ABSTRACT

WOLTER, EMILY MARIE. Consumer Acceptability and Flavor Characteristics of Cucumber Pickles Produced Using an Environmentally-Friendly Calcium Chloride Fermentation. (Under the direction of Dr. Suzanne Johanningsmeier).

Cucumber pickles are traditionally fermented in conditions of high sodium chloride (NaCl) to select for the indigenous lactic acid bacteria necessary to initiate a natural fermentation and prevent spoilage during bulk storage. The pickle industry is looking to adopt a fermentation process that will expel less sodium into waste waters as it is toxic to plants and aquatic organisms. Fermentation of cucumbers with 1.1 % (0.1 M) calcium chloride ($CaCl_2$) looks promising as an environmentally-friendly replacement for traditional 6 % (1.03 M) NaCl fermentations. While NaCl fermented cucumbers are traditionally desalted once prior to processing, two desalting steps are required for $CaCl_2$ fermented cucumbers to reduce the level of $CaCl_2$ to that which is traditionally found in NaCl fermented products. However, it is hypothesized that $CaCl_2$ fermented cucumbers can be desalted once and contain up to 36 mM $CaCl_2$ in finished products without a negative impact on consumer acceptability due to the taste interactions of $CaCl_2$ with the NaCl and acids present in finished products. A difference was observed between $CaCl_2$ and NaCl fermented pickles processed with only one desalting step ($n = 50$, $P < 0.05$). However, a series of pairwise preference tests indicated that the taste of $CaCl_2$ was not the factor affecting consumer preference. Furthermore, a consumer liking test ($n = 73$) showed no difference ($P < 0.05$) in flavor liking between NaCl and $CaCl_2$ fermented products, regardless of the number of pre-processing desalting steps. A threshold test ($n = 52$) demonstrated that the 50% detection

threshold of $CaCl_2$ in hamburger dill chip pickles was 64.1 mM, above the legal limit of 36 mM. In turn, consumer preference of cucumber pickles was likely influenced by other flavor attributes related to individual fermentation variability or textural differences, not $CaCl_2$ taste. A trained descriptive sensory analysis panel (n = 9) evaluated commercially fermented cucumber pickles collected from NaCl fermentations (n= 4) and $CaCl_2$ fermentations (n = 4) after 4 and 8 months of commercial bulk storage in open-top, 10,000 L tanks. Cucumber size, fermentation salt, finished product $CaCl_2$ concentration, bulk storage time, and fermentation variability were evaluated for their effect on 11 taste and flavor attributes, with off-flavors characterized as oxidized, green, earthy/musty, and metallic. The products tasted for descriptive sensory analysis were analyzed by two-dimensional gas chromatography-mass spectrometry, and volatile compounds were correlated with sensory flavor scores. Oxidized flavor was found to increase with bulk storage time ($P < 0.0001$) and was higher in $CaCl_2$ fermented products overall ($P = 0.0106$). The increase in oxidized flavor was associated with a decrease in aldehydes and an increase in the following ketones: (E,E)-3,5-heptadien-2-one, 4-penten-2-one, 1-(2-hydroxy-5-methylphenyl)-ethanone, and 1-cyclopropyl-1-propanone. It was hypothesized that $CaCl_2$ and NaCl fermented pickle products have similar volatile compound profiles due to the similarity in fermentation microorganisms. Differences in volatile compound profiles among the samples in this study were mainly attributed to bulk storage time and individual fermentation variability, but $CaCl_2$ fermented products did tend had higher amounts of compounds correlated to oxidized flavor. Green and earthy/musty flavors were significantly affected by individual fermentation variability ($P < 0.05$). Finished product $CaCl_2$ concentration had an effect on the perception of basic tastes, with 35 mM

$CaCl_2$ resulting in greater bitter and salty tastes than 21 mM $CaCl_2$ ($P < 0.05$). Despite the sensitivity of the trained panel, 35 mM is below the 50% detection threshold of $CaCl_2$ in hamburger dill chip pickles and consumer preference was not affected by 35 mM $CaCl_2$ in finished products. In conclusion, the environmentally-friendly $CaCl_2$ fermentations result in finished products with acceptable flavor characteristics when processed after shorter bulk storage times.

Consumer Acceptability and Flavor Characteristics of Cucumber Pickles Produced Using an Environmentally-Friendly Calcium Chloride Fermentation.

by
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DEDICATION

And whatever you do, in word or deed, do everything in the name of the Lord Jesus, giving thanks to God the Father through Him (Col. 3:17). I thank Jesus Christ, my Lord and Savior, for this wonderful opportunity for advancement. May the research that I completed be used to further advance the field of food science.

Additional thanks to:

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BIOGRAPHY

Emily Wolter was born in Wichita, KS on July 16, 1990 to David and Linda Wolter and moved to Kingwood, TX a few years later. Her childhood days involved wrestling with her big brother Danny, playing with American Girl Dolls, eating a lot of ice cream, and just loving life.

After graduating Kingwood High School, she attended Texas Tech University and received her Bachelor of Science in Food Science under the Science Option. During this time, she obtained an internship at Dynamic Foods, having the chance to work with some of the nicest people and gaining exposure to a wide variety of aspects within the food industry.

Emily left Lubbock, TX with plans to further her education through graduate school. Emily heard great things about the food science program at North Carolina State University and had met many of their students on the Student Association board of the Institute of Food Technologists (IFTSA) where she resided as the Vice President of Development and Communications for 2 years. After visiting the department at North Carolina State University, she packed up her belongings and made the 24 h road trip with her mom and beloved miniature Schnauzer, Molly, to get settled in Raleigh.

Not long after arriving in Raleigh, Emily met Stephen, and it was no secret that sparks flew on their very first date. Emily and Stephen are engaged to be married on January 10, 2014 and will move their lives to Plano, Texas where Emily will begin her career as a product developer under the title of R&D Associate Principal Scientist in the Frito Lay division of PepsiCo.

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CHAPTER 1 – Literature Review

1.1 The Taste of Calcium Salts in Foods: Implications for Sodium-Free Cucumber Fermentations

By: Emily M. Wolter and Suzanne D. Johanningsmeier

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Abstract

Calcium salts perform many functions in foods, including fortification, salt substitution, and texture retention. One example of calcium salt usage in the food industry is the potential use of calcium chloride as an environmentally-friendly alternative for brining cucumbers. The predominately bitter taste associated with calcium salts combined with its increasingly widespread use in foods necessitates research to understand the complex taste interactions that will determine the levels of calcium salts that can be successfully incorporated. This review highlights current research on the taste of calcium salts and the sensory impact in a variety of food applications.

Application of Calcium Salts in Foods

Calcium salts perform a variety of functions in foods, such as fortification, salt substitution, and texture retention. The addition of calcium salts to foods to augment the calcium intake of calcium deficient populations is currently of interest (Bailey and others 2010); the benefits and barriers associated with fortifying foods and beverages with calcium was recently reviewed by Rafferty and others (2007). Several food products fortified by the addition of calcium salts have been tested for consumer acceptability (Table 1.1). Calcium carbonate, calcium sulfate, and tricalcium dicitrate have been successfully added to pita breads at concentrations approximately 35-45% below their threshold in an effort to increase the calcium intake for populations that do not receive adequate intake from other sources (Ziadeh and others 2005). Calcium gluconate, calcium lactate gluconate, and calcium lactate were successfully added to apple juices at concentrations of approximately 31, 33, and 34 mM, respectively (Russel and others 2010). In addition, calcium gluconate and calcium lactate have been used to fortify milk with an optimal concentration of 12.4 mM, based on heat stability and sensory evaluation by 7 trained panelists (Singh and others 2007). A trained panel (n = 11) also found that approximately 12 mM calcium lactate added to fruit yogurt resulted in no negative taste impact (Singh and Muthukumarappan 2008). Calcium ascorbate, calcium chloride ($CaCl_2$), and calcium lactate have been experimentally used in beef marinades at 100, 200, and 300 mM; 100 mM calcium lactate, resulting in approximately 8.8 mM calcium in the finished cooked product, provided the best flavor enhancement and was recommended for inclusion in marinades for beef *longissimus* (Lawrence and others 2003). Marinating with $CaCl_2$ resulted in the greatest development of off-flavors ($P < 0.05$), namely bitter, metallic, sour, soapy, and astringent. Additionally, off-flavor intensity was significantly greater ($P < 0.05$) in 300 mM marinade treatments (calcium concentration in cooked product ranged from 33 to 37 mM) for all calcium salts tested, as compared to the marinades with lower concentrations (Lawrence and others 2003). Lawrence and others (2003) also discovered that calcium ascorbate inhibited lipid oxidation, while calcium lactate and $CaCl_2$ acted as pro-oxidants of lipid oxidation.

Calcium salts are most useful in the fortification of foods when used at concentrations below the detection threshold. The detection threshold (also known as absolute threshold) is defined as the lowest stimulus capable of producing a sensation. The detection threshold of each calcium salt will vary based on the complexity of the food matrix in which it is used. In turn, use of calcium salts in foods is an efficient means to augment consumer calcium intake, but requires additional research for each new food application to ensure no unforeseen interactions occur, such as those observed by Lawrence and others (2003), and that consumer perception of the finished product is not impacted. When used at levels above threshold for a specific food matrix, off-flavors tend to negatively affect the finished product. For example, Ziadeh and others (2005) determined the detection threshold of calcium carbonate, calcium citrate, and calcium sulfate in pita breads and panelists noted a number of objectionable attributes as concentrations increased above the threshold levels, including astringent, bitter, chalky, sour, metallic, irritating, and throat-catching sensations. A variety of food applications employing calcium salts in the fortification of foods without a negative impact on consumer acceptability of finished products are summarized in Table 1.1.

It is important to note that some calcium salts may be used at higher concentrations than others, such as the calcium carbonate and calcium sulfate used in pita breads at concentrations of 125 and 130 mM, respectively (Ziadeh and others 2005). Additionally, 5 panelists trained to evaluate texture of fermented cucumber pickles did not notice any off-flavors in samples with up to 37 mM calcium, which implies that the detection threshold of $CaCl_2$ in fermented pickle products may be greater than 37 mM (Buescher and others 2011). However, the detection thresholds of calcium salts and the impact of varying acid and salt concentrations in pickle products has not been reported. Adding calcium salts to foods requires extensive research as each product allows for different taste interactions and masking effects that influence detection thresholds and ultimately affect consumer perception of the finished product.

In addition to use in fortified foods, $CaCl_2$ (0.17 – 0.50% w/w) has been used in conjunction with magnesium chloride ($MgCl_2$) and potassium chloride (KCl) as a partial replacement for sodium chloride (NaCl) in reduced sodium food applications (Table 1.2). Doyle and Glass (2010) recently published a comprehensive review on the effects of sodium reduction on food safety, food quality, and human health. There is a push by influential organizations such as the American Heart Association, American Medical Association, and the Institute of Medicine for commercial food processors to reduce sodium in their products, but the process is not as simple as just cutting out salt; one must take into account how the reduction will affect the processing capabilities and sensorial properties of the finished product (Thilmany 2010). Berry (2010) also addresses the health-related push to reduce sodium in foods from an industry standpoint, highlighting the importance of understanding the chemistry of salt and other sodium-containing constituents within the food matrix to ensure altering these constituents does not negatively impact processing or consumer perception of the finished product.

In reduced sodium fermented sausages with 40% KCl replacing NaCl, a trained panel found the products to have a significant increase in bitter taste as compared to the control with no KCl (Gelabert and others 2003). However, Hooge and Chambers (2010) reduced the NaCl in chicken broth and tomato soup by approximately 48% with the addition of KCl at levels of 0.6–0.75% and 0.45–0.75%, respectively, with no significant increase in bitterness. Gimeno and others (1998) experimented with an approximately 48% reduction of NaCl in dried fermented sausages using a formulation that combined NaCl, KCl, MgCl, and $CaCl_2$ at levels of 1.00%, 0.55%, 0.23%, and 0.46%, respectively; 12 trained panelists described the resulting product as having a significant increase in color intensity and decrease in saltiness, but no significant off-flavors. Zanardi and others (2010) found a significant decrease in color intensity, decrease in saltiness, and increase in lipid oxidation when replacing 50% of NaCl in Italian salami with a blend of KCl, $CaCl_2$, and $MgCl_2$ at concentrations of 0.42%, 0.24%, and 0.24%, respectively. Horita and others (2011) partially replaced NaCl in reduced-fat mortadella and found that the most acceptable sensory properties were obtained from a combination of 1.0% NaCl, 0.5% KCl, and 0.5% $CaCl_2$. However, the emulsion stability of the product was compromised and further research into methods for stabilizing the emulsion is necessary. For fresh-pack cucumber pickles, a 20% reduction in NaCl was achieved by supplementation with 0.40% KCl, 0.17% $CaCl_2$, and 0.09% $MgCl_2$, along with 0.19% citric acid, and resulted in a product that was preferred over the 2.0% NaCl reference product in a paired comparison test (n = 104) (McFeeters and Fleming 1997). In the same study, a triangle test (n = 34) determined that no significant difference ($P < 0.05$) was detectable for up to a 40% NaCl reduction when 1.20% NaCl was supplemented with 0.80% KCl, 0.33% $CaCl_2$, and 0.19% $MgCl_2$.

For most of the studies mentioned above, the sensory quality of the products was assessed by trained panelists, but to determine the true potential of the formulations in the marketplace, consumer sensory panels would be necessary. An important discovery related to the future of sensory research on reduced sodium products was made by Lucas and others (2011); while reduced sodium hash browns were significantly less liked ($P < 0.05$) when evaluated by consumers in a laboratory/sensory booth setting, no significant difference ($P > 0.05$) in liking was detected when evaluated in a meal conducted in a lunch room-style setting. Additionally, the detection threshold for salt tends to be lower for younger subjects and female subjects (Hatae and others 2009), which points to the importance for gathering demographic information during sensory testing of reduced sodium food products and performing a more comprehensive analysis of the data.

Calcium salts have also been shown to improve texture or assist with texture retention of many food products (Table 1.3). The application of calcium salts for texture retention and shelf life extension of fruits and vegetables was reviewed recently by Martin-Diana and others (2007). Dominguez and others (2001) found a significant improvement in texture upon blanching rehydrated bell peppers in 360 mM $CaCl_2$ for 3 minutes at 65°C, followed by an additional blanch, after a 16 minute hold time, for 3 minutes at 95°C in 180 mM $CaCl_2$. Similarly, blanching jalapeno pepper halves in 300 mM $CaCl_2$ at 65°C for 4 minutes resulted in tissue firmness 4.3 times greater than that of the control (Palma-Zavala and others 2009). Papageorge and others (2003) found an equilibrated concentration of 5 mM $CaCl_2$ in both blanched and unblanched acidified red bell peppers to provide a significantly firmer texture. Further addition of $CaCl_2$ improved firmness in blanched peppers, but did not significantly change firmness of unblanched peppers. Goodarzi (2009) found that immersing fresh strawberries in 25 mM $CaCl_2$ or calcium sulfate resulted in a product with increased firmness and augmented calcium content, yet no difference in taste from the control strawberry receiving no blanching treatment. Furthermore, Martin-Diana and others (2006) found that washing iceberg lettuce in about 69 mM calcium lactate at 50°C for 1 minute resulted in improved crispness, extending the storage time to 12 days.

It is important to note that the inherent calcium content of vegetables varies widely; a study comparing 24 different vegetables found a strong positive correlation ($r = 0.93$, $P < 0.000001$) between bitterness and calcium content (Tordoff and Sandell 2009). Many of the studies summarized in Table 1.3 focused solely on texture and did not objectively evaluate taste. However, the relative inherent bitterness of vegetables (and other foods with inherently bitter constituents) will affect the level to which additional calcium salts can be added without negatively influencing consumer perception. Buescher and others (2011) noted that while 37 mM $CaCl_2$ improved mesocarp crispness of fermented cucumber pickles, the effect of elevated $CaCl_2$ on taste was unknown and in need of further research. Trained panelists described beef longissimus muscle with up to 37 mM $CaCl_2$ as bitter, metallic, sour, soapy, and astringent (Lawrence and others 2003). Furthermore, Goodarzi (2009) noted that bitterness became an issue as the concentration of calcium salts in strawberry blanching solutions applied to improve fruit firmness increased, especially up to 75 mM. The sensory experience involves taste, texture, and appearance. In turn, evaluating one attribute without the others limits the practical application of research in the food industry.

One major application of calcium salts in the food industry involves the use of $CaCl_2$ as a process aid for cucumber pickle fermentations to assist with texture retention; residual calcium concentrations of approximately 17 mM are commonly found in finished products (Buescher and others 2011). Additionally, a sodium-free fermentation process based on the texture retaining properties of $CaCl_2$ is being developed; brine containing $CaCl_2$ has been successfully used for fermentation of cucumbers in the absence of sodium chloride in laboratory trials (McFeeters and Perez-Diaz 2010) and is being experimentally implemented in commercial cucumber fermentations.

Sodium Chloride and Calcium Salts in Cucumber Fermentations

Cucumbers (*Cucumis sativus*) were first fermented in Mesopotamia about 4,500 years ago. Fermentation of vegetables began as a means of preservation for out-of-season use or for long trips at sea (Doyle and Beuchat 2007). 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 cucumbers being one of the primary products (Doyle and Beuchat 2007).

Cucumbers are chosen for pickle manufacturing based on size, maturity, cultivar, and physiology (Fleming 1984). Cucumbers are divided into classes based on the size of their diameter (1A $\leq$ 19 mm, 25 mm $\leq$ 2A $\leq$ 32 mm, 32 mm $\leq$ 2B $\leq$ 38 mm, and 39 mm $\leq$ size 3 $\leq$ 51 mm), with 2A and 2B sizes typically used for commercial fermentations (Lu and others

2002; McFeeters and Perez-Diaz 2010). Pickling cucumbers are commercially fermented in 40,000 L open-top, plastic or fiberglass tanks with a minimum of 5% salt (NaCl) in the fermentation brine. (Franco and others 2012). Most commercial fermentation processes occur under conditions of high salt concentration and oxygen exclusion, 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). Typically, tanks are located outdoors, so the time required for fermentation completion varies from about 3 weeks to 2 months, with a lower temperature corresponding to a longer fermentation time (Hutkins 2006). The finished pH of the fermented cucumbers must be less than 4.6, and typically is much lower around 3.5, with a lactic acid concentration of no less than 0.6% (Hutkins 2006; Fleming and others 1992).

Sodium chloride is essential to the commercial fermentation process for a number of reasons. Primarily, NaCl acts as a selection agent for proper microbial growth and inhibition of spoilage microorganisms during long term bulk storage (Franco and others 2012). In cold climates, to prevent freezing of tanks in the winter, NaCl may be used in concentrations as high as 12% in the fermentation brines (McFeeters and Perez-Diaz 2010). Retention of fruit firmness during processing and storage is attributed partly to NaCl, which helps to inhibit growth of microorganisms responsible for softening (Hutkins 2006). Technology is developing for controlled fermentation with pure starter cultures, allowing for faster fermentations and less added salt, but is not widely used (Fleming and others 2002; Jagannath and others 2012; Johanningsmeier and others 2007; Perez-Diaz and McFeeters 2011).

Only 2% to 3% salt is desired in the finished product, which results in the need to desalt the fermented cucumbers to remove NaCl, and consequently other nutrients and flavor compounds, prior to packing the finished product (Fleming 1984). Replacing some or all of the NaCl in cucumber fermentations has a number of beneficial ramifications. For example, the 2010 Dietary Guidelines for Americans proposed a lower daily sodium intake recommendation than is currently consumed by the average American, 2,300 mg per day as compared to 3,400 mg per day. The lower sodium recommendation was proposed by the Institute of Medicine as the Tolerable Upper Intake Level. Lower sodium consumption is a means to combat the prevalence of high blood pressure and cardiovascular disease in the U.S., since strong evidence supports that lower sodium intake leads to lower blood pressure (U.S. Department of Health and Human Services and U.S. Department of Agriculture 2010). Additionally, since fermented cucumbers must be desalted before processing into finished products due to the elevated NaCl concentrations required in natural fermentations, pickle plants produce large volumes of wastewater with high biological oxygen demand (McFeeters and Perez-Diaz 2010). The Environmental Protection Agency (EPA) has established National Secondary Drinking Water Regulations, declaring the secondary maximum contaminant level of chloride to be 250 mg/L, which is regulated and enforced individually by each state (U.S. Environmental Protection Agency 2009). The EPA checks for chlorides as a method to indirectly test the levels of NaCl expelled in waste water. Sodium, when present in the cell cytoplasm at high concentrations, is toxic to plants (Zhang and others 2010). The mechanism of entry of sodium into plant roots in high salinity environments is still not well defined (Kronzucker and Britto 2011). However, calcium may assist in preventing some degree of sodium toxicity. For example, in tomato plants grown under controlled conditions, receiving a nutrient solution containing 70.4 mM NaCl, addition of 20 mM Ca^{2+} resulted in a 70% reduction of sodium in the leaf tissue. (Montesano and van Iersel 2007). Furthermore, in ‘Picual’ olive cuttings grown in a controlled greenhouse with a nutrient solution, 75 mM NaCl, and varying levels of $CaCl_2$, shoot lengths were greater in plants grown with $CaCl_2$ up to concentrations of 40 mM. The reduced growth at 40 mM $CaCl_2$ is thought to be related to the high external total ion concentration (Melgar and others 2006). Replacing the 6-8% NaCl (equilibrated) used in fermentation brines with 1.1% $CaCl_2$ (equilibrated) would allow for an 80% reduction in the chloride levels found in pickle plant wastewaters (McFeeters and Perez-Diaz 2010), resulting in a more environmentally friendly process that would enable companies to more easily comply with regulations.

One potential drawback to sodium-free fermentations is that $CaCl_2$ fermented cucumbers currently employ an additional pre-processing desalting step (McFeeters and Perez-Diaz 2010), which requires more water and pre-processing time as compared to the single desalting step used with traditional NaCl fermentations. However, the additional desalting step is used as a precaution to lower the $CaCl_2$ content to the level of current commercial products to ensure no off-flavors are detected by consumers in the finished products. Obtaining a finished product with less than the 36 mM $CaCl_2$, the legal limit for pickled vegetable products (U.S. Food and Drug Administration 2011), is possible with one pre-processing desalting step, but the presence of off-flavors from the elevated $CaCl_2$ is uncertain. Future research in the pickle industry should include acceptability testing of pickles containing an equivalent calcium concentration as that of a $CaCl_2$ fermented pickle receiving one pre-processing desalting step to determine if consumers can detect any off-flavors in finished products. In addition, descriptive sensory analysis of $CaCl_2$ fermented pickle products would be beneficial for explaining the impact of elevated $CaCl_2$ on the sensory quality of finished products.

Sensory Characteristics of Calcium Salts

Sodium and calcium salts employ different taste transduction mechanisms, which affects the manner in which they are perceived by the taste buds (Liem and others 2011; McCaughey and Scott 1998; Tordoff and others 2012; Tordoff and others 2008; Tordoff, 2001; Gabriel and others 2009). This difference in perception leads to different taste sensations. In general, calcium salts are predominantly bitter and salty, with astringent, metallic, and irritative sensations contributing to a lesser extent (Lawless and others 2003). The detection of multiple tastes from a single stimulus, such as calcium salts, may be explained by the idea that a single compound is thought to be detected through more than one taste transduction pathway, with each pathway likely responding to multiple classes of stimuli (Herness and Gilbertson 1999). Generally, bitterness and sourness of a salt increase as the atomic weight of the anion or cation increases, dominating salty taste; Murphy and others (1981) discovered that sodium chloride and lithium chloride were the only salts perceived as predominantly salty of 15 salts evaluated. Divalent salts, such as $CaCl_2$, have complex sensory characteristics resulting from a combination of retronasal, gustatory, and tactile sensations (Lim and Lawless 2005).

For most inorganic salts, the cation is the determining factor in the overall sensory profile (Lim and Lawless 2005; Tordoff 1996; Yang and Lawless 2005). In an animal model, Tordoff (1994) compared consumption of water containing varying concentrations of calcium salts and other mineral salts. Consumption of the organic and inorganic calcium salts was most influenced by the common calcium cation and no significant difference in consumption was observed for 5 organic and inorganic calcium salt solutions at equimolar concentrations (2 mM). However, this influential nature of the cation appears to vary based on the salts being employed, the sample matrix, and the resulting interactions. Ball and others (2002) found no significant effect of cation on liking scores in an applied study of reduced sodium soups containing either calcium diglutamate or monosodium glutamate (MSG) at concentrations up to 43 mM. Additionally, Yang and Lawless (2005) have shown that the bitterness of calcium salts is dependent on their anion. In a qualitative study employing multidimensional scaling and cluster analysis, divalent salts with a chloride anion were positioned near quinine, a characteristically bitter compound, implying that the chloride component correlated to bitterness (Lim and Lawless 2005). One study using a descriptive analysis panel and magnitude estimation scaling found a 32 mM solution of $CaCl_2$ in distilled water to be about four times as bitter as salty (Van Der Klaauw and Smith 1995). Additionally, 7 trained panelists described a 20 mM solution of $CaCl_2$ as having a significant bitter characteristic (Yang and Lawless 2005). Tordoff (1994) observed that mineral chlorides with a larger molecular weight were less preferred in a rat study. This may be related to the discovery of Murphy and others (1981) that humans tends to describe salts with heavier cations as more bitter, while those with lighter cations tend to be saltier. It is important to note that detection of bitterness is variable across the population with certain groups being classified as non-tasters, while others are supertasters, capable of detecting bitter taste in what is generally sub-threshold concentrations (Lawless and others 2000). This variability in bitterness will affect decisions by food processors when establishing their threshold for bitter compounds in specific products; for example, processors may choose to use calcium salts below the threshold determined by a consumer threshold test to ensure that the majority of the population, including supertasters, will not perceive a negative impact on flavor. This process is highlighted by Ziadeh and others (2005), who determined through a series of triangle tests that calcium carbonate, calcium citrate, and calcium sulfate should be used in fortified pita breads at concentrations approximately 35 – 45% lower than the calculated detection threshold concentrations to obtain a product similar (P < 0.01) to traditional pita bread as assessed by 108 consumers.

Calcium Chloride Threshold

Concentration of $CaCl_2$ is important when considering the characteristics of the salt in solution. Tordoff (1996) employed a panel of 18 women, thought to be more sensitive to calcium than men, to determine the detection threshold of 5 different calcium salts in deionized water solutions: phosphate (.020 mM), hydroxide (0.011 mM), chloride (0.008 mM), lactate (0.050 mM), and gluconate (0.009 mM). The detection threshold of $CaCl_2$ in distilled water systems has been thoroughly researched; the threshold concentration is quite variable among studies and greater than that found by Tordoff (1996), ranging from 2.3 to 6.1 mM (Anderson, 1955; Neyraud and Dransfield, 2004; as cited in Zoeteman and others 1978). At a subliminal threshold of 1 mM, $CaCl_2$ imparts bitter, sour, and sweet taste sensations, while at a suprathreshold concentration of 100 mM, bitter, salty, and sour are the dominate tastes perceived (Tordoff 1996). The detection threshold of $CaCl_2$ in fermented cucumber pickles has not yet been determined, but is of importance when determining the extent to which $CaCl_2$ fermented cucumbers must be desalted before processing to ensure consumers perceive the finished products as acceptable. Further research is needed to determine the maximum concentration of $CaCl_2$ that can be incorporated in finished pickle products without negatively impacting consumer perception of the product.

Calcium lactate is an alternative ingredient to $CaCl_2$ that provides a somewhat different taste profile. Calcium lactate is generally recognized as safe (GRAS) and can be used in food products without limitation under good manufacturing practices. Calcium chloride has regulatory limitations depending on the food product in which it is used; for processed vegetables, $CaCl_2$ cannot exceed 0.4% or 36 mM (U.S. Food and Drug Administration, 2011). Bitterness is distinctly greater in $CaCl_2$ as compared to calcium lactate, likely due to anionic inhibition in which the larger, organic lactate anion possibly inhibits the attachment of calcium to taste receptors (Yang and Lawless 2005; Lawless and others 2003; Tordoff 1996; Nakamura and Kurihara 1991). Calcium lactate is also characteristically more sour and less salty than $CaCl_2$, which is likely due to the production of lactic acid upon dissociation of calcium lactate in solution (Tordoff 1996). In a study evaluating the basic taste profiles of $CaCl_2$ and calcium lactate at 6 different concentrations within the range of 0 to 100 mM, 100 mM $CaCl_2$ was the only solution that was distinctly salty (Tordoff 1996). Therefore, the chloride ion is thought to play some role in salty taste and tends to increase bitterness (Breslin and Spector 2008; Tordoff 1996), which is in agreement with Lim and Lawless (2005) who found that divalent salts with a chloride ion tend to have a bitter characteristic.

Future threshold work with non-sodium salt replacers should focus on fundamental research that would be beneficial in application studies across the food industry. For example, the detection threshold for a number of potassium salts was determined by Schiffman and others (1995), who also discovered that the threshold for both sodium and potassium salts could be theoretically calculated using the charge mobility of the anion due to the high correlation between the detection thresholds and molar conductivity of the anion. To make fundamental studies such as that conducted by Schiffman and others (1995) more applicable to food systems, it would be beneficial to test the findings in complex media involving other constituents, such as NaCl and acids, to better account for the interactions inherently present in most food systems. For example, Green and others (2010) discovered that for all possible mixtures of sucrose, NaCl, citric acid, and quinine sulfate, sucrose tended to be the least suppressed taste compound and greatest suppressor.

Taste Interactions

Successful application of calcium salts in foods is challenging due to the high degree of flavor interactions taking place in the food matrix. When considering the combination of NaCl, acetic acid, and $CaCl_2$, such as that encountered in a pickle product, it is important to consider the possibility of suppression and masking effects. Suppression is a linear, subtractive process, while masking is a nonlinear process that involves the decrease in intensity of one compound due to the addition of another compound (Breslin 1996). Mixtures of weakly suprathreshold concentrations of acids and salts tend to display enhancing effects on the taste of the two compounds. Alternatively, at strongly suprathreshold concentrations, no obvious interaction or suppression is likely to be observed (Breslin 1996). For example, Hatae and others (2009) found that rice vinegar added to NaCl solutions at subliminal concentrations of half the concentration of the threshold for each panelist intensified salty taste. Additionally, NaCl and MSG at suprathreshold concentrations have been shown to reduce perceived sweetness and bitterness of pure solutions of sucrose and quinine sulfate at concentrations of moderate strength, 0.05 M and 2.5 x 10^{-5} M, respectively (Kemp and Beauchamp 1994). Kemp and Beauchamp (1994) also found that NaCl and MSG at suprathreshold concentrations altered perception of flavoring compounds. Mint flavor (0.67% v/v) decreased as NaCl concentration increased from 0.001 M to 0.171 M NaCl, but NaCl had no significant effect on imitation butter flavor, imitation pistachio flavor, lemon extract, or celery extract (1% v/v). Similarly, at suprathreshold concentrations, MSG showed a significant suppression effect for mint flavor, in addition to lemon flavor. At sub-threshold concentrations, NaCl and MSG had no significant effect on odor, taste, or flavor (Kemp and Beauchamp 1994).

In general, sodium salts have been shown to suppress the bitter taste of compounds, such as urea, with non-sodium chloride salts unable to perform in the same manner (Breslin and Beauchamp 1995). This has also proven true in pharmaceutical applications in which sodium was the most effective cation at suppressing bitterness as compared to 4 other cations when added as acetate salts (Keast and Breslin 2002). In turn, in a food application, such as a pickle containing moderate levels of NaCl in addition to elevated levels of $CaCl_2$, the characteristic bitterness of $CaCl_2$ may be suppressed. However, in a recent study, NaCl at concentrations as high as 20 to 40 mg/100 g was not effective in reducing bitter taste in fruit smoothies containing bitter olive leaf extracts with polyphenol levels of 20 mg/100 g, while sodium cyclamate and sucrose proved successful in reducing bitterness by 39.9% and 24.9%, respectively (Kranz and others 2010). This highlights the importance of product specific research when incorporating bitter compounds in food products, as there is a substantial amount of variation in the taste impact, related to both the food matrix and the chemical properties of the bitter compound. Therefore, a compound that masks bitterness in one food application may not provide the same effect in another food matrix with differing chemical properties.

