ABSTRACT

KUHLMAN, NICHOLAS GRANT. Sensory and Instrumental Analysis of Pasteurized Dill Pickles Made from Acidified, Bulk Stored Cucumbers. (Under the direction of Dr. Roger McFeeters).

Storing fresh cucumbers for an 8 to 12 week period in an acidified, non-fermented environment prior to processing would extend the time for processing locally grown cucumbers into pasteurized pickle products. The effect of acidified storage of fresh cucumbers for up to 120 days prior to their conversion to pasteurized dill pickles was evaluated. Cucumbers stored at 18ºC prior to pasteurization maintained a fresh, non-cured appearance as well as a firm texture. Equilibrated acid concentrations of 3.5% acetic acid also helped to reduce the amount of curing and maintained firmness in cucumber tissue. Dill pickles made from acidified, bulk stored cucumbers maintained a firm texture after pasteurization. However, the cured appearance of bulk stored cucumbers increased by 40% within 30 days of pasteurization so that it was much higher than the cured appearance of dill pickles made directly from the same lot of fresh cucumbers and stored for an equivalent period of time after pasteurization. Sour, sweet, bitter and vinegar intensities were similar for dill pickles prepared from either bulk stored or fresh cucumbers. Firmness, saltiness, and dill flavor while significantly different, differed by less than 1 point on a 15-point scale. The major flavor difference between pasteurized dill pickles made from stored acidified cucumbers and pickles made directly from fresh cucumbers is that pickles made from stored cucumbers had an “other” flavor. The intensity of this flavor was perceived at a similar level regardless of the brine composition or storage time of the cucumbers prior to pasteurization. Descriptors for this “other” flavor were chlorine, pool, barny and metallic. GCxGC-TOFMS analysis of volatile components in dill pickles prepared from bulk stored cucumbers and dill

pickles prepared from fresh cucumbers resulted in detection of 24 components that were significantly different ($p<0.05$) between at least one treatment pair. Individual volatile components did not provide a clear basis for differentiation among different storage brine treatments. However, PCA analysis using the 24 components was able to differentiate most treatments.

Sensory and Instrumental Analysis of Pasteurized Dill Pickles Made from Acidified, Bulk Stored Cucumbers

by
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DEDICATION

To all the people that supported me along the way no matter what I did

BIOGRAPHY

Nicholas Grant Kuhlman, the son of Douglas and Rhonda Kuhlman, was born on October 16, 1982. He was the youngest of three children. His two older sisters were Prudence Tetzloff and Stephanie Ried. He grew up in St. James, Minnesota, where his family still resides. During his childhood he enjoyed activities like cooking, playing with his cousins, playing piano, watercolor painting, and avoiding helping with farm chores at all cost. While in high school Nicholas competed in cross country and track & field for team sports, and he was a drummer and a vocalist in the school band and choir. While in high school, Nicholas had a wide range of jobs. He was a movie theater projectionist, a pizza boy, and a bank teller.

After finishing high school, he moved to Sioux Falls, South Dakota, where he attended Augustana College. During his time at Augie he had the opportunity to travel and study in Ireland and Scotland. He was a participant in the on campus organizations Concert and Lecture, Augustana Coalition for Social Justice, American Chemical Society, and worked off campus at a local bagel shop as the morning baker as well as working part time in at a local hospital in research and development of bioadhesives. In May of 2005 he graduated from college with a bachelor's degrees in chemistry and English. After completing undergraduate, he took a year off and worked at a group home for adults with disabilities. He decided graduate school was the path he wanted to take, packed his bags, and headed to North Carolina.

In July of 2006, Nicholas moved to Raleigh, North Carolina to begin his Master of Science in the Department of Food, Bioprocessing and Nutrition Sciences at North Carolina

State University, under the watchful eye of Dr. Roger McFeeters of the USDA/ARS Food Fermentation Lab. During this time he was an active member of the North Carolina State Food Science Club as participant in committees, as well as, the club treasurer. In July of 2008 he started a 6 month co-op with Campbell's Soup/StockPot in Everett, Washington.

With intentions of returning to North Carolina State and continuing on with his PhD, he had no idea the effect the city of Seattle would have upon him. Putting his plans for his PhD on hold for a few years, he decided to throw caution to the wind and uproot himself and settle in Seattle. As he writes this biography from a coffee shop in the Capitol Hill neighborhood of Seattle, he knows that he is home.

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me from the first day I got here and are a huge reason why I decided to stay. Seattle can be a big, expensive city, but the conversations we had and the food we ate made it livable.

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It's my fear that I've left someone out, but even if you don't see your name, know that you have left an impression upon me. Thank you all for the support you've given me now and in the future.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Literature Review

1.1 Acidified Food Storage

1.1.1 Early preservation by acidification

Predating refrigeration and thermal processing, humans had limited choices when it came to their diet. Meats and fresh produce were eaten fresh, minimally processed by salting or dehydrating, or rotten (Pedersen, 1979). Techniques such as refrigeration and canning, which prolonged meat and produce shelf life, were not used until 1760 and 1795 (Potter and Hotchkiss, 1998). Even with advances in cold storage and canning, these techniques and products were not readily available. Along with dehydration, a technique that has been used for thousands of years is fermentation. The process of storing cucumbers by pickling has been in use for nearly 4000 years, with origins in Mesopotamia (Rose, 1982). Many foods were stored in salted solutions with natural fermentation as a method to preserve foods beyond harvest and for long trips (Breidt et al., 2007; Hutkins, 2006). Before refrigeration and even today in developing countries, people rely on naturally occurring bacteria to ferment fresh produce so they may be stored for extended periods of time (Babcock and Azam-Ali, 1998). Modern processing has allowed for a shift away from fermented foods to acidified, refrigerated, or thermally processed foods (Babcock and Azam-Ali, 1998). Rather than relying on naturally occurring bacteria to lower the pH of a brining solution to a suitable level to prevent growth of spoilage and pathogenic bacteria, producers of acidified foods adjust the pH using concentrated acids. This addition of concentrated acids allows for a quicker processing time, as well as different flavor profiles depending upon the acidulant used. Pasteurization of acidified fresh pack pickles is an example of this approach.

1.1.2 Acidified foods in a modern age

With advances in technology such as refrigeration and rapid transportation, along with advances in processing techniques, consumers have an increased amount and variety of fresh fruits and vegetables in their diets. Tomatoes, brassicas, and onions are the top three cultivated fresh vegetable crops, followed by cucumbers (FAOSTAT, 2004). Brassicas and onions are usually consumed raw or with minimal processing. Tomatoes are available fresh, but also in many processed forms including canned, juice, sauce and paste. While cucumbers (*Cucumis sativus)* are consumed fresh, they have a long history of being consumed as a fermented product. Even with changes in technology, pickles prepared from cucumbers continue to be popular. Sixty percent of cucumbers consumed are eaten fresh while, the remaining forty percent are made into a variety of pickle products (Fleming et al., 2002). Depending upon geographical location, pickling cucumbers in the United States are usually harvested in late spring and early fall (Lerner and Dana, 2001). There are two basic classes of cucumbers: field and greenhouse-grown slicers and pickling cucumbers. The slicer cucumbers are generally eaten raw, are larger, sweeter, and have a thicker skin, which is not suitable for pickling. Pickling cucumbers consist of cultivars that are smaller in size and are produced mainly for this purpose (USDA, 2004).

1.1.3 Pickle definitions: Fermented, Fresh Pack, and Refrigerated

Pickling is a process in which salt and/or vinegar are added in solution as a primary form of preservation (Fleming and Moore, 1983). Acidified foods are defined by the U.S. Code of Federal Regulations (United States Code of Federal Regulations, 2006) as low-acid

foods to which acid(s) or acid foods(s) are added, having a water activity (a_w) greater than 0.85 and an equilibrated pH of 4.6 or below (United States Code of Federal Regulations, 2006). Commercially, cucumbers are pickled using three major processes: fermentation, acidification with vinegar followed by thermal processing (pasteurization), and acidification of refrigerated cucumbers (Fleming and Moore, 1983). These categories account for about 35%, 40% and 25% of pickled cucumber products respectively (Fleming et al., 2002). Fresh pack and refrigerated pickles make up a majority of the pickles consumed in the United States. A shift in consumer preference could be due to changes in consumer taste from a pickle that is preserved with lactic acid to a pickle that is preserved using acetic acid.

Today fermentation and brining of cucumbers is done for two main reasons: 1) a preservation method to obtain a final product with desired qualities and 2) a way in which to extend the processing season (Fleming and Moore, 1983). Fermented pickles are produced from fresh cucumbers stored in open-air 8,000 to 10,000 gallon bulk storage tanks made of fiberglass or plastic. Homofermentative, acid tolerant lactic acid bacteria are found in low levels on the surface of cucumbers. They consume glucose and fructose present in the fresh cucumber to produce lactic acid. The result of this fermentation is that the pH of the brined cucumbers is reduced to 3.6 or lower with a titratable acidity between 0.6% and 1.2% calculated as lactic acid (Breidt, 2006; Breidt et al., 2007; Hutkins, 2006). To prevent the production of bloated cucumbers as a result of excess CO_2 produced during fermentation, the fermenting cucumbers are purged with a gas to sweep out CO_2 as it is produced in the brine by use of a side arm purging device placed in the fermentation tanks (Costilow, et al., 1981). Initially N_2 was recommended as a purging gas (Etchells et al., 1973; Fleming, 1984) but air

is now used due to lower cost (Costilow, et al., 1981; Fleming, 1984; Humphries and Fleming, 1988; Potts and Fleming, 1979). Cucumbers are covered with salt brine to equilibrate at 5% to 6% during fermentation (Hutkins, 2006). After the active fermentation, salt may be increased up to 10 to 12% to minimize freezing in cold climates (Etchells et al., 1973). Along with salt, cover brines also contain 0.1% to 0.4% calcium chloride to prevent loss of firmness, and 0.05% to 0.2% acetic acid to release CO_2 from the brine preventing cucumber bloater formation as well as softening of cucumbers by aerobic molds found near purging outlets (Breidt, 2006; Breidt et al., 2007; Hudson and Buescher, 1985; McDonald et al., 1991; Potts and Fleming, 1982; Tang and McFeeters, 1983). High salt concentrations during fermentation serve to select for homofermentative lactic acid bacteria including *Pediococcus* sp. and *Lactobacillus plantarum* (Breidt, 2006; Breidt et al., 2007, Hutkins 2006). Calcium chloride was added to fermentation tanks to inhibit softening of pickles (Etchells et al., 1977; Fleming et al., 1987; Tang and McFeeters, 1983). The combination of salt, calcium and acidic conditions result in fermented cucumbers with a firm, crisp texture that can be stored for a year or more (Fleming and McFeeters, 1981). Fermented pickles are desalted and further processed to produce shelf stable products like hamburger dill chips, relishes, and other products.

