

# Effect of Brine Acidification on Fermentation Microbiota, Chemistry, and Texture Quality of Cucumbers Fermented in Calcium or Sodium Chloride Brines

Erin K. McMurtrie, Suzanne D. Johanningsmeier<sup>1b</sup>, Fred Breidt Jr.<sup>1b</sup>, and Robert E. Price

**Abstract:** Commercial fermentation for bulk preservation of cucumbers relies on natural microbiota and approximately 1 M sodium chloride (NaCl) brines, resulting in large volumes of high-salt wastewater. An alternative process utilizing 0.1 M calcium chloride (CaCl<sub>2</sub>) as the only salt was developed to eliminate NaCl from fermentation brines for reduced environmental impact. This study determined the effect of brine acidification on the fermentation microbiota and texture quality of cucumbers fermented in CaCl<sub>2</sub> brines. Cucumber fermentations were conducted in sealed glass jars for six independent lots of cucumbers in a randomized complete block design with a full-factorial treatment structure for brine acidification (acetic acid, hydrochloric acid, or nonacidified) and brining salt (1 M NaCl or 0.1 M CaCl<sub>2</sub>). *Enterobacteriaceae spp.* survived longer and were >1 log colony forming units/mL higher in fermenting cucumbers than in brines. Addition of 25 mM acetic acid to fermentation brines (but not the addition of hydrochloric acid at the same pH) reduced *Enterobacteriaceae spp.* in brines and cucumbers ( $P < 0.002$ ) during the initiation of fermentation for both brining salts. However, acidification had no effect on texture quality of fermented cucumbers ( $P = 0.8235$ ). Despite differences in early fermentation microbiota, fermentation of cucumbers in calcium chloride brines under controlled conditions, with or without acidification, resulted in high retention of tissue firmness. These results differ from fermentations in a commercial setting initiated in brines of neutral pH, indicating that production variables, such as air exposure, interact with brining in CaCl<sub>2</sub> to negatively affect the texture quality of fermented cucumbers.

**Keywords:** cucumber, fermentation, food preservation, lactic acid bacteria, texture

**Practical Application:** This study examined the effects of initial brine acidification on the course of lactic acid fermentation and resulting texture quality of cucumbers fermented in calcium or sodium salt brines. Fermentation brines containing acetic acid (the acid in vinegar) reduced the pH of the cucumber and the soil-associated *Enterobacteriaceae spp.* most rapidly, and favored the conversion of sugars to lactic acid. Interestingly, the texture quality was not affected by brine acidification, and all cucumbers fermented in calcium brines in the absence of air retained their firmness during fermentation and bulk storage.

## Introduction

Current commercial practice for the bulk preservation of cucumbers as pickles utilizes the microbiota present on the cucumbers for a natural fermentation. As such, brines that equilibrate to at least 5% sodium chloride (0.86 M NaCl) are used to select for lactic acid bacteria (LAB) and inhibit salt-sensitive spoilage

and pathogenic bacteria on the cucumbers (Franco et al., 2016). However, this concentration of salt puts the wastewater generated by processors in excess of the EPA's Secondary Drinking Water Standards for chloride concentrations (250 mg/L or 7 mM) by several orders of magnitude (United States Environmental Protection Agency, 2009). Recycling of fermentation brines successfully reduces high NaCl wastewater (Palnitkar & McFeeters, 1975) and has been widely implemented on the commercial scale. However, desalting water produced during processing has a NaCl concentration too low to be recycled but high enough to cause concern for the environment. In an attempt to eliminate NaCl from fermentation brines, an alternative fermentation process was developed by McFeeters and Pérez-Díaz (2010) using 100 mM calcium chloride (CaCl<sub>2</sub>) and 25 mM acetic acid in a 55:45 (w/v) cucumber: brine pack ratio inoculated with *Lactobacillus plantarum* LA0445 starter culture. This fermentation brine contributes no sodium ions to wastewaters and a fifth of the concentration of chloride ions compared to the typical commercial fermentations. Commercialization of this alternative fermentation process was completed with several modifications to the lab scale process (Pérez-Díaz et al.,

JFDS-2018-1561 Submitted 9/30/2018, Accepted 2/28/2019. Author McMurtrie is with the Dept. of Food, Bioprocessing and Nutrition Sciences, North Carolina State Univ., 400 Dan Allen Drive, Box 7624, Raleigh, NC 27695, U.S.A. Authors Johanningsmeier, Breidt Jr., and Price are with Food Science Research Unit, U.S. Dept. of Agriculture, Agricultural Research Service, SEA North Carolina State Univ., 322 Schaub Hall, Box 7624, Raleigh, NC 27695-7624, U.S.A. Direct inquiries to author Johanningsmeier (E-mail: suzanne.johanningsmeier@ars.usda.gov).

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

2015). Removal of acetic acid, addition of 6 mM potassium sorbate as a preservative, and introduction of a minimal air-purging routine were implemented. However, several consumer sensory tests of cucumber pickles produced with the modified CaCl<sub>2</sub> fermentation process showed a lower texture quality in the pickles produced in CaCl<sub>2</sub> brine (Pérez-Díaz et al., 2015; Wilson, Johanningsmeier, & Osborne, 2015). This overall reduction in texture quality was shown to be directly related to the CaCl<sub>2</sub> fermentation process in a series of commercial fermentations from the 2013 and 2014 growing seasons (McMurtrie & Johanningsmeier, 2018).

The changes made in the commercial scale-up included the removal of acetic acid from the brine formulation (Pérez-Díaz et al., 2015) because it can serve as a substrate for oxidative yeast and other spoilage microorganisms in the absence of sugar, which can be a problem in open-top commercial tanks (Franco & Pérez-Díaz, 2012). Removing acetic acid from the cover brine results in a fermentation process with an initial brine pH above 7, which may in part explain the higher *Enterobacteriaceae* counts during the first week of fermentation in CaCl<sub>2</sub> brines (Pérez-Díaz et al., 2015). The pH values after 1 day of fermentation were  $5.9 \pm 0.5$  in CaCl<sub>2</sub> fermentation brines compared to  $4.15 \pm 0.1$  in NaCl brines (Pérez-Díaz et al., 2015). The pH is initially higher in the CaCl<sub>2</sub> brines because of the elimination of the acetic acid, low buffer capacity of the brines, and the addition of potassium sorbate. The use of a starter culture without the inhibitory action of NaCl initiates a quick fermentation that rapidly decreases the brine pH to  $4.0 \pm 0.5$  by the third day of fermentation (Pérez-Díaz et al., 2015). However, neutral pH values can affect the rate of softening through a number of direct and indirect mechanisms including nonenzymatic activity, enzymatic activity, changes in microbial ecology, and preservative efficacy. Nonenzymatic softening rates of blanched cucumbers have been shown to be much higher at neutral pH values, presumably from  $\beta$ -elimination reactions (Krall & McFeeters, 1998; McFeeters & Fleming, 1991). Additionally, the neutral pH range of the initial brining conditions is a much more suitable environment for the survival of microorganisms that may exist on the surface of the cucumber such as bacteria in the *Enterobacteriaceae* family as well as yeasts and molds that have been found to produce softening enzymes (Bell, Etchells, & Jones, 1950; Collmer & Keen, 1986; Costilow, Gates, & Lacy, 1980; Etchells, Bell, Monroe, Masley, & Demain, 1958; Walter, 1991). Etchells, Fleming, Hontz, Bell, and Monroe (1975) found that acidification of cover brine was sufficient for reduction of coliform bacteria within the *Enterobacteriaceae* family allowing for a controlled fermentation. Additionally, McDonald, Fleming, and Daeschel (1991) found that brines acidified using acetic acid reduced *Enterobacteriaceae* counts during the initiation of natural fermentation in NaCl brines. Acetic acid (28 mM) has also been shown to prevent enzymatic softening by the inhibition of molds in commercial air-purged fermentations, resulting in significantly firmer cucumbers (Potts & Fleming, 1982). The preservative potassium sorbate was deemed necessary for successful commercial fermentations by the inhibition of spoilage microorganisms, but it is much more effective at low pH values in its conjugate acid form, sorbic acid (Dharmadhikari, 1992; Sauer, 1977). Therefore, it is plausible that in the first few days of the fermentation when cucumbers in CaCl<sub>2</sub> brines are at neutral pH values, the cucumbers could be softening by nonenzymatic softening reactions or due to pectin degrading enzymes produced by increased metabolic activity of *Enterobacteriaceae*, yeasts or molds due to lessened inhibition by potassium sorbate. The objective of this research was to determine the effect of brine acidification on fermenta-

tion microbiota and texture quality of cucumbers fermented in CaCl<sub>2</sub> brines.

