# **Consumer Acceptability of Cucumber Pickles Produced by Fermentation in Calcium Chloride Brine for Reduced Environmental Impact**

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**Abstract:** Fermentation of cucumbers in calcium chloride (CaCl<sub>2</sub>) brine has been proposed as an alternative process to reduce the environmental impact of traditional, high salt fermentations. The objective of this research was to determine whether consumer acceptability of pickle products would be impacted by fermentation and storage of cucumbers in CaCl<sub>2</sub> brine. Cucumbers were fermented and stored with 0.1M CaCl<sub>2</sub> or 1M sodium chloride (NaCl) in open-air, 3000 gal tanks at a commercial facility and processed into hamburger dill chips containing 0.38M NaCl. Cucumbers fermented in CaCl<sub>2</sub> required additional desalting to reduce CaCl<sub>2</sub> concentrations to that of current products. Consumers (n = 101) showed no significant preference for pickles from different fermented pickles were preferred over CaCl<sub>2</sub> fermented pickles stored for 10 mo and desalted only once (P < 0.01). A series of preference tests indicated that the taste of CaCl<sub>2</sub> was not the factor affecting consumer preference, and the 50% detection threshold of CaCl<sub>2</sub> in dill pickle chips was found to be  $61.8 \pm 7.6$  mM, indicating that processors could potentially use CaCl<sub>2</sub> fermentations with a single desalting step. Consumer liking of flavor (n = 73) was not influenced by fermentation in CaCl<sub>2</sub> or by 23 or 35 mM CaCl<sub>2</sub> in finished products (P > 0.05), but variability in texture decreased consumer liking (P < 0.05). Although promising, individual fermentation variability and texture quality of CaCl<sub>2</sub> fermented products should be further evaluated prior to broad implementation of this process.

Keywords: calcium chloride taste, cucumber pickles, detection threshold, sodium-free fermentation, sustainability, vegetable preservation

**Practical Application:** Disposal of high salt waste continues to burden the pickled vegetable industry. Consumer acceptability of pickles produced by commercial fermentation and storage in 1.1% calcium chloride (CaCl<sub>2</sub>) brines and traditional 6% sodium chloride brines was evaluated. In 2 independent trials, consumers displayed no difference in preference for pickles produced from either process. Further investigation of the taste of CaCl<sub>2</sub> in finished products suggested that processors may use the CaCl<sub>2</sub> fermentation with their normal process without significant concern for alterations in the taste profile. However, variability in texture quality impacted consumer acceptability, indicating the need for further process optimization.

# Introduction

In the United States, more than 800 million kg of fermented vegetables are produced annually (Hutkins 2006). In fact, the pickled vegetable market accounts for more than \$2 billion, with fermented cucumber pickles constituting one of the primary products (Doyle and Beuchat 2007). Pickling cucumbers are commercially fermented in 40000 L open-top, plastic, or fiberglass tanks with

a minimum of 5% sodium chloride (NaCl) in the fermentation brine (Franco and others 2012). Most commercial fermentation processes occur under conditions of high salt concentration, which allows for a natural fermentation by indigenous, homofermentative, lactic acid bacteria present on the surface of cucumbers (Etchells and Jones 1946; Fleming and others 1992). Only 2% to 3% NaCl is desired in the finished product, which results in the need to desalt the fermented cucumbers prior to packing the finished product (Fleming 1984). Waste waters from this process are high in sodium chloride and biological oxygen demand representing a significant waste treatment challenge for processors (Little and others 1976).

Fermentation of cucumbers with 0.1 M calcium chloride  $(CaCl_2)$  instead of the traditional 1.03 M (6 %) NaCl has been successfully applied on a controlled, experimental scale as a means to combat the environmental issues and toxic effects associated with the large amounts of NaCl in pickle plant waste waters (McFeeters and Perez-Diaz 2010). Recent research (Pérez-Díaz and others unpublished) demonstrated the potential for stable commercial cucumber fermentations using CaCl<sub>2</sub> and a starter

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culture in place of NaCl. For this process to be implemented on a commercial scale, the quality of finished products must match or exceed that of current products fermented and stored in high salt brines. Cucumbers brined and fermented in 0.1 M CaCl<sub>2</sub> that are processed with the typical, single desalting step will have a residual CaCl<sub>2</sub> concentration of approximately 36 mM in finished products, the legal limit for CaCl<sub>2</sub> in fermented vegetable products (21 CFR 184.1193). Presently, CaCl<sub>2</sub> brined, fermented cucumbers require additional desalting prior to packing into finished products to reduce the level of CaCl<sub>2</sub> to that which is traditionally found in NaCl fermented products. The additional desalting suggested for this process would result in the use of more resources, such as water, time, and labor. Therefore, it is important to determine whether a difference in consumer acceptability exists between the CaCl<sub>2</sub> and NaCl fermented products and whether the additional desalting step is necessary.

The concern for higher residual CaCl<sub>2</sub> levels in finished pickle products stems from the different taste attributes associated with CaCl<sub>2</sub> in comparison to NaCl. CaCl<sub>2</sub> has been described as predominantly bitter and salty, with astringent, metallic, and irritative sensations contributing to a lesser extent (Lawless and others 2003). In fact, Van Der Klaauw and Smith (1995) found a 32 mM solution of CaCl<sub>2</sub> in distilled water to be about 4 times as bitter as salty. However, the potentially offensive tastes associated with CaCl<sub>2</sub> are masked to some degree in the presence of certain organic ions, such as lactate (Lawless and others 2003), which is found in cucumber pickles. In addition, some bitter compounds are known to be suppressed in the presence of NaCl (reviewed by Breslin 1996), a major component in finished cucumber pickle products. The detection threshold of CaCl<sub>2</sub> in pickle products and the degree to which the bitter taste profile of CaCl<sub>2</sub> can be detected at levels of 36 mM and below is unknown, which leads to the uncertainty of whether additional desalting will be necessary for producing finished products from CaCl<sub>2</sub> fermented cucumbers. The objectives of this study were to evaluate consumer acceptability of commercially produced CaCl2 fermented cucumber pickles and determine the detection threshold of CaCl<sub>2</sub> in fermented hamburger dill chips to direct future research related to the broad commercial implementation of CaCl<sub>2</sub> fermentation processes.