Fresh pack pickles are made by packing fresh cucumbers into jars and covering the cucumbers with cover brine consisting of salt, vinegar, colorants, and spices. United States Department of Agriculture (USDA) grade standards specify that fresh pack pickles must contain a minimum of 0.5% acetic acid (USDA, 1966). Fresh pack pickles are considered acidified foods because they have a water activity of greater than 0.85 and a pH lower than

4.6 (U.S. DHEW, 1979). The pH for the fresh pack products is usually in the range of 3.5 to 4.0 with acetic acid concentrations of 0.6% to 2% and salt concentrations of 2 to 4% (Breidt et al., 2007). The cucumbers are pasteurized to create a shelf stable product by killing the natural microflora, including enteric, acid tolerant pathogens (Breidt, 2006). Not only does the pasteurization prevent microbial growth, it is also responsible for the inactivation of enzymes that may contribute to fruit softening (Breidt, 2006) and possibly the development of a cured appearance (Mok, 1992).

Refrigerated pickles are acidified to a pH between 4.0 and 4.6, stored at a temperature between 1ºC and 4ºC and use preservatives such as sodium benzoate (Fleming and Moore, 1983). They do not receive a heat process (Hutkins, 2006). Besides differences in flavor profiles, refrigerated pickles and fresh pack pickles maintain a fresh, crisp appearance and taste. Also, unlike fermented pickles, fresh pack and refrigerated pickles are made from fresh cucumbers.

Because the fermentation process allows for cucumbers to be stored for long periods of time, the pickle industry can produce product from fermented cucumbers year round. Refrigerated cucumber pickles have a limited shelf life so fresh cucumbers must be received and processed year round by contracting for produce grown from the northern United States into southern Mexico. Since fresh pack products are shelf stable, they can be produced and warehoused between seasons. However, it is more economical in terms of equipment and labor if processing can be extended beyond the local season where processing plants are located. On the other hand, it is not economical to transport fresh produce across the continent for fresh pack pickle products. These competing factors mean that it would be

useful to be able to store fresh produce for several weeks and then make fresh pack products from the stored fruit. This would make it possible to extend the processing period with cucumbers that are grown relatively close to the processing plant.

1.2 Cell Structure and Curing

A problem associated with quality of fresh pack pickled products is curing of the cucumber tissue in the jar after pasteurization. Curing occurs when intercellular air pockets in cucumber tissue are filled by liquid (Corey et al., 1983). This results in a change in the appearance of the cucumber flesh from an opaque white appearance to translucent. The opaque appearance of a fresh pack pickle is associated with a fresh quality. It is speculated that tissue curing can be associated with changes in cucumber cell walls. Howard (1989) and Mok (1992) discussed factors that alter cell wall polysaccharide and protein structure in cucumber fruits, and how these factors may lead to curing. It would be useful to processors if a method could be found which would maintain the fresh opaque appearance of cucumbers while they were held in bulk storage for up to 10 to 12 weeks without resulting in curing.

1.2.1 Cell wall polysaccharide and protein composition

Arabidopsis thaliana is a model plant in studying cell wall composition and function (Zablackis et al., 1995). Plant cell walls are a complex structure of polysaccharides built from monosaccharides such as glucose and galactose and also consist of proteins. The main components of plant cell walls are cellulose, hemicellulose, pectic polysaccharides, and proteins, which compromise 15-30%, ca. 25%, ca. 30%, and ca. 10 -20%, respectively (Reiter et al., 1997; Reiter, 1998; Zablackis et al., 1995). The *A. thaliana* leaf cell walls contain proteins that have an amino acid sequence similar to those of other plant tissues (Kieliszewski et al., 1992; Zablackis et al., 1995). The main functions of plant cell walls are to maintain cell shape, give support, add strength, regulate cell volume, participate in

metabolism and act as a physical barrier (Reiter, 1998). The main components of plant cell wall polysaccharides are cellulose, pectin and matrix polysaccharides that are interconnected hemicelluloses bound to cellulose microfibrils. Cellulose (1, 4-β-D-glucan) gives the cell wall rigidity and is a main component in texture (Imam et al., 2005). Cellulose molecules interact with each other through hydrogen-bonding to form fibrils 0.4 to 0.5 μm, further interacting to create microfibrils with a diameter of 5 to 7 nm (Delmer and Amor, 1995; Fahn, 1982). The threadlike composition of fibrils and microfibrils create the cell wall. Along with cellulose, pectin and hemicelluloses give elasticity and stretch to cell walls (Imam et al., 2005). The major hemicelluloses in plant cell walls are xyloglucan β(1→4) linked glucose residues with α(1→6) linked xylose residues (Fry, 1989). The orientation of cellulose and hemicellulose xyloglucans allows for hydrogen bonding interactions. One xyloglucan can bind with more than one cellulose microfibril to create a three dimensional cell wall structure (McCann et al., 1990).

Interwoven throughout the three-dimensional polysaccharide matrix are proteins. Consisting of approximately 10-20% of the cell wall composition, proteins main functions are cell wall expansion and restructuring the cell wall through enzymatic functions such as glycosidases, peroxidases and esterases (Reiter, 1998; Zablackis et al, 1995). Initially enzymes in the plant cell wall were thought to be the major contributors to cell wall degradation during ripening and abscission, but during expansion and growth of the cells glycosidases, peroxidases, esterases, non-hydrolytic proteins and xyloglucan endotransglycosylases are capable of cleaving sections of the cell wall backbone and reattaching cleaved ends helping to aid in cell expansion (Nishitani, 1995; Reiter, 1998).

With a complex composition of polysaccharides and proteins, the two components work to form a complex, tightly interconnected network that maintains cell structure and function. The polysaccharides and proteins interact through a series of covalent bonds, non-covalent bonds or both and hold the matrix polymers together creating an insoluble network (Lamport and Epstein, 1983). While the polymer matrix network creates an insoluble network, intermicellular and intermicrofibrillar spaces, 1 nm and 10 nm respectively, are large enough openings in the surface of the cell to allow for the exchange of water and ions across in and out of the cell (Lauchli, 1976). Throughout cucumber fruit cell development, tiny pockets of air are entrapped in the cucumber flesh. The coherent matrix of polymers allows for air to be trapped within the cells. However, compositional changes in the cell due to plant bruising, enzymatic activity, changes in pH or changes in solubility of matrix constituents allows for the absorption and diffusion of gas and liquid into and out of the cell wall over time. This results in changes in tissue appearance (Mok, 1992).

1.2.2 Curing and its possible causes

The actual mechanisms behind cucumber curing are unknown, but there are several means by which the air trapped in the cucumber cells can escape and become displaced by water. Figure 1.1 shows a cross-sectional diagram of a cucumber slice (McFeeters et al., 1982). There are three areas of the cucumber slice: exocarp, mesocarp, and endocarp. Curing can occur in any part of the cucumber tissue. The rate of curing is dependent upon the liquid and gas diffusion properties of the cell walls and cell pores (Mok, 1992). The composition of the gas in cucumber cells is similar to that of air, but with slightly elevated

carbon dioxide levels (Corey et al., 1983). It is possible that during the storage process, the integrity of the cell wall decreases allowing for the trapped gas inside the cell to escape, ultimately resulting in curing the cucumber tissue.

Curing can be slowed or accelerated during processing. Blanching and pasteurization have been shown to slow the change in the appearance of cucumber tissue from opaque to translucent (Etchells and Ohmer 1941; Fellers and Pflug, 1968,). The high vacuum created inside jars after pasteurization of pickles increases the curing of tissue. Etchells and Ohmer (1941) reported following the release of the vacuum in excess of 10 inches of mercury created by pasteurization, cucumbers lose their white appearance and take on a cured appearance within 5 to 10 minutes due to a gas exchange relationship. This was also seen in cucumbers that were flushed with oxygen. Following an oxygen flush, fresh cucumbers cured at a rapid rate, when the nitrogen found in the pockets is exchanged with oxygen (Fleming et al., 1980). Respiration by the cucumber fruit uses the oxygen and produces carbon dioxide. The carbon dioxide is 85 times more soluble in water compared to nitrogen. The carbon dioxide then readily diffuses through the cell causing a change in intercellular pressure causing the cells to fill with water (Fleming et al., 1980).

Other factors that affect curing are bruising from handling, chiller damage, extreme temperature exposure in the tank yard and exposure to ethylene. Bruises cause immediate alterations and changes in cell wall structure resulting in immediate loss in cell wall integrity (Miller, 1992). Bruising results in rapid ethylene production within 5 to 60 minutes due to conversion of the precursor 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Yang and Hoffman, 1984). Fresh cucumbers are generally held for no longer than 24 hours

before being processed. If cucumbers are held in storage after processing, anywhere from 6 to 25 days, they produce a burst of ethylene (C_2H_4) resulting in a decrease in chlorophyll and seed cavity pH and in increase in polygalacturonase (PG) activity in the seed cavity (Saltveit and McFeeters, 1980). Cucumbers exposed to ethylene developed undesirable texture attributes including gumminess, chewiness, brittleness and increased elasticity within 48 hours of exposure (Poenicke et al., 1977). Bruising of cucumber fruit during harvest resulted in textural changes within 48 hours of harvesting due to increased activities of polygalacturonase, xylanase, and pectin methylesterease in cucumber mesocarp tissue (Miller et al., 1987). Mesocarp tissue cell wall analysis also showed a decrease in the amount of mannose and galactose and an increase in uronic acid to rhamnose, arabinose and galactose ratio signaling changes in tertiary structural changes in pectin (Miller et al., 1989). Bruising is seen in the exocarp extending into the mesocarp region of the fruit as a result of harvesting and handling. Generally bruising is not a dominating effect in tissue curing other than in extreme cases.