## Materials and Methods

### Fermentations and sample collection

Fresh, size 2B (3.5 to 3.8 cm diameter) pickling cucumbers were obtained from a local processor and packed into 32 oz. (946 mL) jars. A full-factorial treatment structure for brining salt (equilibrated concentrations of 1 M NaCl and 0.1 M CaCl<sub>2</sub>) and acidification (equilibrated 0.025 M acetic acid, hydrochloric acid at the same brine pH, and no acidification) was used to create six brining treatments. The levels of NaCl and CaCl<sub>2</sub> were selected based upon commonly used salt levels for industrial fermentation of cucumbers (approximately 6% NaCl) and a low-salt alternative for reduced environmental impact of brining operations (0.1 M CaCl<sub>2</sub>). The level of acetic acid was selected based upon previous studies on cucumber fermentation in NaCl or CaCl<sub>2</sub> (McDonald et al., 1991; McFeeters & Perez-Díaz, 2010; Potts & Fleming, 1982), and the HCl treatment allows us to separate the effects of pH and organic acid content. Before equilibration, the pH values of the brines were  $3.58 \pm 0.07$  in the acidified treatments, while the nonacidified treatments were  $9.58 \pm 0.73$  and  $7.05 \pm 1.10$  for the CaCl<sub>2</sub> and NaCl brines, respectively. Using each treatment, 16 jars were packed at a 55:45 (w/w) cucumber to brine ratio for six independent lots of cucumbers in a randomized complete block design. All treatments included 4 mM potassium sorbate and were inoculated with  $10^6$  colony forming units (CFU)/mL *L. plantarum* (LA0445, USDA-ARS, Food Science Research Unit, Raleigh, NC, USA, culture collection). *L. plantarum* strain LA0445 was selected based on its established suitability for cucumber fermentation in calcium chloride brines in previous studies as well as its use in commercial implementation of this process (McFeeters & Perez-Díaz, 2010). *L. plantarum* starter culture was grown at 30 °C in deMan, Rogosa, and Sharpe (MRS) broth (Difco, Becton, Dickinson and Co., Sparks, MD, USA) to a population of approximately  $10^9$  CFU/mL, centrifuged to pellet cells, and resuspended in saline. After inoculation, the jars were sealed and incubated at 28 °C. Fermentations were monitored for 21 days as well as at 100 days to simulate a typical bulk storage time. At each time point (1, 2, 3, 5, 7, 14, 21, and 100 days), at least one jar was removed for sampling and was not included in the rest of the experiment. At each time point, the cucumbers were aseptically removed from the brines and blended using autoclaved Waring blenders to form a slurry, which was further subjected to stomaching (model 400; Tekmar Co., Cincinnati, OH, USA). Brine from each jar was aseptically collected as a separate sample. The slurry and brine from each fermentation and timepoint were individually analyzed for microbial counts, pH, and fermentation metabolites by high-performance liquid chromatography (HPLC) analysis. Additional samples of cucumber slurry were collected at selected timepoints for culture independent analysis of the microbiota by high throughput 16S ribosomal deoxyribonucleic acid (rDNA) sequencing.

### Microbial enumeration of microorganisms

Samples were serially diluted using 0.85% saline solution and spiral plated using an Autoplate 4000 (Spiral Biotech, Norwood, MA, USA). Enumeration of *Enterobacteriaceae* was done by plating on Violet Red Bile agar (Difco) containing 1% glucose (Sigma-Aldrich, St. Louis, MO, USA) (VRBG) incubated at 37 °C for 24 hr. LAB were enumerated using MRS (Difco) supplemented

with 0.001% cycloheximide (OXOID, Thermo-Fisher, Fair Lawn, NJ, USA) incubated at 30 °C for 3 days. Yeasts and molds were enumerated using yeast and mold agar (YMA, Difco) supplemented with 0.01% chloramphenicol (Sigma-Aldrich) and 0.01% chlortetracycline (Sigma-Aldrich) incubated at 30 °C for 3 days.

### Instrumental texture analysis

Texture measurements were performed using a mesocarp puncture test on a 6.7 mm slice obtained from the center of 30 raw cucumbers to represent the initial cucumber mesocarp firmness on day 0 and on slices obtained aseptically from the center of 15 cucumbers on days 7, 14, 21, and 100 prior to the remaining cucumber being blended into a slurry. The mesocarp puncture test was conducted on a TA.XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) using a 3-mm-diameter stainless steel probe to puncture the mesocarp tissue (Thompson, Fleming, Hamann, & Monroe, 1982). In turn, the mesocarp of one lobe of each slice was centered above a 3.1 mm hole in the base plate and the probe was lowered at a test speed of 2.5 mm/s through the sample (Yoshioka, Horie, Sugiyama, & Sakata, 2009). The test was conducted and data analyzed using Texture Expert software (version 6.1.3.0, Texture Technologies Corp.). The peak force of 15 individual cucumbers per fermentation was averaged and recorded in Newtons (N) as the firmness value.

### Detection of fermentation metabolites

Concentrations of organic acids, residual sugars, and selected spoilage metabolites were measured by HPLC analysis using an Agilent 1260 Infinity HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an HPX-87H ion exchange column for organic acid analysis (Bio-Rad Laboratories, Hercules, CA). Chromatography was conducted with 0.03 N sulfuric acid mobile phase at a flow rate of 0.6 mL/min and using a column temperature of 37 °C. Organic acids were quantified using an Agilent 1260 DAD detector (G4212B, Agilent Technologies Inc.) set at 210 nm. Residual sugars and alcohols were quantified using an Agilent 1260 RI detector (G1362A, Agilent Technologies Inc.) that connected in series after the DAD detector. HPLC analysis was conducted on both the brines and the cucumber slurries. Slurries were produced by aseptically blending the cucumbers in a Waring Blender until homogenous as described earlier. Slurries were frozen at -80 °C and then thawed to lyse the cucumber cells for more accurate sugar measurements. The samples were centrifuged at 10,000 × *g* for 7.5 min prior to HPLC analysis.

### pH and analysis of salts

A Fisher Accumet (model AR25) pH meter calibrated with standard pH 2.00, 4.00, and 7.00 buffers (Thermo-Fisher) was used for pH measurement. Calcium and total chloride concentrations were determined according to Fleming, McFeeters, and Breidt (2001) and Official Methods of Analysis of AOAC International Method 968.31 (2000) for the first replication to determine how quickly the brine components equilibrated with the cucumbers. The calcium method used 0.025 N disodium ethylenediaminetetraacetate dihydrate as a titrant and hydroxy naphthol blue as the indicator. CaCl<sub>2</sub> concentrations were estimated to be equal to the molar concentrations of titrated calcium. The natural calcium (approximately 3.0 ± 0.8 mM) contributed by the fresh pickling cucumbers (McMurtrie & Johanningsmeier, 2018) was considered negligible and not considered in the calculations. Titration of total chlorides was done by a modification of Fajans Method

using 0.171 N silver nitrate as a titrant and dichlorofluorescein as the indicator (Collier, 1936; Fajans & Hassel, 1923). NaCl concentrations were estimated to be equal to the molar chloride concentrations since no other major sources of chloride should be present in the cucumbers fermented in brines with NaCl as the only salt.

### Statistical analysis

All results were analyzed using SAS statistical software (version 9.4, SAS Inst., Cary, NC, USA). A randomized complete block design with a full-factorial treatment structure was performed for the salt and acid levels. A general linear model analysis of variance (ANOVA) was used with least square means. Fermentation time, salt, and acidification treatment were designated as fixed effects. Statistical significance, unless otherwise stated, was indicated at *P* < 0.05. Values were presented as means ± standard deviation in Table 1. Figures represent data for the cucumber portion (unless otherwise indicated) plotted as the means ± standard error.