Keast and others (2003) reported that the taste impact of two bitter compounds is generally additive when combined. Furthermore, Keast and others (2004) evaluated bitter suppression by sodium salts of both singular solutions and binary mixtures of 5 different bitter compounds and made an interesting discovery that the bitterness of the binary mixtures was generally predictable based on the bitterness suppression of the compounds when tested individually. The seemingly additive nature of bitterness suppression is beneficial knowledge for product developers, as it may help to reduce the inherent complications associated with formulating a food product with added bitter compounds. It would be beneficial to know if this additive nature of bitter compounds holds true in more complex food systems. In addition to limiting the concentration of a bitter compound in food products or taking advantage of masking and suppression interactions already present in foods, bitter masking compounds may be added to reduce the bitterness of certain constituents added to food. Ley (2008) recently published a comprehensive review of this topic that highlights how bitter taste compounds are detected within the mouth, possible bitter masking constituents for use in food applications (i.e. strong flavors, polymers/complexing agents, and low molecular weight compounds), and the consequences to food processors of adding bitter masking molecules.

Conclusions

The application of calcium salts in food products is beneficial for a variety of reasons including calcium fortification, NaCl replacement, and texture retention, which explains the increasingly widespread use; but food processors must be aware of the different taste properties and detection thresholds of the varying calcium salts in specific food products, especially when used as a replacement for NaCl. In addition to considering the differing taste properties of each salt, product developers must be knowledgeable of the chemical ramifications and potential processing challenges associated with removing NaCl or adding calcium salts to a food matrix, have an understanding of taste interactions among food constituents, and be equipped to perform adequate sensory testing to determine the ultimate impact these alterations have on consumer perception. Future research in these areas will equip food product developers with knowledge, such that practical sodium reduction strategies and higher quality food products with improved texture or enhanced nutrition can be more readily implemented by commercial processors.

Keywords

Calcium chloride; calcium salts; taste interactions; environmentally friendly fermentation; calcium chloride threshold; sensory attributes

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Table 1.1 – Calcium salts added to various food matrices for the purpose of calcium fortification without significant change in finished product acceptability

Food Matrix	Calcium Salt	Concentration (mM)	Concentration (% w/w)	Reference
Beef *longissimus* Marinades	Calcium lactate	100 (in marinade) 9 (in cooked product)	2.18 0.20	Lawrence and others (2003)
Apple Juice	Calcium lactate Calcium lactate gluconate Calcium gluconate	34 33 31	0.74 1.07 1.33	Russel and others (2010)
Milk	Calcium lactate Calcium gluconate	12 12	0.26 0.52	Singh and others (2007)
Fruit Yogurt	Calcium lactate	12	0.26	Singh and others (2008)
Pita Bread	Calcium carbonate Calcium sulfate Tricalcium dicitrate	125 130 23	1.25 1.77 1.16	Ziadeh and others (2005)

Table 1.2 – Combinations of calcium chloride ($CaCl_2$), potassium chloride (KCl), and magnesium chloride (MgCl) added to various food matrices as a replacement for sodium chloride (NaCl)

Food Matrix	Salt	Concentration (mM)	Concentration (% w/w)	% NaCl Reduction	Reference
Dried Fermented Sausages	$CaCl_2$	41	0.46	48.40	Gimeno and others (1998)
	KCl	149	0.55		
	$MgCl_2$	19	0.23		
	NaCl	171	1.00		
Reduced-Fat Mortadella	$CaCl_2$	45	0.50	50.00	Horita and others (2011)
	KCl	67	0.50		
	NaCl	171	1.00		
Fresh-Pack Cucumber Pickles	$CaCl_2$	30	0.33	40.00	McFeeters and Fleming (1997)
	KCl	107	0.80		
	$MgCl_2$	20	0.19		
	NaCl	205	1.20		
Italian Salami	$CaCl_2$	22	0.24	40.00	Zanardi and others (2010)
	KCl	56	0.42		
	$MgCl_2$	25	0.24		
	NaCl	231	1.35		

Table 1.3 – Calcium salt solutions applied to various food matrices to assist with texture retention or improvement

Food Matrix	Calcium Salt	Concentration (mM)	Concentration (% w/w)	Reference
Fermented Cucumber Pickles	Calcium chloride	37[a]	0.41	Buescher and others (2011)
Jalapeno Pepper Halves	Calcium chloride	300	3.33	Palma-Zavala and others (2009)
Acidified Red Bell Peppers	Calcium chloride	5[a]	0.06	Papageorge and others (2003)
Rehydrated Dried Bell Peppers	Calcium chloride	360, 180	4.0, 2.0	Dominguez and others (2001)
Strawberries	Calcium chloride Calcium sulfate	25 25	0.28 0.34	Goodarzi (2009)
Iceberg Lettuce	Calcium lactate	69	1.50	Martin-Diana and others (2006)
Beef *longissimus* Marinades	Calcium lactate	100	2.18	Lawrence and others (2003)

[a]Calcium chloride concentration in finished product. All other concentrations refer to calcium chloride concentration in washing/blanching solution.

1.2 Sensory Analysis Methods

Product developers share a common goal to create a safe and abundant food supply in an efficient and environmentally-friendly manner that will satisfy the consumer. In turn, consumer testing is a crucial step prior to the implementation of any new product or process on a large scale. One must ensure that consumer acceptability of the product is high enough to create a demand and support growth. To address this, several consumer sensory testing methods have been developed.

Difference Testing

Difference testing is one type of consumer testing in which consumers are provided with samples in one of a variety of test assessments to determine whether or not they can discern a difference. Difference testing can assess either an overall difference between products or focus on the difference of specific attributes. Some of the overall difference tests widely used in sensory testing today include: triangle test, duo-trio test, two-out-of-five test, same/different test, “A”-“Not A” test, and difference-from-control test (Meilgaard and others 2007). Each test caters to different products, scenarios, and research objectives. For example, while the triangle test may be statistically more powerful than the paired comparison or duo-trio tests, the triangle test has limited use with products that are conducive to carry-over, fatigue, or adaptation; the same/different test or “A”-“Not A” test are more appropriate for these types of products. Additionally, some tests require little to no training (triangle, paired comparison, same/different), while other tests (duo-trio, “A”-“Not A”, two-out-of-five, difference-from-control) involve training or familiarization with a reference sample (Meilgaard and others 2007).

The "A"-"Not A" difference test was designed for comparing products with high variability in sample appearance or aftertaste (ISO 8588:1987). Therefore, the "A"-"Not A" difference test is ideal for pickle products, which tend to have chip-to-chip variability, even within the same treatment, and a lingering aftertaste. The test begins with panelists tasting two reference samples labeled "A" and "Not A" to familiarize themselves with the two treatments being compared. When the panelist is ready, the samples are removed and not returned. Then, unknown samples labeled with a random 3-digit code, an equal number of "A" and "Not A" samples, are presented to the panelists in a randomized order for identification as either "A" or "Not A", based on the reference samples that they tasted previously (ISO 8588:1987). The "A"-"Not A" test has been regarded as an extended same/different test that relies heavily on memory and, therefore, if a large number of unknowns are presented, it may be beneficial to provide "A" references at predetermined times during the assessment of the unknowns (Peryam 1958). Furthermore, a variation of the test in which only an "A" reference is provided to panelists exists, but is not recommended because this method encourages use of a τ-cognitive strategy, as opposed to the β-cognitive strategy promoted by the presentation of both references (Meilgaard and others 2007; Santosa and others 2011). A τ-cognitive strategy has been described as a sensory yardstick in which the panelist compares the stimuli to the reference based on the "length" of their yardstick. The length determined by the panelists is uncontrolled by the researcher and, therefore, creates response bias (Santosa and others 2011). Alternatively, the β-cognitive strategy involves tasting both references and drawing an imaginary line between the products. Panelists taste unknown samples and, based on the perceived stimuli, determine which side of the line the unknowns fall on. Once again, the position of the imaginary line in the panelist's mind is uncontrolled by the researcher, so results should be analyzed using statistics based on signal detection theory (Santosa and others 2011, Swets 1996).

Data analysis for the "A"-"Not A" test involves use of the chi-squared test. If the resulting p-value is less than the selected alpha level (i.e. 0.05), the null hypothesis is rejected, indicating that there is evidence of a significant difference between the treatments (Bower 2009).

Preference Testing

In addition to determining if an overall difference exists between samples, obtaining information on consumer preference is also of importance. Preference tests do not provide any information on degree of liking, but are well-suited to situations in which one product is being directly compared to another, such as would be the case with an ingredient substitution (Meilgaard and others 2007). Paired preference tests are well described in the sensory literature; in summary, the test involves giving panelists two randomly coded samples and asking them to choose which sample they prefer (Lawless and Heymann 1998). Preference test data can be analyzed using the binomial test or chi-square test, which provide similar results. However, the chi-square test, in its uncorrected form, is statistically more powerful (Bower 2009).

One important consideration when performing a preference test is whether to make the test forced-choice or to allow a "no preference" option, which enables one to distinguish between a true no preference or a split-preference, but makes data analysis much more complex since the binomial, chi-square, and Z-distributions depend on the normality of the data associated with a forced-choice test (Ennis and Ennis 2012). Alfaro-Rodriguez and others (2005) recommend including the "no preference" option, but Chapman and Lawless (2005) debate whether the "no preference" option is worth the trouble, since it will also result in a decrease in statistical power. There are four options cited in the literature (Chapman and Lawless 2005; Ennis and Ennis 2012; May and Johnson 1997; Quesenberry and Hurst 1964) for statistical analysis of "no preference" responses, with ignoring the "no preference" votes being the most statistically powerful option, provided the "no preference" responses are not too large.

Hedonic Liking Testing

Performing a hedonic consumer liking test, also known as acceptance testing, enables one to gauge how much consumers like a product, either overall or for a specific attribute. Consumers' preferences can be inferred based on which products receive a higher liking score (Meilgaard and others 2007). A liking test is typically performed using a hedonic scale, which ranges from 1 to 9 with anchor labels of "dislike extremely" to "like extremely" and the middle point labeled with a 5 and "neither like nor dislike". Gacula, Jr. and Rutenbeck (2006) performed a simulated consumer study and determined that 40 consumers was sufficient to detect a difference of 0.6 to 1.0 on a 9-point hedonic scale with a power of greater than 80% at a confidence level (α-level) of 0.05. Moeller (2012) determined that the standard deviation for a consumer liking test of hamburger dill chip pickles was approximately 2.2. In turn, to detect a 1-point difference between 4 hamburger dill chip pickle samples in a liking test with 80% power, 72 consumers would be required (Appendix B).

Detection Threshold Testing

In food applications, the detection threshold refers to the lowest concentration of a certain food constituent capable of producing a detectable sensation – taste, flavor, aroma, etc. (Meilgaard and others 2007). Taste and flavor detection thresholds can be difficult to determine due to the high degree of complex interactions in food products. Senthil and Bhat (2011) found the detection threshold of cardamom oleoresin to be significantly greater in milk than in either water or a 2.5% sugar solution. Furtherrmore, Lawless and others (2000) found the detection threshold of capsaicin to be significantly greater in oil than water. While threshold tests are typically performed using an aqueous matrix to allow for controlled experiments with fewer interactions, a more practical approach may also be taken by performing the test directly within the food matrix of interest, such as the work performed by Ziadeh and others (2005) to determine the threshold of calcium salts in fortified pita breads. In addition, certain food ingredients, such as iron salts, may contribute a combination of retronasal, gustatory, and tactile sensations, which further complicates the human perception and resulting detection threshold (Lim and Lawless 2006).

Selection of Consumers and Power Calculations

Demographic information of importance to the study at hand is typically obtained from participants to determine if any specific characteristics (age, gender, ethnicity, consumption frequency, etc.) correlate with results. For example, Omary and others (2009) found age, gender, ethnicity, frequency of eating chocolate chip cookies, daily effort to include fiber in the diet, typical consumption of reduced-fat cookies, and texture preference for cookies to all have an effect on consumer acceptability of chocolate chip cookies prepared with varying levels of high-soluble fiber whole barley flour. Data shows that the largest group of cucumber pickle consumers in the United States is males aged 20 to 59, with senior citizens consuming the least (Lucier and Lin 2000).

To determine the number of consumers required to have sufficient statistical power, it is important to balance four parameters: α-level, β-level, standard error of the experiment, and the expected difference in means (Hough and others 2006). In difference testing, one aims to keep the α-level, probability of detecting a difference when one does not exist, low. This controls for a Type I error, rejecting the null hypothesis given that it is correct (Bower 2009). Alternatively, a similarity test controls for the β-level, probability of missing a difference that truly exists, also described as a Type II error, failing to reject the null hypothesis given that it is false (Meilgaard and others 2007; Bower 2009). One way to evaluate these parameters for a particular experiment is through use of a power test in a statistical program, such as SAS statistical software (version 9.3, SAS Institute Inc., Cary, NC), controlling for the four parameters specified above. Consumer sensory tests typically select a confidence level (α-level) of 95% or 99% and a power of 80% or greater (Mazzocchi 2008).

Descriptive Sensory Analysis

In an effort to better explain consumer preferences and instrumental data, descriptive sensory analysis panels are of particular utility to researchers in the food industry (reviewed by Murray and others 2001). Descriptive sensory analysis uses a trained panel of 6 to 12 people acting as a single instrument to evaluate products for sensory attributes of importance (reviewed by Drake 2007). To ensure a panel will be discriminatory, Heymann and others

(2012) recommended having a minimum of 10 panelists. This recommendation was made after analyzing sample means from smaller subsets of panelists from 3 different trained panels consisting of 14 to 22 panelists and determining if means were significantly different from that of the full panel. Gacula, Jr. and Rutenbeck (2006) provided evidence through a computer simulation study that a minimum of 5 trained panelists can be used to provide sufficient power of greater than 0.99 to detect a difference of 1 point on a 15-point scale at an alpha level of 0.05 or 0.10. However, the standard deviation among panelists was not specified and would greatly affect the required sample size for sufficient power.

There are a number of descriptive analysis methods, each requiring different levels of training and pursuing different objectives; a study focusing on evaluating specific attributes may employ the Flavor Profile Method or Texture Profile Method. To perform comprehensive descriptive analysis using a product specific scale, the Quantitative Descriptive Analysis (QDA) method would be appropriate, while the Spectrum™ Method employs a universal intensity scale to evaluate samples, equipping panelists for evaluation of a variety of products (Lawless and Heymann 1998).

Spectrum™ Descriptive Sensory Analysis Method

The Sensory Spectrum™ Method was created by Gail Civille of General Foods in the 1970’s with inspiration from the Texture Profile Method previously created in the 1960’s by scientists at General Foods (Lawless and Heymann 1998). The Spectrum™ Method makes use of a highly trained panel scaling attributes on a universal 15-point, standardized scale that enables cross-comparison of results across laboratories. The scaling procedure is absolute, anchored by multiple food and chemical references, and uses an intensity scale in which a score of 5 for salty is theoretically of equal intensity to a 5 for sour and all other attributes (Meilgaard and others 2007). The panelist training procedure is rigorous, with the ultimate goal of training panelists to act in unison as an instrument. Descriptive sensory analysis results help to bridge the gap between instrumental and consumer data, allowing for a more comprehensive interpretation of the data (reviewed by Drake 2007). The Spectrum™ Method, including modified versions of the method, is well-established for use in sensory testing of food products and has been used to evaluate a wide variety of products, such as cheese (Drake and others 2001), peanut butter (McNeill and others 2002), soybeans (Krinsky and others 2006), and sweet potatoes (Leksrisompong and others 2012). Panelists are trained to scale taste and flavor attributes using reference solutions and food products.

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1.3 Oxidized Flavor in Cucumber Pickles

Oxidized flavor is an off-flavor of particular concern for pickle products. By definition, oxidation is simply the loss of electrons from a compound (Smith 2008). The oxidation of polyunsaturated fatty acids is of particular concern in flavor chemistry (Andreou and Feussner 2009). Oxidation of unsaturated lipids is the process in which carbon-centered alkyl radicals and peroxy radicals are formed due to the presence of initiators, such as enzymes, light, heat, metals, metalloproteins, and microorganisms. After initiation, propagation in the presence of oxygen leads to the formation of hydroperoxides as the primary products (St. Angelo 1992). In the presence of light, a non-free radical process, known as photooxidation, may form hydroperoxides due to the reaction of unsaturated fatty acids with singlet oxygen resulting from the excitation of oxygen by a photosensitizer, such as chlorophyll (Frankel 1991). For plant tissues, the polyunsaturated fatty acids found in greatest abundance are linoleic and linolenic acid (Baysal and Demirdoven 2007). After completion of cucumber fermentation, Pederson and others (1964) observed a 5-fold increase in free fatty acids and a reduction in phospholipids greater than 90% of that found in the raw cucumber. Linoleic acid was found in the flesh, skin and seed of raw cucumbers at concentrations of 11, 38, and 26 mg/100 g, respectively. After fermentation, linoleic acid in the flesh, skin, and seed changed to 215, 444, and 908 mg/100 g, respectively. Similarly, linolenic acid was found in the flesh, skin and seed of raw cucumbers at concentrations of 10, 255, and 18 mg/100 g, respectively. After fermentation, the concentration of linolenic acid in the flesh, skin, and seed changed to 450, 545, and 1067 mg/100 g, respectively.

During fermentation, cucumbers are susceptible to oxidation due to the nature of commercial fermentations with open tanks exposed to the sunlight and the common practice of purging the tanks with air to mix the contents and remove carbon dioxide (Buescher and Hamilton 2000). Additionally, cucumbers and the salt used for brining contain trace amounts of metals, such as iron, zinc, and copper, which can play a role in promoting oxidation of pigments and flavor compounds (Eisenstat and Fabien 1953).

Volatile Flavor Compounds in Fresh Cucumbers

Cho and Buescher (2011) found that fresh cucumber juice contained ethanol, propanal, (E)-2-pentenal, hexanal, (E)-2-hexenal, and (Z)-6-nonenal. The characteristic aroma of fresh cucumbers has been mainly attributed to (E,Z)-2,6-nonadienal and E-2-nonenal, which are enzymatically synthesized by the action of lipoxygenase during tissue disruption from linolenic and linoleic acid, respectively (Buescher and Buescher 2001). E-2-nonenal has been shown to have approximately 2% of the odor impact of (E,Z)-2,6-nonadienal (Xu and others 2012). These fresh cucumber flavors are not typical of fermented cucumbers due to the inactivation of lipoxygenase under the low pH conditions of fermentation (Wardale and Lambert 1980). Furthermore, Buescher and Buescher (2001) attributed the observed loss of (E,Z)-2,6-nonadienal production when fresh cucumbers were frozen to -20°C or pasteurized to the inactivation of lipoxygenase. Interestingly, significantly less (E,Z)-2,6-nonadienal is produced in the exocarp as compared to the mesocarp and endorcarp tissues, which explains why smaller diameter fruits are associated with less (E,Z)-2,6-nonadienal production (Buescher and Buescher 2001). Exocarp tissues have higher levels of lipoxygenase, hydroperoxide lyase, and unsaturated fatty acids; low (E,Z)-2,6-nonadienal production by exocarp tissues is likely attributed to limited substrate, enzyme inhibitors, or production with concomitant degradation (Wardale and others 1978; Wardale and Lambert 1980; Pederson and others 1964; Buescher and Buescher 2001). Palma-Harris and others (2002) demonstrated that an increase in (E,Z)-2,6-nonadienal resulted in an increase in the intensity of “fresh cucumber flavor” in refrigerated cucumber pickles as assessed by 24 trained panelists.

Linoleic acid is believed to be the precursor of hexanal, (E)-2-heptenal, (E)-2-octenal, and E-2-nonenal; while propanal, (E)-2-hexenal, (E)-2-pentenal, ethanal, and (E,Z)-2,6-nonadienal are formed from linolenic acid (Grosch and Schwarz 1971; Zhou and others 2000). The production of these flavor aldehydes is hypothesized to include breakage of double bonds in the unsaturated fatty acids by a dioxygenase-like reaction and the formation of hydroperoxide intermediates (Grosch and Schwarz 1971; Zhou and others 2000). Of these compounds, (E,Z)-2,6-nonadienal and hexanal were identified in fermented cucumber brines with characteristic aromas of fresh cucumber and green, respectively (Marsili and Miller 2000). Additionally, (E)-2-nonenal and (E)-2-octenal were identified by Cordero and others (2010) in roasted hazelnuts with fatty/green and green aromas, respectively.

Cucumbers have a very high fatty acid α-oxidation activity, in which fatty acids are enzymatically broken down into $C_{(n-1)}$ long-chain fatty aldehydes and carbon dioxide (Borge and others 1998). Pentadecanal has been identified as the product of α-oxidation of palmitic acid in cucumber homogenate (Borge and others 1998). Due to the high water activity of cucumber pickles, oxidation of these products likely involves enzymatic or microbial mechanisms; direct, non-enzymatic oxidation tends to be outcompeted by other modes of deterioration at high water activities (St. Angelo 1992).

Volatile Flavor Compounds in Fermented Cucumbers

A number of secondary fermentation reactions occur in fermented cucumber pickles, which may contribute to the flavor profile of finished products. Fermented cucumbers evaluated straight from the tank yard have been described as silage-like, sour, slightly sweet, and green (Marsili and Miller 2000). Marsili and Miller (2000) identified trans- and cis-4-

hexenoic acid, low volatility compounds, as key flavor compounds with aromas characteristic of fermented cucumber brine by gas chromatography-olfactometry; additional compounds with high odor impact values in fermented cucumber brines included 2-heptanol, cis-2,4-hexadienoic acid (tentative identification), phenyl ethyl alcohol, 2,6-nonadienal, and 2-dodecen-1-al (tentative identification). While pure solutions of trans-4-hexenoic acid were characterized as similar to authentic brine samples, addition of phenyl ethyl alcohol (a rose/floral note) to the pure solution resulted in a closer match. Phenylacetaldehyde was also present in brine samples and is produced by oxidation of phenyl ethyl alcohol (Marsili and Miller 2000). Murray and Whitefield (1975) reported the presence of 3-isopropyl-2-methoxypyrazine in fresh cucumbers; since the compound does not change significantly during fermentation and has an odor threshold of only 2 ppt, it likely plays a role in the overall aroma profile of fermented cucumbers (Zhou and McFeeters 1998; Whitefield and Last 1991).

Previous work by Zhou and McFeeters (1998) identified high levels of linalool in 2% salt fermentation brines, but Marsili and Miller (2000) observed linalool in only 5% of brine samples with the traditional 8-10% salt concentration, indicating linalool is likely not a major aroma impact compound in traditional fermented cucumbers; varying salt levels in fermentations could alter the microflora, which would impact the resulting fermentation by-products that contribute to flavor and aroma (Marsili and Miller 2000). Zhou and McFeeters (1998) compared volatile compounds found in fresh cucumbers to those in fermented cucumbers; ethyl benzene, *o*-xylene, and benzaldehyde were the only compounds identified in fermented cucumbers that were not present in fresh cucumbers. Four compounds of interest were found in lower concentrations in fermented cucumbers, as compared to fresh cucumbers: hexanal, (E)-3,7-dimethyl-1,3,6-octatriene, (E,Z)-2,6-nonadienal, and 2-undecanone (Zhou and McFeeters 1998). Acetic acid is commonly added to fermentation cover brines; while ethyl acetate is a component of acetic acid, the compound also appears to form during fermentation (Zhou and McFeeters 1998).

"Green" is a note sometimes described by descriptive analysis panels evaluating cucumber pickles. To identify the volatile compounds contributing to this flavor attribute, it may be beneficial to consider the 8 volatile compounds characteristic of the green odor emitted by plant leaves: (Z)-3-hexenol, (E)-3-hexenol, (E)-2-hexenol, (Z)-3-hexenal, (E)-3-hexenal, (E)-2-hexenal, hexanol, and hexanal (Hatanaka 1996). In addition, Forss and others (1962) found that (E,Z)-2,6-decadienal was characterized by the flavor notes of "green," "plant-like," and "like cucumbers." After further review of the literature, Forss and others (1962) hypothesized that the cis-nonconjugated unsaturation is characteristic of a compound that produces a "green" or "plant-like" flavor. In addition, Forss and others (1962) evaluated the characteristic cucumber flavor compounds near their threshold concentrations and determined that 2-nonenal and (Z,Z)-2,6-nonadienal were described as "oily" and "tallow", while (Z,E)-2,6-nonadienal was described as "green" or "like cucumbers". As the concentration of the compounds increased, panelists gave the description of "like cucumbers" to all three compounds.

Venkateshwarlu and others (2004) modeled oxidative off-flavors commonly found in fish oil by mixing pure volatile flavor compounds in milk and evaluating the intensity of fishy and metallic off-flavors in these samples. A trained 16-member descriptive analysis panel determined that (E,Z)-2,6-nonadienal and 1-penten-3-one were the 2 main compounds characteristic of metallic and fishy off-flavors; although, the fishy and metallic flavors were not present when the compounds were added to the milk alone, confirming the importance of compound interaction in flavor production. In addition, the metallic odor of penten-3-one was enhanced in the presence of heptenal, while a synergistic relationship between (E,Z)-2,6-nonadienal and (Z)-4-heptenal contributed to fishy off-flavors (Venkateshwarlu and others 2004).

Cleary and McFeeters (2006) demonstrated that introduction of oxygen into fresh-pack dill pickles under anaerobic conditions resulted in increased levels of hexanal, heptanal, and pentanal. In addition, Zhou and others (2000) observed a significant increase in hexanal and (E)-2-heptenal when fermented cucumber slurries were incubated in the presence of oxygen. Compounds initially undetectable in the slurries were observed after oxygen exposure including (E)-2-pentenal, (E)-2-hexenal, and (E)-2-octenal. The concentration of these five aldehydes that increased and/or formed during oxidation was highly correlated to oxidized odor intensity, as assessed by 20 trained sensory panelists. Furthermore, Zhou and others (2000) determined that the aldehydes were not formed due to lipoxygenase activity, since heat treatment of the cucumber slurries to inactivate the enzyme prior to oxygen incubation did not result in a significant reduction of aldehyde production.

In comparing analysis of volatiles from fermentation brine and cucumber slurry, it is important to note the differences between these two sample types. The lipid content of cucumber flesh is much lower than that of the seed or skin, which suggests that some aromatic compounds might partition to the endo- or exocarp (Pederson and others 1964).

Zhou and McFeeters (1998) compared results from gas chromatography-mass spectrometry for fermented cucumber slurry and brine from samples fermented in 2% NaCl. Differences in relative peak area between sample types were less than 2-fold for a majority of the 37 identified compounds; hexanal and ethyl-benzene were found in higher concentrations in the brine, while α-caryophyllene, 2-undecanone, and (E,Z)-2,6-nonadienal were found in higher concentrations in the cucumber slurry. Johanningsmeier and McFeeters (2011) identified 314 volatile compounds in the brine of cucumbers fermented in 6% sodium chloride (NaCl), including hydrocarbons, aldehydes, alcohols, ketones, acids, esters, ethers, furans, pyrans, phenols, nitrogenous compounds, and sulfur-containing compounds.

Two-Dimensional Gas Chromatography – Time-of-Flight Mass Spectrometry

Gas chromatography coupled with mass spectrometry is an analytical technique that allows for the characterization of volatile compounds within a food matrix. The compounds are separated on a column by many techniques, such as size-exclusion, ion-exchange, or polarity (Nielsen 2010). Solid-phase microextraction (SPME) is a technique for sample preparation that involves sampling the headspace of a sample with an adsorbant fiber to collect volatile compounds for desorption onto the column. The SPME technique is known for its high sensitivity, reproducibility, and robustness (Tikunov and others 2005). While one-dimensional gas chromatography is sufficient for many analytical applications, analysis of more complex samples requires additional separation power. In fact, Davis and Giddings (1983) developed a statistical theory using Poisson statistics to estimate that a one-dimensional chromatogram will never contain more than 37% of all possible separated peaks. Two-dimensional gas chromatography (GC x GC) was first demonstrated by Liu and Phillips

(1991) and provides an additional means of separation by introducing the compounds eluting from the first column onto a second column characterized by different chemical or physical properties. The columns are connected by a modulator, which concentrates small portions of the first column's effluent for introduction onto the shorter second column. The most common modulator is a cryogenic trap due to its ease of use and efficiency in conserving volatile compounds (Reviewed by Begnaud and others 2009). In a conventional two-dimensional configuration, the first column separates compounds primarily based on boiling point and the second column provides further separation of compounds based on a different property, such as polarity (Reviewed by Mondello and others 2008). A reversed column configuration exists in which a polar column is used in the first dimension and a somewhat less polar column is used in the second dimension. This configuration typically yields better separation for complex samples containing a large number of polar compounds such as aldehydes, ketones, lactones, acids, and alcohols (Adahchour and others 2004). Once separated, compounds may be introduced into a time-of-flight mass spectrometer (ToF MS), resulting in the detection of a number of charged molecular fragments. A unique fingerprint, or spectrum of molecular fragments, is created for each chemical compound. The fragments have specific masses and a fixed relative abundance that allow for tentative identification (Nielsen 2010). Standard compounds can be subjected to the same process to allow for confirmed identification and quantification of compounds of interest. Two-dimensional gas chromatography has been used for the volatile characterization of many food products, such as ginger (Shao and others 2003), butter (Adahchour and others 2005), pepper (Cardeal and others 2006), Malaysian soursop (Cheong and others 2011), fermented cucumber pickles

(Johanningsmeier and McFeeters 2011), wine (Welke and others 2012), and strawberries (Samykanno and others 2013).

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1.4 Overview of Research

Hypotheses

1. $CaCl_2$ fermented cucumber pickles can contain the maximum legal level of $CaCl_2$ for pickled vegetable products of 36 mM with no significant effect on consumer acceptability due to the taste interactions of $CaCl_2$ with the sodium chloride and acid inherent to pickle products.
2. $CaCl_2$ and NaCl fermented cucumber pickle products develop similar volatile compound profiles due to the use of similar fermentation microorganisms.

Objectives

1. To determine the detection threshold of $CaCl_2$ in traditional hamburger dill chip pickles.
2. To evaluate the overall consumer acceptability of NaCl and $CaCl_2$ fermented cucumber pickle products.
3. To compare the volatile compounds present in finished products of NaCl and $CaCl_2$ fermented cucumbers after bulk storage in the tank yard.

4. To evaluate taste and flavor attributes of finished products of NaCl and $CaCl_2$ fermented cucumbers after bulk storage in the tank yard.

Approach

Samples will be obtained from commercial, open-top fermentation tanks after bulk storage in the tank yard, to account for the inherent variability encountered in commercial processing. To begin, it would be beneficial to know if consumers can detect a difference between $CaCl_2$ and NaCl fermented pickle products. If consumers can detect a difference between products of the two fermentations, paired preference testing should be performed to determine if this detectable difference affects consumer preference. A consumer liking test on finished products of $CaCl_2$ fermented cucumber pickles receiving either one or two desalting steps prior to processing will provide a deeper understanding of the consumer acceptability of products from the environmentally-friendly $CaCl_2$ fermentation process.

To gain a more analytical understanding of the flavor characteristics of finished products of NaCl and $CaCl_2$ fermentations, a descriptive sensory analysis panel will quantitatively evaluate the products for key taste and flavor attributes. In addition, volatile compounds present in finished products will be characterized using two-dimensional gas chromatography – time-of-flight mass spectrometry. Correlation between the intensity of flavor attributes and volatile compounds may provide insight into which compounds are contributing to certain off-flavors.

Impact

The pickle industry is looking to adopt a fermentation process that will expel less sodium, which is toxic to plants and aquatic organisms in high concentrations, into waste waters. While fermentation with 100 mM $CaCl_2$ looks promising, flavor properties and consumer acceptability of the commercially produced pickle products have not yet been determined. This research will focus on consumer perception of pickle products from both NaCl and $CaCl_2$ fermentations receiving either one pre-processing desalting step, as is traditional, or two desalting steps, which may be necessary for $CaCl_2$ fermented products to ensure no change in consumer acceptability of finished products. Processors would benefit economically and environmentally if they could desalt $CaCl_2$ fermented cucumbers only once without concern for the adverse taste of elevated levels of $CaCl_2$ in finished products. The results of this research will help to direct future research in this area and provide the industry with information on how the more environmentally-friendly $CaCl_2$ fermentation procedure will affect flavor properties and consumer perception of finished products.