Curing and damage to cucumber fruit cell walls can also be correlated to chilling damage. Holding a cucumber fruit at too low of a temperature can cause high membrane leakage and ethylene production (Kuo and Parkin, 1989). Nicholas and Pflug (1960) showed that holding cucumbers at high temperatures (>22°C) can cause tissue curing. Storage at cooler temperatures helps to prevent curing in fresh cucumbers (Fellers and Pflug, 1968). Gross and Wang (1984) showed that chilling stress induced a rapid onset of ethylene production through a pathway similar to natural fruit ripening (Wang and Adams, 1980) within 24 hours of fruit being chilled and then rapidly warmed. Chilling stress, chilling

followed by warming, resulted in a rapid change in neutral sugar and galacturonic acid composition in cell call polysaccharides (Gross and Wang, 1984). Miller et al. (1987) showed that storage at cooler temperatures decreased ethylene production. Tissue firmness decreased after 8 hours of storage at 38ºC compared to cucumber fruit stored for the same period of time between 0ºC and 10ºC (Miller et al., 1987). Again, other than in extreme cases of chiller damage, cucumbers are generally brined or pasteurized before they warm up sufficiently for chill injury to develop.

Processing fresh cucumbers during the peak of harvest occurs quickly. Cucumbers arrive to processing plants and are either placed in fermentation tanks for storage or processed into fresh pack or refrigerated pickles the day of arrival or the following morning. Because cucumbers are processed into pickles as immature fruit, fruit maturation and ripening is not a concern for industry. However, examining the effects of maturation and ripening on cucumber cell wall structure may be useful in understanding the mechanisms behind curing. Tissue softening results from changes in pectin and hemicelluloses due to solubilization and depolymerization that can result in cell wall loosening and disintegration (Brady, 1987; Fischer and Bennett, 1991). Late in the ripening of cucumber fruit, citric acid concentrations in the endocarp tissue can be as high as 1% of the wet weight (McFeeters et al., 1982). PG-catalyzed pectin degradation is thought to be the primary process involved with fruit softening (Crookes and Grierson, 1983). PG activity catalyzes the hydrolysis of glycosidic linkages of polymerized galacturonic acids aiding in the solubilization of covalently bound pectin (Rose et al., 1998). Along with changes in pectin, the degree of solubility and molecular mass of hemicelluloses changes during ripening.

Changes in cell walls due to enzymatic activity such as pectin demethylation and changes in solubility characteristics of pectic substances occur in cucumbers during fermentations (Buescher and Hudson, 1986; Lampi et al., 1958; Tang and McFeeters, 1983). The demethylation is most likely due to cucumber pectin methylesterase which is activated after exposing cucumbers to brine solutions (Bell et al., 1951; McFeeters et al., 1985). The addition of high salt and calcium chloride are used to help inhibit the pectinolytic enzymes (Buescher et al., 1979; Buescher and Hudson, 1984; Fleming et al., 1987). Maruvada and McFeeters (2009) found that cucumbers blanched prior to low salt fermentation had a decrease in enzymatic activity. Ferguson (1984) suggests that in the absence cell degrading enzymes, calcium can increase cohesion of cell wall structural components. While the changes in cell wall polysaccharide composition due to enzymatic activity is mostly studied in fermented cucumbers, it might also be a factor influencing curing in fresh pack and refrigerator pickles.

Calcium chloride was added to pickles to reduce the loss of firmness (Breidt, 2006; Breidt et al., 2007; Hudson and Buescher, 1985; Tang and McFeeters, 1983). Firming of plant tissue by calcium is to due to interactions with the 2^{++} charge and galacturonans in the cell wall (Grant et al., 1973). McFeeters and Fleming (1989) found calcium cross-linking was infrequent in conditions in where calcium was an inhibitor of softening. They also found there is no apparent relationship between the affinity of a divalent ion for pectin and its ability to reduce softening (McFeeters and Fleming, 1989). Results suggest that textural effects of calcium on reducing cucumber softening are a result of calcium binding at sites other than pectin and carboxylic groups (McFeeters and Fleming, 1989). McFeeters and

Fleming (1989) suggests Grant's 'egg box' model for calcium cross-linking (Grant et al., 1973) may not be a satisfactory mechanism to explain firmness retention in cucumber fruits in the presence of calcium.

However, some studies have shown that polysaccharide changes may not be the main contributor to curing. Tissue curing was studied in metabisulfite accelerated curing and hydrogen peroxide delayed tissue curing of refrigerator pickles (Howard, 1989). Results showed that changes in polysaccharide composition in plant cell walls was not directly associated with curing, but rather, curing could be related to changes in proteins in plant cell walls (Howard, 1989). Throughout the fermentation process, lactic acid bacteria naturally lower the pH of the cover brine solution. In fresh pack and refrigerated pickles, cucumbers are stored in a cover solution with a pH less than, at or below 4.6. Acidic storage conditions can alter the state of proteins found in the cucumber cell wall. Proteins are generally in their most stable configurations at their isoelectric point (Damodaran, 1996). The reduction of pH could result in alterations of proteins found in the plant cell wall. The acidic conditions result in a higher positive charge altering the protein structure. Mok (1992) found a relationship between pickle tissue curing and changes in protein solubility and distribution. Fermented pickles cured most rapidly, within 30 days, compared to fresh pack and refrigerated pickles, which had curing of 50% and 80% respectively after 5 months. In both situations the degree of curing was directly correlated to protein solubility (Mok, 1992). Exposure of the cucumber tissue to acidic, high ionic strength brine caused precipitation or a reduction in solubility of cucumber proteins. Mok (1992) speculates that changes in cell well protein structure are the main contributing factor to tissue curing. Changes in protein

solubility and distribution throughout the cell wall as well as the depolymerization of proteins during curing, were strongly correlated to changes in cucumber tissue.

1.3 Instrumental and Sensory Analysis of Foods

1.3.1 Texture analysis

Texture is an important sensory attribute to many foods. Consumers prefer a firm, crisp, crunchy pickle. Instrumental and sensory analysis of cucumber firmness can be used to assess pickle quality. Provided that physical or chemical attributes of foods can be correlated with sensory data, instrumental analysis of food quality factors can be done on many more samples in less time and at lower cost than sensory analysis. Objective methods, such as punch and die assemblies, are currently used to measure the amount of force needed to puncture cucumber flesh. Instrumental analysis allows for unbiased results due to the elimination of panelists and creates a common reference for researchers, industry, and consumers (Abbott, 1999). Several methods have been used to measure cucumber firmness. These include Magness-Taylor fruit pressure tester, Chatillon spring Puncture Tester and the Instron UTM (Thompson et al., 1982). This methodology has been used to measure changes in texture induced by heat treatments (Luna-Guzman et al., 1999) to enzymatic softening (Maruvada and McFeeters, 2009; Thompson et al., 1982). Thompson et al., (1982) was able to correlate the measured firmness of fermented cucumber mesocarp tissue to sensory measurements.

1.3.2 Descriptive Sensory Analysis

Descriptive sensory analysis is used to give quantitative scores to assess flavor profiles and physical characteristics of foods. Sensory evaluation is defined as “the scientific method used to evoke, measure, analyze, and interpret those responses to products as

perceived through the senses of sight, smell, touch, taste, and hearing" (Stone and Sidel, 1993). It is a quantitative technique in which collected numerical data is used to develop a relationship between product characteristics and human perception (Lawless and Heymann, 1998). Sensory methods can be applied in several ways from determining consumer liking and preference to descriptive techniques in which lexicons are created to give product descriptions. Descriptive sensory analysis can be applied to products to evaluate product attributes and quality.

There are two main methods used in descriptive sensory analysis, Quantitative Descriptive Analysis (QDA) and the Spectrum™ method. The QDA method is product specific (Stone and Sidel, 1993; Lawless and Heymann, 1998). Panelists are selected from the general public and meet in focus groups to discuss product attributes and descriptors (Lawless and Heymann, 1998). From these discussions, a consensus is met amongst the panelists and a standardized vocabulary is developed to describe differences between samples (Lawless and Heymann, 1998). During this time, panelists also determine the reference standards or verbal definitions that are then used to anchor the descriptive terms (Lawless and Heymann, 1998).

Another common method used in descriptive sensory analysis is the Spectrum™ method. This method was designed for industry and based on procedures designed by Sensory Spectrum Consulting in the 1980s (Lawless and Heymann, 1998). Based upon the Texture Profile Method (TPM), the Spectrum™ method expands beyond textural analysis and uses the same techniques to analyze the full "spectrum" of attributes in a product (Murray et al., 2001). Using the Spectrum™ method multiple attributes and products can be

directly compared using a universal 15-point scale, where 0 is no stimulus to 15 a very strong stimulus. Trained panelists utilize reference anchors at various intensities on the scale to calibrate senses and make product comparisons. Panelists initially undergo 50 to 100 hours in intensive training by a panel leader. Following intensive training and discussion of product attributes, panelists are then able to evaluate characteristics and compare them to a standardized lexicon (Meilgaard et al, 1991). The Spectrum™ method allows for a wide variety of characteristics and products to be analyzed through the use of a universal scale. This method is useful in determining changes in products over time (Lawless and Heymann, 1998). In some instances such as product firmness, which is a human perception, sensory evaluation is a favored practice over the instrumentation (Szczesniak, 2002). The use of a universal scale and trained panelists allows for collected data points to be considered absolute and allows for the use of standard statistical procedures (Lawless and Heymann, 1998). As with all methods, drawbacks to using the Spectrum™ method include panel training, panel maintenance, and panelist changeover.

1.3.3 Food volatile analysis by two-dimensional gas chromatography/mass spectrometry

Instrumentation can be used to analyze volatile components found in foods. In conjunction with descriptive sensory analysis, it can be used to determine the chemical composition of a food. The overall flavor profile of a food is determined by a complex makeup of volatile and non-volatile compounds. Solid-phase microextraction (SPME) is a technique to sample volatile compounds by reversible binding of analyte molecules to a silica fiber coated with an appropriate stationary phase (Zhang et al., 1994). The stationary phase

coated capillary fiber is inserted into the headspace above the analyte sample. Salt is added to the sample to reduce the solubility of analytes so that their concentration in the headspace gas phase will increase. The volatile compounds present in the headspace above the analyte equilibrate and bind reversibly to the stationary phase on the fiber (Zhang et al., 1994). After allowing for sufficient time for the volatile compounds found in the headspace to equilibrate with the fiber, the fiber is removed from the headspace and inserted into a gas chromatograph (GC). High temperatures in the chromatograph injection port drive the analyte molecules off the fiber into the gas phase where they travel through the chromatographic column and are separated based upon differential interaction with the stationary phase of the column.