### Culture-independent fermentation microbiota analysis

DNA was isolated using the MoBio Power Soil DNA Isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). Primers for DNA sequencing targeted variable regions V3 and V4 of the 16S ribosomal ribonucleic acid (rRNA): S-D-Bact-0341-b-S-17 (5'- CCT ACG GGN GGC WGC AG -3'), S-D-Bact-0785-a-A-21 (5'- GAC TAC HVG GGT ATC TAA TCC -3'), generating an approximately 465 bp fragment (Klindworth et al., 2013). Amplification and bidirectional sequencing with overlapping 250 bp sequence reactions was carried out using Fluidigm (Fluidigm Corp., San Francisco, CA, USA) and Illumina MiSeq technology (Illumina Inc., San Diego, CA, USA) by the Univ. of Illinois' Roy J. Carver Biotechnology Center (<https://biotech.illinois.edu/htdna>). DNA sequence data consisting of paired forward and reverse Illumina fastq format (Cock, Fields, Goto, Heuer, & Rice, 2009) files were analyzed using the Mothur software package (Schloss et al., 2009). Assembled sequences were initially filtered to allow one ambiguous base and to select sequences between 400 and 500 bp in length. Chimeras were removed using the Mothur vsearch algorithm (Rognes, Flouri, Nichols, Quince, & Mahe, 2016), and resulting sequences were classified using Ribosomal Database Project's (RDP) 16S database (version 16\_022016) and classifier (Cole et al., 2014; Wang, Garrity, Tiedje, & Cole, 2007). Following classification, chloroplast, mitochondrial, and eukaryotic sequences were removed. Sequences were then sorted by taxonomic classification (with >97% identity to RDP database), combining Mothur-generated taxonomy and treatment group files to make a summary table.

## Results and Discussion

### Fermentation

All cucumber fermentations for each lot of cucumbers were completed within 21 days of fermentation, indicated by the reduction in reducing sugar content (Figure 1) and equilibrated lactic acid concentrations (Figure 2). Reducing sugars were utilized faster in the CaCl<sub>2</sub>-brined cucumbers and significantly more sugars were utilized in these treatments than in the NaCl-brined cucumbers (Figure 1 and Table 1). This is consistent with the higher LAB counts during fermentation in the CaCl<sub>2</sub> brine treatments (Figure 3), which is likely due to NaCl acting as a greater inhibitor of growth than CaCl<sub>2</sub> (Naewbanij,

**Table 1—Effect of initial brine composition on residual sugars and fermentation metabolites in fermented cucumber (21 days).**

Salt	Acid	Fructose (mM) <sup>a</sup>	Glucose (mM) <sup>a</sup>	pH <sup>a</sup>	Lactic acid (mM) <sup>a</sup>	Acetic acid (mM) <sup>a</sup>	Ethanol (mM) <sup>a</sup>
CaCl <sub>2</sub>	No acid	3.8 ± 3.8 <sup>b</sup>	1.8 ± 2.4 <sup>c</sup>	3.14 ± 0.10 <sup>b</sup>	107.4 ± 13.8 <sup>ab</sup>	5.9 ± 1.2 <sup>b</sup>	19.3 ± 4.7 <sup>a</sup>
	Acetic	4.6 ± 4.3 <sup>ab</sup>	2.2 ± 3.5 <sup>c</sup>	3.12 ± 0.07 <sup>b</sup>	119.5 ± 13.7 <sup>a</sup>	31.1 ± 1.4 <sup>a</sup>	11.5 ± 2.3 <sup>bc</sup>
	HCl	6.9 ± 7.2 <sup>ab</sup>	5.1 ± 6.1 <sup>bc</sup>	3.11 ± 0.09 <sup>b</sup>	104.3 ± 18.7 <sup>abc</sup>	5.4 ± 0.5 <sup>b</sup>	19.3 ± 4.3 <sup>a</sup>
NaCl	No acid	8.8 ± 7.0 <sup>ab</sup>	12.3 ± 5.6 <sup>ab</sup>	3.26 ± 0.03 <sup>a</sup>	86.6 ± 20.1 <sup>bc</sup>	3.9 ± 0.5 <sup>c</sup>	12.9 ± 3.0 <sup>b</sup>
	Acetic	7.9 ± 4.7 <sup>ab</sup>	14.9 ± 6.6 <sup>a</sup>	3.25 ± 0.02 <sup>a</sup>	93.7 ± 14.7 <sup>abc</sup>	29.8 ± 0.9 <sup>a</sup>	6.7 ± 4.9 <sup>c</sup>
	HCl	13.2 ± 8.1 <sup>a</sup>	15.1 ± 5.5 <sup>a</sup>	3.29 ± 0.06 <sup>a</sup>	80.7 ± 21.8 <sup>c</sup>	3.6 ± 0.5 <sup>c</sup>	13.9 ± 2.7 <sup>ab</sup>

<sup>a</sup>Means for treatments followed by different lowercase letters are significantly different at  $P < 0.05$ .

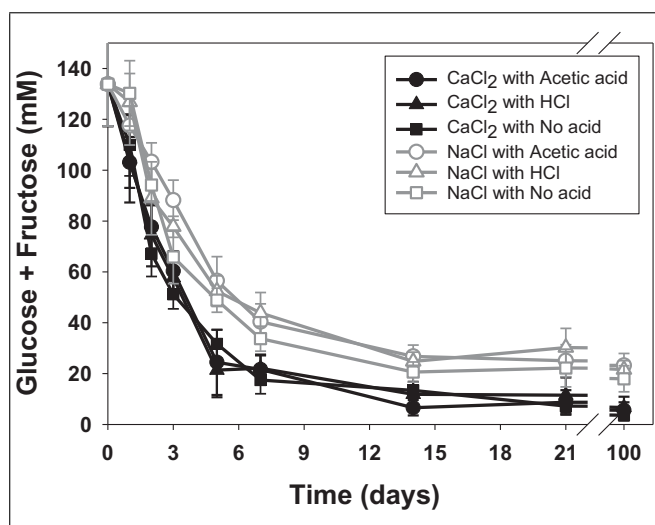


Figure 1—Depletion of glucose and fructose during cucumber fermentation in CaCl<sub>2</sub> or NaCl brines with varying initial brine acidification treatments.

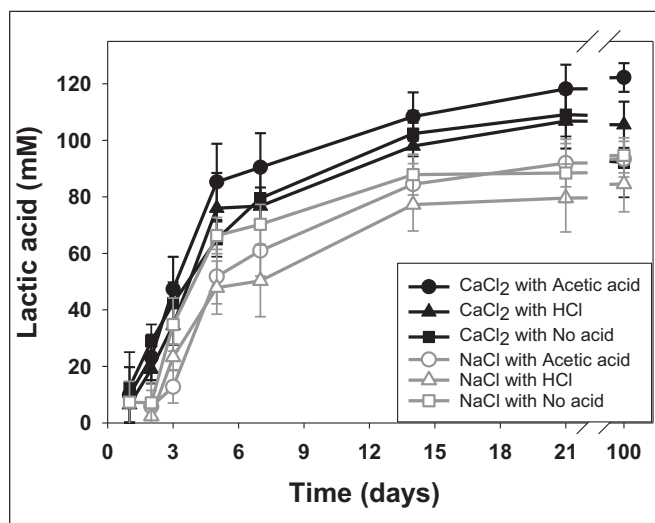


Figure 2—Accumulation of lactic acid in cucumber fermented in CaCl<sub>2</sub> or NaCl brines with varying initial brine acidification treatments.