CHAPTER 2 – Consumer Acceptability of Cucumber Pickles Produced Using an Environmentally- Friendly Calcium Chloride Fermentation

Target Journal: Food Quality and Preference

2.1 Abstract

The pickle industry is looking to adopt a fermentation process that will expel less sodium into waste waters, as it can be toxic to plants and aquatic organisms in high concentrations. Fermentation of cucumbers in 0.1 M calcium chloride ($CaCl_2$) looks promising as an environmentally-friendly alternative to traditional 1.03 M sodium chloride (NaCl) fermentations. NaCl fermented cucumbers are traditionally desalted once prior to processing, but an additional desalting step may be necessary for $CaCl_2$ fermented cucumbers due to concern for the adverse taste of elevated levels of $CaCl_2$ in finished products. The objectives of this research were to determine the detection threshold of $CaCl_2$ in hamburger dill chip pickles and to evaluate consumer acceptability of $CaCl_2$ fermented pickles. A difference was observed between $CaCl_2$ and NaCl fermented pickles processed with one desalting step (n = 50, $P < 0.05$). However, a series of pair-wise preference tests indicated that the taste of $CaCl_2$ was not the factor affecting consumer preference. A consumer liking test (n = 73) showed no difference in flavor liking between NaCl and $CaCl_2$ fermented products ($P < 0.05$). Furthermore, a threshold test (n = 52) demonstrated that the 50 % detection threshold of $CaCl_2$ in hamburger dill chip pickles was 64.1 mM, substantially above the legal limit of 36 mM. This suggests processors could benefit by implementing $CaCl_2$ fermentations and desalting only once without concern for the adverse taste of elevated levels of $CaCl_2$. Prior to broad implementation of this process, flavor stability during

bulk storage, fermentation variability, and texture quality of $CaCl_2$ fermented products should be evaluated further.

2.2 Introduction

In the United States, more than 800 million kilograms of fermented vegetables are produced annually (Hutkins 2006). In fact, the pickled vegetable market accounts for more than $2 billion, with fermented cucumbers being one of the primary products (Doyle and Beuchat 2007). Pickling cucumbers are commercially fermented in 40,000 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% salt is desired in the finished product, which results in the need to desalt the fermented cucumbers to remove NaCl, and consequently other nutrients and flavor compounds, prior to packing the finished product (Fleming 1984).

Fermentation of cucumbers with 100 mM calcium chloride ($CaCl_2$) instead of the traditional 1.03 M 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 amount of NaCl in pickle plant waste waters (McFeeters and Perez-Diaz 2010). $CaCl_2$ fermented cucumbers require additional desalting prior to packing into finished products to reduce the level of $CaCl_2$ to that which is traditionally found in NaCl fermented products. If 100 mM $CaCl_2$ fermented cucumbers receive only one pre-processing desalt step, finished products will have a residual $CaCl_2$ concentration of approximately 36 mM, the legal limit for $CaCl_2$ in fermented vegetable products (21CFR184.1193). The additional desalting suggested for $CaCl_2$ fermented products results in the usage of more resources, such as water, time, and labor.

Recent research (unpublished data) demonstrated the potential for stable commercial cucumber fermentations using $CaCl_2$ in place of NaCl. However, in order for this process to be more broadly 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. Therefore, it is important to determine whether a difference in consumer acceptability of finished products exists between the $CaCl_2$ and NaCl fermented products.

The concern for higher residual $CaCl_2$ levels in finished products stems from the different taste attributes associated with $CaCl_2$ in comparison to NaCl. $CaCl_2$ 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 $CaCl_2$ in distilled water to be about 4 times as bitter as salty. However, the potentially offensive tastes associated with $CaCl_2$ are masked to some degree in the presence of certain organic ions, such as lactate, which is found in cucumber pickles (Lawless and others 2003). In addition, some bitter compounds are known to be suppressed in the presence of NaCl (*Reviewed by* Breslin 1996), a major component in cucumber pickles with a concentration of 2% NaCl in finished products on average. The detection threshold of $CaCl_2$ in pickle products and the degree to which the bitter taste profile of $CaCl_2$ 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 $CaCl_2$ fermented cucumbers.

The objectives of this study were to evaluate consumer acceptability of commercial $CaCl_2$ fermented cucumber pickles, determine the detection threshold of $CaCl_2$ in fermented hamburger dill chips, and direct future research related to the broad commercial implementation of $CaCl_2$ fermentation processes.

2.3 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 1.03 M NaCl or 0.1 M $CaCl_2$ in a commercial tank yard in open-top, 10,000 L plastic tanks. Cucumbers were collected from at least 3 feet below the surface of the tank, sliced into 3 to 6 mm hamburger dill chip rounds, processed in 16-oz glass jars using a traditional commercial brine formula for hamburger dill chips (58:42, cucumber to brine pack-out ratio), pasteurized, and stored at room temperature (23 ± 2 °C) under ambient lighting. Fermented cucumbers were desalted to equilibrium prior to processing using distilled water in a 60:40, cucumber to water, ratio. When needed, a second desalting step at a 60:40, cucumber to water ratio, would follow completion of the first desalting. Calcium chloride was the only constituent of the brine that was intentionally modified for each treatment; concentration of $CaCl_2$ in finished products was determined by EDTA titration (Gindler and King 1972). All finished products contained an equilibrated NaCl concentration of 0.38 M.

Panelist Selection and Testing Environment

Subjects were recruited from the North Carolina State University (NCSU) campus 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. All panelists were 18 years or older and were given the option of an individually wrapped, store-bought food treat as a token of appreciation.

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, IL) labeled with random 3-digit codes. The 3-digit sample codes and sample order were randomized between panelists. Room temperature distilled water and unsalted crackers were provided to cleanse the palate between samples.

Paired Preference Testing of Commercial Products

Cucumbers were collected from two different commercial fermentations after a tank storage time either 2 or 8 months and packed into finished products. All finished products had a shelf storage time of 12 months. Panelists (n=101, 56 female) were distributed across the following age divisions: 18-25 (49%), 26-35 (22%), 36-45 (12 %), 46-55 (12%), and 56-65 (6%). Sixty-eight percent of respondents were Caucasian. Pickle consumption 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 month results, respectively, due to 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 $CaCl_2$ fermentation treatment that contained an equilibrated $CaCl_2$ concentration of 18 mM and 24.5 mM for the 2 and 8 month pair, 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. This experiment was performed over the course of 2 days in 2 different locations. Day 1 testing was performed outside in covered tents (NCSU Brickyard, Raleigh, N.C., U.S.A.). Day 2 testing occurred in a classroom with ambient temperature and lighting (NCSU Department of Food, Bioprocessing, and Nutrition Sciences, Raleigh, N.C., U.S.A.).

Consumer Testing of Small Scale Experimental Products

Commercially fermented cucumbers were processed, after a 9 to 10 month tank yard storage time, in a food grade pilot plant, packed in 16-oz glass jars, pasteurized at 75°C for 15 minutes, and stored on the shelf for 2 months for the A-Not A test and Preference Test 1 and 5 months for Preference Test 2. The 4 treatments, $CaCl_2$ 1 desalt, $CaCl_2$ 2 desalt, NaCl traditional, and NaCl with elevated $CaCl_2$, contained 36 mM, 28 mM, 20 mM, and 34 mM $CaCl_2$, respectively.

A paired A-Not A difference test (Bi and Ennis 2001) was performed in isolated booths at the NCSU sensory testing facility (Department of Food, Bioprocessing, and Nutrition Sciences, 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 two samples included a traditional NaCl fermented treatment (A) and a $CaCl_2$ 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, two samples were presented to the panelists one at a time in a random order, with an equal distribution of two 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 Department of Food, Bioprocessing, and Nutrition Sciences, Raleigh, N.C., U.S.A.). 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 vs. $CaCl_2$ 1 desalt treatment and (2) NaCl traditional treatment vs. $CaCl_2$ 2 desalt treatment. Panelists (n = 50, 32 female) were distributed across the following age divisions: 18-25 (34%), 26-35 (30%), 36-45 (12%), 46-55 (16%), 56-65 (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, due to the panelists expressing no preference.

For Paired Preference Test 2, the 2 sample pairs were: (1) NaCl fermented treatment with elevated $CaCl_2$ vs. NaCl traditional treatment and (2) NaCl fermented treatment with elevated $CaCl_2$ vs. $CaCl_2$ 1 desalt treatment. Panelists (n = 50, 37 female) were distributed across the following age divisions: 18-25 (38%), 26-35 (26%), 36-45 (12 %), 46-55 (14%), 56-65 (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, due to the panelists expressing no preference.

Consumer Liking Test of Commercial Products

Cucumbers were commercially fermented and bulk stored in the tank yard 4 months prior to processing into finished products; finished products had a shelf storage time of 2 months. Panelists (n = 73, 51 female) were distributed across the following age divisions: 18-25 (48%), 26-35 (21%), 36-45 (16 %), 46-55 (10%) and 56-65 (5%) (Figure 2.3). Standard deviation of the liking scores ranged from 1.3 to 2.0. Panelists were presented with four treatments in a randomized complete block design. Treatments differed by fermentation brining salt and the concentration of $CaCl_2$ in finished products: NaCl fermented traditional (23 mM $CaCl_2$), NaCl fermented with elevated $CaCl_2$ added to finished product (35 mM $CaCl_2$), $CaCl_2$ fermented 1 desalt (35 mM $CaCl_2$), and $CaCl_2$ fermented 2 desalt (23 mM $CaCl_2$). Panelists were asked to rate their overall liking, as well as flavor, texture, and appearance liking on a hedonic scale, with 1 and 9 corresponding to dislike extremely and like extremely, respectively. In addition, panelists were given the option of commenting on what they liked most or least about the samples for each of the four categories.

Instrumental Texture Analysis

Mesocarp firmness of fermented hamburger dill pickle chips was measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp, Scarsdale, NY/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). 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/sec. Data was collected and analyzed using

Texture Expert software (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). The force required to puncture the mesocarp was recorded and expressed in Newtons (N). Firmness measurements were done on 15 reps of each treatment. Texture was evaluated at ambient temperature (23 ± 2 ^{o}C).

Color Analysis

The L*, a*, b* values of the hamburger dill pickle chips evaluated in the liking test were measured using a Minolta Chroma Meter model CR-300 (Minolta Co., Ltd., Osaka, Japan). Due to 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 $CaCl_2$ in Hamburger Dill Chip Pickles

Cucumbers were commercially fermented in a NaCl brine and stored in the tank yard 4 months prior to processing into finished products; finished products had a shelf storage time of 2 months. The cucumbers were desalted to equilibrate to a NaCl concentration of 2.2% in finished products. Pickles were packed in a commercial facility with equilibrated $CaCl_2$ concentrations of 26, 36, 50, 70, and 98 mM $CaCl_2$ to allow for performance of a 5-series threshold test with a step-factor of 1.4. A modified blank sample containing a residual $CaCl_2$ level of 21 mM was chosen for use in this test because this concentration is the approximate concentration in current commercial hamburger dill chip pickles. The testing procedure was followed as outlined in ASTM standard E679 (2011) with a 2 minute 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: 18-25 (56%), 26-35 (12%), 36-45 (10 %), 46-55 (13%) and 56-65 (10%). Eighty-one percent of panelists consumed pickles on a monthly or weekly basis.

Data Analysis

Data was collected using paper ballots and compiled in an electronic spreadsheet. All results were analyzed using SAS statistical software (version 9.3, SAS Institute Inc., Cary, NC).

Preference tests were analyzed using a chi-squared 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 was later analyzed by splitting the no preference votes with no significant difference ($P > 0.05$) in the results, supporting that dropping the no preference votes did not affect overall conclusions. Consumer liking data was analyzed by repeated measures ANOVA and Tukey's honestly significant difference post-hoc test. In addition, a statistical power test was performed ($\alpha = 0.05$) prior to 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 (Appendix B). The $CaCl_2$ 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 $CaCl_2$ concentration on the x-axis and interpolating the threshold concentration of $CaCl_2$ at different detection levels. Basing the threshold test on the statistics of a 1-sample t-test, an expected mean deviation of 9 and a standard deviation of 22 would require 52 people to obtain sufficient statistical power of approximately 80% (Appendix B).

2.4 Results

Paired Preference Testing of Commercially Processed Products

No significant preference was observed between $CaCl_2$ 2 desalt treatment and NaCl fermented cucumber pickles, regardless of the tank yard storage time of 2 months ($\chi^2 = 0.10$, $P = 0.75$) or 8 months ($\chi^2 = 0.17$, $P = 0.68$) (Table 2.1). Therefore, despite the potential for variability in commercial processing, these results showed that consumers do not have a significant preference ($P < 0.05$) between products from the two fermentation brining treatments when the finished product $CaCl_2$ concentration was maintained at typical levels of 18 to 25 mM. This finding suggests that cucumbers can be fermented and stored in $CaCl_2$ brines without a negative impact on consumer acceptability. Furthermore, 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 acceptability of finished products. In turn, the question of whether or not only one desalting step could be employed pre-processing for $CaCl_2$ fermented products was addressed in a subsequent difference test.

Consumer Testing of Small Scale Experimental Products

A-Not A Difference Test

Consumers could differentiate between the traditional NaCl fermented pickle ($\chi^2 = 6.48$, $P = 0.01$) and the $CaCl_2$ brined cucumber pickles ($\chi^2 = 15.68$, $P < 0.0001$) (Table 2.1). Due to 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 are able to detect a difference. Therefore, preference tests were performed between the NaCl traditional treatment and either the $CaCl_2$ fermented 1 desalt treatment or the 2 desalt treatment to determine if an additional desalt step for the $CaCl_2$ fermented cucumbers prior to packing into finished products would affect consumer preference.

Paired Preference Tests

The NaCl fermented treatment was preferred over both of the finished products from this particular $CaCl_2$ fermentation, regardless of the extent of desalting ($P < 0.05$) (Table 2.1). The significant preference for NaCl over the $CaCl_2$ 2 desalt treatment was especially unexpected, as it appears to contradict the results from the preference test on commercial products. However, a number of important factors aside from the potential adverse taste of elevated levels of $CaCl_2$ 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 the difference in salt brining treatment, referring to the difference in how fermentation volatiles are produced when comparing the process of NaCl and $CaCl_2$ fermentations.

Fermented cucumbers collected from the same NaCl fermentation tank as above were processed into products in which the only difference was the level of $CaCl_2$ in finished products, either 20 mM or 34 mM, and consumers expressed no significant preference between the products ($\chi^2 = 0.02$, $P = 0.88$) (Table 2.1). However, when the level of $CaCl_2$ in finished products of both NaCl and $CaCl_2$ fermented cucumbers was equivalent, 35 ± 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 (χ^2 = 5.33, P = 0.02). Therefore, it appears that when $CaCl_2$ is present in pickle products at levels around the legal limit of 36 mM, the resulting taste and texture due to the elevated residual $CaCl_2$ does not influence consumer preference; rather, inherent fermentation variability related to long term bulk storage or difference in brining salt treatment was likely influencing consumer preference.

Consumer Liking Test of Commercially Fermented Products

Flavor was the only category in which the liking scores did not differ ($P > 0.05$) among the treatments (Figure 2.1). This finding further supports the results from paired preference testing, in which the elevated level of residual $CaCl_2$ did not affect consumer acceptability of the finished products, regardless of individual fermentation variability or salt brining treatment. However, for the remaining three categories of overall liking, texture, and appearance liking, consumers gave higher scores ($P < 0.05$) to the two NaCl fermented pickles over the $CaCl_2$ fermented pickles (Figure 2.1). In addition, approximately 20% of the respondents commented negatively about the "softness" or "lack of crispiness" for the $CaCl_2$ fermented cucumbers, regardless of $CaCl_2$ concentration in the finished product. The significantly lower firmness of $CaCl_2$ fermented pickles was confirmed through instrumental texture analysis (Table 2.2). The significantly lower liking scores associated with the appearance of the $CaCl_2$ fermented pickles was further evaluated through the use of colorimetry analysis, in which the L* values were found to be greater (P < 0.05) and a* values more negative ($P < 0.05$) for $CaCl_2$ fermented pickles (Table 2.3). The lack of significant 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 off-flavors or to reduce the $CaCl_2$ concentration (Figure 2.1).

Detection Threshold of $CaCl_2$ in Hamburger Dill Chip Pickles

The detection threshold of $CaCl_2$ in NaCl fermented hamburger dill pickle chips as determined by the ASTM E679 calculation method for the best estimate threshold was 53 mM ± 22 mM. Despite the high standard deviation, the statistical power of this test was still above the desired 80% power. Due to the shortcomings of the ASTM method, Lawless (2010) developed an alternative method in which the chance-corrected percent correct for each concentration of $CaCl_2$ 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 is $y = 0.92x + 7.54$, $R^2 = 0.96$, where y is the chance-corrected percent correct and x is the concentration of $CaCl_2$ in finished hamburger dill chip products (Figure 2.2). 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 $CaCl_2$ in hamburger dill chips at a more conservative level, such as the concentration at which 25% of the population will be able to detect $CaCl_2$ in hamburger dill chips. Using this equation, the 10%, 25%, and 50% detection levels were 34.8, 46.0, and 64.1 mM $CaCl_2$, respectively (Figure 2.2). Regardless of method of calculation, the 50% detection threshold of $CaCl_2$ in fermented hamburger dill chip pickles was greater than the highest concentration that is legally allowed in fermented vegetables (36 mM). A small portion of the population, approximately 10%, may be capable of detecting the presence of $CaCl_2$ at a concentration of 36 mM in cucumber pickles.

2.5 Discussion

Calcium chloride 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 taste profile of cucumber pickles is unknown. One of the major concerns for pickle processors when considering a $CaCl_2$ fermentation is the potentially adverse taste related to a higher $CaCl_2$ concentration in finished products. It was hypothesized that no significant difference or preference would be expressed between the traditional NaCl fermented products and the $CaCl_2$ fermented products receiving either one desalting step or two desalting steps prior to processing due to the taste interactions of the main components of pickle brines, acid and NaCl, with $CaCl_2$. This research showed that residual $CaCl_2$ 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 2.1; Figure 2.1). Additionally, the 50% detection threshold of $CaCl_2$ was found to be well above 36 mM in fermented hamburger dill chip pickles (Table 2.4). In turn, processors may choose to adopt the $CaCl_2$ fermentation process with only one desalting step without concern for the effect of elevated $CaCl_2$ 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 $CaCl_2$ concentration of approximately 28 mM is suggested. This quantity was calculated using the alternative threshold method, in which the percent of respondents capable of detection is 0.0% and the chance-corrected percent is 33.3% (Figure 2.2).

Significant preferences were observed in the preference testing of small scale experimental products, but not in relation to residual $CaCl_2$ concentration. When $CaCl_2$ 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 2.1). Likewise, while consumers showed no significant preference ($P > 0.05$) between a NaCl fermented traditional treatment and a $CaCl_2$ 2 desalt treatment in commercially packed products, significant preference ($P < 0.05$) was observed between these two treatments in the trials involving small scale experimental products (Table 2.1). 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 from, the extent of desalting, and the use of alum during desalting. Additionally, commercially packed fermented pickles are processed with certain specifications set by the commercial processor, including pasteurization times and temperatures, tank yard conditions, pack-out ratios, and shelf life estimations. All of these variables likely have some effect on consumer perception of the finished product, but many are hard to determine due to confidentiality and variability when working on a large-scale. In turn, the 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.

While no significant difference ($P > 0.05$) in consumer acceptability of flavor of the $CaCl_2$ and NaCl fermented products was expressed in the consumer liking test of commercially packed products, regardless of residual $CaCl_2$ levels in finished products, texture differed significantly ($P < 0.05$). In contrast to earlier work completed by Buescher and others (2011) in which $CaCl_2$ increased mesocarp crispness, consumers from the liking test noted that both of the $CaCl_2$ fermented treatments were “soft” or “less crisp” and scored the $CaCl_2$ fermented treatments significantly lower in texture liking ($P < 0.05$), regardless of residual $CaCl_2$ levels (Table 2.2). In fact, instrumental texture analysis found that the $CaCl_2$ fermented pickles were significantly less firm than the NaCl fermented pickles (Table 2.2). This discrepancy may be due to a quality issue associated with the particular tank from which the $CaCl_2$ fermented cucumbers for the liking test were collected (fermentation variability) or an inherent characteristic of the $CaCl_2$ fermentation process (salt brining treatment). One possible explanation for the reduced textural quality of the $CaCl_2$ fermented products, related to the influence of salt brining treatment, 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 due to the PG enzyme. Furthermore, while Buescher and others (1979) demonstrated that 100 mM $CaCl_2$ 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. This may be related to the variability in ionic strength of the different fermentation brines. McFeeters and others (1980) found that increasing levels of ionic strength up to 0.2 resulted in increased PG activity, while ionic strength levels greater than 0.2 resulted in precipitation of substrate and loss of enzyme activity. Therefore, the lack of NaCl in the $CaCl_2$ fermentation brines may be associated with the increased activity of the native PG enzyme, resulting in textural defects after long term storage. Lu and others (2002) discovered 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, so as to avoid introducing another variable. However, 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. There is some interest in the pickle industry to remove alum from the desalting waters due to health concerns, but the removal of alum may result in a significant decrease in texture quality of finished products.

It is also possible that consumer acceptability was influenced by the appearance of the cucumber pickles. While the same concentration of yellow 5 was added to all of the treatments, color was significantly different among the treatments. $CaCl_2$ 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 2.3). 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 $CaCl_2$ marinade resulted in steaks that were less red than those marinated in calcium lactate. The pro-oxidation effects seemingly associated with $CaCl_2$ may actually be related to the impurity of $CaCl_2$ being used, in which 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 $CaCl_2$. It is possible that the reduced textural properties were associated with an increase in soluble solids within the matrix, which may result in an increase in light scattering and a lighter color perceived by consumers.

The consumer liking test involved an informative demographic questionnaire, the results of which highlight a few key points that assist in better understanding the consumer base for pickle products (Figure 2.3). Eighty percent of consumers (n=73) 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. When asking consumers to choose all factors that affect their purchase of pickle products, flavor and texture were the top two factors of influence, signifying the importance of maintaining a consistent flavor and texture in products across batches and over storage time. Furthermore, 69% of consumers were within the age range of 18 to 35. This is of importance because if the majority of pickle consumers are younger, as the results of this test suggest, processors should have a special interest in their perception of products, as they will be the consumers in years to come, as well. Furthermore, the impact that products make on these younger consumers in the present will likely affect their future purchase habits.

More information is needed to determine the inherent variability of $CaCl_2$ fermented products and whether it differs significantly from current NaCl fermented products. Potential sources of variability encountered in commercial scale pickle processing that may be influencing consumer acceptability of finished products include: cucumber origin, cucumber size, the season in which fermentation starts, fermentation brining salt, tank material, storage time in the tank yard prior to processing into finished products, the individual fermentation tanks from which cucumbers are collected, the extent of desalting, the use of alum during desalting, pasteurization time and temperatures, and finished product storage time and environment.

2.6 Conclusions

While a difference was observed between $CaCl_2$ and NaCl fermented pickles processed with one desalting step ($n = 50$, $P < 0.05$), pair-wise preference testing showed that the taste of $CaCl_2$ was not the factor affecting consumer preference. This finding was further supported by the calculated 50% detection threshold of $CaCl_2$ in hamburger dill chip pickles ($n = 52$) of 64.1 mM, which is above the legal limit of 36 mM for pickled vegetable products. Additionally, a consumer liking test ($n = 73$) showed no difference in flavor liking between NaCl and $CaCl_2$ fermented products ($P < 0.05$). This suggests that processors could benefit economically and environmentally by implementing $CaCl_2$ fermentations and desalting only once without concern for the adverse taste of elevated levels of $CaCl_2$. Prior to broad implementation of this process, flavor stability during bulk storage, fermentation variability, and texture quality of $CaCl_2$ fermented products should be evaluated further.

2.7 Acknowledgments

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2.8 Keywords

Sodium-free vegetable fermentation, calcium chloride, paired preference, detection threshold, cucumber pickles, sustainability

2.9 References

21CFR184.1193

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Table 2.1 – Consumer sensory results for paired preference and A-Not A difference testing of hamburger dill chip cucumber pickles fermented in either 1.03 M NaCl or 100 mM $CaCl_2$ with varying levels of finished product $CaCl_2$

Number of Panelists	No Pref. Votes	Brining Salt	Tank Yard Storage (Mo.)	Desalt Steps	$CaCl_2$ in Finished Product (mM)	Shelf Storage (Mo.)	Results	χ^2	P-value ($\alpha = 0.05$)
Paired Preference Testing of Commercially Processed Products									
101	10	$CaCl_2$	2	2	18	12	No Significant Preference	0.10	0.75
		NaCl	2	1	18	12			
101	9	$CaCl_2$	8	2	24	12	No Significant Preference	0.17	0.68
		NaCl	8	1	25	12			
A-Not A Difference Testing of Small Scale Experimental Products									
50	N/A	$CaCl_2$	10	1	36	2	Significant	6.48	0.01
		NaCl	9	1	20	2	Difference	15.68	<0.0001
Paired Preference Testing of Small Scale Experimental Products									
50	7	$CaCl_2$	10	1	36	2	NaCl Preferred	8.40	0.004
		NaCl	9	1	20	2			
50	4	$CaCl_2$	10	2	28	2	NaCl Preferred	4.26	0.04
		NaCl	9	1	20	2			
50	3	NaCl	9	1	20	5	No Significant Preference	0.02	0.88
		NaCl	9	1	34	5			
50	2	$CaCl_2$	10	1	36	5	NaCl Preferred	5.33	0.02
		NaCl	9	1	34	5			

Table 2.2 – Evaluation of texture by instrumental analysis and consumer liking testing[1] (n = 73) for fermented hamburger dill chip cucumber pickles[2]

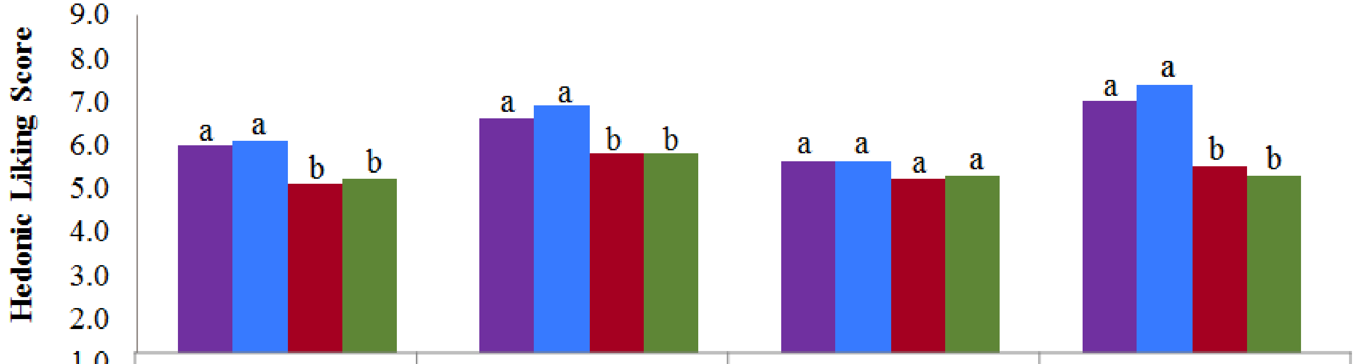
Fermentation Brine	Desalt Steps	Finished Product $CaCl_2$ (mM)	Firmness (N)	Texture Liking	
NaCl	1	23	8.4 ± 0.3^{ab}	7.0 ± 1.4^{a}	Like Moderately
NaCl	1	34	9.6 ± 0.4^{a}	7.4 ± 1.3^{a}	Like Moderately
$CaCl_2$	2	23	7.3 ± 0.1^{bc}	5.5 ± 2.0^{b}	NLND[3]
$CaCl_2$	1	35	7.1 ± 0.3^{c}	5.3 ± 1.9^{b}	NLND

[1] 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, $P < 0.05$).
[2] All treatments stored in the tank yard 4 mo prior to processing, stored on the shelf for 2 mo prior to evaluation, and contained an equilibrated NaCl concentration of 0.38 M.
[3] Neither like nor dislike

Table 2.3 – Evaluation of appearance by color analysis and consumer liking[1] testing (n = 73) for fermented hamburger dill chip cucumber pickles[2]

Fermentation Brine	Desalt Steps	Finished Product $CaCl_2$ (mM)	L*	a*	b*	Appearance Liking	
NaCl	1	23	39.9 ± 1.1^{b}	-7.2 ± 0.2^{b}	21.6 ± 1.4^{a}	6.6 ± 1.6^{a}	Like Moderately
NaCl	1	34	41.8 ± 2.5^{b}	$-7.6 \pm 0.2^{a,b}$	22.9 ± 1.9^{a}	6.9 ± 1.4^{a}	Like Moderately
$CaCl_2$	2	23	49.0 ± 0.0^{a}	-8.2 ± 0.0^{a}	25.7 ± 0.1^{a}	5.8 ± 1.7^{b}	NLND[3]
$CaCl_2$	1	35	49.0 ± 0.0^{a}	-8.0 ± 0.2^{a}	26.0 ± 1.9^{a}	5.8 ± 1.6^{b}	NLND

[1] 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, $P < 0.05$).
[2] All treatments stored in the tank yard 4 mo prior to processing, stored on the shelf for 2 mo prior to evaluation, and contained an equilibrated NaCl concentration of 0.38 M.
[3] Neither like nor dislike



	Overall Liking	Appearance	Flavor	Texture
NaCl (23 mM $CaCl_2$)[3]	6.0 ± 1.7	6.6 ± 1.6	5.6 ± 1.9	7.0 ± 1.4
NaCl (35 mM $CaCl_2$)	6.1 ± 1.5	6.9 ± 1.4	5.6 ± 1.7	7.4 ± 1.3
$CaCl_2$ (23 mM $CaCl_2$)	5.1 ± 1.8	5.8 ± 1.6	5.2 ± 1.8	5.5 ± 2.0
$CaCl_2$ (35 mM $CaCl_2$)	5.2 ± 2.0	5.8 ± 1.7	5.3 ± 1.9	5.3 ± 1.9

Figure 2.1 – Consumer liking[1] (n = 73) of fermented hamburger dill pickle chips[2] produced using traditional 1.03 M sodium chloride (NaCl) and environmentally-friendly 0.1 M calcium chloride ($CaCl_2$) fermentations

[1] 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, $P < 0.05$).
[2] All treatments were stored in bulk in the tank yard 4 mo prior to processing, stored on the shelf as finished products for 2 mo prior to evaluation, and contained an equilibrated NaCl concentration of 0.38 M.
[3] Fermentation brining salt (concentration of $CaCl_2$ in finished products)

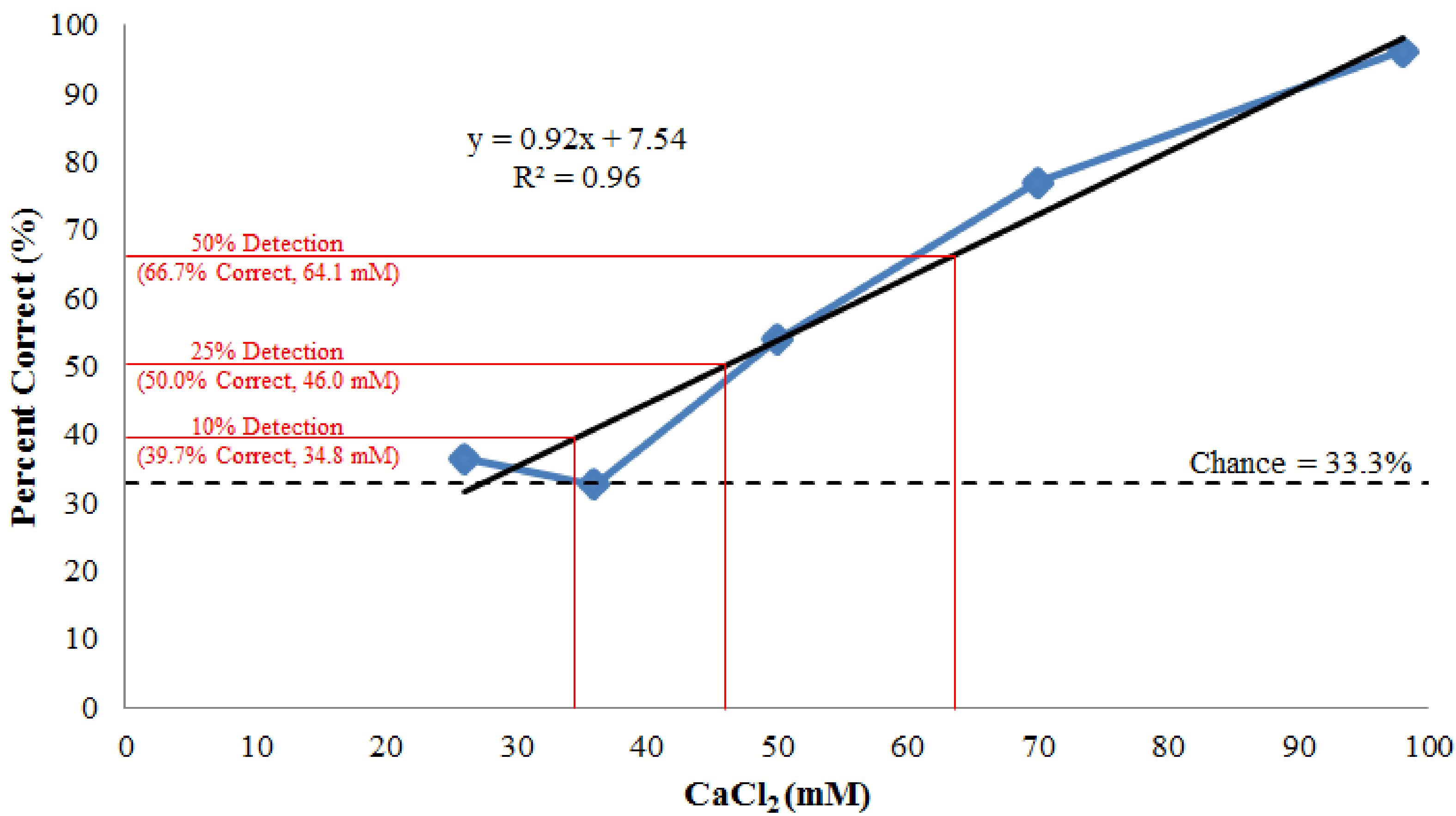


Figure 2.2 – Interpolation at chance-corrected proportions of the detection threshold (n = 52) of calcium chloride ($CaCl_2$) in fermented hamburger dill chip pickles

