2</sub> brine during subsequent storage compared to firmness retention when cucumbers were fermented in brines containing both NaCl and CaCl<sub>2</sub> at concentrations typically used in commercial fermentations. If fermentation and storage of cucumbers with CaCl<sub>2</sub> as the only salt in the cover brine were similar to fermentations with NaCl as the major brining salt in combination with CaCl<sub>2</sub>, then efforts could be made to adapt commercial tank yard fermentations to be done without NaCl.

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## Materials and Methods

### Cucumbers, ingredients, and chemicals

A broad range of cucumber sizes are fermented commercially from 10 mm up to 51 mm diameter. For these experiments sizes based upon fruit diameter (1A  $\leq$ 19 mm, 2A  $\geq$ 25 mm and  $\leq$ 32 mm, 2B  $>$ 32 mm and  $\leq$ 38 mm) from different growing areas in the United States and Mexico were supplied by a local processor. The small size 1A cucumbers have the highest value on a weight basis. The size 2A and 2B cucumbers are sizes fermented in large volume commercially. Cucumber cultivars were not identified. Food grade anhydrous CaCl<sub>2</sub>, vinegar (20% acetic acid), glass jars and lids were obtained from the same processor. Morton's pickling salt was purchased at local grocery stores. Other chemicals were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.).

### Fermentations

Fermentations were done by filling 55% of the volume of glass jars (710 mL for size 1A cucumbers; 1360 mL for size 2A and 2B cucumbers) with cucumbers and 45% of the volume with a cover brine containing all components at concentrations 2.22-fold higher than the intended concentrations after equilibration with cucumbers in the containers. For example, fermentations with 1.03 M NaCl, 40 mM CaCl<sub>2</sub>, which was used as a control for comparison with fermentations carried out without NaCl, were done by preparing cover brine that contained 2.28 M NaCl and 88.9 mM CaCl<sub>2</sub>. After filling jars with cucumbers and brine solution, they were closed with a metal lid that was heated in boiling water to soften the sealing liner and applied to the jars while still hot. A rubber septum was inserted in each lid to allow inoculation of the jars and for sampling the brine with a syringe without opening the containers. The brined cucumbers were inoculated with *Lactobacillus plantarum* LA0445 (USDA-ARS, Food Science Research Unit, Raleigh, NC culture collection), which was grown overnight at 30 °C in MRS (Difco, Detroit, Mich., U.S.A.) broth with 0.342 M NaCl to a population of approximately 10<sup>9</sup> CFU/mL. The bacteria were removed from the broth by centrifugation, re-suspended in an equal volume of physiological saline and then inoculated into the fermentation jars at 10<sup>6</sup> CFU/mL.

### Experiments

**Time course of metabolite changes in fermentations with 100 mM CaCl<sub>2</sub> compared to fermentations with 1.03 M NaCl, 18 mM CaCl<sub>2</sub>.** Twenty 1360 mL jars were packed with size 2A cucumbers and covered with brine to equilibrate at 100 mM CaCl<sub>2</sub>, 25 mM acetic acid. Another 20 jars were packed with cucumbers and covered with brine to equilibrate at 1.03 M NaCl, 18 mM CaCl<sub>2</sub>, 8 mM acetic acid. All jars were inoculated with 10<sup>6</sup> CFU/mL *L. plantarum* and incubated at 30 °C. Duplicate jars fermented with and without NaCl in the cover solution were analyzed at 10 sampling times from 1 to 17 d after inoculation. Sections of all cucumbers from a jar were blended together without added liquid. A sample of the slurry from each jar was frozen for subsequent analysis. Brine from each container was frozen separately. The cucumber and brine samples from each jar were analyzed for pH and for fermentation substrates and products by high-performance liquid chromatography (HPLC) to determine changes inside and outside the cucumbers during the first 17 d of fermentation. The pH and metabolite concentrations inside and outside the cucumbers at each sampling point were compared using a *t*-test to determine when components equilibrated during the fermentation process.

**Effect of CaCl<sub>2</sub> concentration on the concentration of fermentation metabolites and pH after the completion of fermentation.** Triplicate 1360 mL jars were filled with size 2B cucumbers and cover solutions with CaCl<sub>2</sub> that equilibrated to final concentrations of 5 to 100 mM. Vinegar was added to all the cover solutions to equilibrate at 25 mM acetic acid. After inoculation with *L. plantarum* culture at 10<sup>6</sup> CFU/mL, the jars were incubated at 30 °C for 110 d. For comparison with fermentations with NaCl, 3 jars were filled with cucumbers from the same lot and covered with brine solution to equilibrate at 1.03 M NaCl, 40 mM CaCl<sub>2</sub>, and 8 mM acetic acid. At the end of the incubation period, brine from each jar was analyzed for pH and for the major fermentation substrates and products by HPLC. Linear regression was used to evaluate changes in pH and metabolite concentrations related to changes in the concentration of CaCl<sub>2</sub>.