# **Materials and Methods**

Fermentation and sample processing

Cucumbers of size 2B (32 to 38 mm in diameter) or 3A (39 to 51 mm in diameter) were fermented in either 1.03M NaCl or 0.1M CaCl<sub>2</sub> in a commercial tank yard in open-top, 10,000 L polymer tanks. Fermented cucumbers collected from at least 3 feet below the surface of the brine were desalted to equilibrium prior to processing using distilled water in a 60:40, cucumber to water, ratio. A 2nd desalting step at a 60:40, cucumber to water ratio, followed completion of the 1st desalting for selected treatments. Desalted, fermented cucumbers were sliced into 3 to 6 mm hamburger dill chip rounds, packed into 16-oz glass jars with a traditional commercial brine formula for hamburger dill chips (58:42, cucumber to brine pack-out ratio), pasteurized in a commercial tunnel pasteurizer according to manufacturers specifications, and stored at room temperature (23  $\pm$  2 °C) under ambient lighting. All finished products contained an equilibrated NaCl concentration of  $0.38 \pm 0.03$  M,  $96 \pm 10$  mM acetic acid added as vinegar, and  $21 \pm 6$  mM lactic acid from fermentation. The proportion of brine to cucumbers in each jar may vary up to 5% in the commercial setting. However, jar to jar variation was controlled to

<0.5 % in finished products packed by the researchers. CaCl<sub>2</sub> was the only constituent of the brine that was intentionally modified for each treatment; concentration of CaCl<sub>2</sub> in finished products was quantified by titration with EDTA using bromocresol blue as an indicator (AOAC Official Method 968.31).

## Panelist selection and testing environment

Subjects 18 years or older were recruited from the North Carolina State Univ. (NCSU) community for each of the experiments outlined in this study under approval from the NCSU Institutional Review Board (IRB #3092, 2192, and 2971), and informed consent was obtained from all participants. For all experiments, 2 hamburger dill chip slices from each jar were served at room temperature in 2-oz plastic soufflé cups with lids (Solo cup, Highland Park, Ill., U.S.A.) labeled with random 3-digit codes. The 3-digit sample codes and sample order were randomized among panelists. Room temperature distilled water and unsalted crackers were provided to cleanse the palate between samples.

## Paired preference testing of commercial products

The goal of these tests was to determine whether pickle consumers had differences in preference between products from the 2 fermentation brining processes when the finished product CaCl<sub>2</sub> concentration was maintained at typical levels of 18 to 25 mM. Finished cucumber pickle products were obtained from 2 independent, commercial trials comparing the CaCl<sub>2</sub> and NaCl brining processes for fermentation and bulk storage. The fermented cucumbers were stored for either 2 or 8 mo of bulk storage before processing into finished products. All finished products had a shelf storage time of 12 mo before sensory evaluation. Panelists (n =101, 56 female) were distributed across the following age divisions: 18 to 25 (49%), 26 to 35 (22%), 36 to 45 (12%), 46 to 55 (12%), and 56 to 65 (6%). Sixty-eight percent of respondents were Caucasian. Pickle consumption demographics fell mainly into 4 categories: several times per week (21%), once a week (27%), 2 to 3 times per month (32%), and once a month (11%). Results from 10 and 9 panelists were omitted from the 2 and 8 mo preference test results, respectively, because of the panelists expressing no preference. Panelists were presented with 2 pairs of samples using a randomized complete block design. Samples in each pair were commercially processed to reach equivalent salt and acid compositions upon equilibration. The pairs, differing by independent fermentations, storage time in the tank yard, and desalting involved a traditional NaCl fermentation treatment and a CaCl<sub>2</sub> fermentation treatment that contained equilibrated CaCl<sub>2</sub> concentrations of 18.7  $\pm$  2.5 mM and 24.1  $\pm$  1.3 mM for the 2 and 8 mo pairs, respectively. Panelists were instructed to taste the samples from left to right and indicate which sample they preferred in each pair or if they had no preference.

## Consumer testing of small-scale experimental products

Small-scale experimental products were created to evaluate whether higher residual calcium levels would influence consumer preference. Commercially fermented cucumbers were processed, after a 9 to 10 mo bulk storage time, in a food grade pilot plant, packed in 16-oz glass jars, pasteurized at 75 °C for 15 min, and stored under ambient conditions until testing. The 4 treatments, CaCl<sub>2</sub> 1 desalt, CaCl<sub>2</sub> 2 desalt, NaCl traditional, and NaCl with elevated CaCl<sub>2</sub>, contained 36, 28, 20, and 34 mM CaCl<sub>2</sub>, respectively.

A paired, A-Not A difference test (Bi and Ennis 2001) was performed in isolated booths at the NCSU sensory testing facility (Dept. of Food, Bioprocessing, and Nutrition Sciences, NCSU, Raleigh, N.C., U.S.A.). Panelists (n = 50, 34 female) were distributed across the following age divisions: 18–25 (36%), 26–35 (34%), 36–45 (8%), 46–55 (12%), 56–65 (8%), and 66+ (2%). Seventy-four percent of panelists consumed pickles on a monthly or weekly basis. The A-Not A testing procedure was performed as outlined in ISO 8588:1987 (E). The 2 samples included a traditional NaCl fermented treatment (A) and a CaCl<sub>2</sub> fermented 1 desalt treatment (Not A). An A and Not-A reference sample was presented to each panelist for an unlimited amount of time. When prompted by the panelists, the reference samples were removed and not returned. Then, 2 samples were presented to the panelists one at a time in a random order, with an equal distribution of 2 presentations: "A" followed by "Not A" or "Not A" followed by "A."