Stone, & Fung, 1986). During the initiation of fermentation, the environments inside the cucumber and in the brine are quite different for microbial survival and growth (McDonald et al., 1991). Immediately after brining, cucumbers have ample supplies of substrates for microorganisms with minimal inhibition as the brine components have not yet equilibrated, making the cucumber a nutritious environment for the growth of the microorganisms present. The brine is a very different environment containing 2.29 M (13.3%) NaCl or 0.22 M (2.4%) CaCl<sub>2</sub> with 8.9 mM potas-

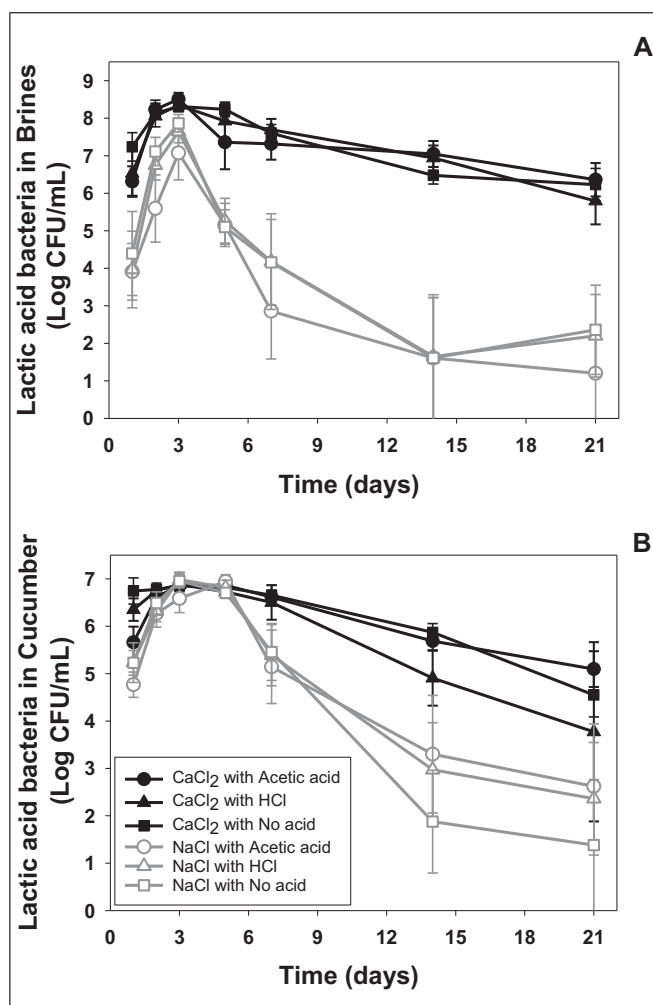
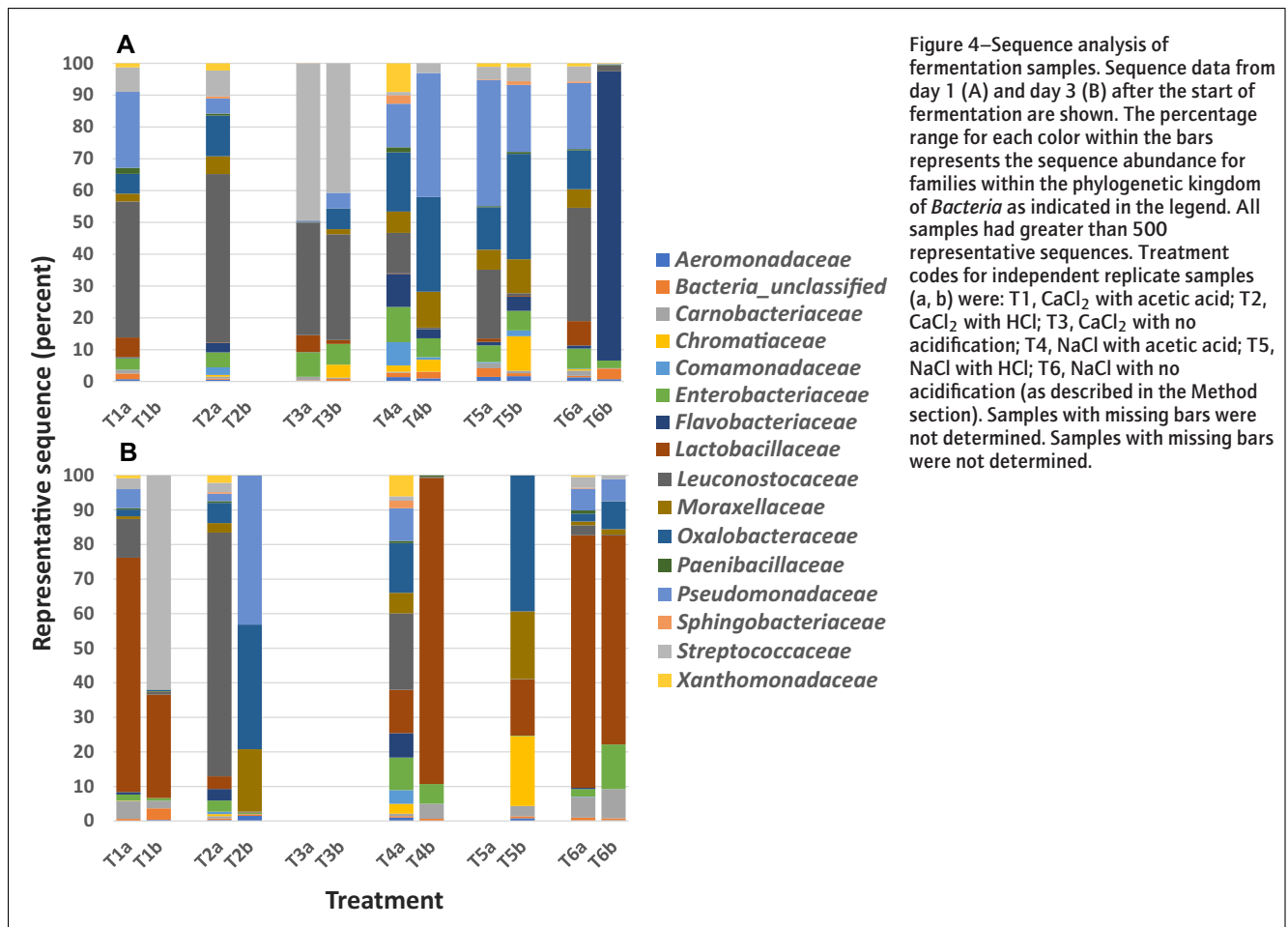


Figure 3—Lactic acid bacteria enumerated in the brine (Panel A) and cucumber (Panel B) during cucumber fermentation in CaCl<sub>2</sub> and NaCl brines with varying initial brine acidification treatments.

sium sorbate and may have a low pH (3.6 to 3.7) depending on the addition of hydrochloric acid (HCl) or 0.055 M acetic acid, thus deterring growth of microorganisms. Acidification of the brines resulted in initial brine pH values of 3.6 to 3.7 and the nonacidified brines started out greater than pH 7, while the cucumber had an initial pH of approximately 6. The *L. plantarum* starter culture was inoculated into the brine at 10<sup>6</sup> CFU/mL, but the harsh brine environment in the treatments with NaCl as the brining salt reduced the LAB counts to approximately 10<sup>4</sup> CFU/mL within the first day (Figure 3A). Nonetheless, the LAB counts in the NaCl brines nearly matched the LAB counts in the CaCl<sub>2</sub> brines by day 3 of fermentation before dramatically decreasing by 2 log CFU/mL



by the end of the first week and by another 2 log CFU/mL by the end of the second week (Figure 3). The LAB counts in the cucumbers (Figure 3) showed a similar pattern but 1.5 to 2 Log CFU/mL lower than in the fermentation brines. LAB populations in the fermentations conducted in CaCl<sub>2</sub> survived longer and utilized more reducing sugars (Figure 3 and Table 1) accounting for the significantly greater production of lactic acid and lower pH values after 21 days of fermentation (Table 1). Significantly higher concentrations of acetic acid and ethanol were also produced in the fermentations in CaCl<sub>2</sub> brines.

Each CaCl<sub>2</sub> treatment contained acetic acid concentrations of about 4.5% to 5.5% of the concentration of lactic acid. The data may be explained in part by the generally higher relative abundance of *Leuconostocaceae* sp in some CaCl<sub>2</sub> treatments during the initiation of fermentation (Figure 4A, gray bars, T1 and T2). These differences in early fermentation microbiota were not observed by plating on MRS, which does not distinguish between heterolactic and homolactic populations of LAB. In general, the relative abundance of the *Lactobacillaceae* population increased between day 1 and day 3, with the exception of the fermentations acidified with HCl, which had reduced representation of *Lactobacillaceae* at 3 days compared to other day 3 samples (T2 a and b, Figure 4B). It is possible that less protonated organic acid as a selective agent for *Lactobacillaceae* combined with the low pH in the HCl treatment influenced the growth and population distributions of the microbiota. *Lactobacillaceae* are better able to grow and survive in the presence of organic acids in fermentation brines compared

to other microorganisms (including *Leuconostocaceae*) due to their ability to lower internal cell pH and reduce intracellular organic acid accumulation (McDonald, Fleming, & Hassan, 1990; Russell, 1992). The sequencing data also showed differences in microbial populations between replicates (designated a and b) for day 1 and day 3 samples (that is, comparing T4 and T6 samples in Figure 4A and 4B). Future studies to determine the influence of initial fermentation conditions on the developing microbiota may require additional replication to take this variation into account.