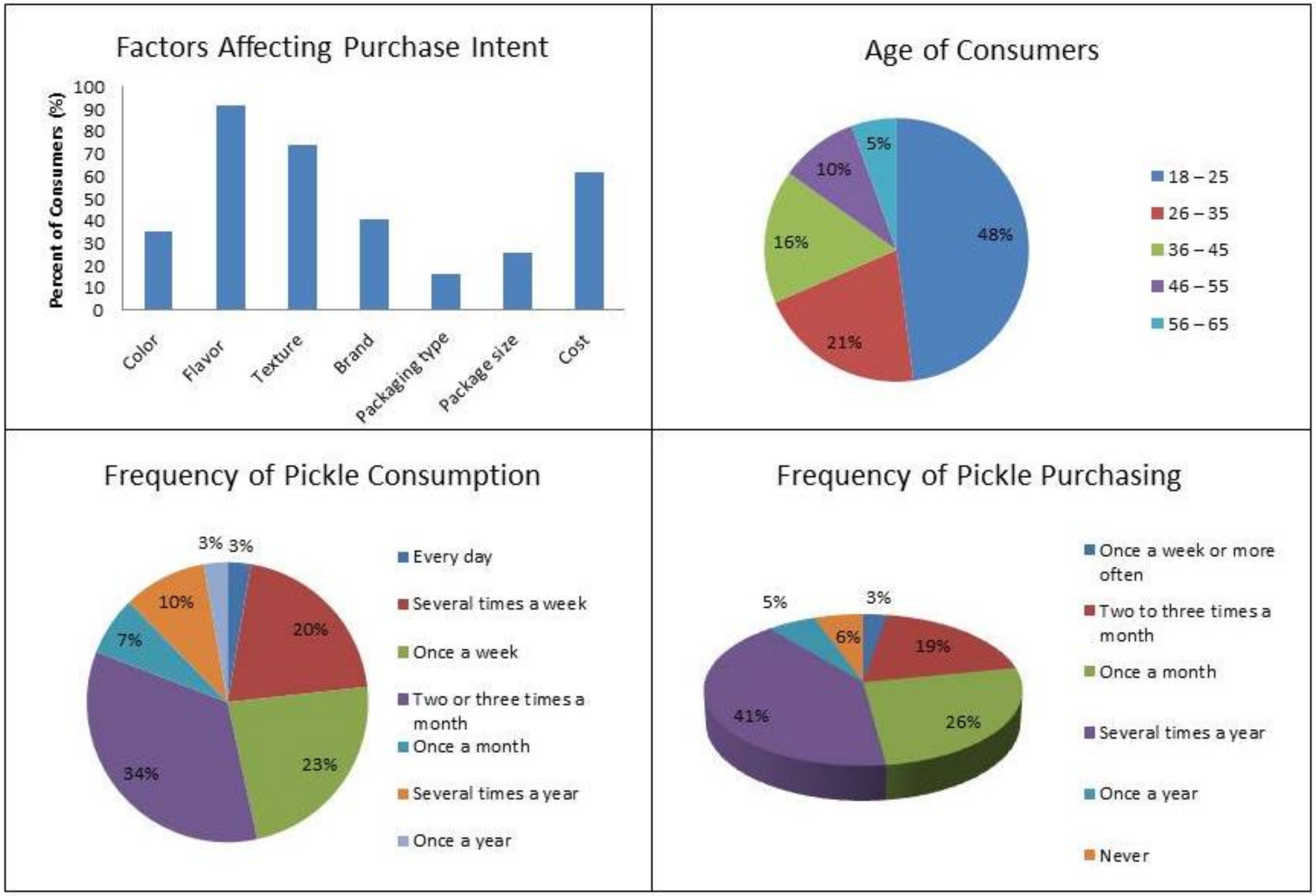


Figure 2.3 – Demographic information of consumers from a consumer liking test evaluating hamburger dill chip cucumber pickles (n=73)

CHAPTER 3 – Characterization of off-flavors in fermented hamburger dill chip cucumber pickles

Target Journal: Journal of Agricultural and Food Chemistry

3.1 Abstract

The pickle industry is looking to adopt a fermentation process that will expel less sodium into waste waters and 0.1 M calcium chloride ($CaCl_2$) looks promising as an alternative salt for fermentation. The objectives of this research were to evaluate the flavor attributes and volatile compounds present in finished pickle products produced from cucumbers fermented and stored in $CaCl_2$ and NaCl brines. Evaluation of products during bulk storage from both $CaCl_2$ and NaCl fermentation brines with either traditional (21 mM) or elevated (35 mM) finished product $CaCl_2$ concentration was performed through quantitative descriptive sensory analysis and volatile compound analysis. Oxidized flavor increased during bulk storage ($P < 0.05$); $CaCl_2$ fermented products had a greater oxidized flavor overall ($P < 0.05$), suggesting that $CaCl_2$ fermentations may more readily or more quickly produce oxidized flavor compounds. Saturated and unsaturated aldehydes previously found in cucumber pickles exposed to oxygen were found to decrease with bulk storage, while a subset of ketones positively correlated to oxidized flavor increased with bulk storage. Earthy/musty and green flavors differed significantly among individual fermentations ($P <$ 0.05). Finished pickle products containing 35 mM $CaCl_2$ were more bitter and salty and less sweet than products with 21 mM $CaCl_2$ ($P < 0.05$). Fermentation and bulk storage in a $CaCl_2$ fermentation brine resulted in products that were detectably more oxidized, bitter, and salty, which may result in the need for an adjustment of cover brine formulations and shorter bulk storage times.

3.2 Introduction

Pickling cucumbers are commercially fermented in 40,000 L open-top, plastic or fiberglass tanks with a minimum of 5% sodium chloride (NaCl) in the fermentation brine (Franco and others 2012). The high salt concentration in brines 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% salt is desired in the finished product, which results in the need to desalt the fermented cucumbers to remove NaCl, and consequently other nutrients and flavor compounds, prior to packing the finished product (Fleming 1984). Sodium-free cucumber fermentations with 100 mM calcium chloride ($CaCl_2$) has been successfully applied on a controlled, experimental scale as a means to combat the environmental issues and toxic effects associated with the large amount of NaCl in pickle plant waste waters (McFeeters and Perez-Diaz 2010). Recent research (unpublished data) demonstrated the potential for stable commercial cucumber fermentations using $CaCl_2$ in place of NaCl. However, in order for this process to be broadly 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. Therefore, it is important to determine whether a difference in flavor development and taste properties exists between the $CaCl_2$ and NaCl fermented products.

A number of secondary fermentation reactions occur in fermented cucumber pickles, which contribute to the flavor profile of finished products; fermented pickles evaluated straight from the tank yard have been described as silage-like, sour, slightly sweet, and "green" (Marsili and Miller 2000). Marsili and Miller (2000) identified trans- and cis-4-

hexenoic acid as key flavor compounds with aromas characteristic of fermented cucumber brine by gas chromatography-olfactometry; additional compounds with high odor impact values in fermented cucumber brines included 2-heptanol, cis-2,4-hexadienoic acid (tentative identification), phenyl ethyl alcohol, 2,6-nonadienal, and 2-dodecen-1-al (tentative identification).

Fermented cucumbers are especially susceptible to oxidized off-flavor development due to the nature of commercial fermentation processes, which typically occur in large open-top tanks exposed to the sunlight, and due to the common practice of purging the tanks with air to mix the contents and remove carbon dioxide (Buescher and Hamilton 2000). Additionally, cucumbers contain trace amounts of metals, such as iron, zinc, and copper, which can play a role in promoting oxidation of pigments and flavor compounds (Eisenstat and Fabien 1953). Cleary and McFeeters (2006) demonstrated that introduction of oxygen into fresh-pack dill pickles under anaerobic conditions resulted in increased levels of hexanal, heptanal, and pentanal. In addition, Zhou and others (2000) observed a significant increase in hexanal and (E)-2-heptenal when fermented cucumber slurries were incubated in the presence of oxygen. Additional compounds initially absent in the slurries were detected after oxygen exposure including (E)-2-pentenal, (E)-2-hexenal, and (E)-2-octenal. The concentration of these five aldehydes that increased and/or formed during oxidation was highly correlated to oxidized odor intensity, as assessed by 20 trained sensory panelists.

Venkateshwarlu and others (2004) modeled oxidative off-flavors commonly found in fish oil by mixing pure volatile flavor compounds in milk and evaluating the intensity of fishy and metallic off-flavors in these samples. A trained 16-member descriptive analysis panel determined that (E,Z)-2,6-nonadienal and 1-penten-3-one are the 2 main compounds characteristic of metallic and fishy off-flavors; although, the fishy and metallic flavors were not present when the compounds were added to the milk alone, confirming the importance of compound interaction in flavor production. In addition, the metallic odor of 1-penten-3-one was enhanced in the presence of heptenal (Venkateshwarlu and others 2004).

"Green" is a note sometimes used by descriptive analysis panels evaluating cucumber pickles. To identify the volatile compounds contributing to this flavor attribute, it may be beneficial to consider the 8 volatile compounds characteristic of the green odor emitted by plant leaves: (Z)-3-hexenol, (E)-3-hexenol, (E)-2-hexenol, (Z)-3-hexenal, (E)-3-hexenal, (E)-2-hexenal, hexanol, and hexanal (Hatanaka 1996). In addition, Forss and others (1962) found that (E,Z)-2,6-decadienal was characterized by the flavor notes of "green," "plant-like," and "like cucumbers."

Varying the NaCl levels in fermentations, or completely removing NaCl in the case of 100 mM $CaCl_2$ fermentations, could alter the microflora, which may impact the resulting secondary fermentation by-products that contribute to flavor and aroma. This is highlighted by previous works studying the content of the volatile compound linalool in fermentation brines. Zhou and McFeeters (1998) found high levels of linalool in 2% NaCl fermentation brines, but Marsili and Miller (2000) observed linalool in only 5% of brine samples with the traditional 8-10% NaCl concentration (Marsili and Miller 2000).

The objectives of this study were to compare the volatile compounds present in finished products of $CaCl_2$ and NaCl fermentations over bulk storage and to characterize the taste and flavor attributes of the finished products through descriptive sensory analysis.

3.3 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 with either 1.03M NaCl or 0.1 M $CaCl_2$ in a commercial tank yard in open-top, 10,000 L plastic tanks for 4 or 8 months prior to processing into finished products. Tank 1-10 was a NaCl fermentation tank that differed in material from the other 7 tanks, being composed of fiberglass, as opposed to plastic. Cucumbers were collected from at least 3 feet below the surface of the tank. Treatments were randomized in a full factorial design to 8 different commercial tanks (Appendix B).

Fermented cucumbers were desalted to equilibrium prior to processing using distilled water in a 60:40, cucumber to water, ratio. When needed, a second desalting step at a 60:40, cucumber to water ratio, would follow completion of the first desalting. Cucumbers were sliced into 3 mm rounds, processed in 16-oz glass jars using a traditional commercial brine formula for hamburger dill chips (58:42, cucumber to brine pack-out ratio), pasteurized, and stored at room temperature (23 ± 2 °C) under ambient lighting for 1.5 months prior to evaluation. Samples were evaluated for descriptive sensory analysis and a brine sample was obtained and stored at -80°C to allow 4 and 8 month brine samples to be analyzed during the same GCxGC-ToF MS run. All finished products contained an equilibrated NaCl concentration of 0.38 M. Calcium chloride was intentionally modified for each treatment to reach an equilibrated concentration of either 21 mM or 35 mM; concentration in finished products was determined by EDTA titration (Gindler and King 1972).

Descriptive Sensory Analysis Panelist Selection and Training

Subjects (n = 9) were recruited from the North Carolina State University (NCSU) campus with approval from the NCSU Institutional Review Board (IRB #2734) and informed consent was obtained from all participants. All panelists were 18 years or older and were given the option of an individually wrapped, store-bought food treat as a token of appreciation after each session.

Products were evaluated after 1.5 months of shelf-storage by a trained descriptive analysis panel using the Spectrum™ scale for the following taste and flavor attributes: polish dill, vinegar, oxidized, green, salty, sour, sweet, bitter, earthy/musty, astringency, and metallic. Panelists were presented with samples using a randomized incomplete block design.

The panel received over 50 hours of flavor evaluation training on the solutions and food references in Table 3.1. Drobna and others (2003) found that alum was the best reference for astringency, due to the lower degree of bitter and sour taste notes, as compared to tannic acid or fruit juice reference options. Concentrations of 0.25, 0.56, 1.1, and 1.5 g/L alum represented 2, 5, 10, and, 15 intensities on the astringency scale and concentrations of 4.2, 8.8, and 38.5 mM acetic acid represented the intensities 2, 5, and 10 for vinegar flavor, respectively (da ConceicaoNeta and others 2007). References for basic tastes (salty, sour, bitter, and sweet) were obtained from Meilgaard and others (2007) and fit to a line (R-squared > 0.99) to extrapolate solution concentrations for all points of intensity on the scale from 0 to 15. An oxidized reference was defined by the panelists using a hamburger dill chip pickle prepared using a commercial brine formulation without dill emulsion. A commercially packed hamburger dill chip pickle was defined by the trained panelists for all attributes, stored at 4°C to best preserve flavor, and used by panelists as a calibration tool prior to each evaluation.

Headspace SPME and GCxGC – ToF MS

Headspace SMPE and comprehensive GCxGC-ToF MS was performed as outlined in Johanningsmeier and McFeeters (2011) with the following modifications: cucumber brines (50 μL) were diluted 1:20 with deionized water (936 μL), 3N H_2SO_4 (4 μL), and 5 ppm deuterated hexanoic acid as an internal standard (10 μL) in 10 mL screw-cap headspace vials (Microliter Analytical Supplies, Inc., Suwanee, Ga., U.S.A.) with 0.4 g sodium chloride added to assist in "salting out" the volatile compounds. Additionally, the second dimension separation time was 2.75 s, instead of 1.5 s.

Data Processing and Analysis

GCxGC-ToF MS data processing and analysis was performed as outlined in Johanningsmeier and McFeeters (2011), with the following modifications: 20 s peak width in the first dimension, 0.05 s peak width in the second dimension, signal to noise ratio of 6, number of apexing masses of 3, a match required to combine of 600, a molecular weight range of 0 to 1000, and a mass threshold of 0. Correlation analysis of volatile compounds to descriptive sensory analysis scores was performed in JMP Pro 10 (SAS Institute, Inc., Cary, NC, USA) (Appendix E). Compounds related to dill emulsion were manually reviewed and removed.

Statistical Analysis

The effect of brining salt (NaCl or $CaCl_2$), cucumber size (2B or 3A), bulk storage time (4 months or 8 months), finished product $CaCl_2$ concentration (21 mM or 35 mM), and all possible interactions on intensity of flavor attributes was tested using repeated measures ANOVA and Tukey's Honestly Significant Difference post-hoc test ($\alpha = 0.05$). Flavor attributes for which there was no significant effect of brining salt or cucumber size were subsequently analyzed to determine if a specific tank had a significant effect on the specific flavor attribute (Appendix B). All ANOVA and post-hoc tests were performed using the proc mixed function in SAS 9.3 (SAS Institute, Inc., Cary, NC, USA) and principal component analysis was performed on panel means averaged over finished product $CaCl_2$ concentration using the proc prinqual function and a custom template. Raw sensory data was analyzed for panel performance, calculating p-values and MSE-values for each panelist and attribute, using Panel Check version 1.4.0 (SourceForge Inc., Mountain View, CA, USA).

3.4 Results and Discussion

Volatile compounds found in finished products of $CaCl_2$ and NaCl fermentations were found to be different, mainly attributed to oxidized off-flavor development. Differences between the two fermentation brining treatments was further observed in the flavor intensity of off-flavor attributes, as assessed by a trained panel ($n = 9$). Principle component analysis of the flavor characteristics of fermented cucumber pickle products, averaged over finished product $CaCl_2$ concentration, showed that the first two principle components explained approximately 90% of the variance in the pickle products (Figure 3.1). Component 1 explained 72% of this variability and was most strongly influenced by oxidized flavor. In general, fermented cucumbers stored in the tank yard 8 months prior to processing loaded strongly in the positive direction on component 1 and were highly associated with oxidized flavor, with $CaCl_2$ fermented products processed after 8 months in bulk storage having the greatest association to oxidized flavor. Products stored in the tank yard 4 months prior to processing loaded negatively on component 1, indicating a lower association with oxidized flavor. Furthermore, NaCl fermented cucumbers with 8 months of bulk storage loaded negatively on component 2 and were most highly associated with green flavor. Alternatively, $CaCl_2$ fermented products with 4 months of bulk storage tended to load positively on component 2 and were less associated with green flavor and more closely associated with metallic and earthy/musty off-flavors.

ANOVA revealed 478 compounds that differed significantly by bulk storage time and fermentation salt ($P < 0.05$), which clustered according to bulk storage time (Figure 3.7). Alternatively, ANOVA revealed 759 compounds that differed among individual fermentations during bulk storage ($P < 0.05$), which clustered mainly according to bulk storage time and to a lesser extent fermentation brining salt. While a large number of compounds were found to vary among products of different individual fermentations across bulk storage, it was unclear to what extent these compounds contributed to flavor. Correlation analysis of these compounds to the sensory intensity scores in finished products revealed specific compounds that were highly correlated, either positively or negatively, to the off-flavors oxidized, green, and metallic (Appendix E).

Oxidized Flavor in Fermented Cucumber Pickles

All products showed an increase in oxidized flavor with longer bulk storage time, but $CaCl_2$ fermented cucumbers resulted in finished products with higher oxidized flavor than NaCl fermented cucumbers ($P = 0.0106$; Figure 3.2). This finding is consistent with Lawrence and others (2003) who identified $CaCl_2$ as a pro-oxidant of lipid oxidation in a study evaluating the effect of $CaCl_2$ marinades on beef *longissimus*. The pro-oxidation effects seemingly associated with $CaCl_2$ may actually be related to the purity of $CaCl_2$ being used, in which metal contaminants could be involved in initiating oxidation. Additionally, calcium is a known co-factor in the oxygen-evolving complex of photosystem II in plants, which is involved in photosynthesis, and the calcium ions from dissociated $CaCl_2$ may play a direct role in the process of water oxidation (Vrettos and others 2001).

Current literature on fresh-pack and fermented cucumbers demonstrated the production of saturated and unsaturated aldehydes as a result of oxygen exposure (Cleary and McFeeters 2006; Zhou and others 2000). However, in this study, there was a general trend for a reduction in saturated and unsaturated aldehydes during bulk storage; correlation analysis revealed that these compounds were negatively correlated to oxidized flavor, as samples with longer periods of bulk storage were characterized as more oxidized ($P < 0.05$) and had a lower relative abundance of these aldehydes (Figure 3.8). The 4 month samples, which had higher amounts of the aldehydes previously detected in cucumber pickles exposed to oxygen, such as pentanal, (E)-2-pentenal, and 2-hexenal, were characterized as oxidized by descriptive analysis, which appears to be in agreement with the previous studies. However, with longer periods of oxygen exposure during bulk storage, up to 8 months, the aldehydes originally thought to be contributing to oxidized flavor are actually found to decrease, indicating that the oxidized flavor of these samples was characterized by another class of volatile compounds. Ketones are one major class of compounds found to correlate to oxidized flavor ($P < 0.025$), with 7 ketones decreasing in relative abundance over bulk storage (Figure 3.9) and 4 ketones increasing in relative abundance over bulk storage (Figure

3.10). Ketones are known to contribute to oxidized off-flavors (Saxby 1996), indicating that the following ketones, which increase over bulk storage and are positively correlated to oxidized flavor, may be of particular importance to oxidized off-flavor in fermented cucumbers after long periods of bulk storage: (E,E)-3,5-heptadien-2-one, 4-penten-2-one, 1-(2-hydroxy-5-methylphenyl)-ethanone, and 1-cyclopropyl-1-propanone. The exact reaction mechanism related to the increase in specific ketones and decrease in aldehydes correlated to oxidized flavor ($P < 0.025$) is difficult to determine due to the large degree of possible reactions that could take place during bulk storage of cucumbers. It is known that aldehydes and ketones can be formed by cleaving the unsaturated bonds of linoleic and linolenic acids in cucumber pickles (Grosch and Schwarz 1971; Zhou and others 2000). However, aldehydes and ketones are very reactive molecules and can further react with other substances, such as lipids or the terminal groups of amino acids and enzymes, leading to the development of a diverse and complex group of compounds possibly contributing to off-flavors (Phillips and Finley 1988). Interactions can involve hydrogen bonding and electrostatic interactions, which may be reversible, or covalent bonds with amino groups, which are irreversible (Fennema 1996). Additionally, it is plausible that the compounds higher in samples with only 4 months of bulk storage and correlating negatively to oxidized flavor were utilized as a substrate in oxidation reactions to produce some of the compounds clustering with 8 month samples and possessing a significant positive correlation to oxidized flavor ($P < 0.025$). For example, an aldehyde could be reduced to a primary alcohol that may then be oxidized to form a ketone, which is one possible mechanism to explain the decrease in (E,E)-2,4-heptadienal and increase in (E,E)-3,5-heptadien-2-one in these fermented cucumber pickles (Figure 3.11).

Of additional interest to the development of oxidized off-flavor in fermented cucumber pickles are furan-containing compounds, including 2-pentyl-furan, 2-ethyl-furan, and 2-(2-propenyl)-furan, which were found in greater relative abundance in samples with 4 months of bulk storage. St. Angelo (1992) suggests that furan-containing compounds may be precursors to off-flavor compounds because the furan ring can be opened by peroxidation to phenolic cyclization products.

A number of the compounds found to negatively correlated to oxidized flavor have been described in the literature as being associated with green flavor, such as (E,E)-2,4-Hexadienal (Qian and Reineccius 2003), 2,4-Hexadienoic acid ethyl ester (Marsili and Miller 2000), 2-Hexenal (Hatanaka 1996), and (Z)-3-Hexen-1-ol (Rowe 2005).This indicates that higher concentrations of these compounds in cucumber pickles are associated with a lower intensity of oxidized off-flavors. Interestingly, these compounds did not appear as significantly positively correlated to green flavor, but this could be due to lack of definition for the particular flavor these compounds generate in cucumber pickles or flavor masking/interaction effects.

Fermentation Flavors that Varied Among Individual Fermentations

Earthy/musty and green were two flavor attributes that were not related to the effect of brining salt, cucumber size, or finished product $CaCl_2$ concentration ($P > 0.05$). In turn, further statistical analysis was performed to determine if differences in these flavors were due to individual fermentation variability. Products from tank 1-16 had a significantly lower earthy/musty flavor than products from tank 1-10 ($P = 0.0392$) or tank 2-11 ($P = 0.0205$) (Figure 3.5). It is hypothesized that the earthy/musty flavor perceived in pickle products is due to individual fermentation variability, which may be influenced by tank location, environmental exposure, and/or differences in microbial interactions as opposed to a variable under control in this study. Fermentation variability also had a significant effect on green flavor (P = 0.0009; Figure 3.6). Green flavor was significantly lower in products from tank 2-11, as compared to those from tank 1-16 (P = 0.0187), tank 1-18 (P = 0.0161), tank 2-21 (P = 0.0077), and tank 1-22 (P = 0.0004). Additionally, products from tank 1-22 had a significantly higher green flavor than products from tank 1-10 (P = 0.0387) and tank 1-17 (P = 0.0201). Fermentation variability was the main factor influencing perception of green flavor, but bulk storage time also had a significant effect, with products stored in the tank yard for longer periods of time possessing higher green flavor intensities (P = 0.0040; Figure 3.3). However, the increase in green flavor with bulk storage was relatively small compared to the increase in oxidized flavor and the significance of this finding is not clear. Compounds correlated to green flavor followed a similar trend to those correlated to oxidized, with 4 month samples clustering with compounds negatively correlated to green flavor and 8 month samples clustering with compounds positively correlated to green flavor (Table 3.3). The four compounds significantly correlated to green flavor ($P < 0.025$), 3-butenenitrile, 2,3-dimethly-1,4-hexadiene, 3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one, and 1-methyl-4-(1-methylethyl)-2-nitro-benzene, were mainly unconjugated, unsaturated compounds, which is in agreement with Forss and others (1962) who suggested these types of compounds may contribute to green flavor. Not surprisingly, hierarchical clustering analysis revealed that volatile compounds significantly correlated to green flavor clustered with compounds significantly correlated to oxidized flavor ($P < 0.05$; Figure E2). Likely, oxidation reactions break down substrates into both green and oxidized flavor compounds, depending on the extent of the reaction, which helps to explain why so many compounds cluster together by their correlation to these flavor attributes.

Metallic flavor was significantly positively correlated to compounds clustering with 4 month samples ($P < 0.05$), while compounds clustering with 8 month samples were negatively correlated to metallic flavor. As found previously in butterfat and dairy products, 1-octen-3-one was positively correlated ($P = 0.0120$) to metallic flavor (Saxby 1996; Forss and others 1962). In addition, 1-penten-3-one was positively correlated ($P = 0.0010$) to metallic flavor and was previously described by Venkateshwarlu and others (2004) as metallic in nature in the presence of heptenal, which was also present in these fermented cucumber pickle products in the form of (Z)-2-heptenal.

Impact of Finished Product $CaCl_2$ Levels on Taste Characteristics

Interestingly, sour taste was greater ($P = 0.0430$) in products stored in the tank yard 8 months prior to processing, which may be due to increased acid production or potential enhancement of sour taste by the off-flavors, oxidized and green, which also increased with bulk storage time. Additionally, firmness tends to decrease with bulk storage time, so it is possible that softer cucumbers resulted in increased perception of sour taste. In contrast, sweet taste and metallic taste decreased with bulk storage time ($P < 0.05$). Likely, these tastes were subdued by the increased oxidized and green flavors through interaction or masking effects. Interestingly, the interaction of $CaCl_2$ fermentation salt and finished product $CaCl_2$ concentration had a significant effect on metallic taste, with 35 mM $CaCl_2$ fermented products significantly more metallic than 21 mM $CaCl_2$ fermented products ($P = 0.0454$).

Interaction or masking effects may also help explain the significantly lower sweet taste in products with 35 mM residual $CaCl_2$ ($P = 0.0073$; Figure 3.5), as those products were also higher in bitter taste ($P = 0.0067$; Figure 3.6). Bitter and sweet taste stimuli are both detected by G-protein coupled receptors, indicating they may be perceived through the same pathway and therefore, as perception of bitter compounds increases, it is plausible that the perception of sweet compounds would decrease (Kovacic and Somanathan 2012). One perplexing matter is why the higher concentration of $CaCl_2$ salt results in a greater intensity of bitter taste. Free calcium ions have been shown to be associated with bitter taste, but research is lacking in the true molecular mechanism of calcium detection (Neyraud and Dransfield 2004). Calcium is thought to be detected by two different types of G-protein coupled receptors (GPCRs), T1R3 and the extracellular calcium-sensing receptor (CaSR). CaSR is most prominently expressed in Type III taste cells, which are responsive to salts, such as $CaCl_2$. However, research has shown cells with CaSR are sometimes also labeled with anti-G-protein alpha-gustducin, which is a bitter taste marker (Gabriel and others 2009). Bitter compounds are known to be detected by T2Rs, a separate family of GPCRs (Neyraud and Dransfield 2004). GPCRs require a dimeric organization to function and it is common to find different types of receptors within the same cell. Receptors can interact physically through oligomerization, allowing direct transfer of information between proteins, or functionally when signal pathways for two receptors interact (Prezeau and others 2010). In turn, it is possible that T1R3 dimerizes with CaSR, as Tordoff and others (2012) speculated. Alternatively, it is possible that T1R3 may dimerize with T2R, contributing to the bitterness associated with many calcium salts, or even another receptor that has not yet been fully defined. This is a brief possible explanation for the bitter taste associated with the ionized calcium from calcium salts, such as $CaCl_2$, but the true molecular mechanism is still not clear. The addition of $CaCl_2$ at 35 mM also imparted an additional degree of saltiness to the pickles because most of the treatments showed a higher salty taste ($P = 0.0465$) for pickles with 35 mM $CaCl_2$. This could be an important finding for pickle processors because if elevated levels of $CaCl_2$ in finished products result in a more intense salty taste, processors may be able to reduce the level of sodium chloride added to their finished products.

Variables without a Significant Effect on Flavor

Cucumber size was the only controlled variable that had no significant effect on the intensity of taste or flavor attributes of finished products ($P > 0.05$), indicating that the difference in cucumber maturity between sizes 2B and 3A does not significantly affect flavor development during fermentation and bulk storage. Additionally, polish dill and astringency were the only two attributes for which the controlled variables had no observed effect ($P > 0.05$), indicating that the intensity of the characteristic dill pickle flavor and astringent sensation are not significantly affected by fermentation variability or off-flavor development (Appendix B).

3.5 Conclusions

Due to the nature of open-top commercial fermentations, longer bulk storage times lead to an increased intensity of oxidized flavor. The increase in oxidized off-flavor during bulk storage was associated with a decrease in saturated and unsaturated aldehydes and an increase in the following four ketones: (E,E)-3,5-heptadien-2-one, 4-penten-2-one, 1-(2-hydroxy-5-methylphenyl)-ethanone, and 1-cyclopropyl-1-propanone. Additionally, green flavor was discovered to increase with bulk storage time. As both oxidized and green appear to be increasing with bulk storage time, interaction effects among volatile compounds may be of importance when considering the impact of green and oxidized off-flavors on finished products. It is hypothesized that oxidation reactions could lead to either green or oxidized flavor compounds, depending on the extent of the reaction. Metallic flavor was more highly associated with products stored in the tank yard for shorter periods of time prior to processing, which may be due to a masking effect associated with the increase in offensive off-flavor compounds associated with oxidized flavor. Alternatively, the compounds correlated to metallic flavor were generally lower in samples stored in the tank yard for 8 months, which suggests that these compounds may undergo oxidation during bulk storage to compounds with different flavor characteristics. Earthy/musty flavor was influenced by fermentation variability with intensities of the flavor attribute in finished products varying based on which fermentation the cucumbers were collected from. Future research should focus on the variables affecting earthy/musty flavor development, such as environmental exposure and secondary fermentation metabolites, as the earthy/musty flavors associated with individual fermentations have the potential to taint the overall flavor profile of finished products. In conclusion, fermentation with $CaCl_2$ results in products that are detectably more oxidized, bitter, and salty, which may result in the need for an adjustment of cover brine formulations and shorter bulk storage times.