Volatile composition of foods is complex, and the volatiles responsible for odor can are commonly present at the ng/g level (Adahchour et al., 2003) or lower (Amerine et al., 1965). One-dimensional capillary gas chromatography is traditionally used for separation of volatile compounds. However, a one-dimensional separation does not in generally provide sufficient separation for either qualitative or quantitative analysis (Adahchour et al., 2003) of complex mixtures. Compounds partially or completely co-elute from the column so that mass spectra contain ions from multiple compounds which interferes both with identification and quantitation of the co-eluting components. To reduce the problem of co-elution, two-dimensional gas chromatography has been developed. Two-dimensional gas chromatography works by applying two independent gas chromatography separations. The first dimension separation usually separates the sample components based upon boiling point followed by a second dimension separation based upon polarity (Adahchour et al., 2002; Adahchour, 2003). Between the two columns is a modulator device that traps, re-

concentrates, and re-injects continuous time slices of 2 seconds to 8 seconds of effluent from the first column (Adahchour, 2008; Mondello et al., 2006). Figure 1.2 (Adahchour, 2002) shows a schematic diagram of a GCxGC-TOFMS in which a cryogenic modulator is used to focus peaks from the first column onto the second column. The run time on the second dimension column is equal to the modulation time (Shellie and Marriott, 2002). Peak widths in the second dimension are very narrow, sometimes only 100 milliseconds (Adahchour et al., 2003; Adahchour et al., 2008; Ryan et al., 2004). The use of two columns allows for a comprehensive chromatographic method, meaning the transfer of effluent from the first column to the second column is qualitatively and quantitatively complete as well as the separation on the first column is preserved on the second column without mass loss (Cardeal et al., 2006). Two-dimensional gas chromatography requires a fast scanning detector and is most commonly paired with time-of-flight mass-spectrometry (TOF-MS). Analytical methods such as two-dimensional gas chromatography are improved when coupled with TOF-MS due to the increased speed and sensitivity of the detector (Adahchour et al., 2003; Williamson and Bartlett, 2007). TOFMS can detect compounds in the low picogram (pg) range (de Hoffmann and Stroobant, 2002). In a food matrix it is not always possible to gain a sufficient separation using 1D-GC-MS and as a result identification usually relies on a limited number of m/z values rather than complete mass spectrum (Dallüge et al., 2003). Depending upon the capabilities of the instrument two-dimensional gas chromatography coupled with time-of-flight mass spectrometry can be operated at high sampling rates, 5-30kHz, allowing for the generation of 500-30,000 mass spectra per second (Dallüge, et al., 2003). Due to TOF-MS high throughput and sensitivity it has been used in combination with

two-dimensional gas chromatography to analyze volatile compounds and pesticides in foodstuffs such as grapes and pineapples (de Koning et al., 2003), herbicides in barley (Diez et al., 2006), volatiles in peppers (Cardeal et al., 2006), and volatiles in coffee beans (Ryan et al., 2004).

REFERENCES

Abbott, J.A. Quality measurement of fruits and vegetables. *Postharvest Biol. Technol.* 1999, 15, 207-225.

Adahchour, M.: Beens, J.; Brinkman, U.A. Th. Recent developments in the application of comprehensive two-dimensional gas chromatography. *J. Chromatogr. A.* 2008, 1186, 67-108.

Adahchour, M.; Beens, J.; Vreuls, R.J.J.; Batenburg, A.M.; Rosing, E.A.E.; Brinkman, U.A.Th. Application of solid-phase micro-extraction and comprehensive two-dimensional gas chromatography (GCxGC) for flavour analysis. *J. Chromatogr. A.* 2002, 55,361-367.

Adahchour, M.; van Stee, L.L.P.; Beens, J.; Vreuls, R.J.J.; Baenburg, M.A.; Brinkman, U.A.Th. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection for the trace analysis of flavour compounds in food. *J. Chromatogr. A.* 2003, 1019,157-172.

Amerine, M.A.; Pangborn, R.M.; Roessler, E.B. Olfaction. In *Principles of Sensory Evaluation of Food*; Stewart, G.F.; Mrak, E.M.; Chichester, C.O.; Scott, J.K.; Hawthorn, J.; von Sydow, E.; Morgan, A.I., Eds.; Academic Press: New York, New York, 1965; pp. 145-219.

Babcock, M.; Azam-Ali, S. *Fermented Fruits and Vegetables: A Global Perspective.* Food and Agriculture Association Bulletin No. 134; 1998, United Nations; Rome.

Bell, T.A.; Etchells, J.L.; Jones, I.D. Pectinesterase in the cucumber. *Biochem. Biophys.* 1951, 31, 431-441.

Brady, C.J. Fruit ripening. *Annu. Rev. Plant Physiol.* 1987, 38, 155-178.

Breidt, F. Safety of minimally processed, acidified, and fermented vegetable products. In *Microbiology of Fruits and Vegetables*; Sapers, G.M.; Gorny, J.R.; Yousef, A.E., Eds.; CRC Press, Inc: Boca Raton, Florida, 2006; pp. 313-335.

Breidt, F; McFeeters, R.F.; Díaz-Muñiz, I. Fermented vegetables. In *Food Microbiology: Fundamentals and Frontiers*; Doyle, M.P.; Beuchat, L.R., Eds.; ASM Press: Washington, D.C., 2007; pp 783-793.

Buescher, R.W.; Hudson, J.M. Softening of cucumber pickles by Cx-cellulase and its inhibition of calcium. *J. Food Sci.* 1984, 49, 954-955.

Buescher, R.W.; Hudson, J.M. Bound cations in cucumber pickle mesocarp tissue as affected by brining and $CaCl_2$. *J. Food Sci*. 1986, 51, 135-137.

Buescher, R.W.; Hudson, J.M.; Adams, J.R. Inhibition of polygalactruonase softening of cucumber pickles by calcium chloride. *J. Food Sci.* 1979, 44, 1786-1787.

Cardeal, Z.L.; Gomes da Silva, M.D.R.; Marriot, P.J. Comprehensive two- dimensional gas chromatography/mass spectrometric analysis of pepper volatiles. *Rapid Commun. Mass Spectrom.* 2006, 20, 2823-2836.

Corey, K.A.; Pharr, D.M.; Fleming, H.P. Pressure changes in oxygen-exchanged, brined cucumbers. *J. Am. Soc. Hort. Sci.* 1983, 108, 61-65.

Costilow, R.N.; Gates, K.; Bedford, C.L. Air purging of commercial salt-stock pickle fermentations. *J. Food Sci*. 1981, 46, 278-282.

Crookes, P.R.; Grierson, D. Ultrastructure of tomato fruit ripening and role of polygalacturonase isoenzymes in cell wall degradation. *Plant Physiol.* 1983, 72, 1088-1093.

Dallüge, J.; Bens, J.; Brinkman, U.A.Th. Comprehensive two-dimensional gas chromatography: a powerful and versatile analytical tool. *J. Chromatogr. A.* 2003, 1000, 69-108.

Damodaran, S. Amino acids, peptides, and proteins. In *Food Chemistry*. Fennema, O.R.; Karel, M.; Sanderson, G.W.; Tannenbaum, S.R.; Walstra, P.; Whitaker, J.R. Eds.; Marcel Dekker, Inc.: New York, New York, 1996; pp. 321-429.

de Hoffmann, E.; Stroobant, V. Tandem mass spectrometry (MS/MS). In *Mass Spectrometry,* 2nd Edition; John Wiley and Sons, Ltd.: Chichester, U.K., 2002: pp. 133-155.

de Koning, S.; Lach, G.; Linkerhagner, M.; Loscher, R.; Horst Tablack, P.; Brinkman, U.A. Trace-level determination of pesticides in food using difficult matrix introduction-gas chromatography-time-of-flight mass spectrometry. *J. Chromatogr. A.* 2003, 1008: 247-252.

Delmer, D.P.; Amor, Y. Cellulose biosynthesis. *Plant Cell*. 1995, 7, 987-1000.

Diez, C.; Traag, W.A.; Zommer, P.; Marinero, P.; Atienza, J. Comparison of an acetonitrile extraction/partitioning and 'dispersive solid-phase extraction' method with classical multi-residue methods for the extraction of herbicide residues in barley samples. *J. Chromatogr. A.* 2006, 1131, 11-23.

Etchells, J.L.; Bell, T.A.; Fleming, H.P.; Kelling, R.E.; Thompson, R.L. Suggested procedure for the controlled fermentation of commercially brined pickling cucumbers—The use of starter cultures and reduction of carbon dioxide accumulation. *Pickle Pak Sci.* 1973, 3, 4-14.

Etchells, J.L. Ohmer, H.B. A bacteriological study of the manufacture of fresh cucumber pickles. *Fruit Prod. J.* 1941, 19, 331.

Etchells, J.L.; Bell, T.A.; Fleming, H.P. Use of calcium chloride to improve the texture of pickles. Advisory statement by *Pickle Packers Int., Inc.*: St. Charles, Illinois. 1977.

Fahn, A. *Plant Anatomy*; 3rd Edition. Pregamon Preson Oxford, U.K. 1982.

FAOSTAT Data, 2004. *Agricultural Production*. Last updated February 2004. Food and Agriculture Organization of the United Nations.

Fellers, P.J.; Pflug, I.J. Loss of whiteness from fresh cucumber pickles. *Food Technol.* 1968, 22, 105-108.

Ferguson, I.B. Calcium in plant senescence and fruit ripening. *Plant Cell. Environ.* 1984, 7, 477-489.

Fischer, R.L.; Bennett, A.B. Role of all hydrolases in fruit ripening. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 1991, 42, 675-703.

Fleming, H.P. Developments in cucumber fermentation. *J. Chem. Technol. Biotechnol.* 1984, 34B, 241-252.

Fleming, H.P.; Humphries, E.G.; Fasina, O.O.; McFeeters, R.F.; Thompson, R.L.; Breidt, F. Bag-in-box technology: pilot system for process-ready, fermented cucumbers. *Pickle Pak. Sci.* 2002, 8, 1-8.