Paired preference testing was performed at individual stations set up in a classroom with ambient temperature and lighting (NCSU, Dept. of Food, Bioprocessing, and Nutrition Sciences). All paired preference tests presented panelists with 2 pairs of samples in a randomized complete block design. In Paired Preference Test 1, the 2 sample pairs were: (1) NaCl traditional treatment versus  $CaCl_2$  one desalt treatment and (2) NaCl traditional treatment versus CaCl<sub>2</sub> 2 desalt treatment. Panelists (n = 50, 32 female) were distributed across the following age divisions: 18 to 25 (34%), 26 to 35 (30%), 36 to 45 (12%), 46 to 55 (16%), 56 to 65 (6%), and 66+ (2%). Eighty-four percent of panelists consumed pickles on a monthly or weekly basis. Results from 7 and 4 panelists were omitted from pair 1 and 2, respectively, because of the panelists expressing no preference. For Paired Preference Test 2, the 2 sample pairs were: (1) NaCl fermented treatment with elevated CaCl<sub>2</sub> versus NaCl traditional treatment and (2) NaCl fermented treatment with elevated CaCl<sub>2</sub> versus CaCl<sub>2</sub> one desalt treatment. Panelists (n = 50, 37 female) were distributed across the following age divisions: 18 to 25 (38%), 26 to 35 (26%), 36 to 45 (12%), 46 to 55 (14%), 56 to 65 (8%), and 66+ (2%). Eighty-eight percent of panelists consumed pickles on a monthly or weekly basis. Results from 3 and 2 panelists were omitted from pair 1 and 2, respectively, because of the panelists expressing no preference.

## Consumer liking of commercial products

A hedonic evaluation of products was used to gather additional information on the acceptability of CaCl2 brined, fermented cucumber pickles finished to varying residual calcium levels. Cucumbers were commercially fermented and bulk stored in the tank yard 4 mo before processing into finished products; finished products were stored under ambient conditions for 2 mo before evaluation. Panelists (n = 73, 51 female) were distributed across the following age divisions: 18 to 25 (48%), 26 to 35 (21%), 36 to 45 (16 %), 46 to 55 (10%), and 56 to 65 (5%). Eighty percent of consumers participating in the liking test consumed pickle products at least monthly, with 46% having a consumption frequency of weekly or greater. However, purchase frequency was lower than consumption frequency, with 41% of consumers purchasing pickle products no more than several times per year. The consumers in this study reported that flavor and texture were the primary factors influencing purchasing intent, more so than cost or brand. Panelists were presented with 4 treatments in a randomized complete block design. Treatments differed by fermentation brining salt and the concentration of CaCl<sub>2</sub> in finished products: NaCl fermented traditional (23 mM CaCl<sub>2</sub>), NaCl fermented with elevated CaCl<sub>2</sub> added to finished product (35 mM CaCl<sub>2</sub>), CaCl<sub>2</sub> fermented 1 desalt (35 mM CaCl<sub>2</sub>), and CaCl<sub>2</sub> fermented 2 desalt (23 mM

(Dept. of Food, Bioprocessing, and Nutrition Sciences, NCSU, CaCl<sub>2</sub>). Panelists were asked to rate their overall liking, as well Raleigh, N.C., U.S.A.). Panelists (n = 50, 34 female) were distributed across the following age divisions: 18–25 (36%), 26–35 1 and 9 corresponding to dislike extremely and like extremely, (34%), 36–45 (8%), 46–55 (12%), 56–65 (8%), and 66+ (2%). respectively. In addition, panelists were given the option of com-Seventy-four percent of panelists consumed pickles on a monthly or weekly basis. The A-Not A testing procedure was performed

#### **Texture analysis**

Mesocarp firmness of fermented hamburger dill pickle chips was measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp, Scarsdale, N.Y., U.S.A./Stable Micro Systems, Godalming, Surrey, UK) equipped with a 3-mm-diameter punch probe. Methodology was performed as outlined in Thompson and others (1982) and Yoshioka and others (2009). Briefly, pickle slices were placed onto a base plate containing a 3.1-mm hole. The punch probe moved at a test speed of 2.5 mm/s. Data were collected and analyzed using Texture Expert software (Texture Technologies Corp./Stable Micro Systems). The peak force required to puncture the mesocarp was recorded and expressed in Newtons (N). Firmness measurements were done at ambient temperature  $(23 \pm 2 \ ^{\circ}C)$  on 15 slices from each of 2 jars of each treatment. Average peak force from the 15 slices was subjected to statistical analysis.

### Color analysis

The  $L^*$ ,  $a^*$ ,  $b^*$  values of hamburger dill pickle chips evaluated in the liking test were measured using a Minolta Chroma Meter model CR-300 (Minolta Co., Ltd., Osaka, Japan). Because of the transparency of pickle chips, 10 pickle chips were stacked on top of each other and measurements were taken of the mesocarp of the top slice. An average of 10 pickle chip measurements per treatment was reported.

## Detection threshold of CaCl<sub>2</sub> in hamburger dill chip pickles

Cucumbers were commercially fermented in a NaCl brine and stored in bulk for 4 mo before processing into finished products. The cucumbers were desalted to equilibrate to a NaCl concentration of 2.2% in finished products. Pickles were packed in a commercial facility with cover brines formulated to deliver equilibrated CaCl<sub>2</sub> concentrations of 26, 36, 50, 70, and 98 mM CaCl<sub>2</sub> to allow for performance of a 5-series threshold test with a step-factor of 1.4. Concentrations were selected to include values surrounding the legal limit for CaCl<sub>2</sub> in pickle products, and the step factor was based on preliminary trials. A modified blank sample containing a CaCl<sub>2</sub> concentration of 21 mM was chosen to represent the typical level in commercially available hamburger dill chip pickles. Finished products were stored under ambient conditions for 2 mo prior to evaluation. The threshold testing procedure outlined in ASTM standard E679 (2011) was followed with a 2 min forced break between each of the 5 rows. The ballot contained an optional section for comments on the description of the taste difference within each of the 5 rows. Panelists (n = 52, 30 female) were distributed across the following age divisions: 18 to 25 (56%), 26 to 35 (12%), 36 to 45 (10%), 46 to 55 (13%), and 56 to 65 (10%). Eighty-one percent of panelists consumed pickles on a monthly or weekly basis.