The same experimental design and analysis was used for fermentation of size 1A cucumbers except that 710 mL jars were used and CaCl<sub>2</sub> was added at concentrations from 50 to 500 mM. Analysis of the fermented cucumber brines was done 95 d after filling the jars and adding starter culture.

**Changes in firmness of cucumbers fermented without NaCl compared to cucumbers fermented with 1.03 M NaCl, 40 mM CaCl<sub>2</sub>.** Size 2A cucumbers were packed into jars, inoculated, and fermented with 100, 200, and 300 mM CaCl<sub>2</sub> along with control jars fermented with 1.03 M NaCl, 40 mM CaCl<sub>2</sub>. Three months after packing, the jars were transferred from a 30 °C incubator to a 45 °C incubator to accelerate softening (McFeeters and Fleming 1990; McFeeters and others 1995). Duplicate jars of cucumbers from each treatment were evaluated for firmness at each sampling time. A 2nd lot of size 2A cucumbers and a lot of size 1A cucumbers were also fermented and evaluated for changes in firmness during storage. Fermentations were only done with 100 and 200 mM CaCl<sub>2</sub> along with control fermentations in which cucumbers were brined to equilibrate at 1.03 M NaCl, 40 mM CaCl<sub>2</sub>. After a 1 mo fermentation period at 30 °C, the fermented jars were transferred to 45 °C and stored for 7 mo.

### Calcium washout

Fermented size 2A cucumbers were removed from duplicate fermentation jars after 6 wk and transferred to a 2nd jar which contained 40% by volume fermented cucumbers and 60% water. A sample of fermented cucumbers from each jar was taken at the time of transfer for calcium and lactic acid analysis. Jars were closed and stored at 30 °C for 48 h and mixed 3 to 4 times each day. Liquid samples were taken after 24 and 48 h for calcium and lactic acid analysis. After 48 h the liquid was discarded and replaced by an equal volume of fresh water. This process was repeated a 3rd time for jars containing 300 mM CaCl<sub>2</sub>. Due to the ratio of water to cucumbers in the jars, at equilibration the concentration of water soluble components that are not bound to insoluble components of the cucumbers should have been reduced to 40% of the initial concentration in the cucumbers with each change of water. After the washout process was completed, slices were cut from each cucumber in each jar for firmness measurement. The remainder of the cucumber pieces from a jar was blended into a slurry for calcium and lactic acid analysis. The firmness of cucumbers in duplicate jars of each treatment that were not desalted was determined at the same time to compare with cucumbers after the washout process. The concentration of calcium and lactic acid in the liquid at each step was compared to the amount expected at equilibrium to determine when equilibration had been reached.

## Cucumber firmness

Firmness measurements were made with a TA.TX2 Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A./Stable Micro Systems, Godalming, Surrey, U.K.) using a 3 mm diameter punch. Data were collected and analyzed using Texture Expert software (Texture Technologies Corp./Stable Micro Systems). The maximum force required to puncture the mesocarp tissue of a cucumber slice was recorded and expressed in Newtons (N). Firmness measurements were done on a single 6-mm thick slice from the center of each cucumber in a jar for size 2A and 2B cucumbers (Thompson and others 1982). Fifteen size 1A cucumbers from a jar were punched from the outside at the center of the cucumber because they were too small to do a punch test on a slice. Duplicate jars of each treatment were measured at each sampling point.

## Chemical analyses

Liquid samples from fermentations were aseptically removed from sealed jars with a syringe and frozen until analysis. For HPLC analysis the liquid and cucumber slurry samples were thawed, mixed, centrifuged at  $13000 \times g$  in a Rainin microcentrifuge (Rainin Instrument, LLC, Oakland, Calif., U.S.A.) to remove particles and then diluted appropriately with water into autosampler vials so peaks were within the ranges of the standards. Changes in major fermentation metabolites were determined by chromatography on a 30-cm HPX-87H column (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) (McFeeters and Barish 2003). The column was heated to  $65^\circ\text{C}$  and eluted with 0.03 N sulfuric acid at a flow rate of 1 mL/min. A Thermo Separations UV6000 diode array detector (Spectra System Thermo Scientific, Waltham, Mass., U.S.A.) set to collect data at 210 nm was used to detect malic acid, lactic acid, acetic acid, propionic acid, and butyric acid. A Waters model 410 refractive index detector (Waters Corp., Millipore Corp., Billerica, Mass., U.S.A.) connected in series with the diode array detector was used to measure glucose, fructose, and ethanol. External standardization of the detectors was done using 4 concentrations of the standard compounds.

