Commercial Scale Cucumber Fermentations Brined with Calcium Chloride Instead of Sodium Chloride

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Abstract: Development of low salt cucumber fermentation processes present opportunities to reduce the amount of sodium chloride (NaCl) that reaches fresh water streams from industrial activities. The objective of this research was to translate cucumber fermentation brined with calcium chloride ($CaCl_2$) instead of NaCl to commercial scale production. Although $CaCl_2$ brined cucumber fermentations were stable in laboratory experiments, commercial scale trials using 6440 L open-top tanks rapidly underwent secondary cucumber fermentation. It was understood that a limited air purging routine, use of a starter culture and addition of preservatives to the cover brine aids in achieving the desired complete cucumber fermentation. The modified process was used for subsequent commercial trials using 12490 and 28400 L open-top tanks packed with variable size cucumbers and from multiple lots, and cover brines containing $CaCl_2$ and potassium sorbate to equilibrated concentrations of 100 and 6 mM, respectively. *Lactobacillus plantarum* LA0045 was inoculated to 10^6 CFU/mL, and air purging was applied for two 2–3 h periods per day for the first 10 d of fermentation and one 2–3 h period per day between days 11 and 14. All fermentations were completed, as evidenced by the full conversion of sugars to lactic acid, decrease in pH to 3.0, and presented microbiological stability for a minimum of 21 d. This $CaCl_2$ process may be used to produce fermented cucumbers intended to be stored short term in a manner that reduces pollution and waste removal costs.

Keywords: sustainable processing, cucumber fermentation, low salt, vegetable preservation, chloride waste reduction

Practical Application: Implementation of the fermentation processes described is estimated to reduce cost of production in the United States in the millions range from waste water treatments, while preventing the disposal of tons of NaCl into fresh water streams annually. Adoption of the technology is been evaluated by pickle processors worldwide.

Introduction

More than 30% of the pickled cucumber market in the U.S. is currently composed of fermented cucumbers. This sector is estimated to have an annual value of 340 million dollars in retail sales with tens of millions of jars of various sizes, and an even higher value in institutional sales. Fresh cucumbers are fermented in 6440 to 37860 L open top tanks using a cover brine solution that may contain 5% to 10% sodium chloride (NaCl) during the active fermentation period. The salt concentration may be increased up to 18% NaCl post-fermentation in northern regions where the winter temperatures are sufficient to freeze the fermented cucumbers in the tanks during long term storage. The production of fermented cucumber pickles allows for steady employment of permanent full-time employees within the pickling industry, which has a direct impact on the communities where such processing plants are located. Additionally, the availability of fermented cucumbers allows companies to appropriately manage the overflow of fresh fruits during the peak of the harvest season that would otherwise spoil before acidification; and for the manufacture of shelf stable products without pasteurization.

Commercially, fermented cucumbers in high salt brines are desalted once or twice by addition of fresh water to bring the NaCl levels down to edible concentrations (~2%) in the finished pickle products. The processing of cucumbers in U.S. tank yards is estimated to generate up to 45 million liters of effluent waste waters annually, containing a minimum of 3.4% NaCl. Additional processing activities to manufacture acidified and fermented cucumbers in the U.S. are estimated to generate 1.3 billion liters of treated waste waters with a minimum average discharge of 2800 ppm chlorides and 12000 tons of salty sludge containing a minimum of 15% solids that are deposited in landfills every year (personal communication with pickle processors).

There are constant environmental pressures to evaluate manufacturing procedures and make operational improvements, particularly in tank yard operations. Regulatory agencies are implementing stricter limits on the permitted levels of chlorides that may be discharged to the fresh water streams annually. Through the years, the pickling industry in the U.S. has been identifying opportunities to improve their processes. The amount of salt used for cucumber fermentations in the 1940s reached levels as high as 18%. The cover brine recycling practice was introduced in

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the pickling industry in the 1970s as an opportunity to reduce the levels of chlorides discharged to the environment, reclaim salt and reduce the amount of water needed to process cucumbers (McFeeters and others 1977). Currently, the targeted salt concentration for cucumber fermentation in the southern region is as low as 5.6%. Unfortunately, the practices implemented in the pickling industry to reduce the use of NaCl and water during processing have created new challenges. The constant reuse of cover brines may induce the development of a different and potentially more variable flavor profile in the fermented fruits and reduced microbial diversity. Long term storage of fermented cucumbers with lower NaCl allows for the development of secondary spoilage fermentation characterized by an increase in pH, disappearance of lactic and acetic acids and formation of propionic and butyric acids, that are associated with manure-like and cheesy aromas (Fleming and others 1989; Franco and Pérez-Díaz 2012, 2013; Franco and others 2012; Johanningsmeier and others 2012; Breidt and others 2013).

A new fermentation technology that could overcome the challenges of waste water containing NaCl is needed. The design of low NaCl cucumber fermentation by Guillou and others in 1992, was hampered by the development of a then uncharacterized spoilage, after primary fermentation was completed. In 1995, Fleming and others attempted the fermentation of cucumbers without NaCl using calcium acetate as a buffer, blanching to prevent microbially induced bloating, and a *Lactobacillus plantarum* starter culture. Pilot scale experiments showed that firmness of the fermented cucumbers was below that observed with the salty counterparts and that incidence of bloating was unacceptable. Such technology remained underdeveloped, given that blanching represents a significant change in the current pickling industry infrastructure. McFeeters and Pérez-Díaz (2010) proposed a low salt fermentation using calcium chloride ($CaCl_2$) as the only salt to maintain firmness and a starter culture to induce a fast fermentation. Laboratory scale testing of cucumber fermentations brined with $CaCl_2$ indicated that a complete conversion of sugars primarily to lactic acid and a decrease in pH proceeds over a range of $CaCl_2$ concentrations and cucumber sizes (McFeeters and Pérez-Díaz 2010). In closed containers, cucumbers were microbiologically stable after the primary lactic acid fermentation was completed. Firmness retention of cucumbers fermented in 100 mM $CaCl_2$ alone was equivalent to that obtained in cucumbers fermented with 1.03M (6%) NaCl and 40 mM $CaCl_2$ (McFeeters and Pérez-Díaz 2010). It was proposed that added $CaCl_2$ could be reduced to edible concentrations by dilution with water in 24 to 48 h, similarly to what is done to remove excess NaCl in current commercial practices. This fermentation process with 100 mM $CaCl_2$ was estimated to reduce chlorides from tank yard wastes by 60% to 80% as compared to the traditional fermentations in 6.0% (1.03 M) NaCl cover brines.

The objective of this research is to determine the viability of and challenges associated with translating cucumber fermentation brined with $CaCl_2$ to commercial scale production. A major consideration during the experimental design was the availability of information that could aid in designing a strategy to prevent secondary fermentation or spoilage in open top tanks, such as the addition of selected preservative at concentrations known to preserve the fruits long-term (Pérez-Díaz and McFeeters 2008, 2010; Pérez-Díaz 2011). Knowing that the oxygen incorporated into cucumber fermentation tanks during the period of active fermentation could accelerate the initiation of spoilage (Franco and Pérez-Díaz 2012), special attention was given to air purging routines in use. Air purging is used in the pickling industry to remove carbon dioxide (CO_2) gas formed during the primary fermentation in the tanks, so cucumbers do not suffer bloating damage (Fleming and others 1975). Supplementation of fermentation tanks with a *Lactobacillus plantarum* starter culture was also considered as part of the technology to encourage the rapid proliferation of the desired homofermentative culture and, thus, the early inhibition of undesirable microorganisms (Pérez-Díaz and McFeeters 2011). The initially proposed process was modified during the course of the study to include the addition of potassium sorbate and a minimum air purging schedule to prevent the proliferation of indigenous yeasts during and after fermentation.