#### Statistical analyses

Data were collected using paper ballots and compiled in an electronic spreadsheet. All results were analyzed using SAS statistical software (version 9.3, SAS Inst. Inc., Cary, N.C., U.S.A.).

Number of panelists	No pref. votes	Brining Salt	Tank yard Storage (mo)	Desalt steps	CaCl <sub>2</sub> in finished product (mM)	Shelf storage (mo)	Results	χ²	P-value
Paired preferen	nce testing of	f commercial	lly processed produ	ıcts					
101	10	CaCl <sub>2</sub>	2	2	18	12	No significant preference	0.10	0.75
		NaCl	2	1	18	12			
101	9	CaCl <sub>2</sub>	8	2	24	12	No significant preference	0.17	0.68
		NaCl	8	1	25	12			
A—Not A dif	ference testir	ng of small-so	ale experimental p	oroducts					
50	N/A	CaCl <sub>2</sub>	10	1	36	2	Significant difference	6.48	0.01
		NaCl	9	1	20	2	-	15.68	< 0.0001
Paired preferen	nce testing of	f small scale (	experimental produ	ucts					
50	7	$CaCl_2$	10	1	36	2	NaCl preferred	8.40	0.004
		NaCl	9	1	20	2			
50	4	CaCla	10	2	28	2	NaCl preferred	4 26	0.04
50		NaCl	9	1	20	2	r a chi preterrea		0.01
50	3	NaCl	9	1	20	5	No significant preference	0.02	0.88
	-	NaCl	9	1	34	5			
50	2	CaCl <sub>2</sub>	10	1	36	5	NaCl preferred	5.33	0.02
	-	NaCl	9	1	34	5		2.000	

Table 1-Consumer preference study of hamburger dill chip cucumber pickles fermented in either 1.03 M NaCl or 0.1 M CaCl<sub>2</sub> with varying levels of finished product CaCl<sub>2</sub>.

Preference tests were analyzed using a  $\chi^2$  test and an  $\alpha$  level of 0.05. For all preference tests, a no preference option was allowed and the most powerful data analysis option of dropping the no preference votes was applied (Ennis and Ennis 2012). Data were later analyzed by splitting the no preference votes with no significant difference (P > 0.05) in the results, supporting the decision to drop no preference votes from the dataset. Consumer liking data were analyzed by repeated measures ANOVA and Tukey's honestly significant difference *post hoc* test. In addition, a statistical power test was performed ( $\alpha = 0.05$ ) before conducting the liking test with an estimated standard deviation of 2.2; 72 panelists were needed to detect a 1 point difference in means with 80% power.

The CaCl<sub>2</sub> detection threshold was determined using both the ASTM E679 method and the alternative method for threshold data analysis developed by Lawless (2010), which involves plotting the chance-corrected proportion of detection on the y-axis and CaCl<sub>2</sub> concentration on the x-axis and interpolating the threshold concentration of CaCl<sub>2</sub> at different detection levels. Subjects (n = 52) were recruited to obtain sufficient statistical power (approximately 80%) based on the statistics of a 1-sample *t*-test, an expected mean deviation of 9 and a standard deviation of 22. In addition, the GLIMMIX procedure in SAS (SAS version 9.3, SAS Inst., Inc.) was used to fit a generalized linear mixed model appropriate for the dichotomous response. This model had fixed quadratic effects for CaCl<sub>2</sub> concentration, random panelist effects, and an intercept fixed at  $\beta_0 = -\log(2)$  to constrain the probability of guessing for undetectable changes in CaCl<sub>2</sub> concentration to  $\pi = 1/3$  at a  $CaCl_2$  concentration of 0.

# **Results and Discussion**

Paired preference testing of CaCl<sub>2</sub> and NaCl fermented cucumber pickles

In 2 independent commercial trials, no significant preference was observed between CaCl<sub>2</sub> and NaCl fermented cucumber pickles, regardless of the tank yard storage time of 2 mo ( $\chi^2 = 0.10$ , P = 0.75) or 8 mo ( $\chi^2 = 0.17$ , P = 0.68; Table 1). These results showed that consumers (n = 101) did not have a significant

cesses when the finished product CaCl<sub>2</sub> concentration was maintained at typical levels of 18 or 25 mM. This finding suggests that it is possible for cucumbers to be fermented and stored in CaCl<sub>2</sub> brines in a commercial setting without a negative impact on consumer acceptability. Samples evaluated in these tests were collected from different fermentation tanks stored for different periods of time in the tank yard, but neither of these variables had an effect on consumer preference of finished products. In contrast, consumers could differentiate between a traditional NaCl fermented pickle ( $\chi^2 = 6.48$ , P = 0.01) and CaCl<sub>2</sub> brined cucumber pickles with higher residual CaCl2 content in an A-Not A Difference Test  $(\chi^2 = 15.68, P < 0.0001;$  Table 1). Because of the inherent variability of pickles, even within the same treatment, the question was posed as to whether or not the consumer would show an overall preference for one treatment over the other, regardless of whether they were able to detect a difference. Therefore, preference tests were performed between the NaCl traditional treatment and the CaCl<sub>2</sub> fermented cucumber pickles processed with 1 or 2 desalting treatments to determine if an additional desalt step for the CaCl<sub>2</sub> fermented cucumbers was necessary for consumer preference. In these paired preference tests, the NaCl fermented treatment was preferred over both of the finished products from this particular CaCl<sub>2</sub> fermentation, regardless of the extent of desalting (P < 0.05; Table 1). The significant preference for NaCl fermented cucumber pickles over the CaCl<sub>2</sub> 2 desalt treatment was especially unexpected, as it appears to contradict the results from the 2 preference tests on commercial products. However, a number of factors aside from the potential adverse taste of elevated levels of CaCl<sub>2</sub> may influence consumer preference, such as the inherent fermentation variability encountered when experimenting with cucumbers fermented on a commercial scale in open-top fermentation tanks or variations in fermented and bulk stored cucumbers because of the difference in salt brining treatment. These particular fermentations had been in bulk storage in the tank yard for 9 to 10 mo. Further testing was conducted to determine whether the observed difference in preference was influenced primarily by the difference in CaCl<sub>2</sub> content in finished products.