Measurements of pH were done with a Fisher Accumet pH meter, model 825MP, (Pittsburgh, Pa., U.S.A.) which was calibrated with pH 4 and 7 buffers.

Calcium determinations were performed directly on liquid samples during the calcium washout procedure using the dye binding method described by Gindler and King (1972). Cucumber slurry samples were mixed with trichloroacetic acid (TCA) to give a 4% TCA concentration to assure release of calcium ions that might be bound to components of the cucumber tissue. The samples were centrifuged to remove slurry particles prior to calcium analysis. TCA was added to standard calcium solutions so that the same concentration of TCA was in the standard solutions for the dye binding assay.

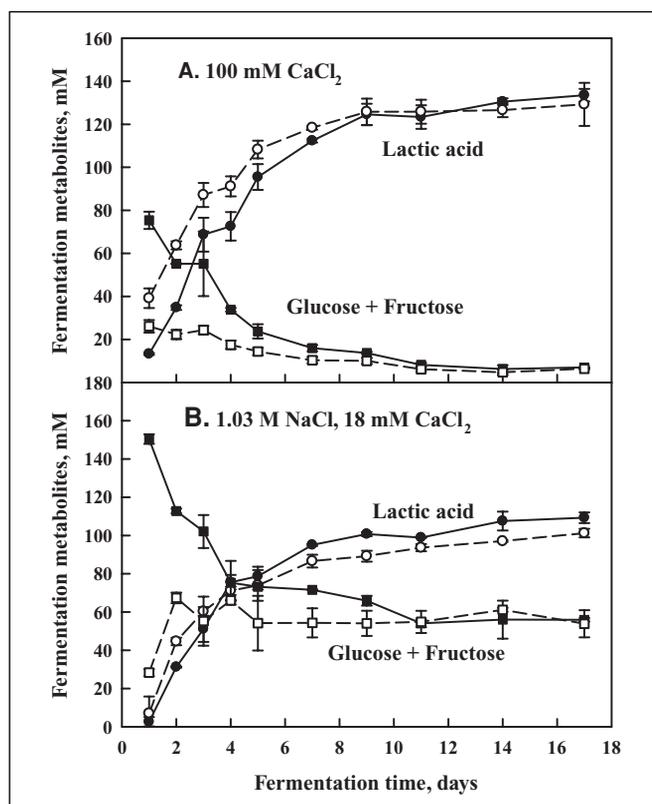
## Statistical analysis

Statistical analysis of experiments was done using Proc GLM in version 9.1.3 of SAS<sup>TM</sup> software (SAS Inst. Inc., Cary, N.C., U.S.A.). This included comparisons among treatments to determine if there were differences in metabolite concentrations and firmness at the  $P \leq 0.05$  level. The effect of  $\text{CaCl}_2$  concentration on changes in pH and fermentation substrates and products was evaluated by doing linear regression and determining if the slope of the regression was different from zero at the  $P \leq 0.05$  level.

## Results and Discussion

During cucumber fermentations there are 2 distinct environments inside and outside the fruit. When the cucumbers are