Materials and Methods

Commercial scale cucumber fermentations

Commercial cucumber fermentations were carried out in 6440 to 28400 L (1700 or 7500 gal) open-top, black polymeric plastic or white fiberglass tanks containing between 50 to 60% whole cucumbers or pre-cut pieces of the fruits, and 50% to 40% cover brine solutions. Table 1 describes the cover brine components and their equilibrated concentrations, tank size and number, fruit sizes used, and tank location for each cucumber fermentation trial. Acetic acid was added vinegar (20% acetic acid) solution. Six trials were run for this study. Cushion cover brine was added into the tanks, prior to the addition of the fruits of different sizes. Fruits tanked were sizes 3A (39 to 51 mm diameter), 2B (27 to 38 mm diameter) or pieces and nubs of variable size fruits that are used for relish. Fresh cucumbers came from multiple farmers primarily located in the United States. For the 5th trial (Table 1 and 2), multiple cucumber sizes were used. In this trial there were 3, 3, and 6 tanks brined with $CaCl_2$ that contained size 3A cucumbers, pieces and nubs, and 2B cucumbers, respectively. As for control NaCl tanks, 1 was packed with size 3A cucumbers, 1 with pieces and nubs, and 2 with size 2B cucumbers. Average trends are presented for the data collected from the samples of the 5th trial, given that no significant differences were observed among the fermentations with different cucumber sizes.

In-tank fruits were immediately covered with wooden boards to prevent them from floating after adding the remaining cover brine volume, so that equilibration between the fruits and cover brine solution components would proceed uniformly. The air purging routine applied to the tanks for each trial is described on Table 2. Rain water was not mixed-in by air purging circulation. Tanks were filled to the very top, so that rain water would run off due to the difference in density. Tanks were replenished with cover brine prepared at the equilibrated concentrations.

Tanks of cucumbers with calcium chloride cover brine were inoculated to 10^6 CFU/mL using a *L. plantarum* LA0045 inocula (USDA-ARS Food Science Research Unit culture collection, Raleigh, N.C., U.S.A.) at 10^9 CFU/mL per 3800 L or 1000 gal of brined cucumbers. Preparation of starter cultures was done as described by Pérez-Díaz and McFeeters (2011). Tanks packed with the traditional NaCl brine formulation were not inoculated.

Sample collection

On each sampling day, approximately 50 mL of cover brine samples were taken from an average of 3.5 ft below the cover brine surface via a perforated pipe placed next to the air purging system in the tank. Cover brine samples were collected after ~100 mL of the cover brines had moved through the sampling tubing. The

Table 1–Description of commercial cucumber fermentation trials. Concentrations listed represent equilibrated levels.

Trial/tank size	# of Tanks/brining process	$CaCl_2$ (mM)	NaCl (mM)	Acetic acid (mM)	Potassium sorbate (mM)	Sodium benzoate (mM)	Cucumbers size	Starter culture (Log CFU/mL)
1 / 6440 L[a]	2 / $CaCl_2$	100	0	20	0	0	2B	None or 6
	1 / NaCl	40	1030	25	0	0	2B	None
2 / 6440 L[a]	2 / $CaCl_2$	100	0	20	0-3	0-3	2B	6
	1 / NaCl	40	1030	25	0	0	2B	None
3 / 12490L	2 / $CaCl_2$	100	0	0	6	0	2B	6
	1 / NaCl	40	1030	8.3	0	0	2B	None
4 / 12490 L	2 / $CaCl_2$	100	0	0	6	0	2B	6
	1 / NaCl	40	1030	8.3	0	0	2B	None
5 / 12490 L	12 / $CaCl_2$	100	0	0	6	0	2B (6), 3A (3), Nubs & Pieces (3)	6
	4 / NaCl	40	1030	25	2	0	2B (2), 3A (1), Nubs and Pieces (1)	None
6 / 28400 L	3 / $CaCl_2$ (1 indoors)	100	0	0	6	0	3A	6
	1 / NaCl	40	1030	25	2	0	3A	None

[a]Tanks used were constructed with black polymeric plastic. All other tanks used were constructed with fiberglass.

Table 2–Description of the air purging routines applied in cucumber fermentation trials conducted for this study.

		Air purging routine		
Trial/tank size	# of tanks/brine type	Estimated rate	Time of application	Pattern of application
1 / 6440 L	2 / $CaCl_2$	57 L/min	1-14 d	Continuously
	1 / NaCl	57 L/min	1-21 d	Continuously
2 / 6440 L	2 / $CaCl_2$	26 L/min	1-10 d	20 h on and 4 h off
	1 / NaCl	57 L/min	1-21 d	Continuously
3 / 12490 L	2 / $CaCl_2$	7 L/min	1-10 d and 11–14 d	2-3 h on 2X /d and 2–3 h on 1X/d
	1 / NaCl	57 L/min	1-21 d	Continuously
4 / 12490 L	2 / $CaCl_2$	7 L/min	1-10 d and 11–14 d	2-3 h on 2X /d and 2–3 h on 1X/d
	1 / NaCl	57 L/min	1-21 d	Continuously
5 / 12490 L	8 / $CaCl_2$	7 L/min	1-7 d and 9, 11, 13 d	2-3 h on 2X /d and 2–3 h on 1X/d
	4 / NaCl	57 L/min	1-21 d	Continuously
6 / 28400 L	3 / $CaCl_2$ (1 indoors)	26 L/min	1-10 d and 11–14 d	4 h on 2X /d and 4 h on 1X/d
	1 / NaCl	57 L/min	1-21 d	Continuously

sampling apparatus consisted of a buffer siphon PVC pump (BSP-1000; CBS Scientific Inc., San Diego, Calif., U.S.A.) connected to a 1/2 inch diameter tygon tubing with a thin wall. The samples were placed in 50 mL sterile conical tubes and immediately transported to our laboratory for same day processing.

Detection of secondary fermentation

Detection of secondary fermentation or spoilage at the commercial scale was based mainly on a rise in pH above 3.3 after the completion of the primary fermentation. In addition to pH, the detection of manure-like and cheesy aromas and visible gas bubbles in the tanks also indicated tanks may be in the process of spoiling. Suspected secondary fermentation was confirmed by HPLC analysis of organic acids and alcohols performed as described below.

Detection of fermentation metabolites

Concentrations of organic acids, and sugars were measured by HPLC analysis using a 30-cm HPX-87H column (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) for the separation of components. The column temperature was held at 37 °C. Components were eluted with 0.03N sulfuric acid at a flow rate of 0.6 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 quantify malic, lactic, acetic, propionic, and butyric acids and potassium sorbate. A Waters model 410 refractive index detector (Waters Corp., Millipore Corp., Milford, Mass., U.S.A.) connected in series with the diode array detector was used to measure glucose, fructose, glycerol, ethanol, propanol, and 1,2-propanediol. External standardization of the detectors was done using 4 concentrations of each standard compound.