3.6 Acknowledgments

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3.7 Keywords

Sodium-free vegetable fermentation, cucumber pickles, off-flavor development, oxidation

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Table 3.1 –Spectrum™ descriptive sensory analysis training references for evaluation of taste and flavor attributes of hamburger dill chip cucumber pickles

Attribute	Definition	Training Reference	Intensity	Reference
Salty	Basic Taste	Sodium chloride in distilled water (0.2 % – 0.7 %)	2 – 15	Meilgaard and others (2007)
Sour	Basic Taste	Citric acid in distilled water (0.05 % – 0.20 %)	2 – 15	Meilgaard and others (2007)
Sweet	Basic Taste	Sucrose in distilled water (1.8 % - 5.0%)	2 – 5	Meilgaard and others (2007)
Bitter	Basic Taste	Caffeine in distilled water (0.05 % – 0.08%)	2 – 5	Meilgaard and others (2007)
Polish Dill	Aromatic characteristic of dill pickles	Polish dill emulsion dispersed in distilled water (0.001% – 0.03 %)	2 – 10	Modified from Moeller and others (2012)
Vinegar	Aromatic associated with vinegar	200 grain vinegar in distilled water (4.2 mM – 38.5 mM)	2 – 10	Modified from da Conceicao Neta and others (2007)
Oxidized	Rancid/ aromatics associated with old fryer oil	Experimental pickle with no polish dill emulsion (intensity set by trained panel)	10	N/A
Green	Sharp, slightly pungent aromatics associated with green plant/ vegetable matter	25 g fresh parsley, rinsed and chopped, soak in 300 ml distilled water for 15 min, filter, taste filtrate	6	Modified from Lee and Chambers (2007)
Earthy/Musty	Humus-like aromatics that may include damp soil, decaying vegetation or cellar-like	Frozen baby lima beans, thawed	3	Talavera-Bianchi and others (2010)
		Sliced white button mushrooms	8.5	Talavera-Bianchi and others (2010)
		Wet dirt (aroma only)	N/A	McNeill and others (2002)

Table 3.1 – Continued

Attribute	Definition	Training Reference	Intensity	Reference
Astringency	Sensation associated with dryness and puckering of the mouth	Alum in distilled water (0.26 g/L – 0.55 g/L)	2 – 5	Modified from da Conceicao Neta and others (2007)
Metallic	Aromatic associated with tin cans or aluminum foil	0.009 g ferrous sulfate / 100 g distilled water	N/A	Modified from Leksrisompong and others (2012)
		Dole unsweetened canned pineapple juice	6	Talavera-Bianchi and others (2010)

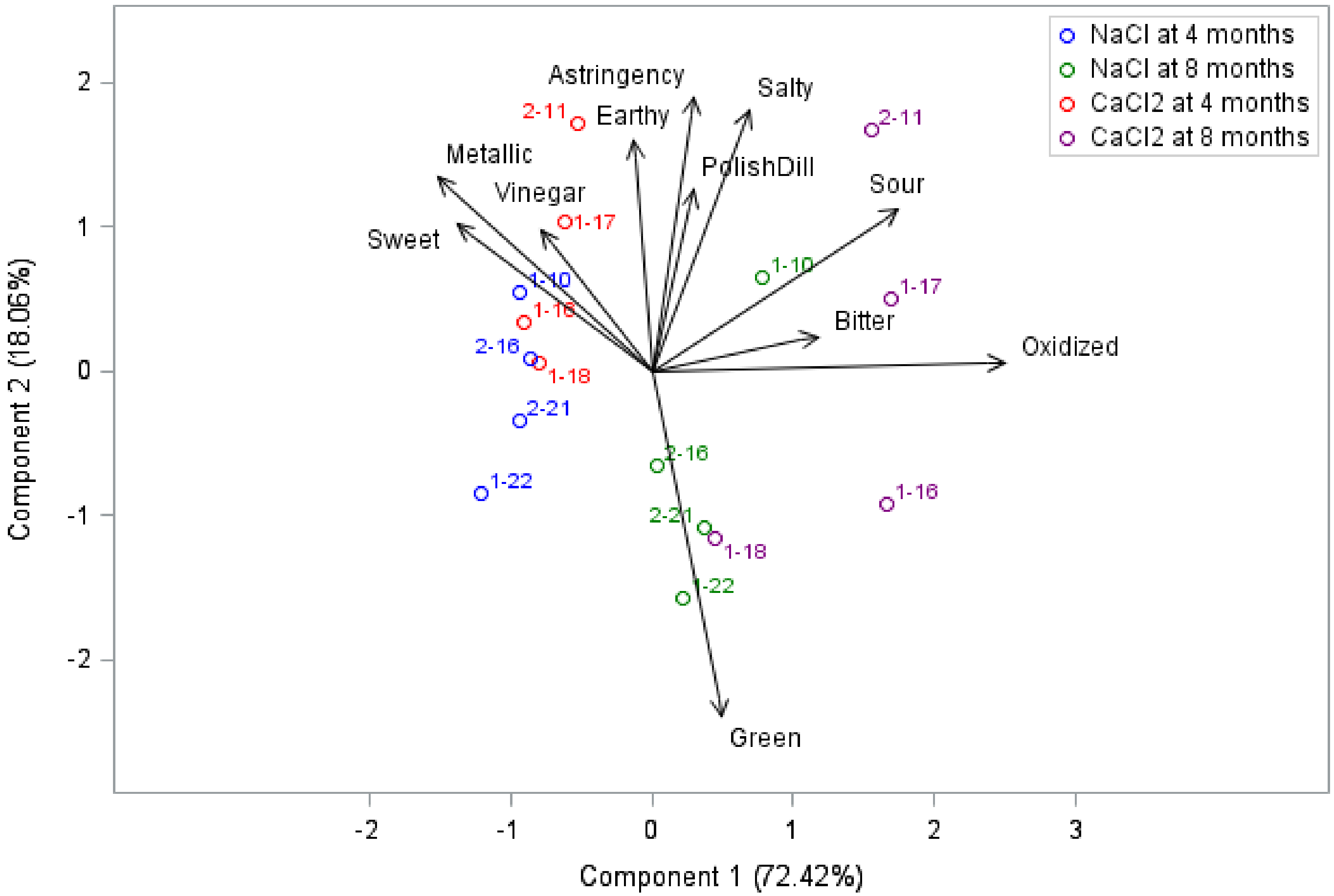


Figure 3.1 – Principal component analysis of descriptive sensory analysis scores of cucumber pickles commercially fermented in 1.03 M NaCl (n = 4) or 100 mM $CaCl_2$ (n = 4) and processed into finished products after 4 or 8 months of bulk storage

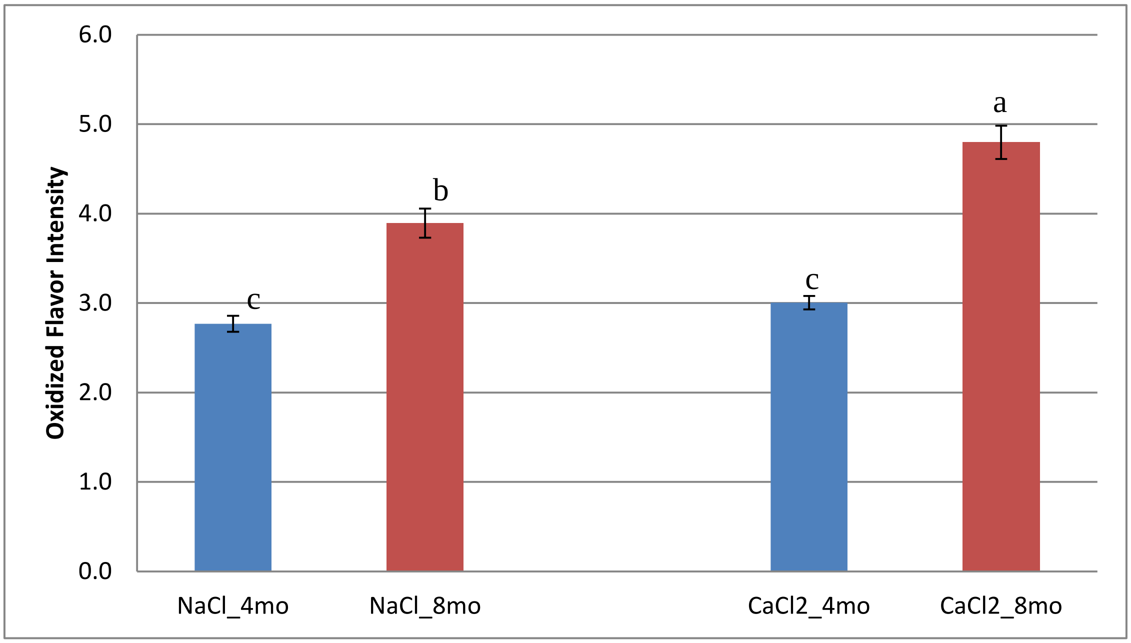


Figure 3.2 – Effect of bulk storage time and fermentation salt on oxidized flavor of hamburger dill chip pickles[1] prepared from cucumbers commercially fermented in either 1.03 M NaCl (n = 4) or 100 mM $CaCl_2$ (n = 4)

[1]Samples evaluated in duplicate by 9 trained panelists; Scores averaged across finished products from 4 tanks, 2 with cucumber size 2B and 2 with cucumber size 3A, as well as finished product $CaCl_2$ of 21 mM and 35 mM ; Standard error bars reported

[2] Means with the same letter (a-b) are not significantly different; Tukey's honestly significant difference, $P < 0.05$

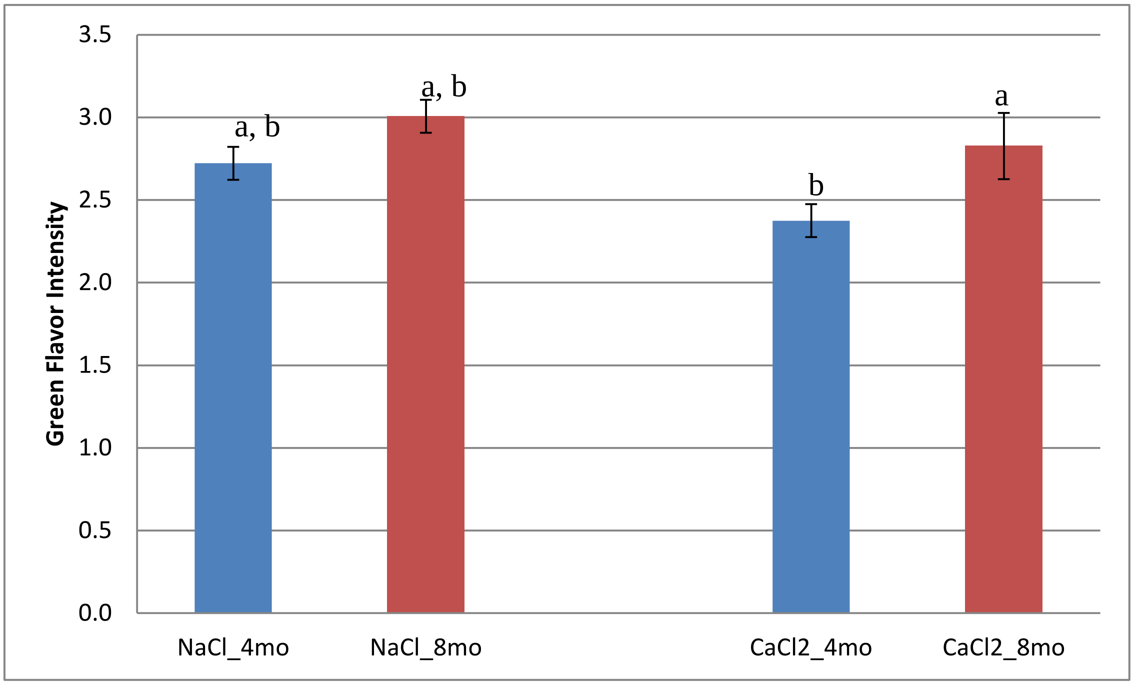


Figure 3.3 – Effect of bulk storage time on green flavor of hamburger dill chip pickles[1] prepared from cucumbers commercially fermented in either 1.03 M NaCl (n = 4) or 100 mM $CaCl_2$ (n = 4)

[1]Samples evaluated in duplicate by 9 trained panelists; Scores averaged across finished products from 4 tanks, 2 with cucumber size 2B and 2 with cucumber size 3A, as well as finished product $CaCl_2$ of 21 mM and 35 mM ; Standard error bars reported
[2] Means with the same letter (a-b) are not significantly different; Tukey's honestly significant difference, $P < 0.05$

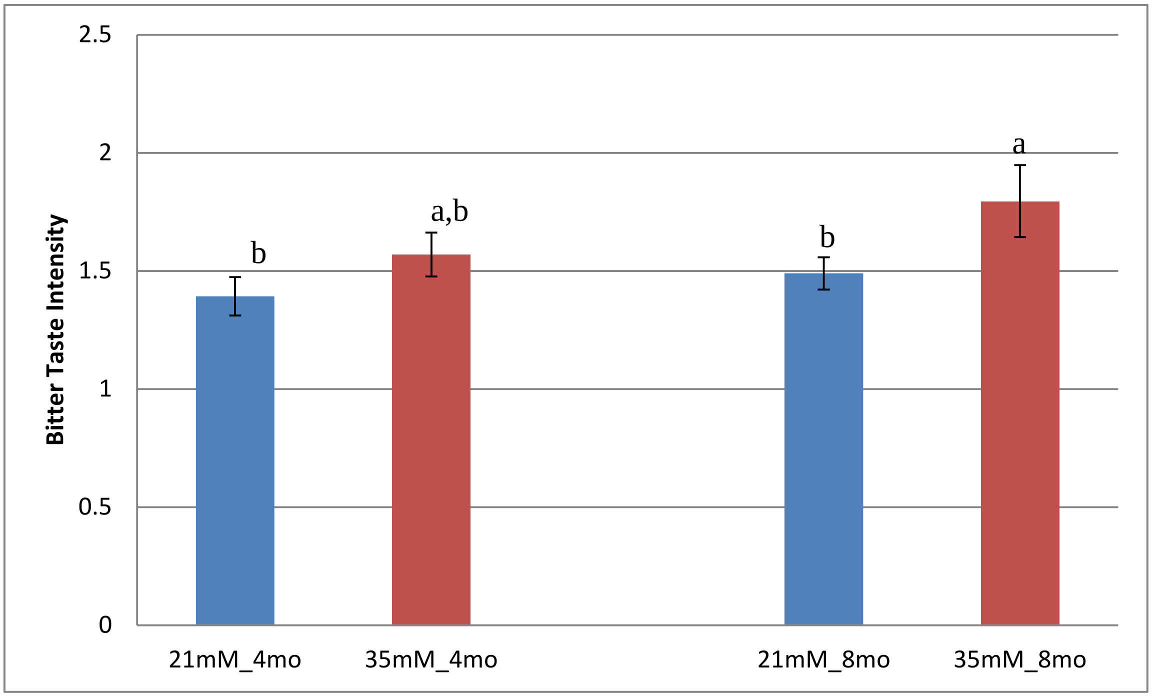


Figure 3.4 – Effect of finished product calcium chloride ($CaCl_2$) and bulk storage on bitter taste intensity of hamburger dill chip pickles[1] with either 21 or 35 mM finished product $CaCl_2$ prepared from cucumbers stored in a commercial tank yard 4 or 8 months prior to processing

[1]Samples evaluated in duplicate by 9 trained panelists; Scores averaged across finished products from 8 commercial fermentation tanks fermented in either NaCl (n=4) or $CaCl_2$ (n = 4) of size 2B or 3A; Standard error bars reported

[2] Means with the same letter (a-b) are not significantly different; Tukey's honestly significant difference, $P < 0.05$

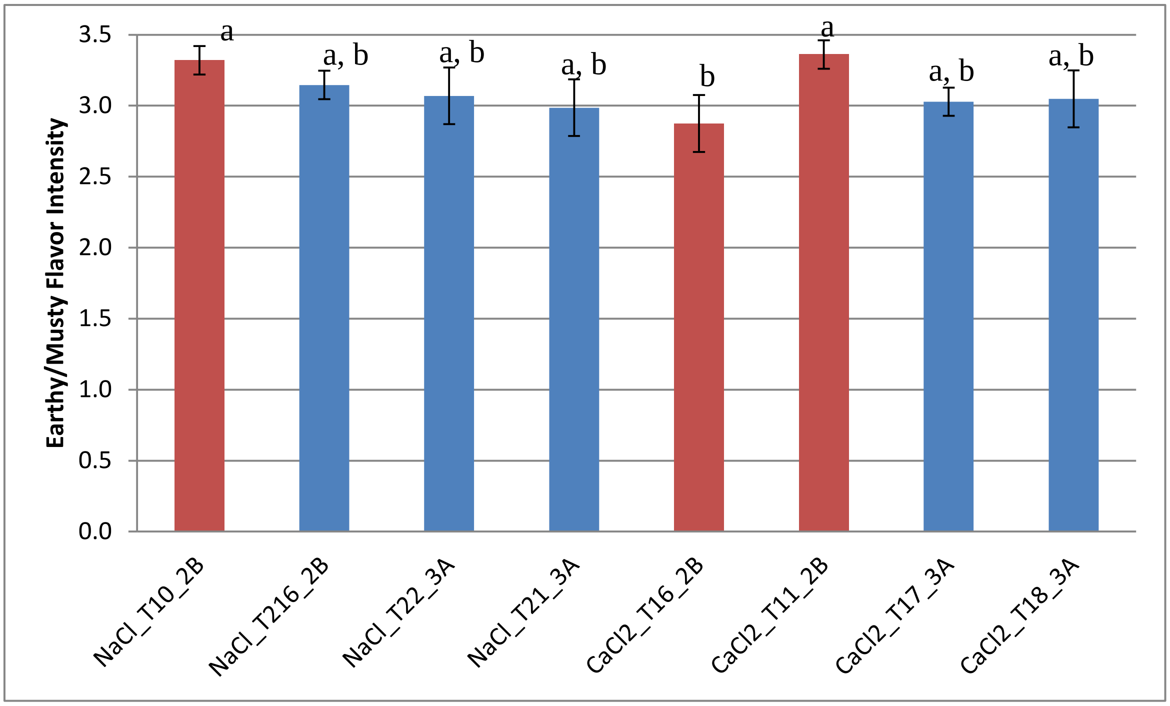


Figure 3.5 – Effect of fermentation variability on earthy/musty flavor of hamburger dill chip pickles[1] prepared from cucumbers commercially fermented in either 1.03 M NaCl (n = 4) or 100 mM $CaCl_2$ (n = 4)

[1]Samples evaluated in duplicate by 9 trained panelists; Scores averaged across finished product $CaCl_2$ of 21 mM and 35 mM and tank yard storage time of 4 months and 8 months; Standard error bars reported
[2] Means with the same letter (a-b) are not significantly different; Tukey's honestly significant difference, $P < 0.05$

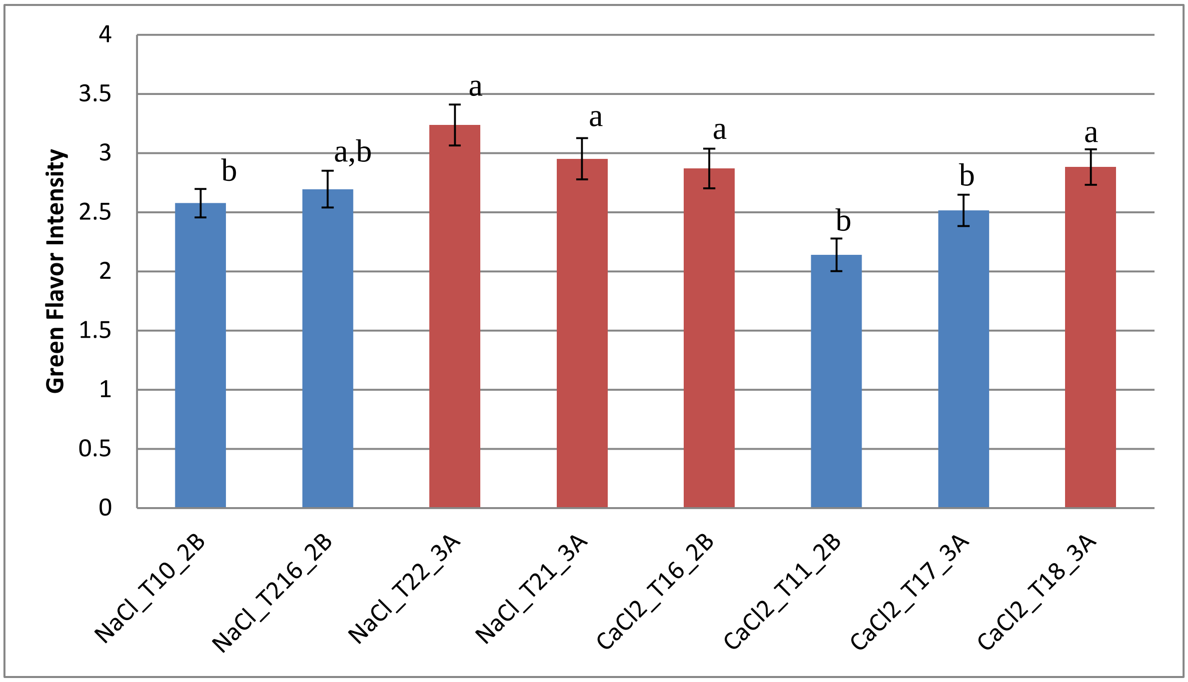


Figure 3.6 – Effect of fermentation variability on green flavor of hamburger dill chip pickles[1] prepared from cucumbers commercially fermented in either 1.03 M NaCl (n = 4) or 100 mM $CaCl_2$ (n = 4)

[1]Samples evaluated in duplicate by 9 trained panelists; Scores averaged across finished product $CaCl_2$ of 21 mM and 35 mM and tank yard storage time of 4 months and 8 months; Standard error bars reported
[2] Means with the same letter (a-b) are not significantly different; Tukey's honestly significant difference, $P < 0.05$

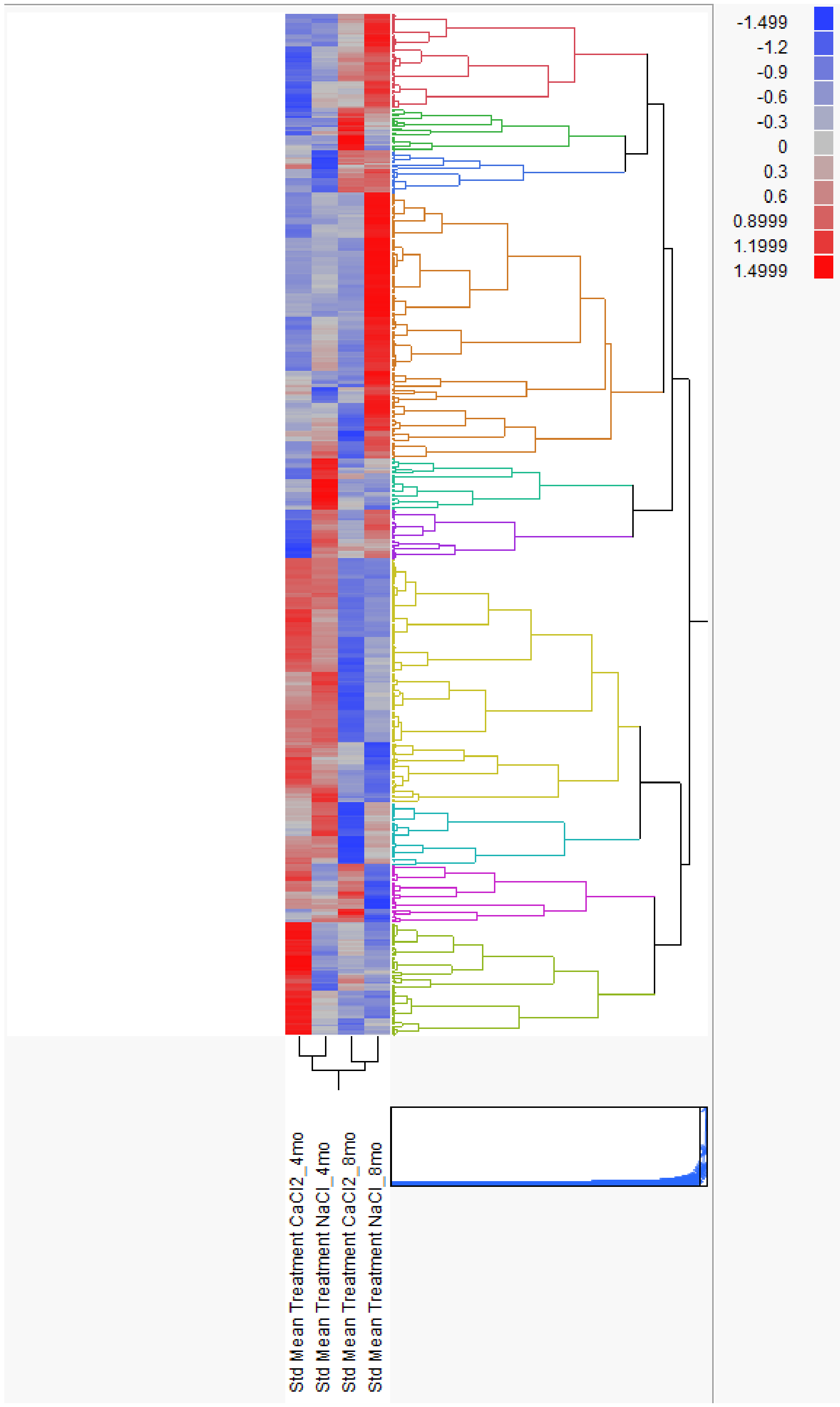


Figure 3.7 – Hierarchical clustering of volatile compounds that changed significantly (*P* < 0.05) in cucumber dill chips commercially fermented in either sodium chloride (NaCl) or calcium chloride ($CaCl_2$) for either 4 months or 8 months prior to processing and shelf storage of 1.5 months

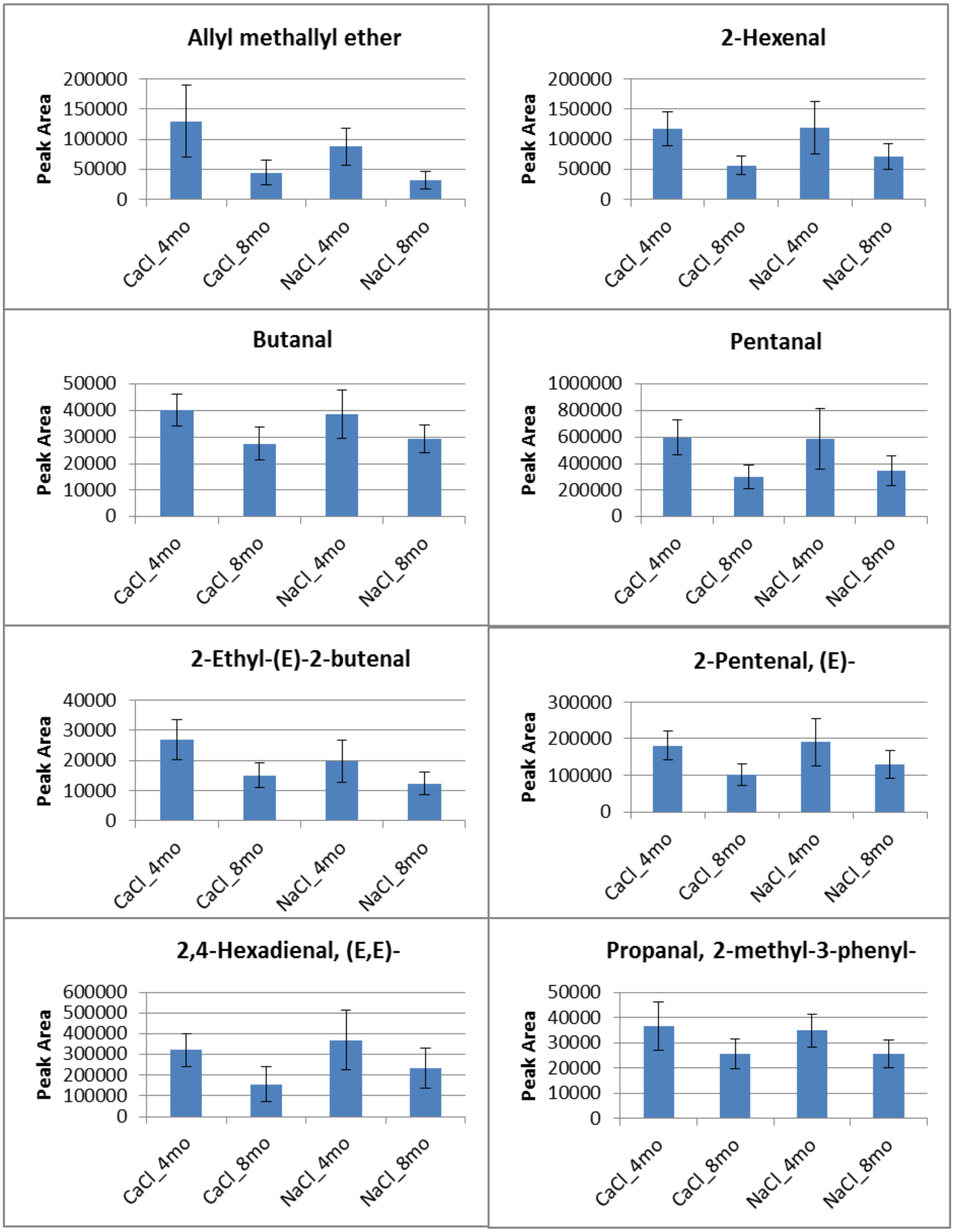


Figure 3.8 – Decrease in relative abundance of saturated and unsaturated aldehydes in finished cucumber pickle products fermented in either sodium chloride (NaCl) or calcium chloride ($CaCl_2$) and processed after 4 or 8 months of bulk storage

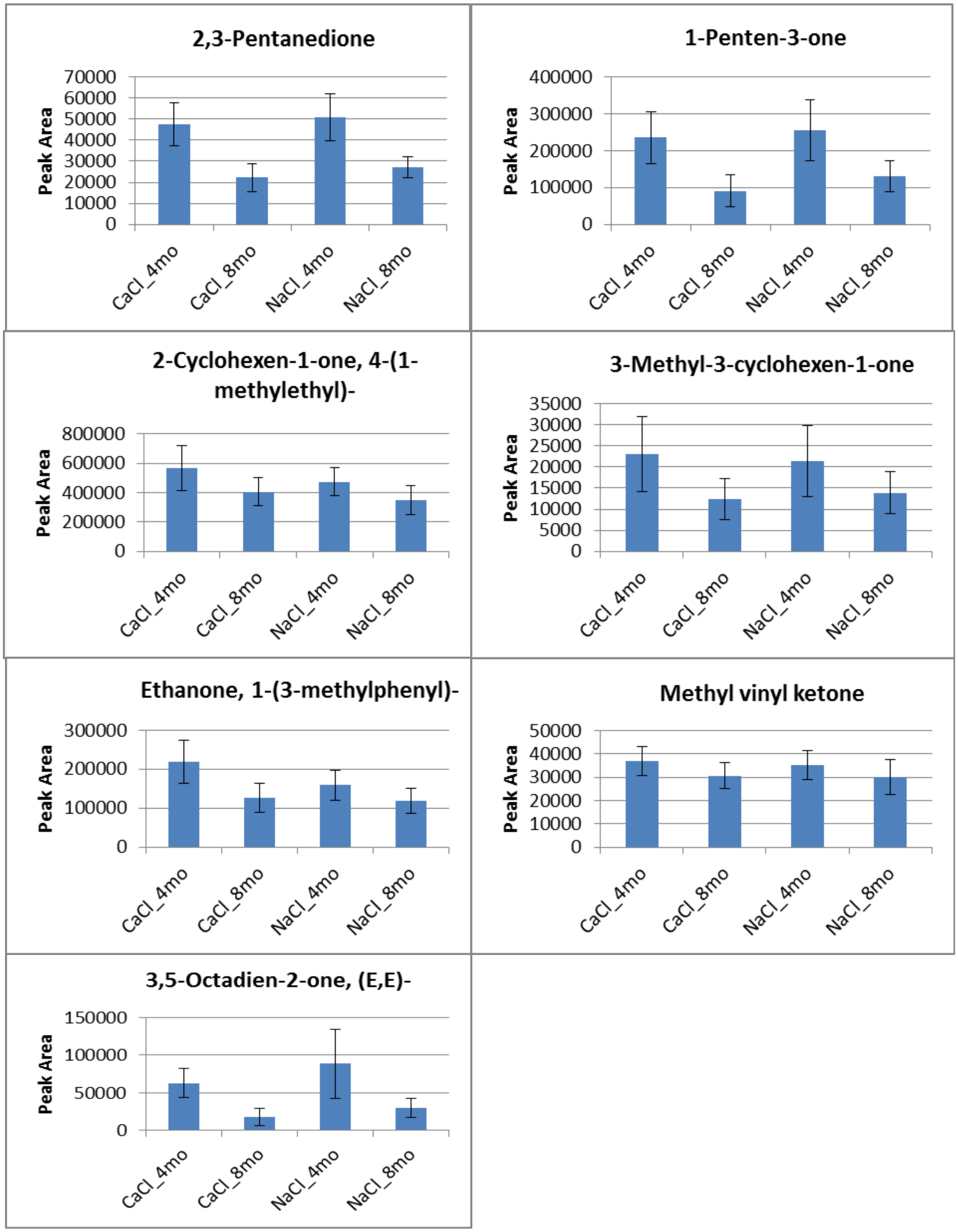


Figure 3.9 – Decrease in relative abundance of ketones in finished cucumber pickle products fermented in either sodium chloride (NaCl) or calcium chloride ($CaCl_2$) and processed after 4 or 8 months of bulk storage