Table 2–Evaluation of texture by instrumental analysis and consumer liking<sup>a</sup> (n = 73) for fermented hamburger dill chip cucumber pickles.

Fermentation process	Desalt steps	Finished product CaCl <sub>2</sub> (mM)	Firmness (N)	Texture liking	
NaCl	1	$22.6 \pm 0.4$	$8.4 \pm 0.3^{ab}$	$7.0 \pm 1.4^{a}$	Like moderately
NaCl	1	$34.3 \pm 0.4$	$9.6 \pm 0.4^{a}$	$7.4 \pm 1.3^{a}$	Like moderately
CaCl <sub>2</sub>	2	$22.6 \pm 0.4$	$7.3 \pm 0.1^{\rm bc}$	$5.5 \pm 2.0^{b}$	NLND <sup>b</sup>
CaCl <sub>2</sub>	1	$35.3 \pm 0.4$	$7.1 \pm 0.3^{\circ}$	$5.3 \pm 1.9^{b}$	NLND

<sup>a</sup>Liking rated on a hedonic scale (1 = dislike extremely, 9 = like extremely); Means with the same letter (a–b) are not significantly different (Tukey's honestly significant difference, P < 0.05).

<sup>b</sup>Neither like nor dislike.

Fermented cucumbers collected from the same NaCl fermentation tank as above were processed into products in which the only difference was the level of CaCl<sub>2</sub> in finished products, either 20 or 34 mM, and consumers expressed no significant preference between the products ( $\chi^2 = 0.02$ , P = 0.88; Table 1). However, when the level of CaCl<sub>2</sub> in finished products of both NaCl and CaCl<sub>2</sub> fermented cucumbers was equivalent,  $35 \pm 1$  mM, and the only difference was the fermentation salt and tank from which the pickles were collected, consumers expressed a significant preference for the NaCl fermented product ( $\chi^2 = 5.33$ , P = 0.02). Therefore, it appears that when CaCl<sub>2</sub> is present in hamburger dill chip pickles at levels around the legal limit of 36 mM, the resulting taste and texture due to the elevated CaCl<sub>2</sub> is not responsible for the observed difference in consumer preference between the traditional hamburger dill chip product and the experimental CaCl<sub>2</sub> fermented product desalted only once. Rather, inherent variability related to fermentation and long-term bulk storage or differences because of brining salt treatment were likely influencing consumer preference in this particular comparison.

# Consumer liking of commercially fermented products

To further investigate the consumer acceptability of the cucumber pickles fermented in 1.1% CaCl<sub>2</sub> brines, a consumer liking test was conducted on hamburger dill chips prepared from NaCl or CaCl<sub>2</sub> fermented cucumbers stored for only 4 mo in bulk

storage before processing into finished products with 23 or 35 mM CaCl<sub>2</sub> and 0.38 M NaCl. Interestingly, the CaCl<sub>2</sub> fermented products were less well liked overall (P < 0.05). Flavor, however, was the only category in which the liking scores did not differ (P > 0.05) among the treatments (Figure 1), supporting the results from paired preference testing, in which the elevated level of residual CaCl<sub>2</sub> did not affect consumer acceptability of the finished products, regardless of individual fermentation variability or salt brining treatment. The lack of difference in consumer liking of flavor within either fermentation brining treatment, regardless of extent of desalting, suggests that there is no practical benefit in additional desalting to reduce fermentation flavors or to reduce the CaCl<sub>2</sub> concentration.

However, consumers gave higher scores for texture and appearance liking (P < 0.05) to the 2 NaCl fermented pickles over the CaCl<sub>2</sub> fermented pickles (Figure 1). In addition, approximately 20% of the respondents commented negatively about the "softness" or "lack of crispiness" for these CaCl<sub>2</sub> fermented cucumbers, regardless of CaCl<sub>2</sub> concentration in the finished product. The significantly lower firmness of CaCl<sub>2</sub> fermented pickles was confirmed through instrumental texture analysis (Table 2), showing a strong trend between reduced mesocarp firmness and consumer liking of texture. The significantly lower liking scores associated with the appearance of the CaCl<sub>2</sub> fermented pickles was further evaluated through the use of colorimetric analysis, in which the



Figure 1–Consumer liking (n = 73) of fermented hamburger dill pickle chips produced using fermentation in 1.03 M sodium chloride (NaCl) or 0.1 M calcium chloride (CaCl<sub>2</sub>) brines. Liking rated on a hedonic scale (1 = dislike extremely, 9 =like extremely). Means with the same letter (a-b) are not significantly different (Tukey's honestly significant difference, P < 0.05). All treatments were stored in bulk in the tank yard 4 mo prior to processing, stored under ambient conditions as finished products for 2 mo prior to evaluation, and contained an equilibrated NaCl concentration of 0.38  $\pm$  0.03 M. Treatments are denoted by fermentation brining salt with concentration of CaCl<sub>2</sub> in finished products in parentheses.

Table 3-Evaluation of appearance by Hunterlab colorimeter analysis and consumer liking<sup>a</sup> (n = 73) for fermented hamburger dill chip cucumber pickles.