Microbiological analysis of fermentation samples

Microbial counts from cover brine samples were determined from serially diluted samples in 0.85% saline solution, and spiral plated using an Autoplate 400 (Spiral Biotech, Norwood, Mass., U.S.A.). Lactic acid bacteria (LAB) enumeration was done using Lactobacilli deMan Rogosa and Sharpe agar (MRS, Becton Dickinson and Co., Franklin Lakes, N.J., U.S.A.) supplemented with 1% cycloheximide (0.1% solution, OXOID, New England) to inhibit the growth of yeasts. MRS plates were incubated anaerobically using a Coy anaerobic chamber (Coy Laboratory Products, Inc., Grass Lakes, Mich., U.S.A.) at 30 °C for 2 to 5 d. Yeasts were enumerated using yeast and molds agar (YMA, Becton Dickinson and Co.) supplemented with 0.01% chloramphenicol (Sigma-Aldrich, St. Louis, Mo., U.S.A.) and 0.01% chlrotetracycline (Sigma-Aldrich) to inhibit bacterial growth. YMA plates were incubated aerobically at 30 °C for 48 h. Differential reinforced clostridial medium (DRCA, Becton Dickinson and Co.) was used for the detection of suspected clostridial species. DRCA plates were incubated anaerobically at 30 °C for 48 h. Black colonies on DRCA plates were tentatively identified as *Clostridium* species. Enterobacteriaceae were enumerated using Violet Red Bile Glucose agar (VRBG, Becton, Dickinson and Co.), and plates were incubated aerobically at 37 °C for 24 h. Purple and pink

colonies, after 24 h incubation on VRBG plates, were recorded as presumptive Enterobacteriaceae.

Finished product quality evaluation

Fermented cucumbers from the 6 tanks in Trials 3 and 4 were processed into hamburger dill chips as described by Wilson and others (2015). Briefly, the fermented cucumbers were desalted using submersion in fresh water (containing only alum as a firming agent) to remove excess sodium or calcium ions, sliced, packed into 16 oz jars to which a fresh cover liquid containing vinegar and flavorings was added, pasteurized, and stored in ambient temperature (25 ± 2 °C) and lighting. Pickle mesocarp firmness was measured using a TA-XT2 Texture Analyzer (Texture Technologies Corp, Scarsdale, N.Y./Stable Micro Systems, Godalming, Surrey, UK) equipped with a 3 mm diameter punch probe moving at a rate of 2.5 mm/s and a base plate with a 3.1 mm diameter hole (Thompson and others 1982; Yoshioka and others 2009). Firmness measurements were conducted at ambient temperature (25 ± 2 °C) for 15 slices from 3 jars from each of the 6 fermentation tanks after 2, 4, 6, 8, 10, 12, and 18 months of finished product shelf storage. Average peak puncture force (N) was recorded as the mechanical firmness value for each jar of hamburger dill chips evaluated. Descriptive sensory analysis was conducted after 18 mo shelf storage. Students and staff ($n = 10$) from North Carolina State Univ., (Raleigh, N.C.) Department of Food Bioprocessing and Nutrition Sciences as well as the USDA Food Science Research Unit, (Raleigh, N.C.) were selected as panelists based upon availability and ability to distinguish flavor and texture attributes in cucumber pickle products. Approval of the North Carolina State Univ. (Raleigh, N.C.) Institutional Review Board (IRB # 2734) was obtained for the use of human subjects. Panelists were trained (50+ h) to use a 15-point intensity scale to assess flavor and texture attributes in cucumber products using a modified Spectrum™ method (Meilgaard and others 2007). Seven texture attributes including hardness, crispness, crunchiness, fracturability, skin and seed awareness, and juiciness were evaluated in fermented cucumber products. Hardness, fracturability, and juiciness were defined as suggested by the Spectrum™ method. Crunchiness and crispness attributes were based upon reference samples suggested by Chauvin and others (2008) with slight modifications in reference materials. Crispness was defined as the multiple, higher-pitched sounds produced as the sample is crushed with the molar teeth. Crunchiness was defined as a single lower-toned noise produced with each chew (Chauvin and others 2008). These sound-related texture attributes were evaluated during the first 3 chews. Flavor attributes included the 4 basic tastes, astringency, metallic, vinegar, dill pickle flavor, and oxidized and musty/earthy off-notes. A single lot of commercial hamburger dill chips was stored at 2 °C for use throughout the study as a reference sample with established attribute intensities. Samples were presented in a randomized complete block design in 2 oz. plastic cups identified with random 3 digit numbers. All samples and the reference pickle were provided at ambient temperature along with ambient temperature distilled water, a 2 oz. sample of Muenster cheese, and salt-free saltine crackers to cleanse the palate between samples. Panelists were presented samples in a randomized order. Panelists were asked to take a 2-min rest period between each sample to avoid palate fatigue. Three replications of sensory analysis were performed on independent days. Sensory panel means for each replication were used for conducting analysis of variance and discrimination of treatment means for significant effects was conducted using Tukey's multiple means comparison. All statistical computations were done in JMP (version 10, SAS® software, SAS Institute, Cary, N.C., U.S.A.).

Results and Discussion

For the purpose of this study, low salt fermentation was defined as the complete absence of NaCl and addition of 100 mM $CaCl_2$ to maintain tissue firmness. Mild acidification with 8.3 to 20 mM acetic acid was applied in selected trials with the purpose of aiding in the release of CO_2 formed during fermentation and, thus, prevent the formation of hollow cavities in the cucumbers (Fleming and others 1975; Fleming 1979). Parallel research was undertaken to study spoilage after primary fermentation, with the purpose of designing strategies to improve the low salt process. Acetic acid was eliminated from the cover brine formulation in subsequent trials in an effort to minimize growth of undesired microbes that could utilize the organic acid (Franco and others 2012).

Half of the expected lactic acid concentration (55 mM) was detected in 1 out of 2 tanks packed with size 2B cucumbers in the first trial. Within a couple of weeks, the lactic acid disappeared and butyric acid formed (data not shown). The second tank was inoculated with *L. plantarum* LA0045 and although lactic acid formed to 75 mM, it was then reduced with a corresponding increase in pH. The decline in lactic acid in both tanks, increase in pH and formation of butyric acid were taken as indicators of the development of secondary cucumber fermentation (Franco and others in 2012). These observations were in agreement with those made by Gulliou and others (1992) and suggested that although $CaCl_2$ brined cucumber fermentations are stable in closed jars (McFeeters and Pérez-Díaz 2010), a direct translation of the process to commercial open top tanks was not possible.

Aside from confirming that secondary cucumber fermentation is an intrinsic problem of low salt cucumber fermentations, the first trial also suggested that acidification with vinegar may augment its development. Once changes in lactic acid were detected in the experimental tanks, it was decided to add vinegar to artificially decline the pH to 3.5 and prevent microbial instability. However, sometime between 1 and 3 mo of storage, the tanks spoiled again suggesting that acidification with vinegar does not prevent secondary cucumber fermentation. Franco and Pérez-Díaz (2012) reported that selected oxidative yeasts isolated from secondary cucumber fermentations are able to utilize acetic and lactic acids to derive energy in the absence of sugars in a cucumber juice model system. Additionally, strictly aerobic bacteria belonging to the *Acetobacter* genus have been isolated from tanks packed with the $CaCl_2$ cover brine formulation during the initiation of secondary cucumber fermentation (unpublished). These microorganisms are commonly used in the commercial production of vinegar and able to convert acetic and lactic acids to ethanol. Thus, it is now understood that the presence of acetic acid in the low salt fermentation tanks may provide an additional substrate for potential spoilage microbes to proliferate and induce an increment in pH.