Ethanol concentrations were 15% to 18.5% of the concentration of lactic acid in the treatments without acetic acid and were reduced by half when acetic acid was added to fermentation brines. Although present on fresh cucumbers, it is unlikely that the ethanol was produced by fermentative yeasts. Potassium sorbate was added to all treatments to inhibit the growth of yeasts and molds and seemed to sufficiently do so in each treatment tested. Initial yeast and mold counts from the cucumbers were approximately 10<sup>5</sup> CFU/mL, but were reduced below 10<sup>2</sup> CFU/mL soon after brining in all treatments except the nonacidified CaCl<sub>2</sub>-brined treatment. This treatment required an additional day to achieve the same reduction in yeast and mold count. The equilibration of sorbate into the cucumber tissue was somewhat slower in brining treatments that were not acidified, as evidenced by the lower cucumber sorbate content of 3.6 ± 1.2 mM after 1 day of fermentation in nonacidified brines compared to 5.2 ± 1.6 mM for cucumbers fermented in brines acidified with HCl or acetic acid ( $P < 0.05$ ). Nonetheless, sorbate concentrations had nearly

**Table 2—Residual sugars and fermentation metabolites in fermented cucumber after 100 days.<sup>a,b</sup>**

Salt	Acid	Fructose (mM)	Glucose (mM)	pH	Lactic acid (mM)	Acetic acid (mM)	Ethanol (mM)	Propionic acid (mM)
CaCl <sub>2</sub>	No Acid	3.6 ± 3.8 <sup>b</sup> (-0.2)	3.8 ± 0.4 <sup>a</sup> (2.0)	3.28 ± 0.34 <sup>a</sup> (0.14)	92.2 ± 30.1 <sup>b</sup> (-15.2)	15.8 ± 13.9 <sup>b</sup> (9.9)	25.6 ± 8.7 <sup>a</sup> (6.3)	10.7 ± 4.3 (10.7)
	Acetic	6.8 ± 3.5 <sup>a</sup> (2.2)	6.5 ± 2.1 <sup>a</sup> (4.3)	3.06 ± 0.05 <sup>a</sup> (-0.06)	122.2 ± 12.4 <sup>a</sup> (2.7)	31.6 ± 1.6 <sup>a</sup> (0.5)	12.4 ± 7.4 <sup>a</sup> (0.9)	ND
	HCl	11.2 ± 2.9 <sup>a</sup> (4.3)	8.8 ± 0.3 <sup>a</sup> (3.7)	3.05 ± 0.10 <sup>a</sup> (-0.06)	105.5 ± 20.0 <sup>b</sup> (1.2)	6.2 ± 2.2 <sup>c</sup> (0.8)	28.1 ± 2.8 <sup>a</sup> (8.8)	ND
NaCl	No acid	8.8 ± 12.1 <sup>a</sup> (0.0)	12.1 ± 6.9 <sup>a</sup> (-0.2)	3.22 ± 0.05 <sup>a</sup> (-0.04)	94.7 ± 15.2 <sup>b</sup> (8.1)	4.4 ± 1.1 <sup>c</sup> (0.5)	14.8 ± 7.0 <sup>a</sup> (1.9)	ND
	Acetic	8.5 ± 5.6 <sup>a</sup> (0.6)	14.7 ± 6.5 <sup>a</sup> (-0.2)	3.20 ± 0.03 <sup>a</sup> (-0.05)	93.4 ± 15.4 <sup>b</sup> (-0.3)	29.5 ± 1.0 <sup>a</sup> (-0.3)	ND (-6.7)	ND
	HCl	9.1 ± 5.4 <sup>a</sup> (-4.1)	12.5 ± 5.5 <sup>a</sup> (-2.6)	3.23 ± 0.09 <sup>a</sup> (-0.06)	84.6 ± 24.1 <sup>b</sup> (3.9)	3.8 ± 1.0 <sup>c</sup> (0.2)	12.2 ± 12.3 <sup>a</sup> (-1.7)	ND

<sup>a</sup>Means for treatments followed by different lowercase letters are significantly different at  $P < 0.05$ . ND = not detected.

<sup>b</sup>Values in parentheses represent the average change in metabolite content during storage (Day 100 mean - Day 21 mean).

reached equilibrium in all treatments by day 2, with concentrations of  $5.9 \pm 1.9$  and  $5.6 \pm 1.8$  mM in brines and cucumbers, respectively.

Despite the presence of residual sugars in the fermented cucumbers, most of the fermented cucumber treatments were microbiologically stable during 100 days of bulk storage as indicated by constant fermentation metabolite concentrations (Table 2). The notable exception was the fermentation treatment in CaCl<sub>2</sub> brines with no initial brine acidification, which showed evidence of secondary fermentation for several lots of cucumbers. Two of the six lots of cucumbers fermented with this process were clearly in secondary fermentation by 100 days (Table S1) as evidenced by significant decreases in lactic acid concentration and the corresponding increases in acetic acid, propionic acid, and pH (Fleming, Humphries, Thompson, & McFeeters, 2002; Franco, Pérez Díaz, Johanningsmeier, & McFeeters, 2012; Johanningsmeier, Franco, Pérez Díaz, & McFeeters, 2012). Two other lots of cucumbers from this treatment were also showing evidence of the initial stage of secondary fermentation (depletion of residual sugars, small decrease in lactic acid content, and slight rise in pH). These data provide the first indication that initial brine acidification could delay the onset of anaerobic secondary fermentation in cucumbers fermented in brines where CaCl<sub>2</sub> is the only salt.

### Rate of cucumber acidification

The rate of pH change in the first 3 days of fermentation was influenced by both salting treatment and initial brine acidification (Figure 5). Fresh pickling cucumbers had an initial pH of  $6.03 \pm 0.27$ . The addition of acetic acid to fermentation brines produced a more rapid decline in pH, resulting in an average cucumber pH of  $4.49 \pm 0.07$  after 1 day of fermentation compared to  $5.55 \pm 0.31$  and  $5.18 \pm 0.23$  for those fermented in nonacidified and HCl acidified brines, respectively. While the pH of the fermenting cucumber was significantly influenced by initial brine acidification, the production of lactic acid was largely influenced by the salt used for brining with 25 mM more lactic acid produced on average in the CaCl<sub>2</sub>-brined cucumbers (Figure 2). The pH values were similar for all acidification treatments by the third day of fermentation (Figure 5), with pHs approximately 4.5 or 4 for the NaCl- or CaCl<sub>2</sub>-brined cucumbers, respectively. If the initial rate of acidification was influencing the enzymatic or nonenzymatic softening of cucumber tissue, we would expect to see some differences in tissue firmness during the first 21 days of fermentation. Since there were no differences in cucumber mesocarp

firmness among the treatments during fermentation (Figure 6), we can conclude that the initial rate of cucumber acidification did not play a direct role in the texture quality of fermented cucumber.

### Role of *Enterobacteriaceae* spp.

Of particular interest in this experiment was the effect of brining salt and acidification on the growth and survival of *Enterobacteriaceae* spp., naturally found on the fresh cucumbers. Counts on fresh pickling cucumber were  $4.8 \pm 0.2$  log CFU/mL and remained more than 1 log CFU/mL higher in the cucumbers than in the brine for the first 3 days (Figure 7). By day 5, when the pH had reached 3.2 to 3.7, the *Enterobacteriaceae* counts were below detection levels in all treatments for both cucumbers and brine. Fermentation brines containing acetic acid significantly reduced *Enterobacteriaceae* spp. counts in brines and cucumbers ( $P < 0.002$ ). In contrast, the addition of HCl at the same pH did not have an effect nor did the use of NaCl compared to CaCl<sub>2</sub> (Figure 7). Bautista-Gallego, Arroyo-Lopez, Duran-Quintana, and Garrido-Fernandez (2010) concluded that CaCl<sub>2</sub> helped reduce *Enterobacteriaceae* in model olive fermentations, but the concentrations of CaCl<sub>2</sub> were much higher than in our studies. In Pérez-Díaz et al. (2015), *Enterobacteriaceae* counts were higher in nonacidified CaCl<sub>2</sub> fermentation brines than the fermentations conducted in recycled NaCl brine. Since the initial brine pH differed as well as the salt content of the two brining systems, it was not previously possible to determine whether the difference in brining salt alone would encourage the persistence of the *Enterobacteriaceae* spp. Our data suggest that it was the organic acids in the recycled brines that were inhibitory to the *Enterobacteriaceae* rather than the differences in the salts between the fermentations in CaCl<sub>2</sub> and NaCl at the commercial scale. The specific inhibitory effects of organic acids have been attributed to acidification of the cell cytoplasm and accumulation of acid anion within cells (Russell, 1992). Treatments with acetic acid added to the brines also significantly reduced the ethanol concentrations (Table 1), suggesting some of the ethanol in the fermentations without acetic acid added may have been produced by uninhibited *Enterobacteriaceae* (Ettchells, Fabian, & Jones, 1945). Although certain *Enterobacteriaceae* species are known to produce softening enzymes (Abbott & Boraston, 2008), cucumber tissue softening was not observed with the presence of higher counts of *Enterobacteriaceae* spp. in this study. The addition of acetic acid to fermentation brines was the only factor found to significantly decrease the *Enterobacteriaceae* populations during the initiation of fermentation. Since the addition of acetic acid did not increase the