Fleming, H.P.; McFeeters, R.F. Shelf life of fresh-pack cucumber pickles. Advisory statement by *Pickle Packers Int., Inc.*: St. Charles, Illinois, 1981.

Fleming, H.P.; Moore, W.R., Jr. Pickling. In *Processing of Horticultural Crops in the United States.* Fuller, G.; Dull, G.G. Eds.; In *CRC Handbook of Processing and Utilization in Agriculture*, Wolff, I.A. Eds., CRC Press, Inc.; Boca Raton, Florida, 1983; Vol. 2, pp. 397-463.

Fleming, H.P.; Pharr, D.M.; Thompson, R.L. Brining properties of cucumbers exposed to pure oxygen before brining. *J. Food Sci.* 1980, 45, 1578-1582.

Fleming, H.P.; McFeeters, R.F.; Thompson, R.L. Effects of sodium chloride concentration on firmness retention of cucumbers fermented and stored with calcium chloride. *J. Food Sci.* 1987, 52, 653-657.

Fry, S.C. Cellulases, hemicelluloses and auxin stimulated growth: a possible relationship. *Physiol. Plant.* 1989, 75, 532-536.

Grant, G.T.; Morris, E.R.; Rees, D.A.; Smith, P.J.C.; Thom, D. Biological interactions between polysaccharides and divalent cations: The egg box model. *FEBS Lett*. 1973, 32, 195-198.

Gross, K.C., Wang, C.Y. Compositional changes in cell wall polysaccharides from chilled and non-chilled cucumber fruit. *Phytochem. Lett.* 1984, 23, 1575-1578.

Howard, L.R. *Factors Affecting Cell Wall Components of Mesocarp Tissue in Fresh Pack Cucumber pickles*. PhD Dissertation; University of Arkansas, Fayetteville, Arkansas. UMI: Ann Arbor, Michigan; 1989.

Hudson, J.M.; Buescher, R.W. Pectic substances and firmness of cucumber pickles as influenced by $CaCl_2$, NaCl and brine storage. *J. Food Biochem*. 1985, 9, 221-299.

Humphries, E.G.; Fleming, H.P. Purging CO_2 from cucumber fermentation and storage tanks. *Appl. Eng. Agr.* 1988, 4, 166-171.

Hutkins, R.W. Fermented foods in human history. In *Microbiology and Technology of Fermented Foods.* Hutkins, R.W. Eds.; Blackwell Publishing: Ames, Iowa, 2006; pp. 3-14; 246-249.

Imam, S.; Shey, J.; Wood, D.; Glenn, G.; Chiou, B.; Inglesby, M.; Ludvik, C.: Klamczynski, A.; Orts, W. Structure and function of complex carbohydrates in produce and their degradation process. In *Produce Degradation-Pathways and Prevention*. Lamikanra, O.; Imam, S.; Ukuku, D., Eds.; CRC Press LLC: Boca Raton, Florida, 2005; pp. 563-597.

Kieliszewski, M. de.; Zacks, R.; Leykam, J.F.; Lamkport, D.T.A. A repetitive proline-rich protein from the gymnosperm Douglas fir is a hydroxyproline-rich glycoprotein. *Plant Physiol.* 1992, 98, 919-926.

Kuo, S.J.; Parkin, K.L. Chilling injury in cucumbers (*Cucumis sativa* L.) associated with lipid peroxidation as measured by ethane evolution. *J. Food Sci.* 1989, 54, 488-491.

Lampi, R.A.; Esselen, W.B.; Thompson, S.L.; Anderson, E.E. Changes in pectic substances of four varieties of pickling cucumbers during fermentation and softening. *J. Food Res.* 1958, 23, 351-363.

Lamport, D.I.A.; Epstein, L. A new model for the primary cell wall: A concatenated extensin-cellulose network. In *Current Topics in Plant Biochemistry and Physiology*. Randall, D.D.; Blevins, D.G.; Larson, R.L.; Rapp, B.J; Parlardy, S.G.; Nelson, C.J.; Polacco, J.C.; George, M.F. Eds.; University of Missouri: Columbia, Missouri, 1983; 2, pp. 73-83.

Lauchli, A. Apoplasmic transport in tissues. In *Transport in Plant II*. Luttge, U. Pitman, M.G. Eds.: Springer-Verglad, New York, New York; 1976; pp 3-53.

Lawless, H.T.; Heymann, H. I. *Sensory Evaluation of Food: Principles and Practices*. Kluwer Academic/Plenum Publisher: New York, New York, 1998.

Lerner, B.R.; Dana, M.N. Growing cucumbers, melons, squash, pumpkins and gourds. Purdue University Cooperative Extension Services. 2001, http://www.ces.purdue.edu/extmedia/hort.htm. H-08-W.

Luna-Guzman, I.; Cantwell, M.; Barrett, D.M. Fresh-cut cantaloupe: Effects of $CaCl_2$ dips and heat treatments on firmness and metabolic activity. *Postharvest Biol. Technol.* 1999, 17, 201-213.

Maruvada, R.; McFeeters, R.F. Evaluation of enzymatic and non-enzymatic softening in low salt cucumber fermentation. *Int. J. Food Sci. Technol.* 2009, 44, 1108-1117.

McCann, M.C.; Well, B.; Roberts, K. Direct visualization of cross-links in the primary plant cell wall. *J. Cell. Sci.* 1990, 96, 323-334.

McDonald, L.C.; Fleming, H.P.; Daeschel, M.A. Acidification effects on microbial populations during initiation of cucumber fermentation. *J. Food Sci.* 1991, 56, 1353-1359.

McFeeters, R. F.; Fleming, H. P. Inhibition of cucumber tissue softening in acid brines by multivalent cations: inadequacy of the pectin "egg box" model to explain textural effects *J. Agric. Food Chem.* 1989. 37, 1053-1059.

McFeeters, R.F.; Fleming, H.P.; Thompson, R.L. Malic and citric acids in pickling cucumbers. *J. Food Sci.* 1982, 47, 1859-1865.

McFeeters, R.F.; Fleming, H.P.; Thompson, R.L. Pectinesterase activity, pectin methylation and texture changes during storage of blanched cucumber slices. *J. Food Sci.* 1985, 50, 201-205.

Meilgaard, M.C.; Civille, G.V.; Carr, B.T. *Sensory Evaluation Techniques,* 2nd Edition; CRC Press: Boca Raton, Florida, 1991.

Miller, A.R.; Dalmasso, J.P.; Kretchman, D.W. Mechanical stress, storage time and temperature influence cell-wall degrading enzymes, firmness and ethylene production by cucumbers. *J. Amer. Soc. Hort. Sci.* 1987, 112, 666-671.

Miller, A.R.; Kelley, T.J.; Mechanical stress stimulates peroxidase activity in cucumber fruit. *HortSci.* 1989, 24, 650-652.

Miller, A.R. Physiology, biochemistry and detection of bruising (mechanical stress) in fruits and vegetables. *Postharvest News Inf.* 1992, 3, 3, 53N-58N.

Mok, Y.N. *Curing of Cucumber Pickle Mesocarp Tissue Affected by Alterations in Protein and Cell Walls*. PhD Dissertation; University of Arkansas, Fayetteville, AR. AR. UMI: Ann Arbor, Michigan; 1992.

Mondello, L.; Casilli, A.; Quinto Tranchida, P.; Lo Presti, M.; Dugo, P.: Dugo, G. Comprehensive gas chromatography coupled to mass spectrometry for the separation of pesticides in a very complex matrix. *Anal. Bioanal. Chem*. 2006, 389, 1755-1763.

Murray, J.M.; Delahunty, C.M. Baxter, I.A. Descriptive sensory analysis: past, present and future. *Food Res. Int.* 2001, 34, 461-471.

Nicholas, R.C. Pflug, I.J. Effect of high temperature storage on the quality of fresh cucumber pickles. *Glass Pack.* 1960, 39, 35.

Nishitani, K. Endo-xyloglucan transferase, a new class of transferase involved in cell wall construction. *J Plant Res.* 1995, 108, 137-148.

Pedersen, C.S. *Microbiology of Food Fermentations,* 2nd Edition; AVI Pub. CO: Westport, Connecticut, 1979.

Poenicke, E.F.; Kays, S.J.; Smittle, D.A.; Willimson, R.E. Ethylene in relation to postharvest fruit quality deterioration in processing cucumbers. *J. Amer. Soc. Hort. Sci.* 1977, 102, 303-306.

Potts, E.A.; Fleming, H.P. Changes in dissolved oxygen and microflora during fermentation of aerated, brined cucumbers. *J. Food Sci.* 1979, 44, 429-434.

Potts, E.A.; Fleming, H.P. Prevention of mold-induced softening in air-purged, brined cucumbers by acidification. *J. Food Sci.* 1982, 47, 1723-1727.

Potter, N.N.; Hotchkiss, J.H. *Food Science*, 5th Edition. An Aspen Publication: Gaithersburg, Maryland, 1998; pp. 113-199.

Reiter, W.D. *Arabidopsis thaliana* as a model system to study synthesis, structure and function of the plant cell wall. *Plant Physiol. Biochem.* 1998, 36, 1-2, 167-176.

Reiter, W.D.; Chapple, C.; Sommerville, C.R. Mutants of *Arabidopsis thaliana* with altered cell wall polysaccharide composition. *Plant J.* 1997, 12, 335-345.

Rose, A.H. History of scientific basis of microbial activity in fermented foods. In *Fermented Foods*. Rose, A.H. Eds.; Academic Press: London, U.K., 1982; pp. 1-12.

Rose, J.K.C.; Hadfield, K.A.; Labavitch, J.M. Bennett, A.B. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. *Plant Physiol.* 1998, 117, 345-361.

Ryan, D.; Shellie, R.; Tranchida, P.; Casilli, A.; Mondello, L.; Marriot, P. Analysis of roasted coffee bean volatiles by using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *J. Chromatog. A.* 2004, 1054, 57-65.

Saltveit, M.E.; McFeeters, R.F. Polygalacturonase activity and ethylene synthesis during cucumber fruit development and maturation. *Plant Physiol*. 1980, 66, 1019-1023.

Shellie, R.; Marriott, P.J. Comprehensive two-dimensional gas chromatography with fast enantioseparation. *J. Anal. Chem.* 2002, 74, 5426-5430.

Stone, H.; Sidel, J.L. *Sensory Evaluation Practices*, 2nd Edition. Academic: Orlando, Florida; 1993.