Microbes associated with acetic acid utilization as part of secondary cucumber fermentations are aerobic (Franco and Pérez-Díaz 2012). Cucumber fermentations with the $CaCl_2$ cover brine formulation gave a normal lactic acid fermentation that was stable during storage when they were fermented in closed 46 oz glass jars in the laboratory (McFeeters and Pérez-Díaz 2010). This circumstantial evidence prompted the reduction in air purging during NaCl free cucumber fermentations, with the goal of preventing spoilage and achieve a complete lactic acid fermentation.

Table 3–Microbial counts, sugars and sorbic acid detected in cover brine samples collected from the 3rd trial of $CaCl_2$ cucumber fermentations. Data represent 1 control fermentation tank packed with the traditional NaCl cover brine formulation and 2 experimental tanks packed with the $CaCl_2$ experimental cover brine.

	Sugars (mM)				
	Glucose		Fructose		Sorbic acid (mM)
Fermentation time (d)	NaCl	$CaCl_2$	NaCl	$CaCl_2$	$CaCl_2$
1	2.3	2.4 ± 0.1	2.4	1.1 ± 1.6	14.5 ± 0.5
3	5.1	BDL	5.9	3.8 ± 0.1	11.3 ± 0.7
4	6.3	BDL	7.6	3.2 ± 0.1	10.2 ± 0.9
8	13.2	2.5 ± 0.1	16.1	2.5 ± 3.6	7.2 ± 0.9
10	11.1	3.9 ± 0.9	19.7	10.1 ± 0.8	7.1 ± 0.5
14	3.2	4.7 ± 0.9	7.1	11.3 ± 0.6	6.4 ± 0.4
24	3.1	3.1 ± 0.1	BDL	5.8 ± 0.4	4.5 ± 0.7

BDL, below detection level.

Table 4–Fermentation metabolites and pH measured in cover brine samples collected from the 3rd trial of $CaCl_2$ cucumber fermentations. Data represent 1 control fermentation tank packed with the traditional NaCl cover brine formulation and 2 experimental tanks packed with the $CaCl_2$ experimental cover brine.

	Formation of metabolic products (mM)							
	Lactic acid		Acetic acid		Ethanol		pH	
Fermentation Time (d)	NaCl	$CaCl_2$	NaCl	$CaCl_2$	NaCl	$CaCl_2$	NaCl	$CaCl_2$
1	BDL	BDL	21.1	BDL	BDL	BDL	3.9	6.4 ± 0.1
3	BDL	2.8 ± 0.2	15.5	2.7 ± 0.2	BDL	44.9 ± 2.3	4.3	5.6 ± 0.1
4	BDL	5.7 ± 1.2	14.7	2.3 ± 0.2	BDL	49.3 ± 3.4	4.4	5.1 ± 0.1
8	BDL	30.2 ± 5.4	12.9	5.4 ± 0.4	BDL	55.5 ± 2.7	4.6	3.9 ± 0.1
10	3.9	51.5 ± 6.1	13.8	7.8 ± 0.2	58.5	56.7 ± 2.7	4.5	3.1 ± 0.1
14	25.4	69.2 ± 4.8	18.3	9.4 ± 0.3	98.4	56.1 ± 3.1	3.9	3.4 ± 0.1
24	46.1	90.9 ± 2.8	24.4	12.3 ± 0.3	97.1	55.6 ± 2.4	3.6	3.3 ± 0.1

BDL, below detection level.

In the second trial, 2 commercial tanks of 6440 L were packed with 2B cucumbers and the experimental $CaCl_2$ cover brine formulation. Both tanks were inoculated with *L. plantarum* LA0045 to encourage a faster completion of the fermentation, as observed on the first trial. One tank was supplemented with 3 mM sodium benzoate and the second with 3 mM potassium sorbate with the goal of preventing growth of aerobic microbes capable of utilizing organic acids (Borg and others 1955; Etchells and others 1961; Pérez-Díaz and McFeeters 2010). These preservatives have been used in pickle processing for decades and are known to be ineffective against lactic acid bacteria at these low concentrations. The air purging routine was also modified (Table 2) to reduce proliferation of aerobic spoilage microbes, in particular yeasts. The lactic acid concentration in the presence of 3 mM potassium sorbate detected after 5 d of fermentation (69.9 ± 0.3 mM) was about 10 mM higher than that detected at the same time point in the tank containing 3 mM sodium benzoate (59.2 ± 0.6). However, after a week of fermentation the lactic acid had decreased to 58.7 and 53.1 in the tanks containing potassium sorbate and sodium benzoate, respectively, which was more than 19 mM lower than the concentration detected in the NaCl control tank (77.7 mM). These observations suggested that while the addition of preservatives traditionally used in pickle products delayed lactic acid disappearance, it was insufficient to stabilize the fermentations. Parallel studies conducted in the laboratory suggested that at a pH of 3.5 and 1.1% $CaCl_2$, it is necessary to add 12 mM sodium benzoate and higher concentrations of potassium sorbate to achieve cucumber preservation (Pérez-Díaz and McFeeters 2008; Pérez-Díaz and McFeeters 2010; Pérez-Díaz 2011). Thus, it was considered that the 3 mM of preservatives added in these tanks was insufficient to have a significant impact in stabilizing the tanks long term.

Additionally, in the second trial a significant amount of bloating was observed in the cucumbers on top of the tanks, presumably due to the application of a reduce air purging rate from 57 to 26 L/min. Thus, it was also considered that a reduction in yeast growth, as expected from the addition of the preservatives, would also result in less cucumber bloating.

Table 3 and 4 show results obtained from the third trial at the commercial scale in which 6 mM potassium sorbate was used to enhanced inhibition of yeasts and indirectly reduce the bloating defect. These tanks were packed during the month of November, which has average temperatures between 6 and 18 °C (43 to 65°F) at the trial location (Mount Olive, N.C., U.S.A.), thus, a delayed fermentation course was expected. While it was confirmed that a slow fermentation proceeded in the NaCl control fermentation tanks, numbers of LAB reached the maximum expected levels (8.3 ± 0.2 Log CFU/mL) after 4 d in the tanks containing the $CaCl_2$ experimental formulation. Viable cells of yeasts (3.3 ± 0.6 Log CFU/mL) and Enterobacteriaceae (4.4 ± 0.1 Log CFU/mL) were detected on day 4, confirming that 6 mM potassium sorbate was not sufficient to eradicate them. However, ethanol formed to higher concentrations in the NaCl control tanks, suggesting that potassium sorbate was controlling microbes that would normally be active in the natural and traditional high salt (NaCl) fermentations at temperatures between 6 and 18 °C. Residual sugars (Table 3) and maximum concentrations of lactic acid (Table 4) were detected in all tanks after 24 d of fermentation, presumably due to the expected decrease in ambient temperatures commonly registered in North Carolina at the beginning of December (35 to 55 °F or 2 to 13 °C). However, more lactic acid and a lower pH were detected in the experimental tanks (Table 4) that had been inoculated with *L. plantarum* LA0045. These observations

Table 5–Lactic acid formed (top panel) and cover brine pH (bottom panel) measured from commercial cucumber fermentations brined with $CaCl_2$ or NaCl at variable seasonal stages with particular range in temperatures.