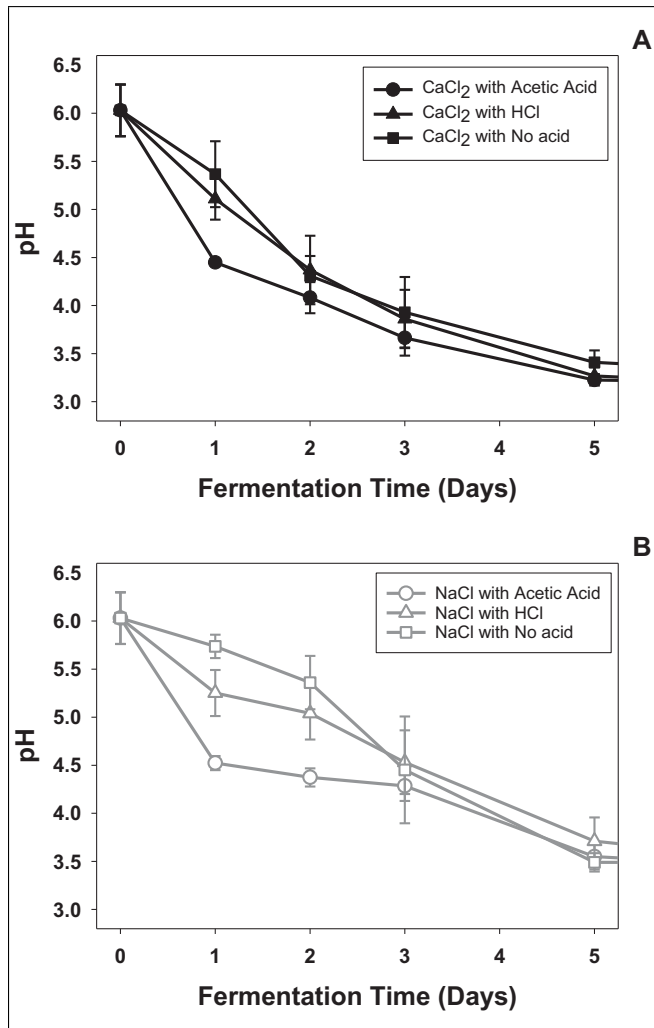


Figure 5—Cucumber pH change during the first 5 days of fermentation in CaCl<sub>2</sub> (Panel A) or NaCl (Panel B) brines with varying initial brine acidification treatments.

mesocarp firmness, it is unlikely that these *Enterobacteriaceae* spp. are causative agents in cucumber softening.

### Texture quality of fermented cucumber

Fermented cucumber mesocarp firmness was unaffected by the different brining treatments during the fermentation period. However, after 100 days at 28 °C to simulate a typical bulk storage period, there was a large difference in cucumber tissue firmness between the brining salt treatments. The average peak force of fresh pickling cucumber mesocarp was  $9.5 \pm 0.7$  N, increased by 1.5 to 2 N after brining in all treatments, and remained at that level during fermentation for 21 days (Figure 6). However, after 100 days, the cucumbers fermented in NaCl brines had average peak force values significantly lower than the fresh cucumbers, while those fermented and stored in CaCl<sub>2</sub> brines maintained their firmness. The cucumbers brined in NaCl had an average firmness of 7.5 N, while the CaCl<sub>2</sub>-brined counterparts were significantly higher at approximately 10.4 N ( $P < 0.0001$ , Figure 6). Firmness values derived from the mesocarp puncture test were previously shown to be highly correlated with important consumer sensory attributes of crunchiness and crispness (Buescher,

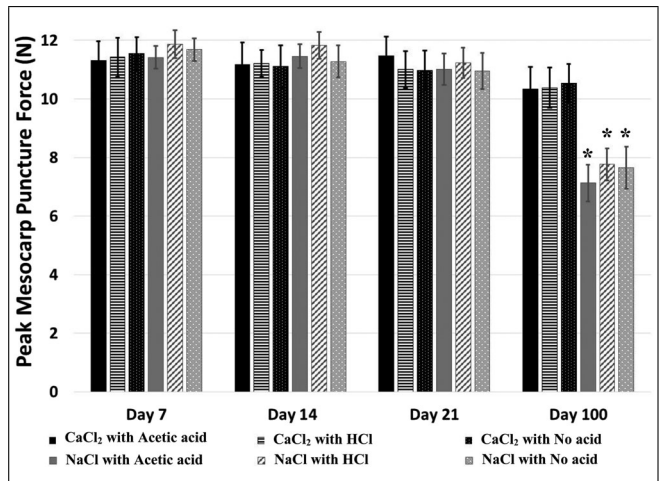


Figure 6—Cucumber mesocarp firmness (N) during fermentation for 21 days and after a typical bulk storage time of 100 days. Bar heights are the mean values for six independent lots of size 2B pickling cucumbers fermented in brines that varied in salt and acid content. Error bars represent the standard error of the mean. Asterisks above bars designate mean firmness values that were significantly different than those of fermented cucumbers on day 7 ( $\alpha = 0.05$ ).

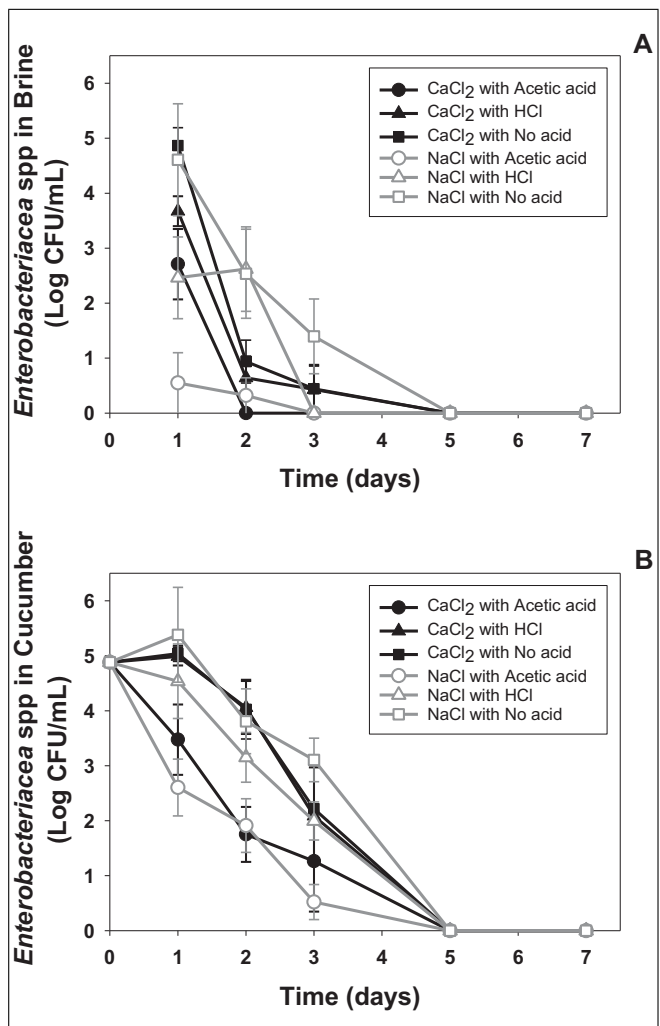


Figure 7—*Enterobacteriaceae* spp. enumerated in the brine (Panel A) and cucumber (Panel B) during the first week of fermentation in CaCl<sub>2</sub> and NaCl brines with varying initial brine acidification treatments.