Fermentation	Desalt	Finished product	<i>I</i> *	a*	<i>h</i> *	Appearance	
NaCl	1	$22.6 \pm 0.4$	$39.9 \pm 1.1^{b}$	$-7.2 \pm 0.2^{b}$	$21.6 \pm 1.4^{a}$	$6.6 \pm 1.6^{a}$	Like moderately
NaCl	1	$34.3 \pm 0.4$	$41.8 \pm 2.5^{b}$	$-7.6 \pm 0.2^{a,b}$	$22.9 \pm 1.9^{a}$	$6.9 \pm 1.4^{a}$	Like moderately
CaCl <sub>2</sub>	2	$22.6 \pm 0.4$	$49.0 \pm 0.0^{a}$	$-8.2 \pm 0.0^{a}$	$25.7 \pm 0.1^{a}$	$5.8 \pm 1.7^{b}$	Like slightly
CaCl <sub>2</sub>	1	$35.3 \pm 0.4$	$49.0 \pm 0.0^{a}$	$-8.0 \pm 0.2^{a}$	$26.0 \pm 1.9^{a}$	$5.8 \pm 1.6^{\mathrm{b}}$	Like slightly

<sup>a</sup>Liking rated on a hedonic scale (1 = dislike extremely, 9 = like extremely); means with the same letter (a-b) are not significantly different (Tukey's honestly significant difference, P < 0.05)

 $L^*$  values were found to be greater (P < 0.05) and  $a^*$  values more negative (P < 0.05) for CaCl<sub>2</sub> fermented pickles (Table 3).

Detection threshold of CaCl<sub>2</sub> in hamburger dill chip pickles

The detection threshold of CaCl<sub>2</sub> in NaCl fermented hamburger dill pickle chips as determined by the ASTM E679 calculation method for the best estimate threshold (BET) was 53  $\pm$ 22 mM. Despite the high standard deviation, the statistical power of this test was still above the desired 80% power. Because of the limitations of the ASTM method, Lawless (2010) developed an alternative method in which the chance-corrected percent correct for each concentration of CaCl2 in the 5 rows is plotted using an ordinary least squares regression (OLS). In turn, the threshold concentration at varying detection levels can be interpolated. The OLS equation for this data set was  $\gamma = 0.92x + 7.54$ ,  $R^2 =$ 0.96, where y is the chance-corrected percent correct and x is the concentration of CaCl2 in finished hamburger dill chip products (Figure 2). This method of threshold calculation is particularly useful, as it allows one to determine the detection threshold at any given level of detection, not just 50%. For example, pickle processors can determine the detection threshold of CaCl<sub>2</sub> in hamburger dill chips at a more conservative level, such as the concentration at which 25% of the population will be able to detect CaCl<sub>2</sub> in hamburger dill chips. Using this equation, the 10%, 25%, and 50% detection levels were 34.8, 46.0, and 64.1 mM CaCl<sub>2</sub>, respectively

> 50% Detection 7% Correct, 64.1 mM)

25% Detection

(50.0% Correct, 46.0 mM) 10% Detection

(39.7% Correct 34.8 mM)

10

20

30

100

90

80

70

60

50

40

30 20

10 0 0

Percent Correct (%)

(Figure 2). The ability to estimate confidence intervals accounting for the panelists as random variables was accomplished using a logistic regression model with a quadratic fit and forced intercept to represent the chance probability of 1/3 for undetectable levels of CaCl<sub>2</sub>. This model produced similar estimates for 10%, 25%, and 50% detection levels, 39, 49.2, and 61.8 mM, respectively, with the greatest difference observed at the low levels of detection that are near the legal limit for CaCl<sub>2</sub> in finished pickle products (Table 4). Regardless of method of calculation, the 50% detection threshold of CaCl<sub>2</sub> in fermented hamburger dill chip pickles was greater than the highest concentration that is legally allowed in fermented vegetables (36 mM). However, the modeling of the taste threshold for CaCl<sub>2</sub> in fermented pickle products has not been previously reported, and a small proportion of the population, approximately 10%, may be capable of detecting the presence of elevated CaCl<sub>2</sub> concentrations of 36 mM in cucumber pickles.

CaCl<sub>2</sub> is a commonly used minor ingredient in both fermented and fresh-pack cucumber pickles. According to Buescher and others (2011), the residual calcium concentration in traditional commercial dill pickles has increased to about 17 mM on average. However, the effect of elevated calcium concentration on the consumer acceptability of cucumber pickles was previously unknown. One of the major concerns for pickle processors when considering a CaCl<sub>2</sub> brine for fermentation is the potentially adverse taste re-



Figure 2–Interpolation at chance-corrected proportions of the detection threshold (n = 52) of calcium chloride (CaCl<sub>2</sub>) in fermented hamburger dill chip pickles.

50

CaCl<sub>2</sub>(mM)

60

70

80

40

y = 0.92x + 7.54

 $R^2 = 0.96$ 

90

100

Table 4-Estimates for detection thresholds of calcium chloride	e (CaCl <sub>2</sub> ) in fermented	hamburger dill chip pickle
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Chance-corrected % detection	BET <sup>a</sup> (mM)	OLS interpolated detection threshold (mM)	Quadratic logistic regression model, estimate (95% CI) (mM)
10	N/A	34.5	39.0 (25.7, 52.3)
25	N/A	46	49.2 (39.7, 58.8)
50	$53 \pm 22$	64.1	61.8 (54.2, 69.4)