Lactic acid formed (mM)								
	$CaCl_2$ cover brine				NaCl cover brine			
Number of tanks	2	2	2 (MN)	12	1	1	1 (MN)	4
	Ambient temperature range (°C) (Trial ID)							
Fermentation time (d)	6-18 (3)	12-27 (4)	18-25 (6)	21-31 (5)	6-18 (3)	12-27 (4)	18-25 (6)	21-31 (5)
1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
3	2.8 ± 0.2	34.6 ± 0.7	20.1 ± 5.7	37.8 ± 5.1	BDL	0.4	BDL	15.7 ± 8.7
7 to 8	30.2 ± 5.4	77.3 ± 1.9	77.8 ± 2.8	77.9 ± 3.4	BDL	36	32.9	32.0 ± 5.5
14	69.2 ± 4.8	94.3 ± 4.8	100.9 ± 1.3	82.9 ± 3.8	25.4	61.6	25.1	85.1 ± 5.8
	Cover Brine pH Measured							
1	6.4 ± 0.1	5.58 ± 0.1	5.9 ± 0.5	6.5 ± 0.4	3.9	4.1	4.15	4.1 ± 0.1
3	5.6 ± 0.1	3.53 ± 0.1	4.0 ± 0.5	3.6 ± 0.1	4.3	4.5	3.88	4.2 ± 0.2
7 to 8	3.9 ± 0.1	3.22 ± 0.1	3.6 ± 0.2	3.2 ± 0.1	4.6	3.4	3.55	3.3 ± 0.1
14	3.4 ± 0.1	3.04 ± 0.1	3.3 ± 0.5	3.2 ± 0.1	3.9	3.2	3.42	3.1 ± 0.1

BDL, below detection level.

Table 6–Sensory texture attributes of hamburger dill chips prepared from cucumbers brined and fermented in 1.03 M NaCl or 0.1 M $CaCl_2$ with 6 mM potassium sorbate and an *L. plantarum* starter culture.

			Sensory attribute[a,b]				
Trial (no. tanks)	Brining process	Tankyard storage (mo)	Firmness (N)	Hardness	Fracturability	Crispness	Crunchiness
4 (1)	1.03M NaCl	2	9.45 ± 0.2[a]	7.6 ± 0.2[a]	5.3 ± 0.2[a]	4.1 ± 0.5[a]	8.5 ± 0.4[a]
4 (2)	0.1 M $CaCl_2$	2	8.42 ± 0.5[a]	7.1 ± 0.4[b]	4.8 ± 0.3[b]	3.3 ± 0.2[b]	7.7 ± 0.3[b]
3 (1)	1.03M NaCl	8	8.33 ± 0.7[a]	7.3 ± 0.4[b]	4.8 ± 0.1[b]	3.5 ± 0.1[b]	7.7 ± 0.3[b]
3 (2)	0.1 M $CaCl_2$	8	6.95 ± 0.6[b]	6.1 ± 0.4[c]	4.2 ± 0.4[c]	2.4 ± 0.4[c]	6.2 ± 0.4[c]
N/A	Reference Pickle	N/A	8.89 ± 0.9	7.4 ± 0.4	5.2 ± 0.3	3.1 ± 0.1	7.8 ± 0.4

[a]Means in the same column not sharing a common super script are significantly different ($P < 0.05$). Standard deviations represent the variation between independent evaluation of individual jars of product.
[b]Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity.

suggested that the higher potassium sorbate concentration added to the $CaCl_2$ cover brine, removal of vinegar from the formulation and the minimized air purging had a positive impact in achieving a full fermentation prior to secondary fermentation, which was not detected after 24 d at the lower ambient temperature (2 to 13°C). Significant cucumber bloating was observed in the fermented stock brined with NaCl, but not in the tanks brined with $CaCl_2$.

Given that the third trial was conducted at the end of the 2010 season, it was not possible to observe if a complete fermentation would have proceeded, should the tanks be exposed to higher temperatures. The fourth and fifth trials at the commercial scale were conducted during the months of May and June of 2011 through October, respectively, with ambient temperatures fluctuating between 12 and 31 °C (Table 5). The sixth trial was run during the month of August of 2014 with temperatures fluctuating between 18 and 25 °C (Table 5). Trials 3 to 6 together provided an opportunity to observe fermentations proceeding at temperatures between 6 and 32 °C (43 to 90 °F). No significant differences were observed in lactic acid production and acidification of the brine, except when cucumber fermentations proceeded at temperatures between 6 and 18 °C in the trial initiated in November of 2010 (Table 5).

Fermented cucumbers from Trials 3 and 4 were processed into hamburger dill chips (HDC) in July 2011 to compare finished product quality of pickles produced from the 2 processes in a commercial environment. No significant differences in flavor were detected by the trained descriptive sensory panel after 18 mo shelf storage ($P > 0.05$, data not shown). In contrast, significant differences in the firmness of hamburger dill chips was related to both the brining process and independent trials. Averaged across the 2 trials, the processed products that were fermented with the current commercial process in 1.03 M NaCl brine had a higher firmness value of 8.4 ± 0.1 N compared to 7.8 ± 0.1 N for those fermented with a starter culture in 0.1 M $CaCl_2$ ($P = 0.0005$). It was also observed that HDC produced from fermented cucumbers in Trial 4 that were stored for only 2 mo in the tankyard had a higher firmness than those from Trial 3 which were fermented late in the season and stored for 8 mo before being processed into finished products ($P = 0.0002$). These differences were apparent regardless of brining treatment and may have been due to the longer bulk storage time or differences in quality of the incoming raw materials between the 2 trials. No changes in HDC firmness (N) were observed for 18 mo of shelf storage ($P > 0.05$, *data not shown*), indicating that texture quality of this type of product is primarily impacted during fermentation and bulk storage. Descriptive sensory texture attributes of hardness, crispness, and crunchiness correlated well with mechanical firmness values of the processed product, $R^2 = 0.70$, 0.85, and 0.80, respectively. The $CaCl_2$ process resulted in reduced sensory perception of firmness, crispness and crunchiness intensities compared to the NaCl controls (Table 6). However, in Trial 4 with fermentations starting in May and a limited bulk storage time of 2 mo, the texture quality attribute values were well within the range of normal commercial products. Despite these perceptable differences in texture quality, no difference in consumer preference was detected for these hamburger dill chips produced from cucumbers fermented in $CaCl_2$ brines (Wilson and others 2015), suggesting that this novel brining process is commercially feasible and warrants further study.

Given that the third and fourth trial were conducted with a limited number of tanks, the data collected was not representative of

the diversity of cucumber loads projected to undergo such a process in any commercial tank yard. The fifth trial at the commercial scale consisted of twelve 12490 L tanks packed over the course of the 2011 season. This made it possible to observe differences among lots of fresh cucumbers exposed to common transportation conditions and conduct a more in-depth study of the process as a function of time. The microbial counts during primary fermentation in the $CaCl_2$ experimental tanks were in agreement with the expectations for fermentations initiated by a starter culture and no significant differences were observed among cucumber loads.

Initial LAB counts from the fresh cucumbers used in trial 5 were 5.4 ± 0.9 log CFU/mL, which is 1 log unit higher than expected. The corresponding freshly prepared $CaCl_2$ cover brine showed an average count on MRS of 3.0 ± 0.6 log CFU/mL; while the counts for the fresh NaCl brines were below the detection limit (2.4 log CFU/mL). The *L. plantarum* inocula had an average count of 8.2 ± 0.6 log of CFU/mL prior to addition in the tanks, which generated initial in-tank counts of 6.5 ± 0.2 log CFU/mL (Figure 1). Thus, the inoculated levels of *L. plantarum* were at least 1 log higher than the population of the indigenous LAB in the 5th trial. It was observed that those inocula added to the tanks prior to them reaching 9 log CFU/mL in the preparation media, initiated the fermentation faster (data not shown). LAB usually achieved their maximum numbers within 18 h in the inoculated $CaCl_2$ fermentations as compared to 4 d in the noninoculated NaCl fermentations (Figure 1).