initially covered with brine, diffusion of water soluble components both from the brine into the cucumbers and from the cucumbers into the brine occurs. Figure 1 and 2 show the time course of changes in pH, lactic acid, malic acid, and sugars inside and outside whole size 2A cucumbers with a brine made up to equilibrate at 100 mM  $\text{CaCl}_2$  compared to cucumbers packed in brine to equilibrate with 1.03 M NaCl and 18 mM  $\text{CaCl}_2$ . There were substantial differences between fermentations with and without NaCl. In the first day of fermentation less than 10 mM lactic acid was produced with the brine containing NaCl compared to 40 mM lactic acid in the 100 mM  $\text{CaCl}_2$  brine. It took 5 d for lactic acid inside the cucumbers to equilibrate with lactic acid in the brine in the  $\text{CaCl}_2$  fermentations. In the NaCl fermentations lactic acid was not significantly different between the inside and outside of the cucumbers throughout the first 17 d of fermentation. As would be expected, sugars (glucose and fructose) inside the cucumbers declined. Differences in sugar concentrations between the inside and outside of the cucumbers were not significant 4 d into the fermentations with NaCl and 5 d into the fermentations with  $\text{CaCl}_2$ . The sugars remaining after 17 d were over 50 mM in the presence of 1.03 M NaCl, but less than 10 mM in the 100 mM  $\text{CaCl}_2$ . Figure 2 shows the change in pH in the 1.03 M NaCl and 100 mM  $\text{CaCl}_2$  fermentations. In the first day, pH declined to 4.2 inside the cucumbers fermented with  $\text{CaCl}_2$  compared to pH 4.8 in the cucumbers fermented with NaCl. It took 3 d for the pH inside and outside the fermenting cucumbers to equalize for both the  $\text{CaCl}_2$  and NaCl fermentations. After 17 d pH was 2.9 and 3, respectively. Figure 2 also shows the utilization



**Figure 1**—Changes in fermentation metabolites inside and outside size 2A cucumbers fermented in 100 mM  $\text{CaCl}_2$  brine solution (Panel A) and in 1.03 M NaCl, 18 mM  $\text{CaCl}_2$  brine solution (Panel B). Filled symbols show concentrations of lactic acid (●) and glucose + fructose (■) inside fermenting cucumbers. Open symbols show concentrations of lactic acid (○) and glucose + fructose (□) in the brine surrounding the cucumbers. Error bars show the SD from the analysis of duplicate jars at each sampling time.

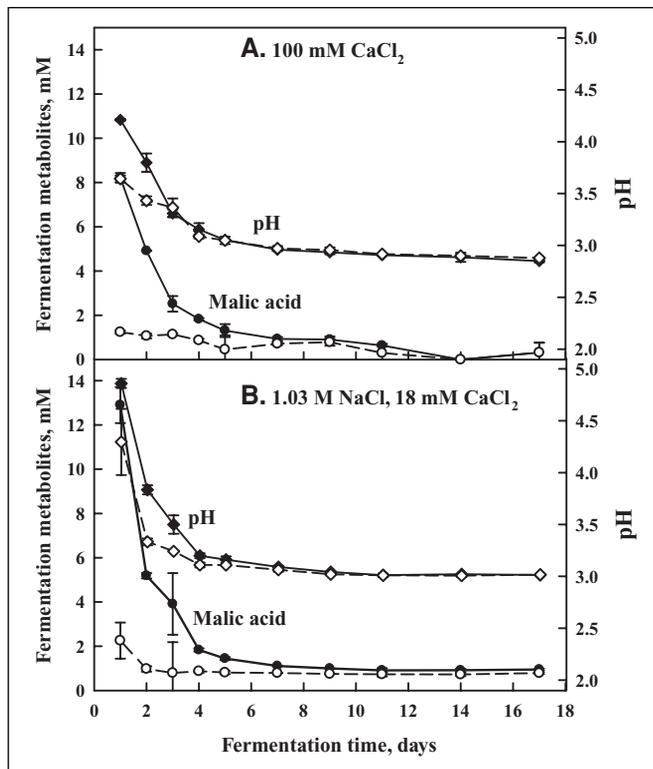
of malic acid, an important source of carbon dioxide gas during cucumber fermentations (McFeeters and others 1984). It took 5 d for the malic acid to equalize in concentration at 1 to 2 mM in both the NaCl and CaCl<sub>2</sub> fermentations.

CaCl<sub>2</sub> concentrations from 5 to 100 mM in the fermentation brines had no significant effect upon the final concentrations of lactic acid, ethanol, and acetic acid 110 d after initiation of fermentations using size 2B (32 to 38 mm diameter) cucumbers (Figure 3). The slope of the linear regression of metabolite concentration as a function of CaCl<sub>2</sub> concentration was not significantly different from zero for all of these components. The mean lactic acid production was 132 ± 6 mM in these CaCl<sub>2</sub> fermentations. The concentration of residual sugars was 1.6 ± 0.6 mM. No detectable propionic acid or butyric acid was detected in any of the fermentations, which indicated that microbial spoilage did not occur regardless of the concentration of CaCl<sub>2</sub> used (Fleming and others 1989). In comparison, the fermentations with cucumbers from the same lot in 1.03 M NaCl, 40 mM CaCl<sub>2</sub> produced 99.4 ± 2.7 mM lactic acid and had 19.0 ± 2.8 mM residual sugars after fermentation and storage for 110 d. Acetic acid (9 ± 1.1 mM) and ethanol formation (5.6 ± 1.1 mM) were also lower than when cucumbers were brined with CaCl<sub>2</sub> alone. The final pH of fermentations with CaCl<sub>2</sub> declined from 3.4 to 3 as the CaCl<sub>2</sub> concentration increased even though the production of acids did not change. This may be related to the fact that dissociation constants for carboxylic acids increase as the ionic strength increases (Butler and Cogley 1998).