Hamilton, Thorne, & Mi, 2011; Perez-Diaz et al., 2015; Yoshioka et al., 2009; Yoshioka, Horie, Sugiyama, Sakata, & Tamaki, 2010) as well as consumer liking of texture (Wilson et al., 2015). Comparison of the instrumental firmness measurements in this study to what is known indicates that the cucumbers fermented in  $\text{CaCl}_2$  brines would have a high degree of crunchiness as well as high consumer liking of texture. Initial brine acidification using acetic acid or HCl had no effect on texture quality of fermented cucumbers for either brining salt ( $P = 0.8235$ ), despite the differences observed in the initial rate of cucumber acidification during fermentation (Figure 5). Furthermore, the cucumbers fermented in  $\text{CaCl}_2$  brines were significantly firmer than cucumbers fermented in NaCl brines, which is in contrast to the softening that was observed in commercial trials in  $\text{CaCl}_2$  brines (McMurtrie & Johanningsmeier, 2018; Pérez-Díaz et al., 2015; Wilson et al., 2015). Based on our findings, we conclude that the exclusion of acetic acid from fermentation brines during commercialization was not directly responsible for the observed softening. Commercial fermentation of cucumbers involves filling large (10,000 to 40,000 L) fermentation tanks with cucumbers and then adding a concentrated cover brine prepared to equilibrate to the desired concentrations of NaCl,  $\text{CaCl}_2$ , acetic acid, and/or potassium sorbate. There are several variables that were controlled in this sealed jar laboratory experiment that are uncontrolled in open-top tanks used in commercial production. These production variables include oxygen that is introduced through air-purging operations and solubilization at the surface of open-top tanks, rainwater that freely falls into open tanks, introduction of new microbes from the air, and evaporation of water or volatile organic compounds from the brine causing varying concentrations of brine components. The commercial use of open-top fermentation tanks could have resulted in greater activity of aerobic pectinolytic organisms such as yeasts and molds (Costilow et al., 1980; Etchells et al., 1958) or softening due to oxidative mechanisms (Duan & Kasper, 2011; Fry, 1998; Miller, 1986). We also controlled other variables that are less precise in commercial fermentation, including exact pack out ratios of 55:45 cucumbers: brine (w/w) resulting in uniform concentrations of brine components, constant incubation temperature of 28 °C, and the use of a starter culture instead of a natural fermentation in the NaCl-brined treatments. Since minimal firmness changes were observed in the treatments with  $\text{CaCl}_2$  brines in this study, we conclude that the reduced firmness documented in commercial trials for  $\text{CaCl}_2$ -brined cucumbers (McMurtrie & Johanningsmeier, 2018) was due to an interaction between the  $\text{CaCl}_2$  brining process and a production variable that was controlled for in these experiments. On the other hand, some softening did occur in the NaCl-brined, fermented cucumbers in this study. Commercial fermentations commonly include approximately 0.03 M  $\text{CaCl}_2$  as a firming agent (McFeeters, Balbuena, & Fleming, 1995). Calcium was not included in the NaCl brining treatments in this experiment to be able to examine the differences between the brining salts individually. The lack of  $\text{CaCl}_2$  as a firming agent in these NaCl brines explains the observed softening during extended storage (Buescher & Burgin, 1988; Fleming, McFeeters, & Thompson, 1987; Guillou & Floros, 1993; Guillou, Floros, & Cousin, 1992; Tang & McFeeters, 1983; Thompson, Monroe, & Fleming, 1979). Taken together, we conclude that an interaction between the  $\text{CaCl}_2$  brining process and air exposure or environmental contamination led to the previously observed degradation of texture quality. Future studies are needed to examine the potential interaction between initial brine acidification and air exposure with regard to product quality.

## Conclusion

In this study, we demonstrated that initial brine acidification with acetic acid facilitated a more rapid decline in the *Enterobacteriaceae* population in both NaCl- and  $\text{CaCl}_2$ -brined cucumber fermentations. Acidification of brines with acetic acid reduced the cucumber pH to below 4.6 within 24 hr, favored conversion of sugars to lactic acid by LAB, and improved the microbial stability of cucumbers fermented in  $\text{CaCl}_2$  brines. Despite the clear differences in early fermentation microbiota and rate of acidification, brine acidification did not affect cucumber mesocarp firmness during fermentation or after 100 days storage. Therefore, we conclude that the native cucumber *Enterobacteriaceae spp.* microbiota do not significantly impact the texture quality of fermented cucumber. Interestingly, cucumbers fermented and stored in  $\text{CaCl}_2$  brines with or without initial brine acidification were found to be significantly firmer than NaCl-brined cucumbers in this study, further demonstrating that high-quality fermented cucumbers can be produced using the  $\text{CaCl}_2$  brining process if controlled conditions are employed.

## Acknowledgments

This study was funded by Mount Olive Pickle Co., Mt. Olive, NC (Project number 58-6645-4-0002) and the USDA-ARS, Southeast Area, Food Science Research Unit, Raleigh, NC (ARS project number 6070-41000-008-00D) with partial support from Pickle Packers Int. (Washington, DC, USA). This research used high-performance computing resources provided by the SCINet project of the USDA Agricultural Research Service, ARS project number 0500-00093-001-00-D. Authors gratefully acknowledge Mrs. Sandra Parker for excellent administrative assistance and editorial review of the manuscript.

## Author Contributions Statements

Erin McMurtrie designed and performed all experiments, analyzed data, and drafted the manuscript. Suzanne D. Johanningsmeier contributed to the conception and design of the study, advised on fermentation, analytical measurements, and data analysis, and revised the manuscript for publication. Fred Breidt Jr. performed the culture-independent fermentation microbiota analysis and contributed to editing the manuscript. Robert E. Price advised on microbiological analysis and performed DNA extractions. All authors participated in reviewing the intellectual content of the manuscript and approved the final version.

## Conflict of Interest Statement

Authors declare no conflict of interests.

## References

- Abbott, D. W., & Boraston, A. B. (2008). Structural biology of pectin degradation by *Enterobacteriaceae*. *Microbiology and Molecular Biology Reviews*, 72(2), 301–316.
- Bautista-Gallego, J., Arroyo-Lopez, F. N., Duran-Quintana, M. C., & Garrido-Fernandez, A. (2010). Fermentation profiles of Manzanilla-Aloreña cracked green table olives in different chloride salt mixtures. *Food Microbiology*, 27, 403–412.
- Bell, T. A., Etchells, J. L., & Jones, I. D. (1950). Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. *Food Technology*, 4(4), 157–163.
- Buescher, R. W., & Burgin, C. (1988). Effect of calcium chloride and alum on fermentation, desalting, and firmness retention of cucumber pickles. *Journal of Food Science*, 53(1), 296–297.
- Buescher, R. W., Hamilton, C., Thorne, J., & Mi, J. C. (2011). Elevated calcium chloride in cucumber fermentation brine prolongs pickle product crispness. *Journal of Food Quality*, 34(2), 93–99.
- Cock, P. J. A., Fields, C. J., Goto, N., Heuer, M. L., & Rice, P. M. (2009). The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. *Nucleic Acids Research*, 38, 1767–1771.
- Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., . . . Tiedje, J. M. (2014). Ribosomal database project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Research*, 42, D633–D642. <https://doi.org/10.1093/nar/gkt1244>.
- Collier, V. (1936). Determination of chlorides in biological materials. *Journal of Biological Chemistry*, 115(1), 239–245.