Cucumber fermentations in the tanks with the $CaCl_2$ cover brine proceeded to completion in an average of 7 d as compared to 21 d in the NaCl fermentations in the 5th trial, as judged by the disappearance of detectable levels of the primary sugars, glucose and fructose (Figure 2). As shown in Figure 2 to 4, sugar utilization, acidification and lactic acid production proceeded more rapidly in the $CaCl_2$ fermentations as compared to the NaCl fermentations under commercial conditions. The use of a starter culture and the absence of NaCl, which reduces the rate of microbial growth, explain such differences in the speed of the 2 types of fermentations.

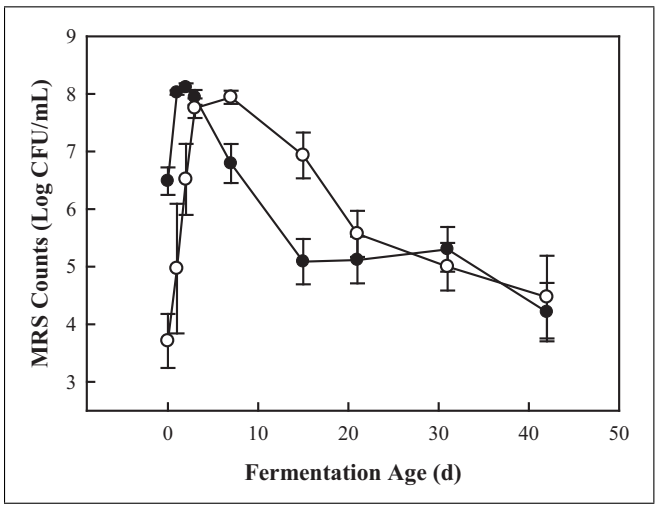

Figure 1–Counts of LAB from MRS detected in the 5th commercial scale trial. Data presented are the means and standard error of 12 experimental tanks with the $CaCl_2$ cover brine (●) and 4 control tanks with the NaCl (○) cover brine. No significant differences were observed among the different cucumber sizes packed.

Significant differences were noticed in the trends of yeasts growth for the 2 types of fermentation brines. Yeasts increased to detectable numbers after day 7 in the fermentation tanks with the $CaCl_2$ cover brine, when the community of LAB was already declining and only low levels of residual sugars remained. Thus, the maximum counts for the community of yeasts averaged at 3.5 ± 0.4 Log CFU/mL, which is 1 log unit below that observed for the fermentations with NaCl (Figure 3). The limited growth of yeasts in the fermentations with the $CaCl_2$ cover brine could have been a combination of the lack of sugars, the limited air purging routine applied and the presence of potassium sorbate that was added for this purpose (Figure 4). Maximum yeasts counts in the NaCl fermentations were detected by day 14 after a short lag phase and with a log phase proceeding earlier than that for the $CaCl_2$ fermentations (Figure 3). As intended, the levels of potassium sorbate added to the NaCl fermentations were 60% less than that present in the $CaCl_2$ fermentations, to represent the concentrations normally added to the standard NaCl fermentations (Figure 4), which apparently were insufficient to prevent yeast growth in the presence of 6% NaCl. Formation of ethanol proceeded concomitantly with yeasts growth in the NaCl fermentations (Figure 5 and 3), but not in the cucumber fermentations brined with $CaCl_2$.

Similarities between the 2 systems studied include a decrease in pH to 3.0 ± 0.3 to 3.3 ± 0.2 and a reduction in counts

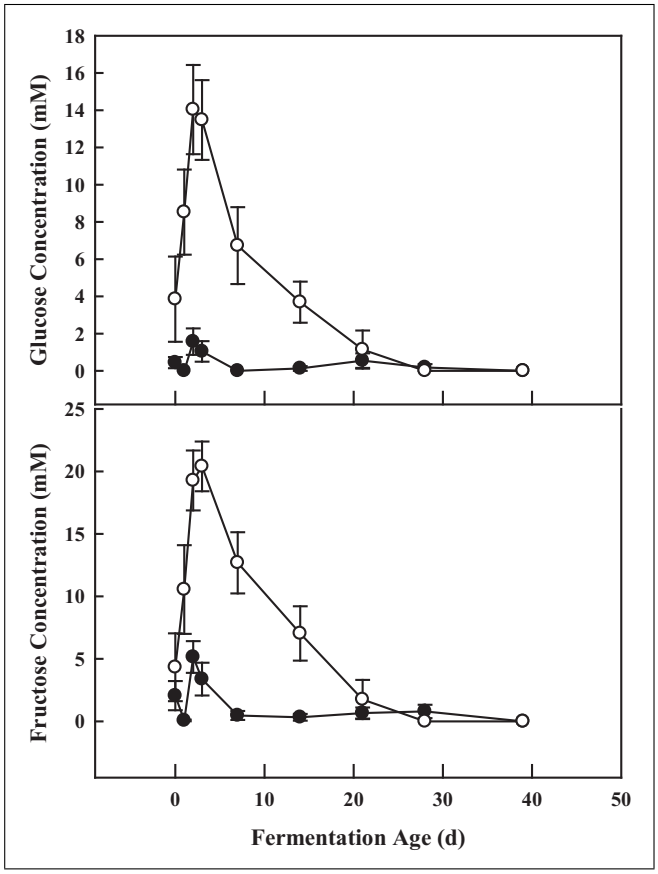

Figure 2–Sugars utilization in commercial scale $CaCl_2$ cucumber fermentations. The means and standard error of the sugar concentrations in fermentations packed with the $CaCl_2$ ($n = 12$) (●) and NaCl ($n = 4$) (○) cover brines, respectively, are shown.

of Enterobacteriaceae within the first week of the fermentation (Figure 6 and 7). A rapid reduction in pH has been reported as the primary event inducing the die off of pathogens of public health significance in fermented cucumbers (Breidt and Caldwell 2011). These observations suggest that the $CaCl_2$ fermentation provides the same or similar assurance of microbiological safety as fermentations with NaCl.

The analysis of the fermentations by high performance liquid chromatography showed that over 80 mM lactic acid was produced in both $CaCl_2$ and NaCl brines within the first week after brining. In addition, an average of 15 ± 4 mM acetic acid was produced along with 25.1 ± 5.5 mM ethanol in the $CaCl_2$ fermentations. Since *L. plantarum* LA0045 is a homofermentative lactic acid bacterium and yeast numbers were very low, this data suggests that other microbes naturally present in the fermentation, such as *Lactobacillus brevis* (Pérez-Díaz and others, submitted) and other LAB (Singh and Ramesh 2008), as well as *Acetobacter* spp. (unpublished) and Enterobacteriaceae (Etchells and others 1945) are metabolically active during the first week of fermentation. This finding suggests that a more in-depth study of the microbiology of the primary stage of commercial scale cucumber fermentations brined with $CaCl_2$ is needed.