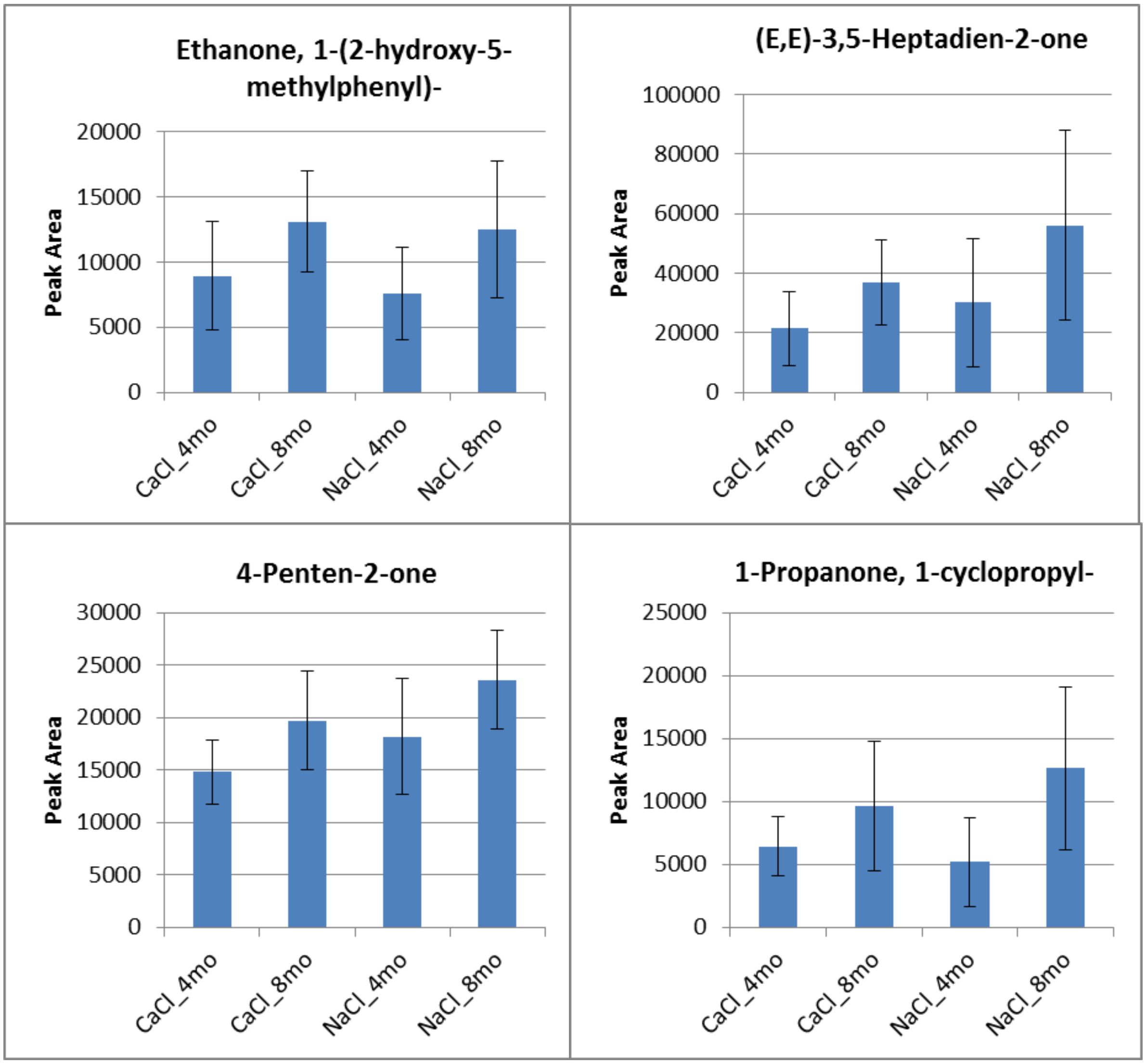


Figure 3.10 – Increase in relative abundance of ketones in finished cucumber pickle products fermented in either sodium chloride (NaCl) or calcium chloride ($CaCl_2$) and processed after 4 or 8 months of bulk storage

4 months Bulk Storage | **8 months Bulk Storage**

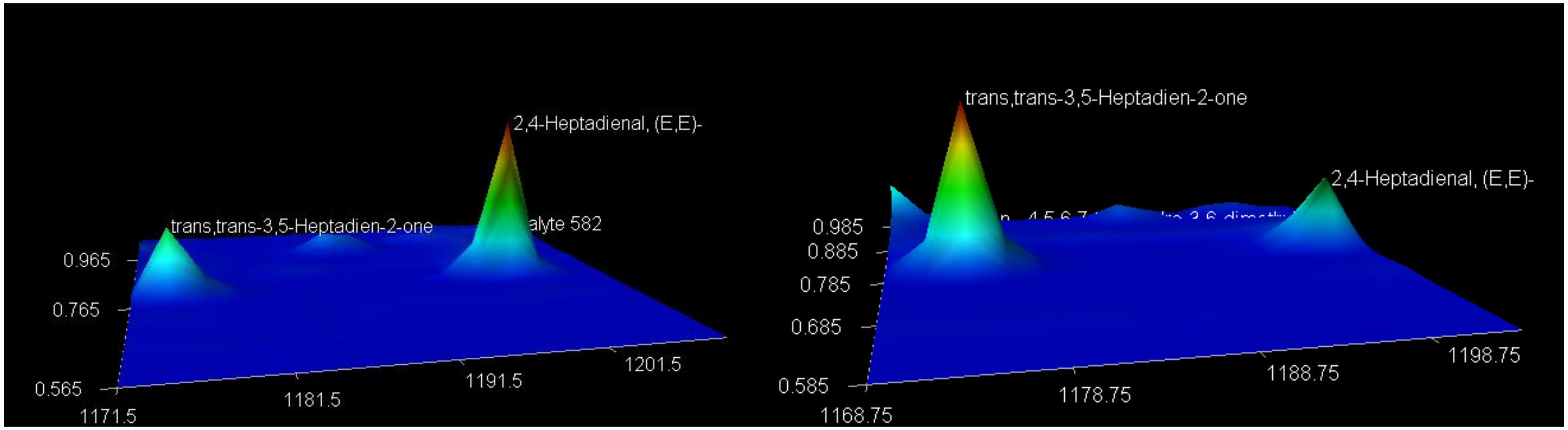


Figure 3.11 – Decrease in (E,E)-2,4-Heptadienal and increase in (E,E)-3,5-Heptadien-2-one in hamburger dill chip cucumber pickle samples processed after 4 or 8 months bulk storage and stored on the shelf for 1.5 months prior to evaluation

Chapter 4: Conclusions

Fermentation of cucumbers with calcium chloride ($CaCl_2$) as an environmentally-friendly alternative to the traditional sodium chloride (NaCl) salt was shown to result in a stable fermentation process with similar chemical changes by the fermentative microorganisms and similar firmness retention on a laboratory scale (McFeeters and Perez-Diaz 2010). However, the sensory quality of the finished products was not determined. Additionally, the appropriate method for commercial processing of $CaCl_2$ fermented cucumbers was uncertain. While one desalting step is applied to traditional NaCl fermented cucumbers to obtain the proper concentration of NaCl in the finished product, producers were unsure of whether $CaCl_2$ fermented cucumbers would need one or two desalting steps prior to processing to reduce the level of $CaCl_2$ in finished products to an acceptable level that would not impart an adverse taste to finished products.

This research evaluated consumer acceptability of products from commercial $CaCl_2$ cucumber fermentations and found that consumers can detect a difference ($P < 0.05$) between $CaCl_2$ fermented cucumbers and NaCl fermented cucumber pickles. Through a series of pairwise preference tests, it was hypothesized that consumer preference was influenced by either fermentation variability or an inherent characteristic related to the fermentation salt, but not $CaCl_2$ taste. This hypothesis was further supported by a consumer liking test (n= 73) in which no difference in flavor liking was found among treatments with and without elevated product $CaCl_2$ concentration, but a significant difference in texture liking was found, with $CaCl_2$ fermented products receiving lower liking scores for texture. Instrumental analysis revealed that consumers disliked the significantly lower firmness ($P > 0.05$) of the

$CaCl_2$ fermented products. Descriptive sensory analysis found that products with 35 mM finished product $CaCl_2$ were more bitter and salty, and less sweet, than products with 21 mM finished product $CaCl_2$ ($P < 0.05$). This finding was expected with a trained panel, but the 50 % detection threshold of $CaCl_2$ (n = 52) in fermented hamburger dill chip pickles was found to be 64.1 mM, which is well above the legal limit of 36 mM. This finding in conjunction with the results of the consumer liking test support that the adverse taste typically associated with elevated levels of $CaCl_2$ in food products is likely not of concern in relation to consumer acceptability of fermented cucumber pickles, up to the legal limit of 36 mM.

Descriptive sensory analysis further revealed that bulk storage time had a significant effect on flavor, with oxidized and green flavors increasing during bulk storage ($P < 0.05$). Volatile compound analysis supported this finding, with compounds significantly positively correlated to oxidized and green flavors clustering with samples stored in the tank yard for longer periods of time. In general, $CaCl_2$ fermented products had a greater oxidized flavor, which suggests that $CaCl_2$ fermentations may more readily or more quickly produce oxidized flavor compounds. The increase in oxidized off-flavor during bulk storage was associated with a decrease in saturated and unsaturated aldehydes and an increase in the following four ketones: (E,E)-3,5-heptadien-2-one, 4-penten-2-one, 1-(2-hydroxy-5-methylphenyl)-ethanone, and 1-cyclopropyl-1-propanone. Earthy/musty and green flavors were found to be significantly affected ($P < 0.05$) by individual fermentations, indicating these particular off-flavors are highly influenced by tanking variability, such as cucumber source, environmental exposure, or microbial growth.

Future research should focus on in-depth texture analysis of $CaCl_2$ fermented products and evaluate possible ways to retard the deterioration of texture. In addition, there is a need to better understand the biochemical and microbiological reactions occurring in $CaCl_2$ fermented products, during fermentation and after bulk storage, and how they differ from traditional NaCl fermented products. This may assist in understanding why $CaCl_2$ fermented cucumbers tend to result in finished products with a higher intensity of oxidized flavor.

In conclusion, data supports that $CaCl_2$ fermented products are a viable option as an environmentally-friendly replacement for traditional NaCl fermented products when processed using short periods of bulk storage. However, more research is recommended prior to implementing $CaCl_2$ fermentations using the long-term bulk storage and shelf storage that many pickle processors are accustomed to.

APPENDICES

Appendix A: Validation of Method for Threshold Calculations

When using the ascending forced choice method of threshold testing, the common method of calculating the threshold involves the American Society of Testing and Materials' (ASTM) best estimate threshold (BET) (E679-91; Table A1). However, an alternative method has also been proposed by Lawless (2010). The alternative method involves interpolation of the chance-corrected data and has many advantages over the traditional method. The ASTM method:

- Has no formal consideration of the potential for panelists to be correct by guessing.
- Discounts low, correct detections if a panelist loses detection at any higher concentration, possibly missing out on effects such as fatigue, adaptation, or an overwhelmed palate.
- Estimates some BET's without actual data if a panelist gets the last row in the test wrong or the first one right.

Furthermore, the alternative analysis enables interpolation of detection levels other than 50%, which could be particularly beneficial in the food industry with regards to regulations or interest in the detection threshold of more sensitive individuals.

To compare the two methods of calculation, a threshold test was performed on $CaCl_2$ in a model brine system with 0.2% salt (NaCl) and 0.5% 200 grain vinegar. The ASTM group BET (approximately 50% detection level) was calculated as 16.4 mM $CaCl_2$, while the alternative method gave a threshold value of 19.1 mM $CaCl_2$ for a 50% detection level. The ASTM method does not claim to provide a strict 50% detection threshold, but a value "not far therefrom". The ASTM method appears to underestimate the threshold in comparison to the alternative method, which may be preferred. However, the alternative method is a more logical method of calculation that can be utilized more widely and does not differ greatly from the 50% detection level estimated by the ASTM method.

Table A1 – Example of ASTM group Best Estimate Threshold (BET) Calculation of a 5-series Threshold Test with a Step Factor of 1.4

Panelist	**$CaCl_2$ Concentration (mM)**					**Individual BET**	**Log_{10}(BET)**	**Key**	
	26	**36**	**50**	**70**	**98**				
1	0	1	0	0	1	=SQRT(70*98)	1.92	0	Incorrect
2	2	0	1	2	1	=SQRT(36*50)	1.63	1	Correct, Sure
3	1	1	1	1	1	=SQRT((26/1.4)*26)	1.34	2	Correct, Unsure
4	2	0	2	1	1	=SQRT(36*50*1.4)	1.70		
						Average	1.65		
						Anti-Log / Group BET (mM)	44.4		

A 3-AFC test has a chance probability of 33.3%, requiring 2/3 of the panel to correctly detect the presence of $CaCl_2$ to obtain a 50% detection level. To determine the true proportion correct that must be observed to obtain a 50% detection threshold, the following formula can be used to take into account the probability of guessing (Lawless 2010).

$$P_{detection} = (P_{observed} - P_{chance})/(1 - P_{chance})$$

To create the graph used to calculate the alternative threshold, the percent correct at each concentration step is plotted on the x-axis against the concentration of $CaCl_2$ (mM) on the y-axis, allowing for the concentration at a specific level of detection to be interpolated (Figure A1).

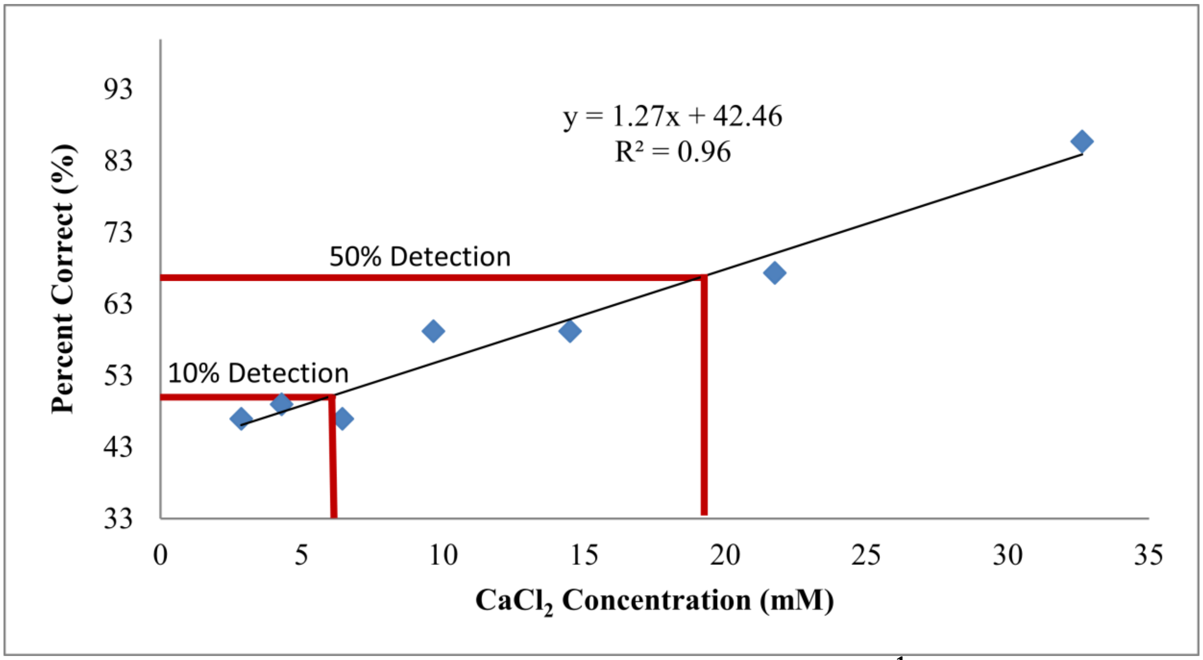


Figure A1 – Detection threshold of $CaCl_2$ in a model brine system[1] as determined by an alternative threshold calculation method[2] displaying interpolation at chance-corrected[3] proportions and the corresponding estimated detection levels.

[1] 0.2% salt (NaCl) and 0.5% 200 grain vinegar in distilled water
[2] Lawless 2010
[3] Chance = 33.3%

Appendix B: Supplementary Statistical Information

Supplemental Material to Chapter 2

Power Analysis

Power is the ability of a significance test to detect a real effect or the probability of rejecting the null hypothesis, given that it is false. While discrimination testing protects against the alpha risk, in which the chance of declaring a false difference is alpha, similarity testing involves stricter control of the beta risk. Similarity testing is different in that it must keep the probability of missing a true difference low. In turn, while a power of 80% is typically considered sufficient for discrimination testing, a power of 95% is typically used when performing a similarity test.

Power Analysis for Threshold Testing

Description of Test: One-sample t-test; H_o: $\mu = 36$; H_a: $\mu \neq 36$

A sample size of 52 assessors provides 82.5% power to detect a 9.0 mean deviation with a standard deviation of 22, which is a conservative estimate for this test, in which the difference between the middle concentration of 36 mM and the next higher concentration of 50 mM was 14 (Figure B1). To detect the same mean deviation of 9.0 under the same conditions, but with a power of 95%, as would be necessary for similarity testing, a sample size of 80 would be required.

Power Analysis for Preference Testing

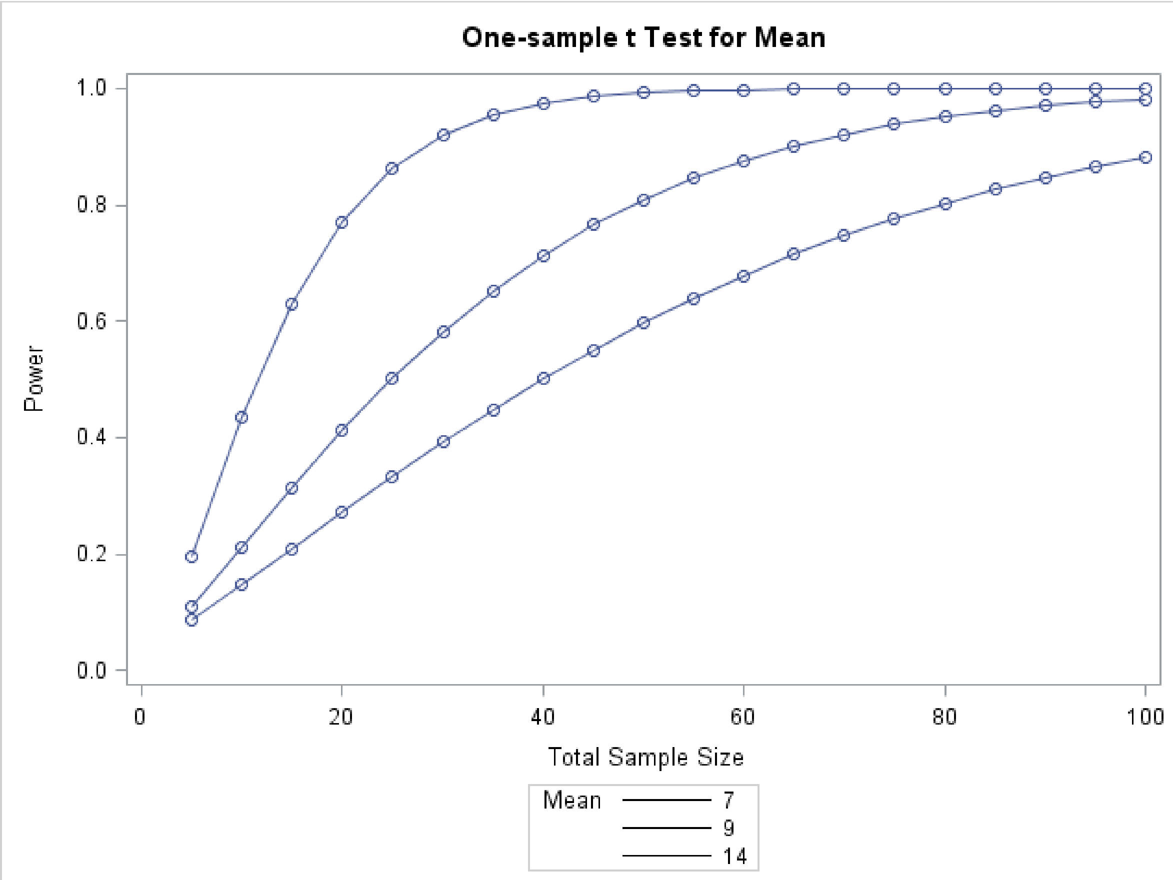
Description of Test: Binomial Proportion; H_o: $\mu = 0.50$; H_a: $\mu \neq 0.50$

A paired preference test has a null hypothesis that the proportion of respondents preferring sample A as opposed to sample B will be 0.50. In turn, the appropriate power test for preference testing would involve the exact test of a single binomial proportion with a null proportion of 0.50. A sample size of 50 would enable one to detect a proportion of 0.30 with 78.2% power (Figure B2). To detect a mean deviation of only 0.10 from the null proportion of 0.50 with a similar power of 78.7%, 200 assessors would be needed.

Power Analysis for Liking Testing

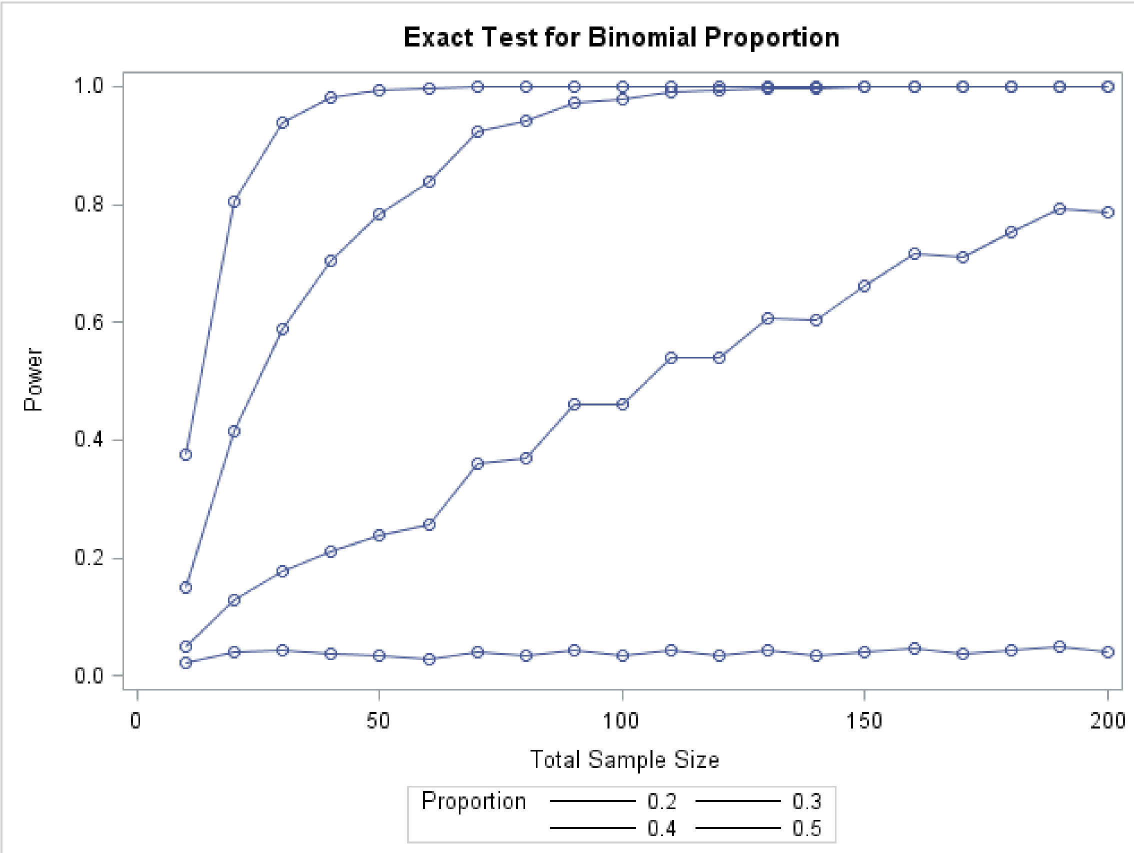
Description of Test: ANOVA F-Test; H_o: all means are equal; H_a: at least one mean differs

Figure B3 was the power curve used in determining the number of consumers needed to complete the liking test. The standard deviation of 2.2 was obtained from a previous liking test on hamburger dill chip pickles (Moeller 2011). To obtain a power of approximately 80% with the ability to detect a difference in liking of 1 point among 4 samples, one would need to have 72 consumers complete the test. However, the actual standard deviation in the liking test (n = 73) was no greater than 2.0, which results in an increased statistical power of approximately 88%.



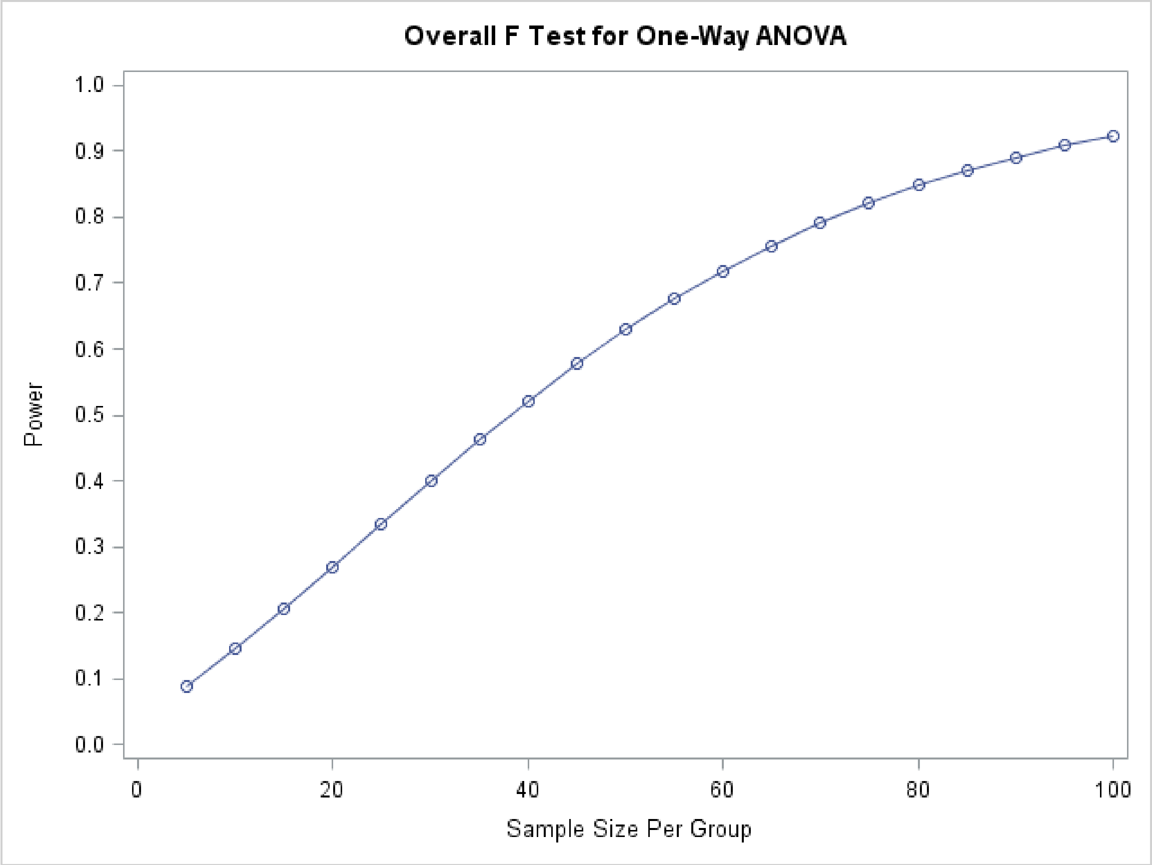
Power for n = 52		
Index	**Mean Deviation**	**Power**
1	7	0.615
2	9	0.825
3	14	0.994

Figure B1 – Power analysis showing relationship between sample size and power for a one-sample t-test with a standard deviation of 22 and a mean deviation of 7, 9, or 14.



Proportion	N Total	Power
0.2	50	0.994
0.2	100	>.999
0.3	50	0.782
0.3	100	0.979
0.4	50	0.237
0.4	100	0.462
0.5	50	0.033
0.5	100	0.035

Figure B2 – Power analysis showing the relationship between the sample size and statistical power of a binomial distribution with a null proportion of 0.50 and a hypothetical binomial proportion of either 0.20, 0.30, 0.40, or 0.50.



Fixed Scenario Elements	
Method	Exact
Group Means	5 5 5 6
Standard Deviation	2.2
Sample Size Per Group	72
Alpha	0.05
Power	0.804

Figure B3 – Power analysis showing the relationship between the sample size and statistical power of an ANOVA F-test to detect a difference of 1.0 point between 4 means with an average standard deviation of 2.2.

Supplemental Material to Chapter 3

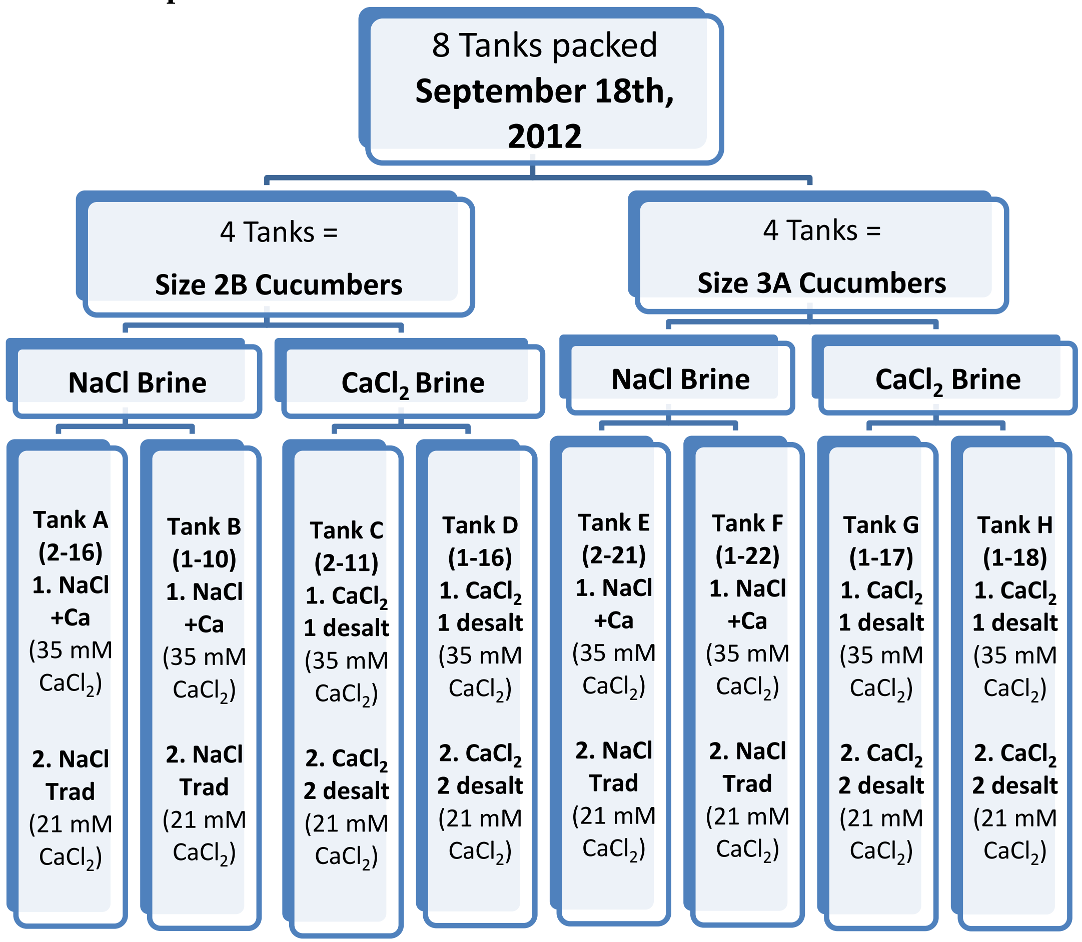


Figure B4 – Completely randomized full factorial design of commercial cucumber fermentation tanks (n=8) assigned to treatments by cucumber size and fermentation brining salt and processed into products with an equilibrated sodium chloride (NaCl) concentration of 0.38 M and either 21 mM or 35 mM calcium chloride ($CaCl_2$).