- Collmer, A., & Keen, N. T. (1986). The role of pectic enzymes in plant pathogenesis. *Annual Reviews in Phytobiology*, 24, 383–409.
- Costilow, R. N., Gates, K., & Lacy, M. L. (1980). Molds in brined cucumbers: Cause of softening during air-purging of fermentations. *Applied and Environmental Microbiology*, 40(2), 417–422.
- Dharmadhikari, M. (1992). Sorbic acid. *Vineyard & Vintage View*, 7(6), 1–5.
- Duan, J., & Kasper, D. L. (2011). Oxidative depolymerization of polysaccharides by reactive oxygen/nitrogen species. *Glycobiology*, 21(4), 401–409.
- Etchells, J. L., Bell, T. A., Monroe, R. J., Masley, P. M., & Demain, A. L. (1958). Populations and softening enzyme activity of filamentous fungi on flowers, ovaries, and fruit of pickling cucumbers. *Applied Microbiology*, 6(6), 427–440.
- Etchells, J. L., Fabian, F. W., & Jones, I. D. (1945). The Aerobacter fermentation of cucumbers during salting. *Michigan Agricultural Experiment Station Technical Bulletin No. 200*, 1–56.
- Etchells, J. L., Fleming, H. P., Hontz, L. H., Bell, T. A., & Monroe, R. J. (1975). Factors influencing bloater formation in brined cucumbers during controlled fermentation. *Journal of Food Science*, 40(3), 569–575.
- Fajans, K., & Hassel, O. (1923). A new method for titration of silver and halogen ions with organic dyestuff indicators. *Zeitschrift für Elektrochemie und Angewandte Physikalische Chemie*, 29, 495–500.
- Fleming, H. P., Humphries, E. G., Thompson, R. L., & McFeeters, R. F. (2002). Acidification of commercially fermented cucumbers in bulk tanks to increase microbial stability. *Pickle Pak Science*, 8(1), 38–43.
- Fleming, H. P., McFeeters, R. F., & Breidt, F. (2001). Fermented and acidified vegetables. In F. P. Downes & K. Ito (Eds.), *Compendium of methods for the microbiological examination of foods* (4th ed., pp. 521–532). Washington, DC: American Public Health Association.
- Fleming, H. P., McFeeters, R. F., & Thompson, R. L. (1987). Effects of sodium chloride concentration on firmness retention of cucumbers fermented and stored with calcium chloride. *Journal of Food Science*, 52(3), 653–657.
- Franco, W., Johanningsmeier, S. D., Lu, J., Demo, J., Wilson, E., & Moeller, L. (2016). Cucumber fermentation. In S. Paramithiotis (Ed.), *Lactic acid fermentation of fruits and vegetables* (Chapter 7, pp. 107–155). Boca Raton, FL: CRC Press.
- Franco, W., & Pérez-Díaz, I. M. (2012). Role of selected oxidative yeasts and bacteria in cucumber secondary fermentation associated with spoilage of the fermented fruit. *Food Microbiology*, 32(2), 338–344.
- Franco, W., Pérez-Díaz, I. M., Johanningsmeier, S. D., & McFeeters, R. F. (2012). Characteristics of spoilage-associated secondary cucumber fermentation. *Applied and Environmental Microbiology*, 78(4), 1273–1284.
- Fry, S. C. (1998). Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. *Biochemistry Journal*, 332, 507–515.
- Guillou, A. A., & Floros, J. D. (1993). Multiresponse optimization minimizes salt in natural cucumber fermentation and storage. *Journal of Food Science*, 58(6), 1381–1389.
- Guillou, A. A., Floros, J. D., & Cousin, M. A. (1992). Calcium chloride and potassium sorbate reduce sodium chloride used during natural cucumber fermentation and storage. *Journal of Food Science*, 57(6), 1364–1368.
- Johanningsmeier, S. D., Franco, W., Pérez Díaz, I. M., & McFeeters, R. F. (2012). Influence of sodium chloride, pH, and lactic acid bacteria on anaerobic lactic acid utilization during fermented cucumber spoilage. *Journal of Food Science*, 77(7), M397–M404.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glockner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41(1), e1 (1–11). <https://doi.org/10.1093/nar/gks808>.
- Krall, S. M., & McFeeters, R. F. (1998). Pectin hydrolysis: Effect of temperature, degree of methylation, pH, and calcium on hydrolysis rates. *Journal of Agricultural and Food Chemistry*, 46(4), 1311–1315.
- McDonald, L. C., Fleming, H. P., & Daeschel, M. A. (1991). Acidification effects on microbial populations during initiation of cucumber fermentation. *Journal of Food Science*, 56(5), 1353–1356, 1359.
- McDonald, L. C., Fleming, H. P., & Hassan, H. M. (1990). Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, 56(7), 2120–2124.
- McFeeters, R. F., Balbuena, M. B., & Fleming, H. P. (1995). Softening rates of fermented cucumber tissue: Effects of pH, calcium, and temperature. *Journal of Food Science*, 60(4), 786–788.
- McFeeters, R. F., & Fleming, H. P. (1991). pH effect on calcium inhibition of softening of cucumber mesocarp tissue. *Journal of Food Science*, 56(3), 730–732, 735.
- McFeeters, R. F., & Pérez-Díaz, I. (2010). Fermentation of cucumbers brined with calcium chloride instead of sodium chloride. *Journal of Food Science*, 75, C291–C296.
- McMurrrie, E. K., & Johanningsmeier, S. D. (2018). Quality of cucumbers commercially fermented in calcium chloride brine without sodium salts. *Journal of Food Quality*, 2018. <https://doi.org/10.1155/2018/8051435>.
- Miller, A. R. (1986). Oxidation of cell wall polysaccharides by hydrogen peroxide: A potential mechanism for cell wall breakdown in plants. *Biochemical and Biophysical Research Communications*, 141(1), 238–244.
- Naewbanij, J. O., Stone, M. B., & Fung, D. Y. C. (1986). Growth of *Lactobacillus plantarum* in cucumber extract containing various chloride salts. *Journal of Food Science*, 51, 1257–1259.
- Official Methods of Analysis of AOAC International Method 968.31 2000. 17th Edition. AOAC International Gaithersburg, MD, USA.
- Palnitkar, M. P., & McFeeters, R. F. (1975). Recycling spent brines in cucumber fermentations. *Journal of Food Science*, 40(6), 1311–1315.
- Pérez-Díaz, I. M., McFeeters, R. F., Moeller, L., Johanningsmeier, S. D., Hayes, J., Fornea, D. S., . . . Bass, D. (2015). Commercial scale cucumber fermentations brined with calcium chloride instead of sodium chloride. *Journal of Food Science*, 80(12), M2827–M2836.
- Potts, E. A., & Fleming, H. P. (1982). Prevention of mold-induced softening in air-purged, brined cucumbers by acidification. *Journal of Food Science*, 47(5), 1723–1727.
- Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahe, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, 4, e2584 (1–22). <https://doi.org/10.7717/peerj.2584>
- Russell, J. B. (1992). Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. *Journal of Applied Bacteriology*, 73, 363–370.
- Sauer, F. (1977). Control of yeast and molds with preservatives. *Food Technology*, 31, 66.
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., . . . Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), 7537–7541.
- Tang, H. C. L., & McFeeters, R. F. (1983). Relationships among cell wall constituents, calcium and texture during cucumber fermentation and storage. *Journal of Food Science*, 48(1), 66–70.
- Thompson, R. L., Fleming, H. P., Hamann, D. D., & Monroe, R. J. (1982). Method for determination of firmness in cucumber slices. *Journal of Texture Studies*, 13(3), 311–324.
- Thompson, R. L., Monroe, R. J., & Fleming, H. P. (1979). Effects of storage conditions on firmness of brined cucumbers. *Journal of Food Science*, 44(3), 843–846.
- United States Environmental Protection Agency. (2009). National Primary Drinking Water Regulations. EPA 816-F-09-004.
- Walter, R. H. (1991). *The chemistry and technology of pectin*. San Diego, CA: Academic Press, Inc.
- Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73(16), 5261–5267.
- Wilson, E. M., Johanningsmeier, S. D., & Osborne, J. A. (2015). Consumer acceptability of cucumber pickles produced by fermentation in calcium chloride brine for reduced environmental impact. *Journal of Food Science*, 80(6), S1360–S1367.
- Yoshioka, Y., Horie, H., Sugiyama, M., & Sakata, Y. (2009). Quantifying cucumber fruit crispness by mechanical measurement. *Journal of Breeding Science*, 59(2), 139–147.
- Yoshioka, Y., Horie, H., Sugiyama, M., Sakata, Y., & Tamaki, Y. (2010). Search for quantitative indicators of fruit texture for breeding in cucumber. *Acta Horticulture*, 871, 171–178.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Residual sugars and fermentation metabolites in fermented cucumber after 100 days for individual lots of cucumbers fermented in calcium chloride brines with or without acetic acid.<sup>1,2</sup>