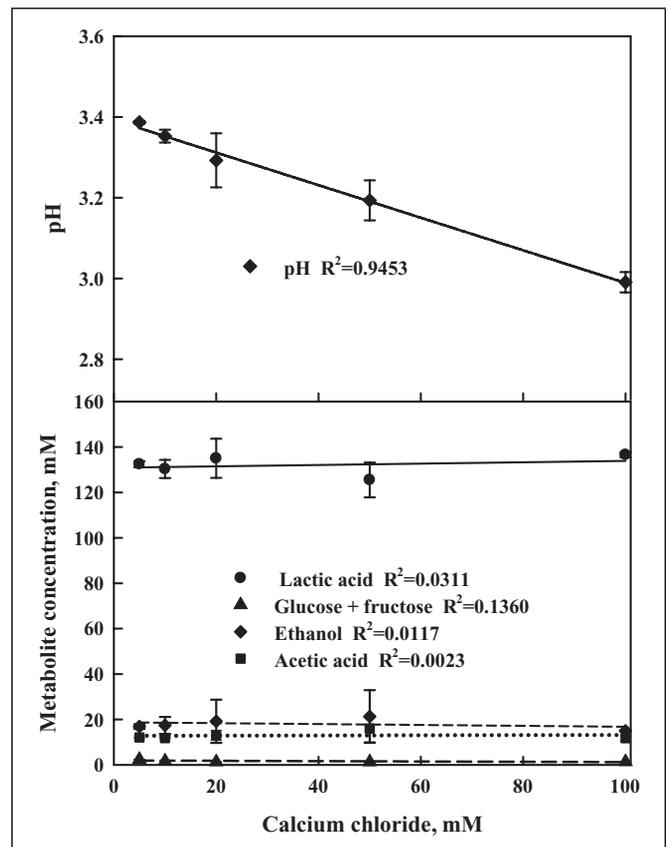
The smallest size cucumbers used for commercial fermentations (size 1A, < 19 mm diameter) were fermented over a range of CaCl<sub>2</sub>

concentrations from 50 to 500 mM. No residual sugars were detected in these fermentations. As was the case with the larger cucumbers, the slopes of the linear regression of lactic acid acetic and ethanol concentrations produced were not significantly different from zero (Figure 4). The final pH of the fermentations also did not change significantly in relation to the concentration of CaCl<sub>2</sub> in the cover solution. The mean pH was 3.74 ± 0.21. The mean lactic acid production was 96 ± 15 mM over the range of 50 to 500 mM CaCl<sub>2</sub> in the cover solutions for the fermentations. Fermentations with 1.03 M NaCl, 40 mM CaCl<sub>2</sub> had a mean pH of 3.50 ± 0.09, a lactic acid concentration of 105 ± 17 mM and no detectable residual sugars. The absence of any detectable residual glucose or fructose regardless of the NaCl or CaCl<sub>2</sub> concentrations in the cover solutions was due to the low initial sugar concentrations that are typical in the 1A size cucumbers. For example, the fresh size 1A cucumbers contained only 83 mM glucose and fructose compared to 154 mM sugars in the size 2A cucumbers fermented in the experiment reported in Figure 1.

Fermentations carried out in brines with CaCl<sub>2</sub> and no NaCl resulted in nearly complete fermentation of the sugars present in fresh cucumbers of different sizes even without addition of acetate buffer to enhance sugar utilization (Etchells and others 1974). This is beneficial for cucumber fermentations because residual sugar, as was present in the 1.03 M NaCl fermentations of larger sizes of cucumbers, can serve as a substrate for undesirable yeast fermentations after the lactic acid fermentation has been finished.