In contrast to fermentation in NaCl brines, additional changes in metabolites occurred after the initial stage of the fermentation in tanks brined with $CaCl_2$. After 14 d of fermentation, the $CaCl_2$ brined cucumbers went through a period in which the lactic acid and ethanol produced began to decline (Figure 5), brine pH increased (Figure 6), and yeasts numbers began to rise (Figure 3) as sorbic acid disappeared (Figure 4). Colonies of LAB on MRS plates began to show multiple morphologies instead of a single

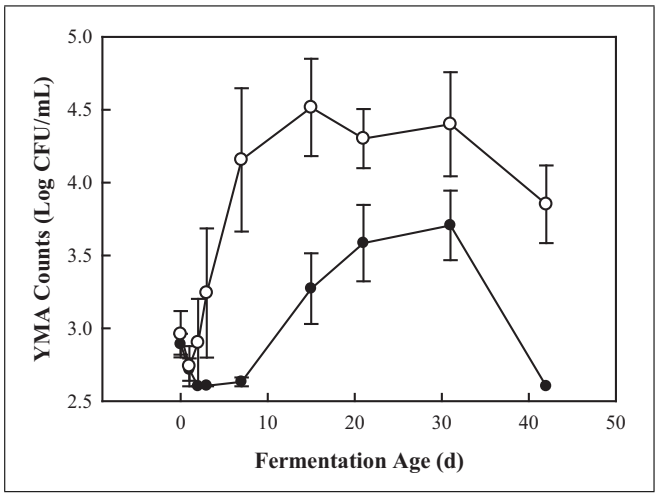

Figure 3–Yeasts counts as a function of time in the commercial scale $CaCl_2$ cucumber fermentations. YMA counts presented are the means with standard error of the 12 $CaCl_2$ (●) and 4 NaCl (○) cover brine fermentations. Detection limit for the spiral plating technique used is 2.6 Log CFU/mL.

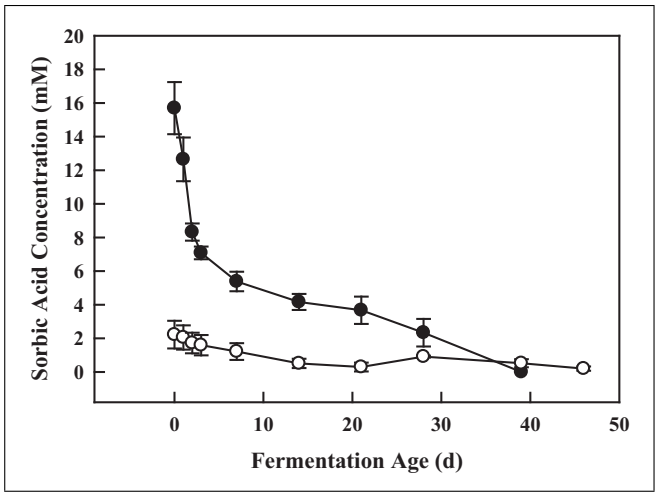

Figure 4–Equilibration and disappearance of sorbic acid from the cover brines of the $CaCl_2$ and NaCl cucumber fermentations. Trends presented are the means with standard error of the 12 $CaCl_2$ (●) and 4 NaCl (○) cover brine fermentations.

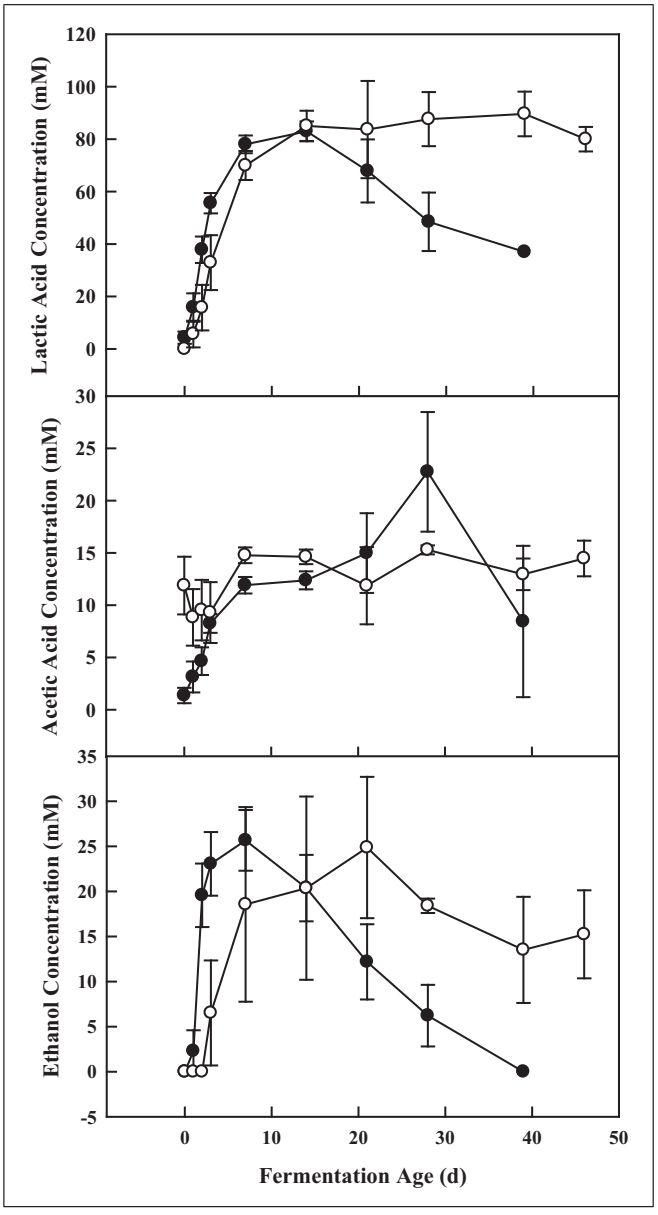

Figure 5–Trends of lactic and acetic acids and ethanol concentrations in the commercial scale $CaCl_2$ and NaCl cucumber fermentations. Values presented are the means with standard error of 12 and 4 fermentations packed with the $CaCl_2$ (●) and NaCl (○) cover brines, respectively.

Table 7–Chemistry and microbiological data corresponding to cover brines collected from the 6th trial of cucumber fermentations without sodium chloride. Data presented represents averages and standard deviations from the 3 independent cucumber fermentations brined with $CaCl_2$ and averages and standard deviations for technical replicates for samples collected from the fermentation brined with NaCl.

	Lactic acid (mM)		Acetic acid (mM)		pH		Counts on MRS (Log CFU/mL)		Total dissolved solids (mg/L)	
Fermentation age (d)	$CaCl_2$	NaCl	$CaCl_2$	NaCl	$CaCl_2$	NaCl	$CaCl_2$	NaCl	$CaCl_2$	NaCl
1	BDL	BDL	BDL	72.9	5.9 ± 0.5	4.15 ± 0.1	7.5 ± 1.0	3.2	23.4 ± 0.3	71.8
3	20.1 ± 5.7	BDL	3.3 ± 0.9	48.7	4.0 ± 0.5	3.88 ±0.1	8.1 ± 0.2	5.9	18.7 ± 0.6	74.9
7	77.8 ± 2.8	32.9	6.4 ± 0.3	38.3	3.6 ± 0.2	3.55 ± 0.2	7.8 ± 0.6	6.9	15.9 ± 0.4	73.5
14	100.9 ±1.3	25.1	9.9 ± 0.4	BDL	3.3 ± 0.5	3.42 ± 0.4	7.7 ±0.3	7.6	15.0 ± 0.3	71.7
19	106.7 ± 1.2	64.1	10.5 ± 0.5	12.5 ± 2.7	3.20 ± 0.4	3.5 ±0.2	NA	NA	NA	NA

BDL, below detection level; NA, not available.

morphology in the early stages of the fermentation (Franco and others 2012), which suggests that LAB other than *L. plantarum* LA0045 increased in numbers. These changes were accompanied by the formation of propionic acid, butyric acid and n-propanol (Figure 8). All of these observations are in agreement with the characteristics of secondary cucumber fermentations described by Franco and others (2012) and with those observed in the earlier trials. These changes indicate that cucumbers fermented in $CaCl_2$ will either need to be processed into final products after completion of the primary fermentation phase or practical procedures to prevent development of the secondary fermentation would have to be implemented.