Szczesniak, A.S. Texture is a sensory property. *Food Qual. Pref.* 2002, 13, 215-225.

Tang, H.C.L.; McFeeters, R.F. Relationship among cell wall constituents, calcium and textures during cucumber fermentation storage. *J. Food Sci*. 1983, 48, 66-70.

Thompson, R.L.; Fleming, H.P.; Hamann, D.D.; Monroe, R.J. Method for determination of firmness in cucumbers slices. *J. Text. Stud.* 1982, 13, 311-324.

United States Code of Federal Regulations. 2006. *Acidified Foods*. 21 CFR Part 114. U.S. Department of Health and Human Services, Washington, D.C.

USDA. National Agricultural Statistics Service, Vegetable Yearbook 2004. "U.S. cucumbers for pickles: Supply, utilization, and price, farm weight, 1979-2004."; Lucier, G.; Plummer, C.; Jerardo, A.; 2004.

USDA. U.S. Standards for grades of pickles. Fed. Reg 31:102231-10305. Office of Federal Registry. National Archives and Records Service, General Services Admin. Washington, D.C.; 1966.

U.S. DHEW. Acidified foods and low acid canned foods in hermetically sealed containers. Food and Drug Administration. Fed. Reg. 44:16204-16238. Office of Federal Registry. National Archives and Records Service, General Services Admin. Washington, D.C.; 1979.

Wang, C.Y. Adams, D.O. Ethylene production by chilled cucumbers (*Cucumis sativus* L.). *Plant Physiol.* 1980, 66, 841-843.

Williamson, L.N.; Bartlett, M.G. Quantitative liquid chromatography-time of flight mass spectrometry. *Biomed. Chromatogr*., 2007, 21, 567-576.

Yang, S.F.; Hoffman, N. E. Ethylene biosynthesis and its regulation in higher plants. *Annu. Rev. Plant Physiol.* 1984, 35, 155-189.

Zablackis, E.; Huang, J.; Muller, B.; Darvill, A.G.; Albersheim, P. Characterization of the cell-wall polysaccharides of *Arabidopsis thaliana* leaves. *Plant Physiol.* 1995, 107, 1129-1138.

Zhang, Z.; Yang, M.; Pawliszyn, J. Solid phase microextraction: A new solvent-free alternative for sample preparation. *Anal. Chem.* 1994, 66, 844A-853A.

Figures

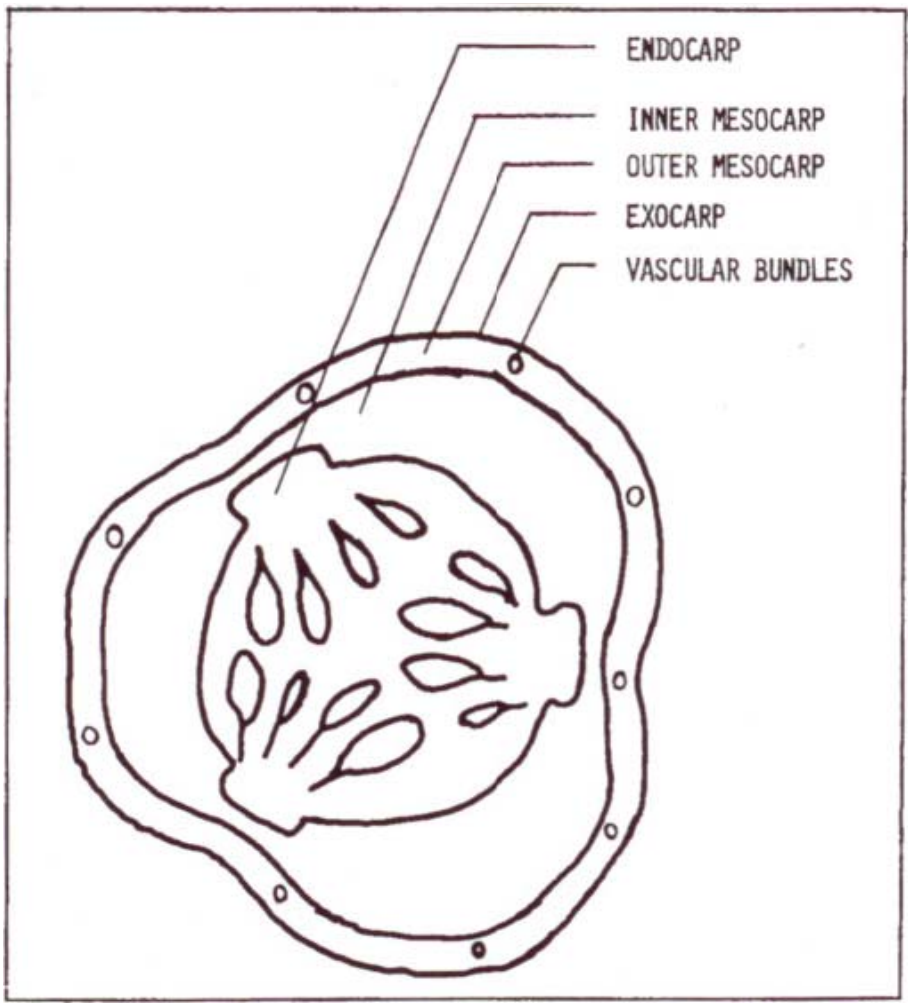

Figure 1.1 A cross sectional view of a cucumber fruit. Figure adapted from McFeeters (1982).

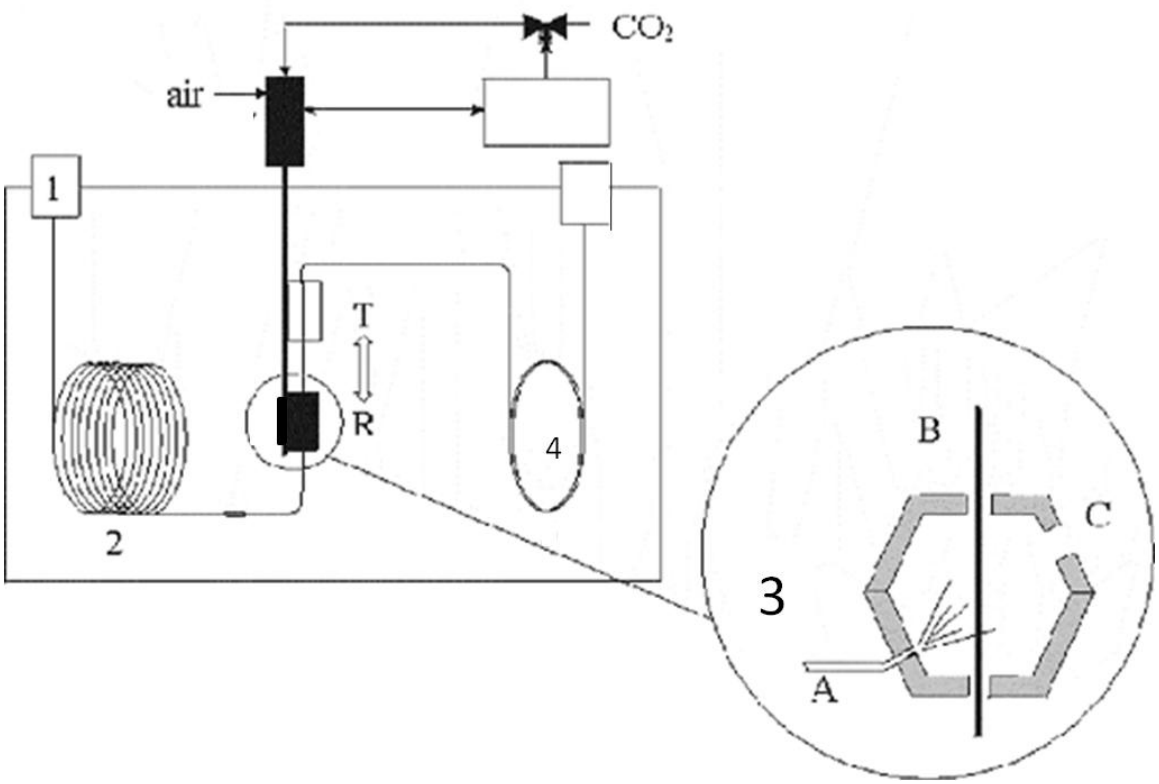

Figure 1.2 Schematic diagram of GCxGC-TOFMS system with a cryogenic modulator. 1) Injector port. 2) First dimension column. 3) Modulation chamber. 4) Second dimension column. T) Trapping position. R) Release position. Insert: A) N_2 inlet. B) Top of second dimension GC column. C) N_2 outlet. Figure adapted from Adahchour (2002).

Chapter 2

Sensory and Instrumental Analysis of Pasteurized Dill Pickles Made from Acidified, Bulk Stored Cucumbers

2.1 Abstract

Storing fresh cucumbers for an 8 to 12 week period in an acidified, non-fermented environment prior to processing would extend the time for processing locally grown cucumbers into pasteurized pickle products. The effect of acidified storage of fresh cucumbers for up to 120 days prior to their conversion to pasteurized dill pickles was evaluated. Cucumbers stored at 18ºC prior to pasteurization maintained a fresh, non-cured appearance as well as a firm texture. Equilibrated acid concentrations of 3.5% acetic acid also helped to reduce the amount of curing and maintained firmness in cucumber tissue. Dill pickles made from acidified, bulk stored cucumbers maintained a firm texture after pasteurization. However, the cured appearance of bulk stored cucumbers increased by 40% within 30 days of pasteurization so that it was much higher than the cured appearance of dill pickles made directly from the same lot of fresh cucumbers and stored for an equivalent period of time after pasteurization. Sour, sweet, bitter and vinegar intensities were similar for dill pickles prepared from either bulk stored or fresh cucumbers. Firmness, saltiness, and dill flavor while significantly different, differed by less than 1 point on a 15-point scale. The major flavor difference between pasteurized dill pickles made from stored acidified cucumbers and pickles made directly from fresh cucumbers is that pickles made from stored cucumbers had an "other" flavor. The intensity of this flavor was perceived at a similar level regardless of the brine composition or storage time of the cucumbers prior to pasteurization. Descriptors for this "other" flavor were chlorine, pool, barny and metallic. GCxGC-TOFMS analysis of volatile components in dill pickles prepared from bulk stored cucumbers and dill pickles prepared from fresh cucumbers resulted in detection of 24 components that were

significantly different ($p<0.05$) between at least one treatment pair. Individual volatile components did not provide a clear basis for differentiation among different storage brine treatments. However, PCA analysis using the 24 components was able to differentiate treatments.