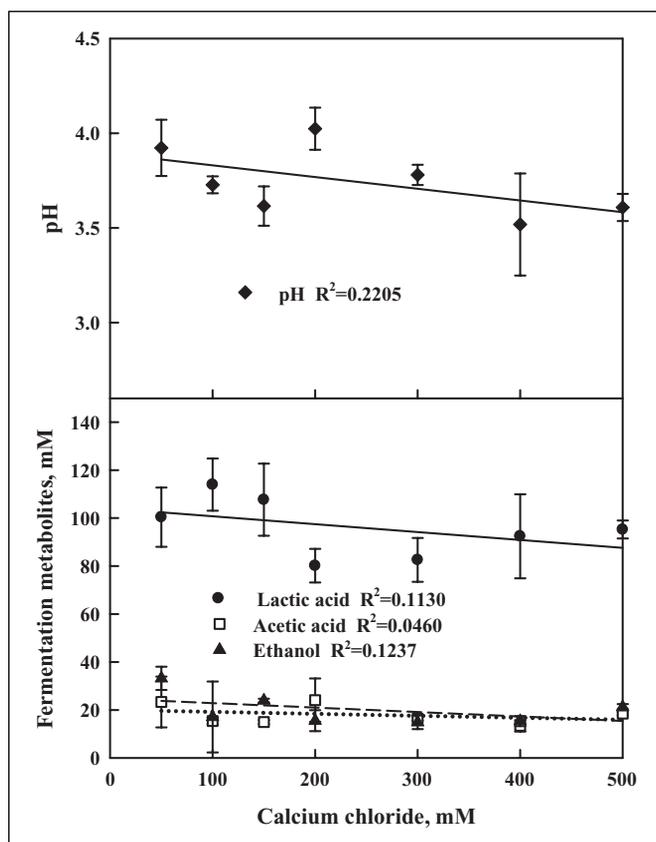
**Figure 2—Changes in malic acid and pH inside and outside size 2A cucumbers fermented in 100 mM CaCl<sub>2</sub> brine solution (Panel A) and in 1.03 M NaCl, 18 mM CaCl<sub>2</sub> brine solution (Panel B). Filled symbols show concentrations of malic acid (●) and pH (◆) inside fermenting cucumbers. Open symbols show concentrations of malic acid (○) and pH (◇) in the brine surrounding the cucumbers. Error bars show the SD from the analysis of duplicate jars at each sampling time.**



**Figure 3—Effect of CaCl<sub>2</sub> brine concentration on the pH and fermentation metabolite concentrations 110 d after the beginning of the fermentations. Lines show the linear regression of metabolite concentrations and pH related to the equilibrated concentrations of CaCl<sub>2</sub> in the fermentation cover solutions. Error bars show the SD from the analysis of triplicate fermentations at each CaCl<sub>2</sub> concentration.**

Lactic acid production was higher and the final pH was slightly lower than cucumbers fermented with 1.03 M NaCl in the larger cucumber sizes, which are the cucumbers fermented in greatest volume in the United States. The fact that the lactic acid concentrations and pH remained stable for several months at 30 °C and there was no detectable production of propionic acid or butyric acid, indicated that viable spoilage microorganisms were either absent or not able to grow in cucumbers fermented with CaCl<sub>2</sub> and no NaCl and stored in closed containers. This is the first step toward evaluating whether cucumber fermentations without use of NaCl may be possible in commercial tank yards.

In addition to the absence of microbial spoilage after the primary fermentation, commercial fermentation requires the firmness of cucumbers to be maintained for a year or more of storage in the fermentation tanks before they are converted into consumer products. Fleming and others (1987) found that cucumbers fermented with 1.03 M NaCl, 18 mM CaCl<sub>2</sub> maintained sufficient firmness for a full year at room temperature so they would be suitable for conversion to consumer products. Therefore, the texture retention of cucumbers fermented without salt was compared to cucumbers fermented with 1.03 M NaCl, 40 mM CaCl<sub>2</sub>, which is a concentration of CaCl<sub>2</sub> in line with current commercial fermentation operations. Given the previous results of Fleming and others (1987), cucumbers stored at elevated temperatures that maintain their firmness during storage equal to or better than cucumbers fer-