<sup>a</sup>BET, best estimate threshold calculated using the ASTM method

hypothesized that no significant difference or preference would be found between the traditional NaCl fermented products and the CaCl<sub>2</sub> fermented products receiving either one desalting step or 2 desalting steps before processing because of the taste interactions of the main components of pickle products, acetic acid and NaCl, with CaCl<sub>2</sub>. This research showed that residual CaCl<sub>2</sub> levels up to the legal limit of 36 mM in pickled vegetable products did not significantly influence consumer preference or consumer liking of flavor in fermented hamburger dill chip pickles (Table 1; Figure 1). In addition, the 50% detection threshold of CaCl<sub>2</sub> was found to be well above 36 mM in fermented hamburger dill chip pickles (Table 4). In turn, processors may choose to adopt the CaCl<sub>2</sub> fermentation process with the typical desalting practices without concern for the effect of elevated CaCl<sub>2</sub> concentrations up to the maximum legal concentration of 36 mM. However, the detection threshold of consumers tends to be extremely variable around what is considered to be sub-threshold or threshold concentrations. If a processor wanted a more conservative estimate to target even the most sensitive consumers, a finished product CaCl<sub>2</sub> concentration of approximately 28 mM is suggested, using the alternative threshold method (Figure 2), or 25 mM to be below the 95% confidence interval for detection by 10% of the population. Significant preferences were observed in the preference testing of small-scale experimental products, but not in relation to residual CaCl<sub>2</sub> concentration. When CaCl<sub>2</sub> concentration was kept constant in both the small-scale experimental products and large-scale commercially packed products, seemingly conflicting results were obtained for consumer preference (Table 1). Similarly, although consumers showed no significant preference (P > 0.05)between a NaCl fermented traditional treatment and a CaCl<sub>2</sub> 2 desalt treatment in commercially packed products, significant preference (P < 0.05) was observed between these 2 treatments in the trials involving small-scale experimental products (Table 1). Samples varied with regards to cucumber origin, the season in which fermentation began, storage time in the tank yard prior to processing into finished products, the individual fermentation tanks from which the cucumbers were collected, the extent of desalting, and the use of alum during desalting. The conflicting preference test results highlight the degree to which the inherent fermentation variability in commercial tank yards may influence the quality of finished products and ultimately may affect consumer preference.

Although no significant difference (P > 0.05) in consumer acceptability of flavor of the CaCl<sub>2</sub> and NaCl fermented products was expressed in the consumer liking test of commercially packed products, regardless of residual CaCl<sub>2</sub> levels in finished products, texture differed significantly (P < 0.05). In contrast to earlier work completed by Buescher and others (2011) in which CaCl<sub>2</sub> increased mesocarp crispness, consumers from the liking test noted that both of the CaCl<sub>2</sub> fermented treatments were "soft" or "less crisp" and scored the CaCl<sub>2</sub> fermented treatments significantly lower in texture liking (P < 0.05), regardless of residual CaCl<sub>2</sub> levels (Table 2). One possible explanation for the reduced texture quality of these CaCl<sub>2</sub> fermented products involves the

potential role of polygalacturonase (PG), a ripening/softening enzyme known to be present in cucumber fruits (Bell and others 1950; McFeeters and others 1980; Cho and Buescher 2012). Bell and Etchells (1961) showed that firmness of cucumber pickles with a NaCl concentration of 0% was reduced by more than 80% after only 1 week of incubation at 30 °C because of the PG enzyme. Furthermore, although Buescher and others (1979) demonstrated that 100 mM CaCl<sub>2</sub> can inhibit the softening activity of the PG enzyme in both high salt (9.0% NaCl) and low salt (4.5% NaCl) fermentation brines, a lower firmness was observed in the low salt treatments. Therefore, the lack of NaCl in the CaCl<sub>2</sub> fermentation brines may be allowing increased activity of the native PG enzyme, resulting in textural defects after long term storage. Lu and others (2002) showed that the composition and structure of the cucumber fruit itself is variable among different sizes and cultivars, which may further contribute to variations in texture quality of finished products. Lastly, despite the common use of alum in the desalting waters of commercial pickle plants, the research samples did not include alum, to avoid introducing another variable. There is some interest in the pickle industry to remove alum from the desalting waters because of health concerns, but the removal of alum may result in a significant decrease in texture quality of finished products. Further studies are needed to determine if including alum in desalting waters plays a crucial role in retaining the texture of finished pickle products fermented and stored in the absence of NaCl.

Consumer acceptability was also somewhat influenced by the appearance of the cucumber pickles. Although the same concentration of yellow 5 was added to all of the treatments, the resulting color was significantly different among the treatments. CaCl<sub>2</sub> fermented products were found to have significantly higher  $L^*$  values (P < 0.05), indicating a lighter colored product, and significantly more negative  $a^*$  values (P < 0.05), indicating a stronger green color (Table 3). Lawrence and others (2003) hypothesized that a high concentration of calcium ions may induce pro-oxidation effects similar to iron or copper ions after discovering that a 100 to 300 mM CaCl<sub>2</sub> marinade resulted in steaks that were less red than those marinated in calcium lactate. The pro-oxidation effects seemingly associated with CaCl<sub>2</sub> may actually be related to the impurity of CaCl<sub>2</sub> being used (94 % pure in these commercial trials), in which trace metal contaminants could be involved in initiating oxidation. Another possible explanation for the difference in color may be related to the reduction in texture quality associated with these particular cucumbers fermented in CaCl<sub>2</sub>. The physicochemical changes that resulted in reduced texture quality may also impact the interaction between the product and light, resulting in changes in color perception by consumers. Further research is needed to understand the basis for the observed differences in appearance of CaCl<sub>2</sub> fermented cucumber pickles.

## Conclusion

Although a difference was observed between CaCl<sub>2</sub> and NaCl fermented pickles processed with one desalting step, pair-wise

preference testing showed that the taste of CaCl<sub>2</sub> was not the factor affecting consumer preference. This finding was further supported by the calculated 50% detection threshold of CaCl<sub>2</sub> in hamburger dill chip pickles of  $61.8 \pm 7.6$  mM, which is well above the legal limit of 36 mM for pickled vegetable products. In addition, there was no difference in flavor liking between NaCl and CaCl<sub>2</sub> fermented products finished with varying levels of calcium chloride, suggesting that processors could employ CaCl<sub>2</sub> fermentations with a single desalting step without concern for the adverse taste of elevated levels of CaCl<sub>2</sub>. Variability in consumer acceptability of CaCl<sub>2</sub> fermented pickles indicated that flavor stability during bulk storage, differences in product appearance, and texture quality of CaCl<sub>2</sub> fermented products should be further evaluated before broad implementation of this process.