2.2 Introduction

Pickled cucumbers are categorized as fermented, fresh pack (pasteurized, shelf stable), or refrigerated pickles. These categories account for about 35%, 40% and 25% of pickled cucumber products respectively (Fleming et al., 2002). Fermented cucumbers are produced when cucumbers are placed in cover brine containing NaCl, $CaCl_2$, and 0.05% acetic acid (Breidt, 2006; Breidt et al., 2007; Hutkins, 2006). Salt tolerant and acid tolerant homofermentative lactic acid bacteria (LAB) present in low numbers on the surface of cucumbers are able grow in these conditions while other microorganisms are inhibited. As a result lactic acid bacteria increase in numbers and consume the glucose and fructose in the cucumber to produce primarily lactic acid. The acid production results in titratable acidity between 0.6% and 1.2% as calculated as lactic acid and lowers the pH into the range of 3.2 to 3.6 (Breidt, 2006; Breidt et al., 2007; Hutkins, 2006). Because the fermentation process allows for cucumbers to be stored for long periods of time, pickle products can be made from the fermented cucumbers year round.

Accounting for an estimated 40% of the pickle industry (Fleming et al., 2002), fresh pack pickles are made by acidification of fresh cucumbers that are packed in jars with hermetically sealed closures, covered with a solution containing vinegar, salt, calcium chloride, yellow color, and flavoring, and pasteurized at 74ºC (165ºF) for 15 minutes to kill vegetative microorganisms and inactivate enzymes in the cucumber fruit to make shelf stable pickle products (Breidt, 2006). Pasteurization also contributes to the preservation of a fresh white opaque appearance of the cucumber flesh instead of a translucent 'cured' appearance that is the characteristic of fermented cucumbers (Howard, 1989; Mok, 1992). Acidified

foods are defined by the U.S. Code of Federal Regulations (21 CFR part 114) as low-acid foods to which acid(s) or acid foods(s) are added, having a water activity (a_w) greater than 0.85 and an equilibrated pH of 4.6 or below (United States Code of Federal Regulations, 2006). USDA grade standards specify that fresh pack pickles must contain a minimum of 0.5% acetic acid (USDA, 1966). The pH for the fresh pack products is usually in the range of 3.5 to 4.0 with acetic acid concentrations of 0.6% to 2% and salt concentrations of 2 to 4% (Breidt et al., 2007).

Fresh cucumbers must be processed within a week of harvest if they are refrigerated or within a day or two if they are held at ambient temperature. Two important quality factors for pasteurized cucumber pickles are a firm, crisp texture similar to fresh cucumbers and retention of the white, opaque appearance of the cut fresh cucumbers. Both of these quality characteristics are influenced by the cell walls of the cucumber tissues. Plant cell walls maintain cell shape, give support, add strength, regulate cell volume, participate in metabolism and act as a physical barrier (Reiter, 1998). Cucumber fruit cell walls are a complex structure of polysaccharides built from several monosaccharides, including glucose, galactose, galacturonic acid, xylose, mannose and others (McFeeters and Lovdal, 1987) plus some poorly characterized cell wall proteins. The main polysaccharides of plant cell walls are cellulose, hemicellulose, pectic polysaccharides, which compromise 15-30%, ca. 25%, and ca. 30% of the dry weight of cell walls, respectively (Reiter et al., 1997; Reiter, 1998; Zablackis et al., 1995). Proteins constitute the remaining 10-20% of the walls (Reiter et al., 1997; Reiter, 1998; Zablackis et al., 1995). The matrix of cell wall polymers allows air to be trapped within the cells and in the intracellular spaces of cucumber fruit. These air spaces,

which constitute about 7% of the volume of fresh cucumbers (Corey et. al., 1983), are responsible for the white, opaque appearance of fresh cucumber tissue.

A problem associated with quality of pasteurized pickled products is curing. Curing occurs when inter- and intra-cellular air pockets in cucumber tissue are filled by liquid (Corey et al., 1983). This results in a change in the appearance of the cucumber flesh from a fresh opaque white appearance to translucent. This change in appearance occurs gradually over a period of months in pasteurized pickles. It is speculated that tissue curing can be associated with changes in cucumber cell walls. Mok (1992) and Howard (1989) discussed factors that alter cell wall polysaccharide and protein structure in cucumber fruits, and how these factors may lead to curing.

Since pasteurized pickle products are shelf stable, they can be produced and warehoused between seasons. However, it would be more economical in terms of equipment and labor if processing could be extended in time beyond the local season where processing plants are located. On the other hand, it is not economical to transport fresh produce across the continent to produce pasteurized products year round. These competing factors mean that it would be useful to be able to store fresh produce for up to10 to 12 weeks in bulk containers while maintaining the texture and appearance of the fruit so that high quality pasteurized pickles could be made from the stored cucumbers. Small cucumbers are grown in Asia and then stored in high acid solution in 55-gallon drums for shipment to the United States and other countries to be used in pasteurized pickle products. The use of sulfite to assure microbial stability results in rapid development of a ‘cured’ appearance in those cucumbers (McFeeters et al., 2002). It would be useful to processors if a method could be found which

would maintain the fresh opaque appearance of bulk stored cucumbers that would be maintained in pasteurized products made from those cucumbers. This would make it possible to extend the processing period with cucumbers that are grown relatively close to the processing location. The objective of this research was to evaluate how factors such as acid concentration, preservatives and storage temperature affect the degree of cured appearance, texture and flavor attributes of pasteurized pickle products prepared from bulk stored cucumbers.

2.3 Materials and Methods:

2.3.1 Evaluation of firmness, curing and sensory attributes of acidified, bulk stored cucumbers

Cucumbers and ingredients for bulk storage of cucumbers and pasteurized dill pickles. Fresh size 2A (25-32 mm diameter) pickling cucumbers, 200 grain vinegar (20% acetic acid), anhydrous $CaCl_2$, high fructose corn syrup (Isomerose^{TM}), yellow #5 food color, Polish dill flavor concentrate, benzoate with color, Antifoam C mixture, lids and glass jars were obtained from a local processor. Morton's pickling salt (NaCl) was purchased from a local grocery store. Food grade sodium benzoate and concentrated HCl was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Food grade calcium fumarate trihydrate ($CaC_4H_2O_4 \bullet 3H_2O$) was purchased from Bartek Chemicals (Sontey Creek, ON, Canada).

Acidified, bulk storage of cucumbers. Cucumbers were acidified and stored in glass jars to evaluate the effects of storage on tissue firmness, cucumber curing, and final pickle product sensory attributes. Non-washed size 2A (25-32 mm) cucumbers were packed in 46 oz jars with a 55:45 cucumber:brine ratio (748 g cucumbers: 602 g cover brine and 10 mL 1.632 M sodium benzoate). Acidified, bulk storage cover brines (Table 2.1) contained acetic acid, NaCl, and anhydrous calcium chloride ($CaCl_2$) at the concentrations given in Table 2.1 to obtain the intended equilibrated concentrations of these ingredients. Following the packing and brining, jars were sealed by placing lids in boiling water (30 seconds) to heat the plastisol liner ensuring an air-tight seal between the lid and the jar. The jars were then sealed by hand. Cucumbers used in bulk storage were blanched or non-blanched and stored at

18ºC, 25ºC, 30ºC or 45ºC for 7, 30, 60, 90 or 120 days before evaluation of tissue firmness and tissue curing.

Blanching procedure. To test the effect of heat on cucumber tissue curing, cucumbers were blanched at 70ºC for 15 minutes. Whole size 2A (25-32 mm) cucumbers were placed in a steam jacketed kettle and covered with enough water so cucumbers could freely move in the kettle. Cucumbers were gently agitated throughout the blanching to ensure the cucumbers were covered with water. Following blanching cucumbers were placed in circulating cold water (5ºC to 10ºC) for 10 minutes with agitation. The cucumbers were drained of excess water and immediately packed into jars.

Cure analysis. A 6 mm slice from the middle of the size 2A (25-32 mm) cucumber was removed. Curing was determined visually, assuming the seed cavity comprised 30% of the cucumber slice surface area (Figure 2.1). Percent cure was evaluated and recorded from 15 slices per jar. Jars were analyzed in duplicate. An overall average was recorded for the replicate jars.

Firmness measurements. Firmness measurements were taken using a TA.XT2 Texture Analyzer (Texture Technologies Corp, Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) equipped with a 3 mm diameter punch. Pickle slices (6 mm thick) were placed onto a base plate containing a 3.1 mm hole. The punch moved at a test speed of 0.80 mm/sec to a rupture test distance 4 mm into the cucumber slices. Data were collected and analyzed using *Texture Expert* software (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). The force required to puncture the cucumber mesocarp was recorded and expressed in Newtons (N). Firmness measurements

were done on 15 slices from each of two jars from each treatment at each sampling time (Maruvada and McFeeters, 2009; Thompson et al., 1982).

Preparation of reference and control pasteurized dill pickles for sensory analysis. Fresh size 2A (25-32 mm diameter) pickling cucumbers were obtained from a local processer washed, and sliced to 6 mm thickness using a food processor (Hobart, Model PF150, Troy, OH) and packed into 24 oz (720 ml) jars. The jars were filled with cucumber slices and appropriate cover brine solution to give a 60:40 (426 g of cucumbers: 284 g of brine) pack out ratio. A commercial dill pickle cover brine formula was used to make the reference and control pickle product. Cover brine was prepared with final product equilibrated concentrations of 2.4% NaCl, 0.24% anhydrous $CaCl_2$, 0.9% acetic acid, 0.1% sodium benzoate, high fructose corn syrup (Isomerose^{TM}), FD&C No.5 yellow food coloring, Polish dill spice concentrate and antifoam C mixture. The jars containing pickles were pasteurized at 74ºC (center of the jar) for 15 minutes in a steam jacketed kettle, then cooled to room temperature. After pasteurization pickle jars were stored at 25ºC for 30 days to allow for equilibration of components. After 30 days, half of the jars were stored at 25ºC (control pickles) and half in refrigerated storage at 4ºC (reference pickles) for the duration of the experiment. The reference pickles stored at 4ºC would undergo minimal changes. Control pickles stored at 25ºC simulated conditions during storage in a warehouse. Pasteurized dill pickles prepared from the cucumbers stored in acidified, bulk storage were then compared to the reference and control samples.