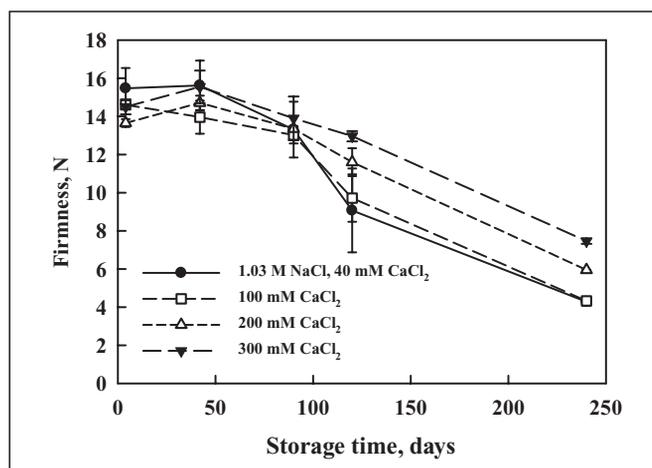


**Figure 4**—Effect of CaCl<sub>2</sub> brine concentration on the pH and fermentation metabolite concentrations of small size 1A cucumbers 95 d after the beginning of fermentation. Lines show the linear regression of metabolite concentrations and pH related to the equilibrated concentrations of CaCl<sub>2</sub> in the fermentation cover solutions. Error bars show the SD from the analysis of triplicate fermentations at each CaCl<sub>2</sub> concentration. Note: Glucose and fructose were not detectable in any of the treatments.

mented with the combination of NaCl and CaCl<sub>2</sub> should retain sufficient firmness during storage for at least 1 y at normal ambient temperatures. Figure 5 shows changes in the firmness of the mesocarp tissue of cucumbers fermented with CaCl<sub>2</sub>. Fermentation with 100, 200, and 300 mM CaCl<sub>2</sub> resulted in retention of firmness not significantly different from that of the cucumbers fermented and stored with 1.03 M NaCl, 40 mM CaCl<sub>2</sub> for 8 mo of storage, including 5 mo at 45 °C. The outcomes of the fermentations were very similar to those shown in Figure 3. Lactic acid production was higher in the CaCl<sub>2</sub> fermentations with pH of  $2.94 \pm 0.05$  compared to the 1.03 M NaCl, 40 mM CaCl<sub>2</sub> fermentations where the final pH was  $3.11 \pm 0.01$ . Both the lactic acid concentration and pH were stable with no detectable formation of propionic acid or butyric acid from 6 wk after the beginning of fermentation until the end of the storage period at 8 mo, indicating the cucumbers fermented without NaCl in closed jars did not spoil microbiologically for an extended period of time (Fleming and others 1989).

A 2nd lot of size 2A cucumbers and size 1A cucumbers fermented with 100 and 200 mM CaCl<sub>2</sub> also retained firmness not significantly different from cucumbers fermented in 1.03 M NaCl, 40 mM CaCl<sub>2</sub> and then stored for 7 mo at 45 °C after a 1-mo fermentation period at 30 °C. These results showed that 100 mM CaCl<sub>2</sub> or higher was sufficient to maintain the firmness of fermented cucumbers as well as cucumbers fermented in 1.03 M NaCl, 40 mM CaCl<sub>2</sub>.

The ability of calcium to maintain cucumber firmness at low pH is related to its ability to bind to cell wall polysaccharides (Buescher and Hudson 1986; McFeeters and Fleming 1990). Generally the maximum concentration of calcium in pickled vegetable products is less than about 25 mM because an increasing proportion of people detect an undesirable chalky flavor as the concentration of calcium is increased. Since calcium can bind to plant cell walls, it was of interest to determine if calcium in the fermented cucumbers would readily washout so the concentration could be reduced to levels suitable for consumer products using a 24-h washout process similar to that currently used commercially to remove NaCl. If, for example, a desalting tank is filled with 60% water and 40% cucumbers containing 1.03 M NaCl, the salt in the cucumbers would equilibrate to 0.41 M in 24 h or less. Table 1 shows the predicted and measured final calcium concentration inside the cucumbers after 2 or 3 steps of calcium washout using a 60% water : 40% cucumber



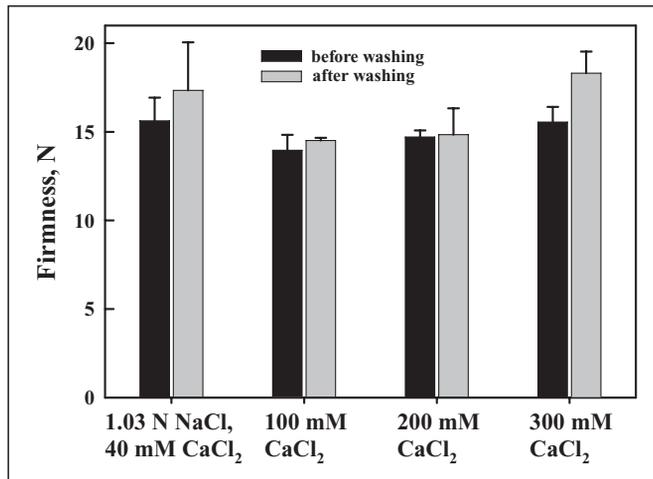
**Figure 5**—Firmness retention in size 2A cucumbers fermented and stored in brines containing only CaCl<sub>2</sub> compared to brining in 1.03 M NaCl, 40 mM CaCl<sub>2</sub>. Cucumbers were fermented and stored for 90 d at 30 °C and then the temperature was increased to 45 °C for the remaining 150 d of storage. Error bars show the SD of the analysis of duplicate fermentations at each sampling time.