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# Authors' Contributions

E.M. Wilson designed the experiments, conducted the sensory research, analyzed the data, and drafted the manuscript. S.D. Johanningsmeier collaborated in the experimental design, supervised the research, assisted in interpretation of data, and reviewed and revised the manuscript. J.A. Osborne generated the statistical model for threshold data and provided general statistical consultation for other parts of the study.

## References

ASTM Intl. 2011. Standard practice for determination of odor and taste thresholds by a forcedchoice ascending concentration series method of limits. E679-04. 1-7.

- Bell TA, Etchells JL. 1961. Influence of salt (NaCl) on pectinolytic softening of cucumber. J Food Sci 26:84–90.
- Bell TA, Etchells JL, Jones ID. 1950. Softening of commercial cucumber salt-stock in relation to polygalacturonase activity. Food Tech 4:157–163.
- Bi J, Ennis DM. 2001. Statistical models for the A-Not A method. J Sens Stud 16:215–37. Breslin PAS. 1996. Interactions among salty, sour and bitter compounds. Trends Food Sci Technol 7:390–9.

- Buescher RW, Hamilton C, Thorne J, Cho MJ. 2011. Elevated calcium chloride in cucumber fermentation brine prolongs pickle product crispness. J Food Qual 34:93–9.
- Buescher RW, Hudson JM, Adams JR. 1979. Inhibition of polygalacturonase softening of cucumber pickles by calcium chloride. J Food Sci 44:1786–7.
- Cho MJ, Buescher RW. 2012. Potential role of native pickling cucumber polygalacturonase in softening of fresh pack pickles. J Food Sci 77:C443–7.
- Code of Federal Regulations, Title 21, Food and drugs (21CFR184.1193). 2011. Calcium chloride. Office of the Federal Register, National Archives and Records Service, General Services Administration.
- Doyle MP, Beuchat LR. 2007. Fermented vegetables. In: Breidt F, McFeeters RF, Díaz-Muñiz I, editors. Food microbiology fundamentals and frontiers. 3rd ed. Washington, D.C.: ASM Press. p 793.
- Ennis JM, Ennis DM. 2012. A comparison of three commonly used methods for treating no preference votes. J Sens Stud 27:123–9.
- Etchells JL, Jones ID. 1946. Characteristics of lactic acid bacteria from commercial cucumber fermentations. J Bacteriol 52(5):593–9.
- Fleming HP. 1984. Developments in cucumber fermentation. J Chem Tech and Biotech 34(4):241–52.
- Fleming HP, McFeeters RF, Daeschel MA. 1992. Fermented and acidified vegetables. In: Vanderzant C, Splitstoesser DF, editors. Compendium of methods for the microbiological examination of foods. 3rd ed. Washington, D.C.: American Public Health Assn. p 929–52.
- Franco W, Perez-Diaz IM, Johanningsmeier SD, McFeeters RF. 2012. Characteristics of spoilage-associated secondary cucumber fermentation. Appl Environ Microbiol 78(4):1273– 84.
- Gindler EM, King JD. 1972. Rapid colorimetric determination of calcium in biological fluids with methylthymol blue. Am J Clin Path 58:376–82.
- Hutkins RW. 2006. Fermented vegetables. In: Anonymous microbiology and technology of fermented foods. 1st ed. Ames, Iowa: Blackwell Publishing. p 233–59.
- Intl. Organization for Standardization. 1987. Sensory analysis methodology 'A' 'not A' test. ISO 8588:1987 (*E*).
- Lawless HT. 2010. A simple alternative analysis for threshold data determined by ascending forced-choice methods of limits. J Sens Stud 25:332–46.
- Lawless HT, Rapacki F, Horne J, Hayes A. 2003. The taste of calcium and magnesium salts and anionic modifications. Food Qual Pref 14:319–25.
- Lawrence TE, Dikeman ME, Hunt MC, Kastner CL, Johnson DE. 2003. Effects of calcium salts on beef longissimus quality. Meat Sci 64(3):299–308.
- Little LW, Lamb JC, Horney LF. 1976. Characterization and treatment of brine wastewaters from the cucumber pickle industry. UNC Wastewater Research Center, Dept. of Environmental Sciences and Engineering, School of Public Health, Univ. of North Carolina at Chapel Hill. ESE Publication No 399.
- Lu Z, Fleming HP, McFeeters RF. 2002. Effects of fruit size on fresh cucumber composition and the chemical and physical consequences of fermentation. J Food Sci 67:2934–9.
- McFeeters RF, Bell TA, Fleming HP. 1980. An endo-polygalacturonase in cucumber fruit. J Food Biochem 4:1–16.
- McFeeters RF, Perez-Diaz I. 2010. Fermentation of cucumber brined with calcium chloride instead of sodium chloride. J Food Sci 75:291–6.
- Official Methods of Analysis of AOAC Intl. Method 968.31 2005. 18th Edition. AOAC Intl. Gaithersburg, Md., U.S.A.
- Thompson RL, Fleming HP, Hamann DD, Monroe RJ. 1982. Method for determination of firmness in cucumber slices. J Texture Stud 13:311–24.
- Van Der Klaauw NJ, Smith DV. 1995. Taste quality profiles for fifteen organic and inorganic salts. Physiol Behav 58:295–306.
- Yoshioka Y, Horie H, Sugiyama M, Sakata Y. 2009. Quantifying cucumber fruit crispness by mechanical measurement. J Breeding Sci 59:139–47.