Table B1 – Results of repeated measures ANOVA modeling brining salt (NaCl or $CaCl_2$), cucumber size (2B or 3A), bulk storage time (4 months or 8 months), and $CaCl_2$ concentration in finished products (21 mM or 35 mM) as fixed effects, TankID nested within bulk storage as random, and bulk storage as repeated

Attribute	Type III test of Fixed Effects – Significant Effects	P-Value ($\alpha = 0.05$)	Least Square Means Significance	P-value (Tukey's Adjustment)
Polish Dill	NONE			
Vinegar	Brining Salt	0.0389	$CaCl_2$ > NaCl	0.0389
	Bulk Storage Time	0.0136	4 mo > 8 mo	0.0136
	Brining Salt*$CaCl_2$*Bulk Storage Time	0.0051	$CaCl_2$*35*4mo > NaCl* 21*8 mo	0.0294
			NaCl* 21*4mo > NaCl*21*8mo	0.0467
Oxidized	Brining Salt	0.0106	$CaCl_2$ > NaCl	0.0106
	Bulk Storage Time	< 0.0001	8 mo > 4 mo	< 0.0001
Green	Bulk Storage Time	0.0040	8 mo > 4 mo	0.0040
	Brining Salt *$CaCl_2$	0.0404		
Salty	$CaCl_2$	0.0465	35 > 21	0.0465
	Brining Salt *$CaCl_2$* Bulk Storage Time	0.0494	None	
Sour	Bulk Storage Time	0.0430	8 mo > 4 mo	0.0430
Sweet	$CaCl_2$	0.0073	21 > 35	0.0073
	Bulk Storage Time	0.0015	4 mo > 8 mo	0.0015
	$CaCl_2$ * Bulk Storage Time	0.0254	21*4mo > 21*8 mo	0.0033
			21*4 mo > 35*4mo	0.0091
			21*4mo > 35*8mo	0.0017
Bitter	$CaCl_2$	0.0067	35 > 21	0.0067

Table B1 – Continued

Earthy	NONE			
Astringency	NONE			
Metallic	Bulk Storage Time	0.0050	4 mo > 8 mo	0.0050
	Salt*$CaCl_2$	0.0251	$CaCl_2$*35 > $CaCl_2$*21	0.0454

Table B2 – Results of repeated measures ANOVA modeling TankID[1], bulk storage time (4 months or 8 months), and the interaction as fixed effects

Attribute	Type III test of Fixed Effects – Significant Effects	P-Value ($\alpha = 0.05$)	Least Square Means Significance	P-value (Tukey's Adjustment)
Polish Dill	NONE			
Earthy	TankID	0.0168	1-10 > 1-16	0.0392
			2-11> 1-16	0.0205
Astringency	NONE			
Sour	Bulk Storage Time	0.0435	8 mo > 4 mo	
Green	TankID	0.0009	1-22 > 1-10	0.0387
			1-16 > 2-11	0.0187
			1-22 > 1-17	0.0201
			1-18 > 2-11	0.0161
			1-22 > 2-11	0.0004
			2-21 > 2-11	0.0077
	Bulk Storage Time	0.0010	8 mo > 4 mo	0.0010
Bitter	NONE			

[1]This analysis was only performed for attributes which did not display significance for brining salt or cucumber size, which were nested within TankID. TankID refers to an individual fermentation tank from which samples were collected from.

Appendix C: Supplementary Sensory Materials

“A” – “Not A” Test

Panelist No. ________
Date________

Test Procedure
Unsalted crackers and water have been provided as palate cleansers between samples.

1. You will be given 2 reference sample cups labeled “A” and “Not A”. Familiarize yourself with these samples before the test begins. Reference samples will be **removed and not returned**.

   When you are ready, turn the table to open the window.

2. Taste sample 027. Is this sample the same as reference “A” or “Not A”? Circle one.

Sample Number	**The Sample is…**	
027	**A**	**Not A**

   Turn the table to open the window.

3. Taste sample 914. Is this sample the same as reference “A” or “Not A”? Circle one.

Sample Number	**The Sample is…**	
914	**A**	**Not A**

   Turn the table to open the window.

Give your ballot to the attendant and you may choose a food treat before leaving.

Thank You!

Paired Preference Test

Panelist No. _____
Date________

Instructions:

- Cleanse palate with water and unsalted crackers, as needed.

- Taste your first pair of samples.

Circle the number of the sample you prefer, or indicate if you do not have a preference.

____ ____

No Preference

- Cleanse palate with water and unsalted crackers, as needed.
- Repeat with your second pair of samples.

Circle the number of the sample you prefer, or indicate if you do not have a preference.

____ ____

No Preference

Demographic Questions Date:_____________

What is your gender?
_____Male _____Female

What is your age?
_____18 to 25 _____26 to 35 _____36 to 45 _____46 to 55 _____56 to 65 ____66+

What is your ethnicity?
____Caucasian ____African American ____ Hispanic ____Asian/Pacific Islander
____Other___________

How often do you eat pickles?
_____Every day
_____Several times a week
_____Once a week
_____Two or three times a month
_____Once a month
_____Several times a year
_____Once a year
_____Never

What do like most about cucumber pickle products (Choose ONE!!)?
_____Crunchiness
_____Firmness
_____Salty taste
_____Sour taste
_____Sweet taste
_____Dill flavor

Do you make the purchasing decisions for your household? ____Yes ____No

How often do you buy pickles for your home?
_____Once a week or more often
_____Two to three times a month
_____Once a month
_____Several times a year
_____Once a year
_____Never

What brand do you prefer to buy when buying pickles?
____Claussen ____Miss Jenny's ____Mt. Olive ____Vlasic ____Other__________

Which of the following affects your decisions when buying pickles?(Mark ALL that apply).
_____Color
_____Flavor
_____Texture
_____Brand
_____Packaging type
_____Package size
_____Cost

Date _______ Panelist No____

Consumer Taste Test
Sample Number XXX

Please rinse out your mouth with cracker and water before starting.
Use cracker and water throughout the test, as needed.

Taste the sample and indicate your OVERALL LIKING of the pickle by checking <u>one box</u> below.

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely				Neither Like nor Dislike				Like Extremely

What did you like **<u>most</u>** about this sample?____
What did you like **<u>least</u>** about this sample? ____

Now, *only LOOK* at the pickle sample and check which box corresponds to how much you like the APPEARANCE of the sample.

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely				Neither Like nor Dislike				Like Extremely

What did you like **<u>most</u>** about the appearance? ____
What did you like **<u>least</u>** about the appearance? ____

How much do you like the FLAVOR of this pickle sample? Check ONE box below.

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely				Neither Like nor Dislike				Like Extremely

What did you like **<u>most</u>** about the flavor? ____
What did you like **<u>least</u>** about the flavor? ____

How much do you like the TEXTURE of this pickle sample? Check ONE box below.

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike Extremely				Neither Like nor Dislike				Like Extremely

What did you like **<u>most</u>** about the texture? ____
What did you like **<u>least</u>** about the texture? ____

Threshold Determination on Pickles

Date______________________

Demographic Information

Age:
___18-25 ___26-35 ___36-45 ___46-55 ___56-65 ___66+

Gender:
___ Female ___ Male ___ Prefer not to answer

How often do you eat pickles? (Check ONE)
_____Every day _____Several times a week _____Once a week _____2-3 times per month
_____Once a month _____Several times a year _____Once a year _____Never

Test Procedure

There are a set of samples in each test row (series of 3) for you to evaluate. You will start by tasting the samples closest to you from left to right; this is row 1. **You may swallow or expectorate the sample into the designated cup**, rinse your mouth well with water, and expectorate the rinse water before moving to the next sample. Unsalted crackers have also been provided to assist in cleansing your palate between samples. Within each row (series of 3), write the random three-digit number of the sample which is *different* from the others. WAIT 2 MINUTES BETWEEN ROWS. Then, proceed to the next row (series of 3) and repeat the tasting sequence.

FOCUS ON DIFFERENCE IN **TASTE**

Row	**Odd Sample (3-digit #)**	**Are you sure? (Yes or No)**	**Description of taste difference *(Optional)***
1 **2 Minute Break**			
2 **2 Minute Break**			
3 **2 Minute Break**			
4 **2 Minute Break**			
5 **2 Minute Break**			

North Carolina State University
INFORMED CONSENT FORM for RESEARCH

Title of Study
Sensory evaluation of $CaCl_2$ and NaCl fermented cucumbers

Principal Investigator	Faculty Sponsor (if applicable)
Emily Wolter	Dr. Suzanne Johanningsmeier

What are some general things you should know about research studies?

You are being asked to take part in a research study. Your participation in this study is voluntary. You have the right to be a part of this study, to choose not to participate or to stop participating at any time without penalty. The purpose of research studies is to gain a better understanding of a certain topic or issue. You are not guaranteed any personal benefits from being in a study. Research studies also may pose risks to those that participate. In this consent form you will find specific details about the research in which you are being asked to participate. If you do not understand something in this form it is your right to ask the researcher for clarification or more information. A copy of this consent form will be provided to you. If at any time you have questions about your participation, do not hesitate to contact the researcher(s) named above.

What is the purpose of this study?

The purpose of this study is to determine the effects of different fermentation treatments on the finished fermented cucumber pickle products.

What will happen if you take part in the study?

If you agree to participate in this study, you will be asked to partake in a sensory test that involves tasting samples, all of which are prepared with FDA-approved food ingredients. After signing this consent form, you will be given a demographic sheet and ballot that outlines the exact test procedure, step-by-step. You will follow those directions for sampling and tasting and record your answers. At any time, you can ask questions or raise concerns. Each voluntary test session should take about 10 minutes of your time and will be held in an available room on the first floor of Schaub Hall, NC State University, 400 Dan Allen Dr., Raleigh, NC 27695.

Risks

Some samples in this study may or may not contain yellow 5, a common colorant in commercial pickle products, which some individuals may have adverse reactions to. Individuals who may be allergic to yellow 5 should not participate.

Benefits

This study will provide valuable information for the pickling industry. Information from this study could assist the pickling industry in adopting a more environmentally friendly fermentation process, while still providing the traditional, high quality product that is expected by consumers.

Confidentiality
The information in the study records will be kept confidential to the full extent allowed by law. Data will be stored securely in a confidential file on the researcher's computer. The paper ballots and consent forms will be stored in a confidential folder in a locked file cabinet. No reference will be made in oral or written reports which could link you to the study.

Compensation
For participating in this study you will receive the option of an individually packaged, store-bought food treat. If you withdraw from the study prior to its completion, you will receive the option of an individually packaged, store-bought food treat.

What if you are a NCSU student?
Participation in this study is not a course requirement and your participation or lack thereof, will not affect your class standing or grades at NC State.

What if you are a NCSU employee?
Participation in this study is not a requirement of your employment at NCSU, and your participation or lack thereof, will not affect your job.

What if you have questions about this study?
If you have questions at any time about the study or the procedures, you may contact the researcher, Emily Wolter, at North Carolina State University, Department of Food, Bioprocessing, and Nutrition Sciences, Schaub Hall Rm 330, Raleigh, NC 27695, or at 281-923-2718.

What if you have questions about your rights as a research participant?
If you feel you have not been treated according to the descriptions in this form, or your rights as a participant in research have been violated during the course of this project, you may contact Deb Paxton, Regulatory Compliance Administrator, Box 7514, NCSU Campus (919/515-4514).

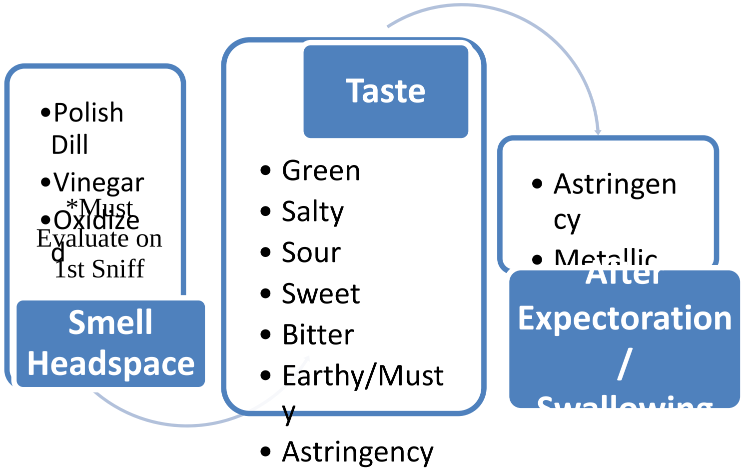
Consent To Participate
"I have read and understand the above information. I have received a copy of this form. I agree to participate in this study with the understanding that I may choose not to participate or to stop participating at any time without penalty or loss of benefits to which I am otherwise entitled."

Subject's signature_________________________________ **Date** ____________

Investigator's signature______________________________ **Date** _____________

Descriptive Analysis Panel Ballot Example
Pickle Panel DA 4 Mo Eval.
03/11/13, Monday
9:30-11:30, Room 232

PanelistNo:____



*****EVALUATE SLOWLY*****

	FLAVOR											
Sample	**PD**	**V**	**Ox**	**Green**	**Sa**	**Sr**	**Sw**	**B**	**E/M**	**A**	**M**	**Other**
Reference Pickle (2 slices)	6	4	6.5	2	12	8	1	1.5	4.5	2	3	
869												
571												

Appendix D: Supplemental Data to Chapter 3 – Descriptive Sensory Analysis and Volatile Compound Analysis of Commercially Fermented Cucumbers Evaluated after Long Term Shelf Storage

Introduction

In Chapter 3, descriptive sensory analysis and volatile compound analysis were used to evaluate the effect of tank yard storage over the course of 4 and 8 months on the flavor profile of finished pickle products. All of the products evaluated were stored on the shelf under ambient lighting and temperature for 1.5 months prior to evaluation. To assess the effect of long term shelf storage, another study was performed on hamburger dill chip pickles stored in the tank yard either 9 or 10 months prior to processing. Samples were evaluated after 1 year of shelf storage for both descriptive sensory analysis and volatile compound analysis. Aside from tank yard storage and shelf storage time, materials and methods were followed as outlined in Chapter 3.

Results/Conclusions

Hierarchical cluster analysis found 291 compounds that were significantly present in the treatments, as compared to a blank sample ($P < 0.05$; Figure D1). However, not many significant differences were observed among volatile compounds in the four treatments. Upon removing the blank artifact compounds from the sample set, a second hierarchical cluster analysis found 52 compounds to differ among the treatments ($P < 0.05$). The two NaCl fermented treatments grouped together with 12 compounds of significant difference from the other treatments, most of which are likely related to oxidation (Figure D2). However, the descriptive sensory analysis panel found the traditional NaCl treatment to be less oxidized ($P < 0.05$) than the $CaCl_2$ fermented product with elevated product $CaCl_2$

(Table D1). The panel also evaluated the $CaCl_2$ fermented treatments as more sour ($P < 0.05$) than the NaCl fermented treatments. The two treatments with elevated levels of $CaCl_2$ (36 mM) were significantly more astringent ($P < 0.05$), but no significant difference ($P > 0.05$) in bitterness was found, as might be expected. The results of this study support that the bitter taste of $CaCl_2$ is likely not of concern in fermented cucumber pickles, up to the maximum level of 36 mM. In addition, while significant differences in oxidation were found in the studies outlined in chapter 3, in which tank yard storage varied while shelf storage remained constant at 1.5 months, it appears that after a year of shelf storage, the flavor profiles of the four different treatments tend to equilibrate, as a much lower degree of differences in the volatile compounds was detected. This is an interesting find because, while products of different fermentations may appear to have different flavor profiles after only 1.5 months of shelf storage, this may not prove true throughout the entire shelf life of the product.

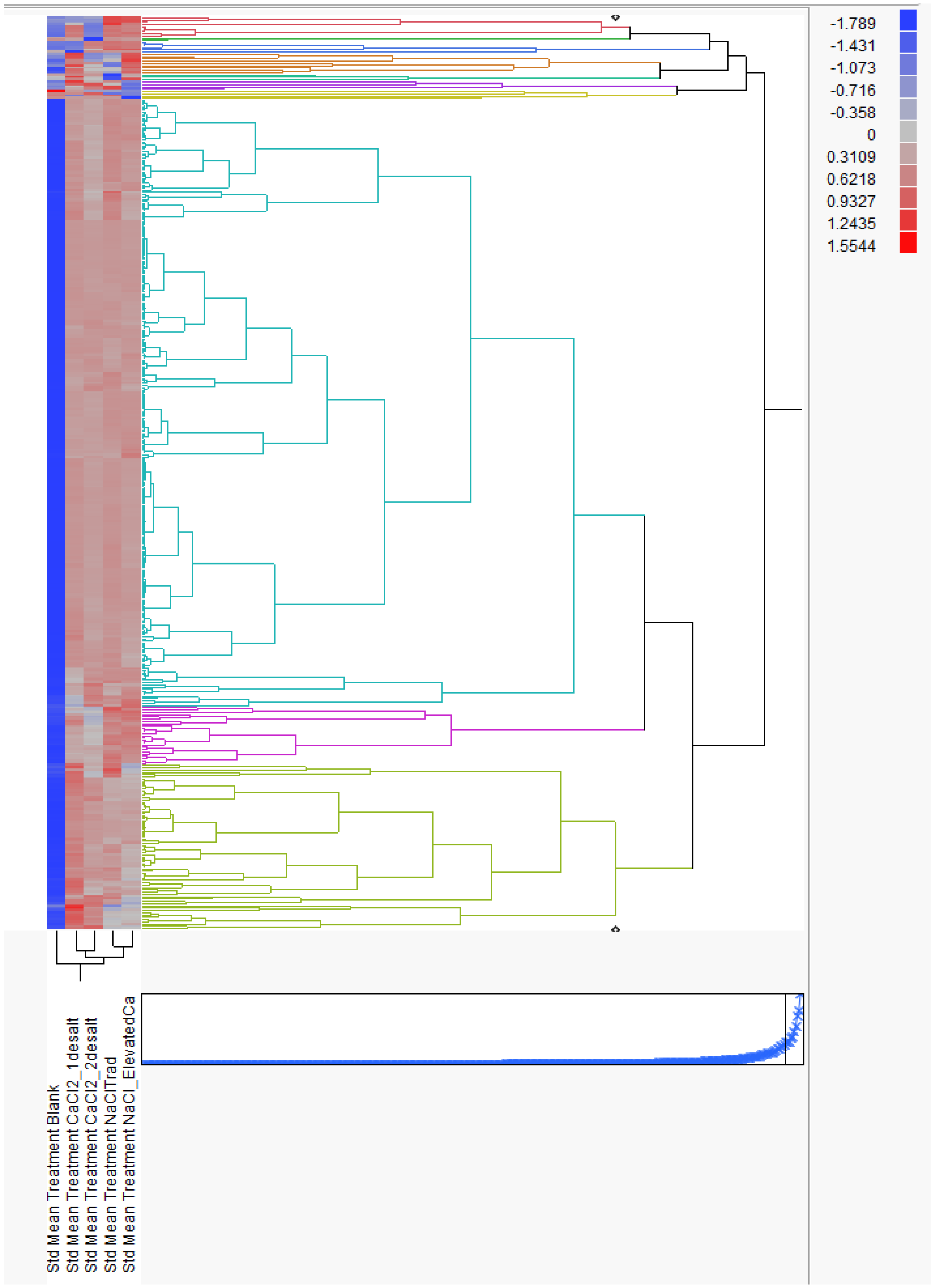


Figure D1 – Hierarchical cluster analysis of volatile compounds in fermented cucumber pickles[1] after 1 year of shelf storage

[1]All fermented cucumbers were processed into hamburger dill chips with an equilibrated NaCl concentration in finished products of 0.38 M and stored under ambient temperature and lighting.

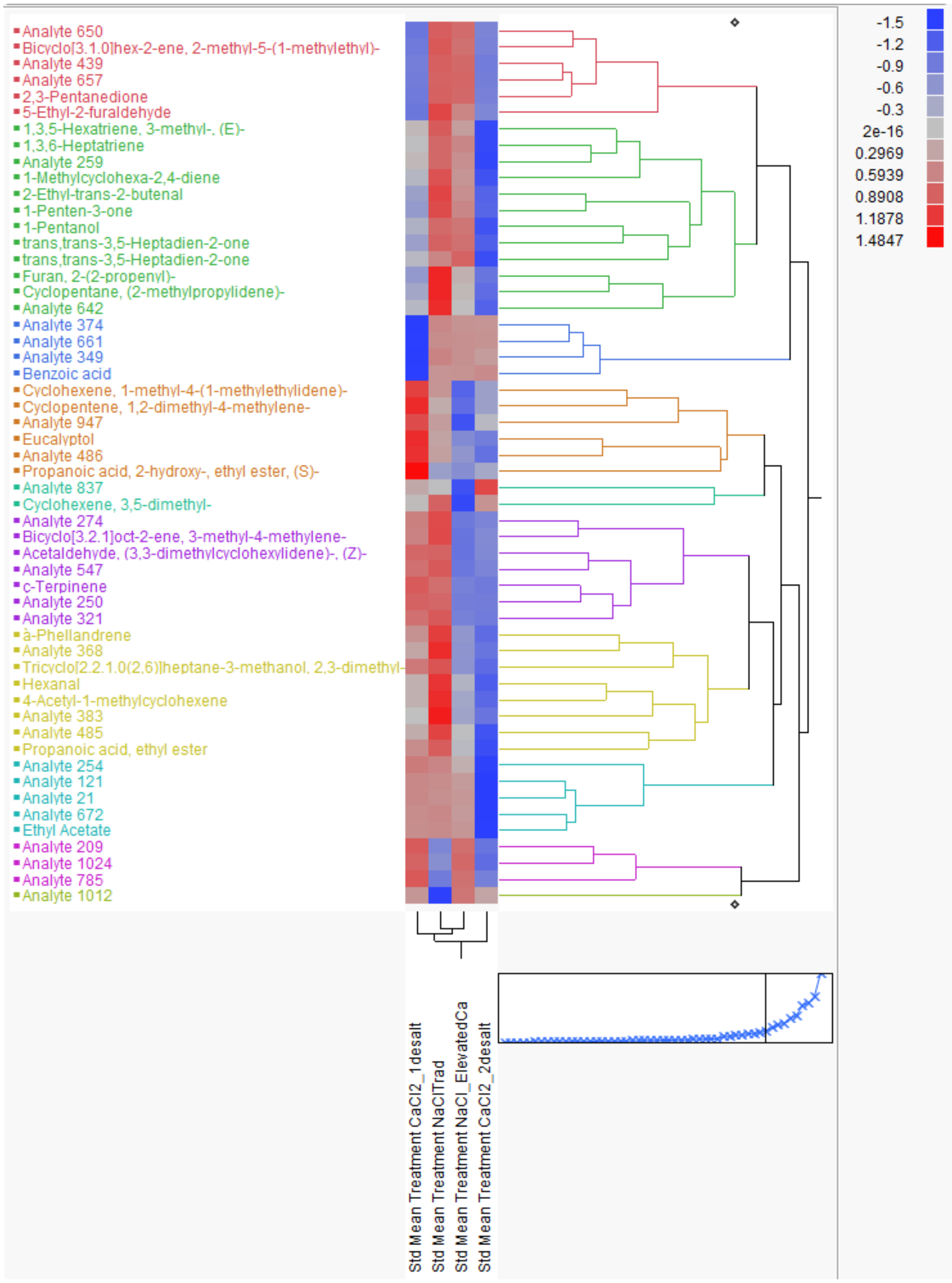


Figure D2 – Hierarchical cluster analysis performed after removal of artifact compounds found in blanks to determine significant volatile compounds in fermented cucumber pickles[1] after 1 year of shelf storage

[1]All fermented cucumbers were processed into hamburger dill chips with an equilibrated NaCl concentration in finished products of 0.38 M and stored under ambient temperature and lighting.

Table D1 – Spectrum™ descriptive analysis results[1] of fermented cucumber pickles[2] after 1 year of shelf storage

Fermentation Salt	Finished Product $CaCl_2$ (mM)	Tank Yard Storage (mo)	PD[3]	Vin	Ox	Sa	Sr	Sw	Bitter	Earthy	Astrin	Me
$CaCl_2$	24	10	5.9[a]	4.4[a]	5.18[a,b]	12.40[a]	8.28[a]	0.55[a]	1.63[a]	3.70[a]	2.03[a]	2.50[a]
			0.09	*0.16*	*0.57*	*0.22*	*0.15*	*0.12*	*0.16*	*0.17*	*0.16*	*0.25*
$CaCl_2$	36	10	6.2[a]	4.5[a]	5.75[a]	13.10[a]	8.33[a]	0.53[a]	1.88[a]	3.78[a]	2.25[b]	2.65[a]
			0.07	*0.13*	*0.68*	*0.28*	*0.18*	*0.11*	*0.21*	*0.21*	*0.14*	*0.30*
NaCl	24	9	6.0[a]	4.2[a]	4.28[b]	11.98[b]	7.58[b]	0.70[a]	1.43[a]	3.73[a]	1.83[a]	2.53[a]
			0.11	*0.19*	*0.34*	*0.24*	*0.15*	*0.11*	*0.14*	*0.20*	*0.14*	*0.24*
NaCl	36	9	6.0[a]	4.2[a]	4.68[a,b]	12.18[a]	7.53[b]	0.68[a]	1.80[a]	3.85[a]	2.30[b]	2.78[a]
			0.09	*0.13*	*0.27*	*0.23*	*0.18*	*0.11*	*0.20*	*0.16*	*0.17*	*0.35*

[1]Samples evaluated on a 15-point intensity scale; Means with the same letter (a-b) are not significantly different (Tukey's honestly significant difference, $p < 0.05$). Standard error designated in italics.

[2]All fermented cucumbers were processed into hamburger dill chips with an equilibrated NaCl concentration in finished products of 0.38 M and stored under ambient temperature and lighting.

[3]Abbreviated attributes include: PD (polish dill), Vin (vinegar), Ox (oxidized), Sa (salty), Sr (sour), Sw (sweet), Earthy (Earthy/Musty), Astrin (astringency), and Me (metallic)

Appendix E: Supplemental Data to Chapter 3 – Mean Intensity Scores for Descriptive Sensory Analysis and Correlation and Cluster Analysis of Volatile Compounds

Table E1 – Spectrum™ sensory descriptive analysis scores[1] of hamburger dill chip pickles with either 21 or 35 mM finished product calcium chloride ($CaCl_2$) prepared from cucumbers commercially fermented in either 1.03 M NaCl (n = 4) or 100 mM $CaCl_2$ (n = 4) and stored in the tank yard 4 months prior to processing

Sample[2]	PD[3]	Vin	Ox	Gr	Sa	Sr	Sw	Bitter	Earthy	Astrin	Me
NaCl_T10_2B_21mM	5.9	4.4	2.4	2.9	12.3	8.2	0.9	1.6	3.4	2.3	2.1
	0.2[4]	*0.2*	*0.3*	*0.3*	*0.2*	*0.2*	*0.1*	*0.1*	*0.3*	*0.1*	*0.3*
NaCl_T10_2B_35mM	6.1	4.2	3.1	2.3	12.4	7.8	0.7	1.5	3.2	2.2	2.1
	0.1	*0.2*	*0.3*	*0.2*	*0.2*	*0.3*	*0.1*	*0.1*	*0.2*	*0.1*	*0.2*
NaCl_T216_2B_21mM	6.1	4.3	2.8	2.9	12.3	7.8	0.8	1.4	3.2	2.1	2.2
	0.1	*0.2*	*0.2*	*0.4*	*0.3*	*0.2*	*0.2*	*0.2*	*0.2*	*0.2*	*0.3*
NaCl_T216_2B_35mM	5.8	3.9	3.0	2.3	11.8	7.3	0.6	1.5	3.3	2.1	2.1
	0.2	*0.1*	*0.3*	*0.3*	*0.4*	*0.2*	*0.1*	*0.2*	*0.3*	*0.2*	*0.3*
NaCl_T22_3A_21mM	5.8	4.3	2.6	3.0	12.0	7.6	0.9	1.5	3.3	2.1	2.2
	0.2	*0.2*	*0.3*	*0.4*	*0.3*	*0.3*	*0.2*	*0.2*	*0.3*	*0.1*	*0.2*
NaCl_T22_3A_35mM	6.3	4.1	2.5	3.1	12.0	7.6	0.7	1.6	3.1	2.2	2.1
	0.2	*0.2*	*0.4*	*0.4*	*0.4*	*0.2*	*0.2*	*0.2*	*0.3*	*0.1*	*0.3*
NaCl_T21_3A_21mM	5.9	4.5	2.8	3.1	11.6	7.9	0.8	1.4	2.8	2.1	2.0
	0.2	*0.2*	*0.3*	*0.4*	*0.5*	*0.2*	*0.1*	*0.1*	*0.3*	*0.1*	*0.3*
NaCl_T21_3A_35mM	6.0	4.3	2.9	2.3	12.1	7.6	0.7	1.5	3.1	2.1	2.2
	0.2	*0.2*	*0.3*	*0.3*	*0.3*	*0.2*	*0.1*	*0.2*	*0.4*	*0.1*	*0.2*
CaCl2_T16_2B_21mM	6.0	4.3	2.7	2.5	12.0	7.7	0.7	1.4	2.7	2.0	2.0
	0.2	*0.2*	*0.3*	*0.3*	*0.2*	*0.2*	*0.2*	*0.1*	*0.3*	*0.1*	*0.2*
CaCl2_T16_2B_35mM	6.2	4.7	3.0	2.5	12.2	8.0	0.6	1.8	3.1	2.5	2.2
	0.1	*0.2*	*0.3*	*0.3*	*0.4*	*0.3*	*0.1*	*0.2*	*0.3*	*0.1*	*0.3*
CaCl2_T11_2B_21mM	6.1	4.3	3.3	1.8	12.1	7.9	0.7	1.3	3.3	2.2	2.3
	0.1	*0.2*	*0.3*	*0.3*	*0.2*	*0.2*	*0.2*	*0.2*	*0.2*	*0.1*	*0.3*
CaCl2_T11_2B_35mM	6.1	4.4	3.1	2.3	12.6	8.2	0.7	1.5	3.3	2.3	2.4
	0.1	*0.2*	*0.3*	*0.3*	*0.2*	*0.2*	*0.2*	*0.1*	*0.3*	*0.1*	*0.3*

Table E1 – Continued

Sample	PD	Vin	Ox	Gr	Sa	Sr	Sw	Bitter	Earthy	Astrin	Me
CaCl2_T17_3A_21mM	6.1	4.5	3.3	2.3	12.4	8.0	0.7	1.5	3.0	2.3	2.0
	0.1	*0.2*	*0.4*	*0.3*	*0.3*	*0.2*	*0.1*	*0.2*	*0.3*	*0.1*	*0.3*
CaCl2_T17_3A_35mM	6.2	4.4	2.9	2.4	12.2	8.2	0.7	1.6	3.1	2.4	2.4
	0.2	*0.2*	*0.3*	*0.3*	*0.3*	*0.3*	*0.1*	*0.2*	*0.3*	*0.2*	*0.3*
CaCl2_T18_3A_21mM	6.1	4.5	3.0	2.7	11.4	7.8	0.8	1.1	2.9	2.0	1.8
	0.1	*0.2*	*0.4*	*0.4*	*0.5*	*0.2*	*0.2*	*0.2*	*0.3*	*0.1*	*0.2*
CaCl2_T18_3A_35mM	6.1	4.4	2.9	2.5	12.4	7.8	0.6	1.7	3.3	2.3	2.2
	0.2	*0.1*	*0.3*	*0.3*	*0.3*	*0.2*	*0.1*	*0.2*	*0.3*	*0.2*	*0.3*

[1]Average of 2 replications for 9 trained panelists

[2] Labeled as "Fermentation Salt_Tank Number_ Cucumber Size_ Finished Product $CaCl_2$"

[3] Abbreviated attributes include: PD (polish dill), Vin (vinegar), Ox (oxidized), Gr (green), Sa (salty), Sr (sour), Sw (sweet), Earthy (Earthy/Musty), Astrin (astringency), and Me (metallic)

[4] Standard error of the mean

Table E2 – Spectrum™ sensory descriptive analysis scores[1] of hamburger dill chip pickles with either 21 or 35 mM finished product calcium chloride ($CaCl_2$) prepared from cucumbers commercially fermented in either 1.03 M NaCl (n = 4) or 100 mM $CaCl_2$ (n = 4) and stored in the tank yard 8 months prior to processing