**Table 1 – Washout of lactic acid and calcium ions from size 2A fermented cucumbers.**

Fermentation brine	Calcium in cucumbers, mM			Lactic acid in cucumbers, mM		
	After fermentation	After washout	Predicted	After fermentation	After washout	Predicted
1.03 M NaCl, 40 mM CaCl <sub>2</sub>	41 ± 0.0	7 ± 0.0	6.6 <sup>a</sup>	80.0 ± 0.4	12.1 ± 0.9	12.8 <sup>a</sup>
100 mM CaCl <sub>2</sub>	88 ± 1.4	15 ± 0.0	14 <sup>a</sup>	130.4 ± 2.1	20.8 ± 0.9	20.9 <sup>a</sup>
200 mM CaCl <sub>2</sub>	181 ± 2.1	30 ± 0.7	29 <sup>a</sup>	104.9 ± 2.0	16.0 ± 1.0	16.8 <sup>a</sup>
300 mM CaCl <sub>2</sub>	259 ± 9.2	17 ± 0.7	16.6 <sup>b</sup>	70.7 ± 4.4	4.6 ± 0.2	4.5 <sup>b</sup>

<sup>a</sup>Expect an 84% reduction after 2 successive equilibrations with 40% cucumbers : 60% water.

<sup>b</sup>Expect a 93.4% reduction after 3 successive equilibrations with 40% cucumbers : 60% water.



**Figure 6 – Firmness of size 2A cucumbers fermented in different brine solutions before and after washing out salt and CaCl<sub>2</sub> from the fermented cucumbers. Error bars show the SD of the analysis of firmness of duplicate jars of cucumbers before and after washout of calcium ions.**

ratio at each step. The predicted final concentration was based upon the assumption that the calcium would completely equilibrate with the water added to the cucumbers after each addition of fresh water. If some calcium was so tightly bound to the cucumber cell walls that it did not equilibrate, the calcium remaining in the cucumber would be greater than the predicted amount. It can be seen that the calcium remaining inside the cucumbers was very close to predicted. Lactic acid, which is very water soluble and which is not known to bind substantially to plant tissues, also was removed from the cucumbers in the predicted proportions. This washout procedure was repeated with a 2nd lot of size 2A cucumbers and a lot of size 1A cucumbers with similar results. For the washout experiments cucumbers were held in water for 48 h at each step. Analysis of calcium and lactic acid in the wash water after 24 and 48 h showed that both components were equilibrated after 24 h.

Figure 6 shows the firmness of the cucumbers before and after calcium removal. There was no significant change in firmness as a result of the washout procedure.

Fermentation of cucumbers in CaCl<sub>2</sub> brine containing no sodium chloride resulted in fermented cucumbers with high concentrations of lactic acid, low pH, and low or no residual sugars. Firmness of the stored, fermented cucumbers was retained at least as well as cucumbers stored in 1.03 M NaCl, 40 mM CaCl<sub>2</sub>, which suggests that they would retain commercially acceptable firmness for at least 1 y at ambient temperatures. Calcium was not tightly bound in fermented cucumbers so excess calcium could be removed by a washout procedure such as is currently used in the industry to remove sodium chloride. The advantage to using 100 to

200 mM CaCl<sub>2</sub> for cucumber fermentations would be a reduction of chlorides from tank yard wastes by 60% to 80% compared to doing fermentations in 1.03 M NaCl. While these results from laboratory fermentations show cucumbers can be fermented and stored without the use of NaCl in the cover brine, it will be a challenge to apply these results to brining in commercial tank yards. Major consideration will be the ability to prevent spoilage in open top tanks, which are air purged during the period of active fermentation to remove carbon dioxide gas from the fermentations tanks so cucumbers do not suffer bloating damage.

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