The sixth trial provided an opportunity to observed commercial cucumber fermentations in an independent location. Cucumber fermentations brined with $CaCl_2$ in this independent location proceeded as expected (Table 7) reaching pH values of 3.60 ± 0.2 on the seventh day as compared to 3.55 ± 0.2 in the fermentation brined with NaCl.

Collectively, the data presented for trials 3 thru 6, suggest that it is possible to ferment cucumbers at the commercial scale in open top tanks, and store them for a short period of time in a cover brine without NaCl. Longer term storage of the fermented fruits may be possible if preservatives are added after the primary fermentation is completed. The proposed NaCl-free technology will require the availability of large volumes of starter cultures that can meet Kosher requirements for the production of vegetables

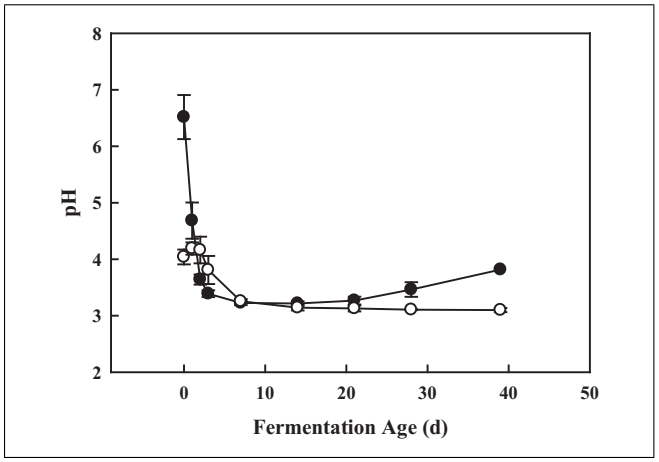

Figure 6–Changes in pH in the cover brines during commercial scale cucumber fermentations. Data presented are the means and standard error of 12 experimental tanks with the $CaCl_2$ cover brine (●) and 4 control tanks with the NaCl (○) cover brine.

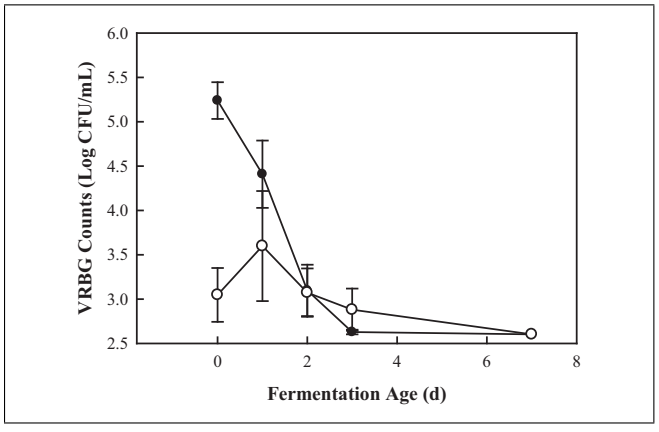

Figure 7–Counts of enterobacteriacea as a function of time in the commercial scale $CaCl_2$ and NaCl cucumber fermentations. Trends presented are the means with standard error of the 12 $CaCl_2$ (●) and 4 NaCl (○) cover brine fermentations. Detection limit for the spiral plating technique used was 2.6 Log CFU/mL.

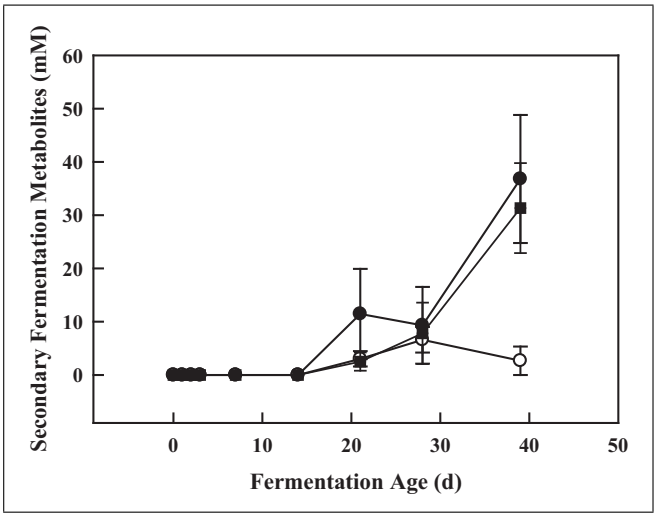

Figure 8–Production of butyric (■) and propionic (●) acids and n-propanol (○) during secondary fermentation of cucumbers brined with the $CaCl_2$ cover brine. Data presented represents means and standard error of 12 tanks packed with the $CaCl_2$ cover brine.

(Pérez-Díaz and McFeeters 2011) and may increase the demand for water if fermentation cover brines are not recycled.

This proposed technology has significant economic advantages due to the reduction of chloride discharges and waste removal costs. Additional advantages include the reduction of pollution from the disposal of high NaCl brines, the elimination of the need to store the cover brine for recycling and potential elimination of the carry-over of undesired flavors, enzymes, and high acid levels from the recycled cover brines.

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Author Contributions

I.M. Pérez-Díaz and R.F. McFeeters: conceptualized the technology, designed trials, conducted experiments at the commercial tank yards, processed and interpreted data, and completed troubleshooting; I.M. Pérez-Díaz: wrote the manuscript; L.A. Moeller: provided advisement regarding the practicality of the proposed technology for the pickling industry in the United States, identified necessary modifications for the proposed technology to be functional at the commercial scale, served as the link between the lead PIs and the tank yard, accommodated for supplies and equipment needed at the tank yard in a timely fashion, collected samples for analysis and conducted preliminary testing at the tank yard; S.D. Johanningsmeier: Collaborated in conducting trial 5 of the commercial scale fermentations, analyzed fermentation biochemistry data and conducted the quality analysis for trials 3 and 4; J. Hayes: collected cover brine samples and delivered to the laboratory for same day processing, completed the microbiological analysis for all samples processed for this study and prepared starter cultures; D. S. Fornea: conducted trials at the tank yard, collected cover brine samples and delivered to the laboratory for same day processing, conducted HPLC analysis for sugars and organic acids; Lisa Rosenberg: conducted quality analysis studies for trials 3 and 4; C. Gilbert, N. Messer, J. Cook: coordinated initial trials at their respective locations and accommodated for supplies and equipment needed; N. Custis: assisted with the trial at her location; K. Beene: designed pipes for sample collection and doors on cover boards for fruits samples collection; D. Bass: aided in locating supplies and tools needed in the tank yard. All authors reviewed and corrected the manuscript.

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