Sample[2]	PD[3]	Vin	Ox	Gr	Sa	Sr	Sw	Bitter	Earthy	Astrin	Me
NaCl_T10_2B_21mM	6.3	4.2	4.3	2.6	11.8	8.0	0.8	1.4	3.4	2.2	2.0
	0.1[4]	*0.1*	*0.3*	*0.2*	*0.2*	*0.1*	*0.1*	*0.1*	*0.3*	*0.1*	*0.3*
NaCl_T10_2B_35mM	6.2	4.3	4.3	2.5	12.4	8.1	0.6	2.5	3.3	2.3	1.8
	0.1	*0.1*	*0.4*	*0.2*	*0.3*	*0.2*	*0.1*	*0.8*	*0.2*	*0.2*	*0.3*
NaCl_T216_2B_21mM	5.8	4.0	3.3	2.7	11.6	7.9	0.6	1.5	2.8	2.2	1.7
	0.2	*0.2*	*0.3*	*0.3*	*0.3*	*0.3*	*0.1*	*0.1*	*0.2*	*0.2*	*0.3*
NaCl_T216_2B_35mM	6.0	4.3	4.0	2.9	12.1	7.6	0.6	1.8	3.3	2.2	1.9
	0.2	*0.1*	*0.3*	*0.3*	*0.2*	*0.4*	*0.1*	*0.1*	*0.3*	*0.1*	*0.3*
NaCl_T22_3A_21mM	5.9	4.0	3.2	3.4	11.7	7.5	0.6	1.4	2.9	1.9	1.9
	0.2	*0.1*	*0.3*	*0.3*	*0.3*	*0.3*	*0.1*	*0.1*	*0.3*	*0.2*	*0.3*
NaCl_T22_3A_35mM	6.1	4.3	4.4	3.5	12.3	8.2	0.5	1.6	3.1	2.4	1.9
	0.2	*0.1*	*0.5*	*0.2*	*0.3*	*0.1*	*0.1*	*0.1*	*0.3*	*0.2*	*0.3*
NaCl_T21_3A_21mM	6.1	4.2	3.9	3.2	11.9	8.2	0.5	1.7	3.0	2.1	2.0
	0.2	*0.1*	*0.4*	*0.3*	*0.1*	*0.2*	*0.1*	*0.2*	*0.4*	*0.1*	*0.3*
NaCl_T21_3A_35mM	6.0	4.3	3.8	3.3	12.0	7.9	0.6	1.9	3.1	2.0	1.8
	0.2	*0.1*	*0.4*	*0.3*	*0.3*	*0.2*	*0.1*	*0.2*	*0.3*	*0.1*	*0.3*
CaCl2_T16_2B_21mM	6.0	4.4	5.1	3.1	12.1	8.3	0.6	1.3	2.9	2.2	1.7
	0.1	*0.1*	*0.7*	*0.3*	*0.3*	*0.2*	*0.1*	*0.1*	*0.3*	*0.1*	*0.3*
CaCl2_T16_2B_35mM	5.9	4.1	4.9	3.3	12.3	8.0	0.5	1.8	2.8	2.1	1.9
	0.2	*0.1*	*0.5*	*0.3*	*0.2*	*0.2*	*0.1*	*0.2*	*0.3*	*0.2*	*0.3*
CaCl2_T11_2B_21mM	6.1	4.2	5.1	2.4	12.1	8.0	0.7	1.6	3.5	2.0	2.2
	0.2	*0.1*	*0.4*	*0.2*	*0.2*	*0.2*	*0.1*	*0.1*	*0.2*	*0.1*	*0.3*
CaCl2_T11_2B_35mM	6.0	4.3	5.0	2.1	12.6	8.4	0.6	1.8	3.5	2.5	2.1
	0.1	*0.2*	*0.4*	*0.3*	*0.2*	*0.2*	*0.1*	*0.2*	*0.3*	*0.2*	*0.3*
CaCl2_T17_3A_21mM	6.2	4.3	4.7	2.5	12.6	8.1	0.6	1.6	3.0	2.3	1.7
	0.1	*0.1*	*0.5*	*0.2*	*0.2*	*0.2*	*0.1*	*0.1*	*0.3*	*0.2*	*0.3*

Table E2 – Continued

Sample	PD	Vin	Ox	Gr	Sa	Sr	Sw	Bitter	Earthy	Astrin	Me
CaCl2_T17_3A_35mM	6.0	4.3	5.4	2.9	12.2	8.4	0.6	1.6	3.0	2.3	1.9
	0.0	*0.2*	*0.5*	*0.2*	*0.2*	*0.2*	*0.1*	*0.1*	*0.3*	*0.1*	*0.3*
CaCl2_T18_3A_21mM	6.2	4.3	3.9	3.1	11.9	8.0	0.7	1.4	3.1	2.0	2.0
	0.1	*0.1*	*0.4*	*0.3*	*0.1*	*0.2*	*0.1*	*0.1*	*0.3*	*0.1*	*0.3*
CaCl2_T18_3A_35mM	5.9	4.2	4.1	3.3	11.6	7.9	0.7	1.5	2.9	2.1	2.1
	0.2	*0.1*	*0.4*	*0.2*	*0.2*	*0.3*	*0.1*	*0.2*	*0.4*	*0.2*	*0.3*

[1]Average of 2 replications for 9 trained panelists
[2] Labeled as "Fermentation Salt_Tank Number_ Cucumber Size_ Finished Product $CaCl_2$"
[3] Abbreviated attributes include: PD (polish dill), Vin (vinegar), Ox (oxidized), Gr (green), Sa (salty), Sr (sour), Sw (sweet), Earthy (Earthy/Musty), Astrin (astringency), and Me (metallic)
[4] Standard error of the mean.

Table E3 – Volatile compounds significantly correlated (*P* < 0.025) to oxidized flavor in hamburger dill chip pickles fermented in either 100 mM $CaCl_2$ or 1.03 M NaCl

Compound	CAS[1]	Correlation to Oxidized Flavor	P-value	Similarity	Reverse	Probability	RI[2]	t_{R1} (sec)	t_{R2} (sec)	Mass[3]
Acids										
2,4-Hexadienoic acid, ethyl ester	110318-09-7	-0.5321	0.0017	852	887	7264	1501.8	1221.13	0.86	67
3-Hexenoic acid, (E)-		0.5238	0.0021	820	827	7298	2000.8	1656.88	0.87	44
Alcohols										
(S)-(+)-2-Pentanol	6032-29-7	-0.545	0.0013	832	907	4280	1114.5	605.08	0.71	45
3-Hexen-1-ol, (Z)-	95123-47-0	-0.4649	0.0073	898	903	2986	1411.4	1081.92	0.69	41
3-Penten-1-ol, (Z)-	764-38-5	-0.4304	0.0139	845	901	3394	1298.7	899.08	0.67	55
Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1α,3α,5α)-		-0.4279	0.0146	894	900	6501	1924	1670.82	0.66	92
7-Octen-2-ol, 2,6-dimethyl-	25279-08-7	0.6454	<.0001	653	764	3104	1467.8	1160.46	0.87	68
Furan-Containing Compounds										
Furan, 2-ethyl-	3208-16-0	-0.5873	0.0004	943	944	8257	912.32	371.29	0.73	81
Furan, 2-pentyl-	64079-01-2	-0.4244	0.0155	875	905	7884	932.68	784.82	0.97	81

Table E3 – Continued

Ketones										
2,3-Pentanedione	600-14-6	-0.5805	0.0005	865	871	8766	998.75	511.5	0.72	43
2-Cyclohexen-1-one, 4-(1-methylethyl)-	500-02-7	-0.5764	0.0006	872	888	4163	1679.3	1446.25	0.83	96
Ethanone, 1-(3-methylphenyl)-	585-74-0	-0.5748	0.0006	924	924	3144	1778.5	1548.25	0.71	119
3,5-Octadien-2-one, (E,E)-	30086-02-3	-0.5542	0.001	874	877	5909	1570.4	1311.83	0.85	95
Methyl vinyl ketone	78-94-4	-0.5337	0.0017	905	908	9458	907.19	363	0.66	55
1-Penten-3-one	1629-58-9	-0.526	0.002	887	889	7371	964.86	456.5	0.73	55
3-Methyl-3-cyclohexen-1-one	65489-63-6	-0.5031	0.0033	837	850	4796	1433.3	1108.25	0.83	67
trans,trans-3,5-Heptadien-2-one	3916-64-1	0.4307	0.0139	712	825	4117	1476.9	1174.25	0.81	67
4-Penten-2-one	13891-87-7	0.4466	0.0104	709	837	5947	985.19	489.58	0.72	43
Ethanone, 1-(2-hydroxy-5-methylphenyl)-	1450-72-2	0.496	0.0039	768	844	2541	1927.9	1672.04	0.69	135
1-Propanone, 1-cyclopropyl-	6704-19-4	0.6017	0.0003	860	900	5549	1013.2	522.54	0.81	69
Phenol Compounds										
Phenol, 3-methyl-	3019-89-4	-0.4625	0.0077	862	879	4649	2070	1773.03	0.6	107

Table E3 – Continued

Saturated Aldehydes										
Propanal, 2-methyl-3-phenyl-	5445-77-2	-0.6059	0.0002	852	875	3055	1786.8	1556.5	0.74	105
Butanal	123-72-8	-0.5217	0.0022	876	882	8343	837.11	294.21	0.67	43
Pentanal	495-85-2	-0.4246	0.0154	845	901	3394	1298.7	404.21	0.77	44
Saturated Cyclic Alkanes										
1,6-Dioxaspiro[4.4] nonane, 2-ethyl-	38401-84-2	-0.4464	0.0104	831	843	8683	1350.6	979.09	1	127
Unsaturated Aldehydes										
Allyl methallyl ether	14289-96-4	-0.7071	<.0001	822	841	1023	1207.5	750.75	0.9	41
2,4-Hexadienal, (E,E)-	142-83-6	-0.5558	0.001	890	894	5374	1402.3	1061.58	0.78	81
2-Ethyl-trans-2-butenal	63883-69-2	-0.5474	0.0012	843	853	1479	1156.7	671	0.85	41
2-Hexenal	73543-95-0	-0.5201	0.0023	930	936	6674	1217.8	767.21	0.85	41
2-Pentenal, (E)-	764-39-6	-0.5193	0.0023	906	906	4712	1125.1	621.5	0.78	55
Unsaturated Alkanes										
1,3,8-p-Menthatriene	21195-59-5	-0.6082	0.0002	863	885	1649	1212.7	759	1.02	91
2,4-Hexadiene, 2,5-dimethyl-	764-13-6	-0.4208	0.0165	800	851	753	1860.9	1619.75	0.71	95

Table E3 – Continued

2,4-Dimethyl-1-heptene	19549-87-2	-0.4066	0.0209	898	900	6964	1229.9	299.75	1.06	55
1-Methylcyclohexa-2,4-diene	19656-98-5	0.4742	0.0061	816	871	1174	910.67	367.87	0.82	79
Octane	50985-84-7	0.5299	0.0018	846	908	5339	776.82	239.29	0.95	43
3-Butenenitrile	109-75-1	0.7828	<.0001	923	937	5053	1174.1	698.5	0.66	41
Unsaturated Cyclic Alkanes										
4-Acetyl-1-methylcyclohexene	9/1/6090	-0.7136	<.0001	869	870	6733	1557.2	1292.5	0.91	43
Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-	49826-53-1	-0.6463	<.0001	801	821	1615	1300.6	815.35	1.02	91
Cyclohexene, 4-methyl-3-(1-methylethylidene)- 2		-0.5551	0.001	915	917	1704	1288.6	880	1.09	93
Cyclohexene, 1-methyl-5-(1-methylethenyl)-	1461-27-4	-0.5327	0.0017	878	880	1358	1200.7	739.75	1.1	68
Cyclopentene, 3-ethylidene-1-methyl-	62338-00-5	-0.4743	0.0061	806	875	1607	988.86	495	1.18	93
1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	23963-70-4	-0.428	0.0145	906	907	6207	1731.4	1501.5	0.83	109
Cyclopropylacetonitrile	6542-60-5	0.7788	<.0001	891	894	5322	1271.1	852.5	0.73	41

[1] Chemical Abstracts Service registry number; Identification based on mass spectral match to the NIST 05 library with > 800 similarity
[2] Retention indices based on first dimension retention of components on SOL-GEL-WAX (polyethylene glycol) column using SPME GCxGC-ToF MS
[3] Mass selected by ChromaTOF software during automated data processing to represent an interference free mass for each analyte. The unique mass for each analyte was used for calculation of peak area.

Table E4 – Volatile compounds significantly correlated (P < 0.025) to green flavor in hamburger dill chip pickles fermented in either 100 mM $CaCl_2$ or 1.03 M NaCl

Compound	CAS[1]	Correlation to Green Flavor	P-value	Similarity	Reverse	Probability	RI[2]	t_{R1} (sec)	t_{R2} (sec)	Mass[3]
Acids										
2,4-Hexadienoic acid, methyl ester, (E,E)-	689-89-4	-0.4782	0.0056	865	865	6183	1453.3	1138.5	0.82	67
Hexanoic acid	142-62-1	-0.4272	0.0147	857	868	4720	1837.4	1600.5	0.6	60
Alcohols										
2-Hexen-1-ol, (E)-	928-95-0	-0.4987	0.0037	901	903	5538	1424.1	1099.33	0.69	57
1-Penten-3-ol	67928-92-1	-0.4539	0.0091	886	908	6789	1154.8	668.17	0.66	57
1-Octanol	72-69-5	-0.4477	0.0102	808	886	2742	1596.7	1354.53	0.74	56
3-Hexen-1-ol, (Z)-	95123-47-0	-0.4384	0.0121	898	903	2986	1411.4	1081.92	0.69	41
1-Pentanol	71-41-0	-0.4096	0.0199	888	910	6978	1267.7	848.17	0.69	42
Aldehydes										
Hexanal	9012-63-9	-0.5519	0.0011	907	907	7615	1049.3	569.25	0.93	41
Butanal, 3-methyl-	590-86-3	-0.5393	0.0014	873	879	8327	879.61	332.75	0.74	41
2-Ethyl-trans-2-butenal	63883-69-2	-0.5217	0.0022	843	853	1479	1156.7	671	0.85	41
2-Pentenal, (E)-	764-39-6	-0.4948	0.004	906	906	4712	1125.1	621.5	0.78	55
Propanal, 2-methyl-	78-84-2	-0.4665	0.0071	801	902	7769	785.56	247.54	0.64	72
Pentanal	495-85-2	-0.4482	0.0101	875	905	7884	932.68	404.21	0.77	44
2,6-Nonadienal, (E,Z)-	557-48-2	-0.4195	0.0168	866	887	5905	1589.3	1336.97	0.89	41
2-Pentenal, (E)-	764-39-6	-0.4131	0.0188	902	903	4939	1125.1	585.75	0.77	83

Table E4 – Continued

2-Hexenal	73543-95-0	-0.4119	0.0192	930	936	6674	1217.8	767.21	0.85	41
Butanal	123-72-8	-0.4076	0.0206	876	882	8343	837.11	294.21	0.67	43
Furan-containing Compounds										
Furan, 2-pentyl-	64079-01-2	-0.4895	0.0045	898	900	6964	1229.9	784.82	0.97	81
Furan, 2-ethyl-	3208-16-0	-0.4703	0.0066	943	944	8257	912.32	371.29	0.73	81
Ketones										
2-Heptanone	29308-56-3	-0.5341	0.0016	881	900	6975	1179.5	706.75	0.94	43
Methyl vinyl ketone	78-94-4	-0.5016	0.0034	905	908	9458	907.19	363	0.66	55
3-Pentanone, 2-methyl-	565-69-5	-0.4982	0.0037	822	832	2759	946.3	426.21	0.86	57
Ethanone, 1-(3-methylphenyl)-	585-74-0	-0.4621	0.0078	924	924	3144	1778.5	1548.25	0.71	119
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-		0.4239	0.0156	887	888	7214	1736.9	1507	0.81	82
Saturated Alkanes										
Nonane, 2,6-dimethyl-	17302-28-2	-0.5355	0.0016	811	864	598	1054.7	550.04	1.83	43
1-Iodo-2-methylundecane	73105-67-6	-0.4425	0.0112	872	895	1095	2001.6	1652.33	1.19	57
Undecane	61193-21-3	-0.3997	0.0234	873	883	991	1070.5	541.75	1.82	43

Table E4 – Continued

Saturated Cyclic Alkanes										
1,6-Dioxaspiro[4.4]nonane, 2-ethyl-	38401-84-2	-0.5784	0.0005	831	843	8683	1350.6	979.09	1	127
Cyclopentane, (2-methylpropylidene)-	53366-58-8	-0.5523	0.001	811	818	1186	1418.8	1086.25	0.9	124
Unsaturated Alkanes										
2,4-Dimethyl-1-heptene	19549-87-2	-0.4106	0.0196	840	847	3235	843.59	299.75	1.06	55
1,4-Hexadiene, 2,3-dimethyl-	18669-52-8	0.4688	0.0068	806	882	748	1874.1	1631.24	0.64	95
Unsaturated Cyclic Alkanes										
4-Acetyl-1-methylcyclohexene	5259-65-4	-0.4138	0.0185	869	870	6733	1557.2	1292.5	0.91	43
1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	23963-70-4	-0.4127	0.0189	906	907	6207	1731.4	1501.5	0.83	109
Benzene, 1-methyl-4-(1-methylethyl)-2-nitro-	943-15-7	0.4315	0.0137	789	796	6858	1985.6	1713.25	0.74	162

[1] Chemical Abstracts Service registry number; Identification based on mass spectral match to the NIST 05 library with > 800 similarity
[2] Retention indices based on first dimension retention of components on SOL-GEL-WAX (polyethylene glycol) column using SPME GCxGC-ToF MS
[3] Mass selected by ChromaTOF software during automated data processing to represent an interference free mass for each analyte. The unique mass for each analyte was used for calculation of peak area.

Table E5 – Volatile compounds significantly correlated (P < 0.025) to metallic flavor in hamburger dill chip pickles fermented in either 100 mM $CaCl_2$ or 1.03 M NaCl

Compound	CAS[1]	Correlation to Metallic Flavor	P-value	Similarity	Reverse	Probability	RI[2]	t_{R1} (sec)	t_{R2} (sec)	Mass[3]
Acids										
2,4-Hexadienoic acid, ethyl ester	110318-09-7	0.5636	0.0008	852	887	7264	1508.1	1221.13	0.86	67
3-Hexenoic acid, ethyl ester, (Z)-	64187-83-3	0.5029	0.0034	839	868	3274	1292	885.5	0.93	60
Alcohols										
3-Penten-1-ol, (Z)-	764-38-5	0.5325	0.0017	845	901	3394	1298.7	899.08	0.67	55
(S)-(+)-2-Pentanol	6032-29-7	0.4795	0.0055	832	907	4280	1114.5	605.08	0.71	45
1-Penten-3-ol	67928-92-1	0.4767	0.0058	886	908	6789	1154.8	668.16	0.66	57
3-Hexen-1-ol, (Z)-	95123-47-0	0.4458	0.0105	898	903	2986	1411.4	1081.92	0.69	41
Methyl Alcohol	67-56-1	0.4213	0.0163	916	926	9164	873.33	329.959	0.54	31
(6Z)-Nonen-1-ol	35854-86-5	-0.4304	0.0139	857	879	1129	1761.8	1540.27	0.68	67
Aldehydes										
2-Hexenal	73543-95-0	0.6206	0.0002	930	936	6674	1217.8	767.21	0.85	41
2-Pentenal, (E)-	764-39-6	0.6068	0.0002	902	903	4939	1125.1	585.75	0.77	83
Benzaldehyde, 4-ethyl-	4748-78-1	0.5975	0.0003	719	788	2652	1714.6	1484.37	0.74	134

Table E5 – Continued

2,4-Heptadienal, (E,E)-	4313-03-5	0.54	0.0014	872	873	4093	1495.1	1201.75	0.82	81
Pentanal	495-85-2	0.5051	0.0032	875	905	7884	932.68	404.21	0.77	44
2,4-Hexadienal, (E,E)-	142-83-6	0.4894	0.0045	901	915	6328	1397	1061.58	0.78	81
2-Ethyl-trans-2-butenal	63883-69-2	0.4836	0.005	843	853	1479	1156.7	671	0.85	41
Methacrolein	78-85-3	0.4596	0.0081	867	894	7201	840.1	297	0.64	70
Propanal, 2-methyl-3-phenyl-	5445-77-2	0.4562	0.0087	852	875	3055	1786.8	1556.5	0.74	105
Hexanal	9012-63-9	0.4553	0.0088	907	907	7615	1049.3	569.25	0.93	41
2,6-Nonadienal, (E,Z)-	557-48-2	0.4016	0.0227	866	887	5905	1589.3	1336.97	0.89	41
Ethers										
Allyl methallyl ether	14289-96-4	0.4701	0.0066	822	841	1023	1207.5	750.75	0.9	41
Furan-containing Compounds										
Furan, 2-ethyl-	3208-16-0	0.5613	0.0008	943	944	8257	912.32	371.29	0.73	81
Furan, 2-(2-propenyl)-		0.5471	0.0012	872	887	7707	1203.9	1375.13	0.74	79

Table E5 – Continued

Ketones										
3-Hexen-2-one	4376-23-2	0.6099	0.0002	821	918	4418	1214.3	759.95	0.83	83
3,5-Octadien-2-one, (E,E)-	30086-02-3	0.5831	0.0005	874	877	5909	1570.4	1311.83	0.85	95
2,3-Pentanedione	600-14-6	0.5604	0.0009	865	871	8766	998.75	511.5	0.72	43
1-Penten-3-one	1629-58-9	0.554	0.001	887	889	7371	964.86	456.5	0.73	55
3-Octen-2-one	1669-44-9	0.533	0.0017	838	841	3982	1407.9	1069.75	0.93	55
3-Methyl-3-cyclohexen-1-one	65489-63-6	0.5161	0.0025	837	850	4796	1433.3	1108.25	0.83	67
2-Heptanone	29308-56-3	0.4419	0.0113	881	900	6975	1179.5	706.75	0.94	43
1-Octen-3-one	4312-99-6	0.4387	0.012	855	876	5470	1302.3	902	0.93	55
Ethanone, 1-(3-methylphenyl)-	585-74-0	0.4136	0.0186	924	924	3144	1778.5	1548.25	0.71	119
3-Buten-2-one, 3-methyl-	814-78-8	0.4108	0.0195	823	867	3668	941.13	418	0.73	69
Pyran-containing Compounds										
2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	7392-19-0	0.578	0.0005	815	819	4713	1104.2	586	1.16	43
Saturated Cyclic Alkanes										
1,6-Dioxaspiro [4.4]nonane, 2-ethyl-	38401-84-2	0.5799	0.0005	831	843	8683	1350.6	979.09	1	127

Table E5 – Continued

Cyclopentane, (2-methylpropylidene)-	53366-58-8	0.5466	0.0012	811	818	1186	1418.8	1086.25	0.9	124
Unsaturated Alkanes										
2,4-Dimethyl-1-heptene	19549-87-2	0.4226	0.016	840	847	3235	843.59	299.75	1.06	55
3-Butenenitrile	109-75-1	-0.5462	0.0012	923	937	5053	1174.1	698.5	0.66	41
Unsaturated Cyclic Alkanes										
4-Acetyl-1-methylcyclohexene	5259-65-4	0.583	0.0005	869	870	6733	1557.2	1292.5	0.91	43
Cyclopentene, 3-ethylidene-1-methyl-	62338-00-5	0.545	0.0013	806	875	1607	988.86	495	1.18	93
4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	1203-08-3	0.5035	0.0033	878	887	8574	2008.7	1729.71	0.76	43
Cyclohexene, 1-methyl-5-(1-methylethenyl)-	1461-27-4	0.4945	0.004	878	880	1358	1200.7	739.75	1.1	68
Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-		0.4681	0.0069	820	835	1287	1931.8	1674.75	0.73	107
Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4	0.4444	0.0108	826	848	7816	1431.7	1104.87	1.23	57
Benzene, 1-methyl-3-(1-methylethyl)-	535-77-3	0.4213	0.0163	822	868	1277	1491.5	1196.17	0.94	119

[1] Chemical Abstracts Service registry number; Identification based on mass spectral match to the NIST 05 library with > 800 similarity
[2] Retention indices based on first dimension retention of components on SOL-GEL-WAX (polyethylene glycol) column using SPME GCxGC-ToF MS
[3] Mass selected by ChromaTOF software during automated data processing to represent an interference free mass for each analyte. The unique mass for each analyte was used for calculation of peak area.

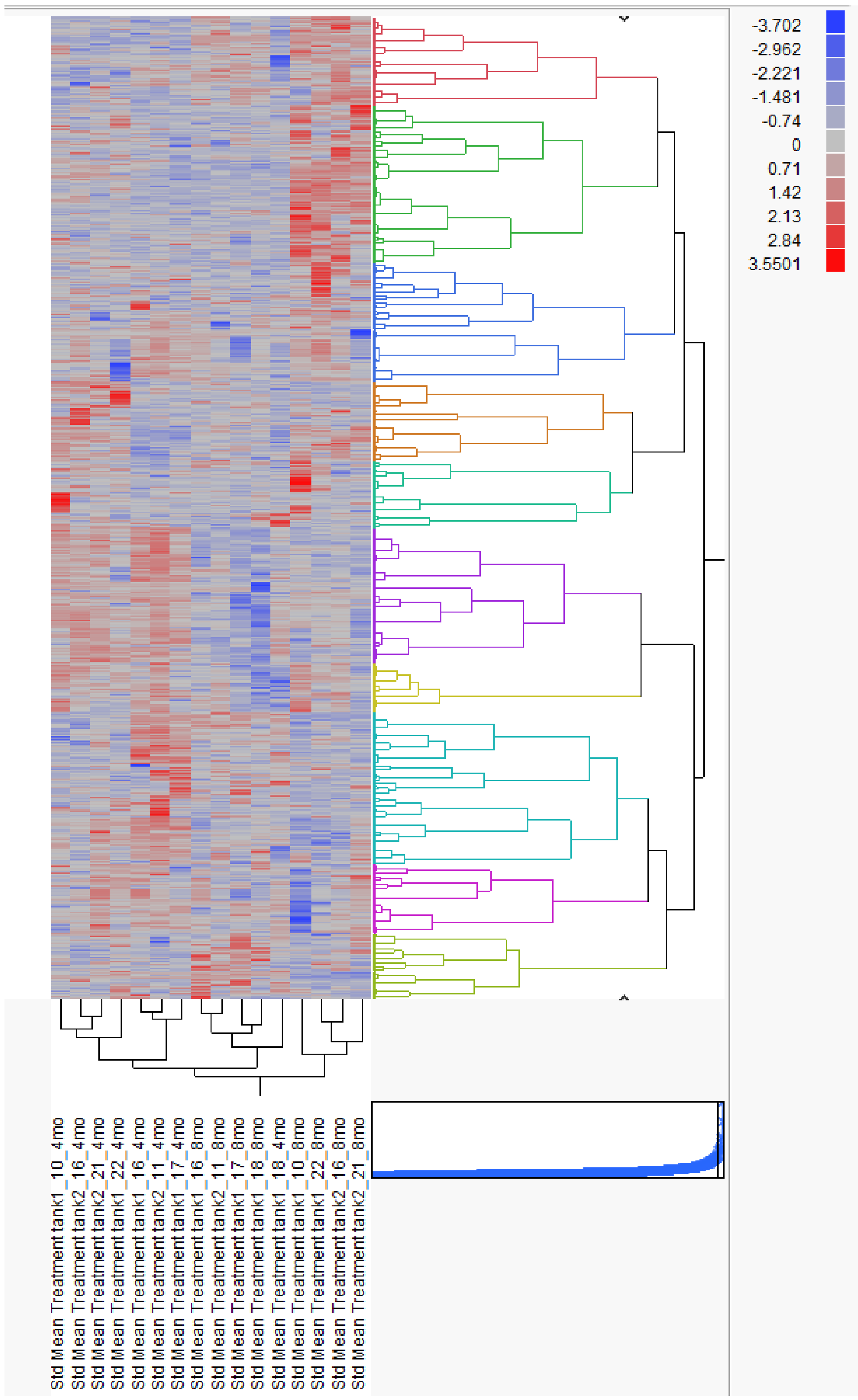


Figure E1 – Hierarchical clustering of volatile compounds that changed significantly (*P* < 0.05) in cucumber dill chips commercially fermented in either sodium chloride (NaCl) tanks (n = 4) or calcium chloride ($CaCl_2$) tanks (n = 4) for either 4 months or 8 months prior to processing and shelf storage of 1.5 months

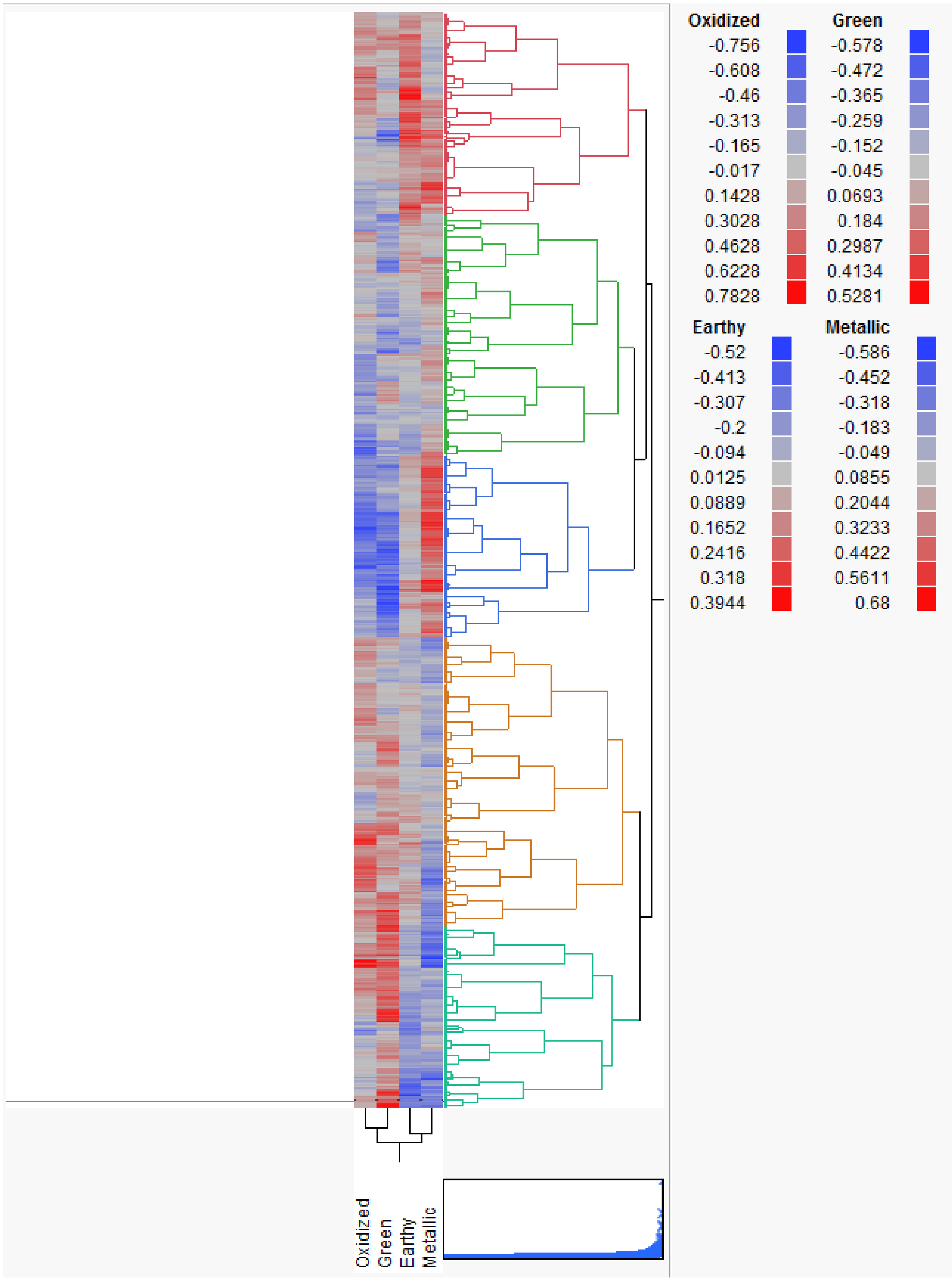


Figure E2 – Hierarchical clustering of volatile compounds significantly clustered (*P* < 0.05) to off-flavor attributes in cucumber hamburger dill chips commercially fermented in either sodium chloride (NaCl) or calcium chloride ($CaCl_2$) for either 4 months or 8 months prior to processing and shelf storage of 1.5 months