Preparation of pasteurized dill pickles from acidified, bulk stored cucumbers. Fresh cucumbers from the same lot were acidified and bulk stored at 25ºC from 30 to 120

days prior to conversion to pasteurized dill pickles. Fresh cucumbers were packed in 1 gallon jars (3700 ml) with a 55:45 cucumber: brine ratio (2035 g cucumbers:1665 g cover brine plus 20 ml of 320 mg/ml sodium benzoate solution). Concentrations of acetic acid, NaCl, $CaCl_2$, calcium fumarate trihydrate ($CaC_4H_2O_4 \bullet 3H_2O$) in cover solutions for each experimental treatment along with the concentrations of these brine components after equilibration are given in Table 2.2.

To make pasteurized dill pickles from stored, acidified cucumbers the concentrations of acetic acid, salt, sodium benzoate, calcium chloride, and sugars in the stored cucumbers were adjusted so that concentrations of these components in the pasteurized dill pickles would be the same as pasteurized pickles made directly from fresh cucumbers. Cucumbers stored in 3.5% acetic acid were equilibrated with a sufficient volume of water to reduce the acetic acid level to 1.5% prior to processing. Acetic acid is removed from 3.5% acetic acid cucumbers when bulk stored cucumbers (1,665 g) are stored in tap water (2,114 g) at 25ºC for 48 hour (42.9:57.1 packout ratio) lowering the acid concentration to 1.5% acetic acid.

The washed cucumbers are sliced and packed in a jar and covered with 40% by weight of a brine solution containing no acetic acid. The pasteurized pickles will contain 0.9% acetic acid, the same acetic acid concentration as in pickles made directly from fresh cucumbers. The equilibrated concentrations of other brine components must be similarly considered so they will also be the same in the dill pickle product made from stored cucumbers as the controls. Since sugar present in the fresh cucumbers will be removed during bulk storage and in any subsequent washing steps, additional high fructose corn syrup was added to the cover solution of the 24 oz jars to compensate for the sugar loss.

Cucumbers stored in 1.5% acetic acid or in 1.3% acetic acid plus calcium fumarate trihydrate did not require removal of acid or other components prior to conversion to pasteurized dill pickles. Packing 60% by weight cucumbers (426 g) and 40% cover solution (284 g) in 24 oz jars resulted in an equilibrated acetic acid concentration of 0.9%, the intended concentration for the pasteurized dill pickles. Likewise, calcium chloride and sodium benzoate were not added to the cover solution for dill pickle products because the concentrations of these components in the stored cucumbers were such that after equilibration with the cover solution they were at the intended concentration in the product. On the other hand, high fructose corn syrup was added to the cover solution so that the equilibrated sugar concentrations would be the same as dill pickles prepared directly from fresh cucumbers.

All jars were hermetically sealed with metal lids and pasteurized at 74ºC at the cold point in the jar for 15 minutes. Following pasteurization jars were cooled to room temperature and then stored at 25ºC and evaluated using a trained sensory panel. Sensory evaluation was preformed at predetermined time periods.

Sensory evaluation. Students and staff (n=9) from North Carolina State University, (Raleigh, North Carolina) Department of Food, Bioprocessing and Nutrition Sciences, were selected as panelists. Panelists were selected based upon availability and the individual panelist's ability to distinguish basic tastes.

Sensory Training. Approval of the North Carolina State University (Raleigh, North Carolina) Institutional Review Board was obtained for the use of human subjects in this study. Panelists were trained in descriptive sensory analysis using the Spectrum^{TM} method

(Meilgaard et al., 1991).

Panelists were for initially trained (40 hours) to use a 15-point scale to assess the intensity of the basic tastes sour, salty, sweet, bitter and then trained to rate a series of attributes for pasteurized dill pickles including cure, appearance difference, firmness, crunchiness, sweet, sour, salty, bitter, astringency, dill intensity, vinegar flavor, oxidized flavor and "other". During the training panelists were individually evaluated to determine the overall panel mean and to assure that all panelists were able to scale the attributes of interest.

Aqueous solutions of citric acid, sucrose, sodium chloride, caffeine, alum, Polish dill flavor and acetic acid were used as reference samples for sour, sweet, salty, bitter, astringency, dill intensity and vinegar flavor, respectively. Most aqueous solution concentrations were based on reference samples used in Spectrum^{TM} method (Meilgaard, et al., 1991). Concentrations of alum and vinegar flavor were based upon previous research on dill pickle sensory analysis (da Conceicao Neta et al., 2007). Polish dill flavor reference sample concentrations were determined during panel training. Concentrations of 10 µL/L, 75 µL/L, 200 µL/L, and 500 µL/L Polish dill flavor were scaled at 2, 5, 10 and 15, respectively, on the intensity scale. Panelists estimated cure as the percent of the surface area of the pickle slice with cured appearance. Panelists were instructed to consider the seed cavity of the pickle slice to be 30% of the surface area. Based on this assumption panelists were asked to determine the overall cured area of pickle slices, using a chart that showed pickle slice curing at 0%, 25%, 50%, 75%, 90% and 100% as a guide (Figure 2.1). Differences in appearance from reference was based on the overall color and appearance of the sample and was not

based upon cure. Panelists were trained to rate firmness based upon reference samples suggested by the Spectrum™ method. Firmness was described as the force required to compress the sample upon first bite with anchors of 0 for soft to 15 for firm. Crunchiness was described as the noise level produced by the food with anchors of 0 for not crunchy to 15 for very crunchy. Panelists were trained on various foods based upon recommendations of Meilgaard et al., 1991.

Following training, (Meligaard et al., 1991) panelists met as a group to assign a consensus score for each attribute to the pasteurized dill pickles prepared directly from fresh cucumbers and stored at 4ºC that was provided as a reference sample for calibration of panelist ratings at each sensory analysis session during the study.

Sensory analysis. Panelists were presented samples in 2 oz plastic cups identified with randomized 3 digit numbers. All samples were at room temperature along with room temperature water and low sodium saltine crackers to cleanse the palate between samples. Panelists were presented samples in a randomized order to avoid bias based upon the order of sample presentation. Three to five pickle slices were in each sample cup. Panelists evaluated a group of 5 to 7 samples per session. Included in the sample set were the reference dill pickles stored at 4ºC and control dill pickles stored at 25ºC. They were also given a cup identified as the reference sample to calibrate scoring of taste attributes on the 15-point scale. Individuals were instructed to first taste the reference sample, cleanse their palate followed by tasting a sample. Panelists were asked to take as much time as needed and to taste their samples slowly to avoid tasting fatigue. They were given the option to swallow or expectorate their samples. Triplicate evaluations of each sample were done at

each sampling time during the study. Sensory analysis of a series of experiments was done according to the experimental designs shown in Figures (2.2-2.4). Sensory analysis was conducted on pickle samples made from cucumbers that were acidified and bulk stored in 3.5% acetic acid (Table 2.2) to determine the effects of 30, 60, 90 or 120 days in acidified, bulk storage, followed by pasteurization and 30 days of final product storage (Figure 2.2). The effect of storage time at 25ºC for pasteurized dill pickles prepared from acidified, bulk stored cucumbers was evaluated by placing cucumbers in acidified bulk storage for 60 days, followed by pasteurization and then doing sensory evaluations after 60, 120, 180 and 240 days storage (Figure 2.3). Sensory analysis of pickle products made from different acidification/blanch treatments (Table 2.2) was completed (Figure 2.4) after the acidified cucumbers were stored for 60 days, followed by conversion to pasteurized dill pickles which were evaluated by the descriptive sensory panel after 30, 60, 90 and 120 days storage at 25ºC in 24 oz glass jars.

Statistical analysis of sensory data. Firmness measurements were analyzed using a general linear model (PROC GLM) using SAS (version 9.1.3 SAS® software, SAS Institute, Cary, NC).

Pickle products were evaluated in a randomized complete block design with three sensory replications. Panel means were evaluated using an ANOVA using SAS (version 9.1.3 SAS® software, SAS Institute, Cary, NC).

2.3.2 Analysis of volatile components in acidified, bulk stored dill pickles

Two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-

TOFMS) analysis of volatile components from pasteurized dill pickles made from acidified, bulk stored cucumbers was preformed based upon a procedure developed by Johanningsmier and McFeeters (unpublished). Dill pickles from the same jars used for sensory analysis were blended using a Waring blender (2 min) to a uniform slurry. A 25 mL sample of the blended slurry was stored at -80ºC until analysis. Frozen samples were thawed, diluted (1 part sample: 9 parts deionized water) plus 4 μL 3 N H_2SO_4. A 1 mL sample of the diluted slurry was put in a 10 mL screw-cap headspace vial (Microliter Analytical Supplies, Inc., Suwanee, GA). A composite sample containing 1 g from each dill pickle slurry that was to be analyzed was made, and then diluted in the same manner. Sodium chloride (0.40 g) was added to each vial to "salt out" volatile compounds so as to increase their concentration in the sample vial headspace. The vials were placed in the refrigerated (2ºC) tray on the CombiPal autosampler (LEAP Technologies, Carrboro, NC) in randomized order calculated with PROC PLAN, version 9.1.3 SAS® software, SAS Institute, Cary, NC. A sample vial was moved to the heater/shaker on the autosampler and heated with shaking for 30 min at 40ºC with 100 rpm agitation (5 s on and 2 s off). Headspace volatiles were collected by insertion of a 1 cm 50/30 μm DVB/CarboxenTM/PDMS StableFlex^{TM} SPME fiber (Supelco, Bellefonte, PA) into the sample vial. Volatile compounds were desorbed from the SPME fiber after the fiber was inserted into the GC inlet at 250ºC for 15 min. Blank samples (996 deionized water, 4 μL 3 N H_2SO_4, and 0.40 g NaCl) were run between each dill pickle sample to reduce carryover of compounds on the SPME fiber.

Comprehensive, two-dimensional gas chromatography/mass spectrometry. A LECO® Pegasus III® time-of-flight mass spectrometer (Model# 614-100-700, Leco

Corporation, St. Joseph, MI) was connected to an Agilent GC (Model # 6890N, Agilent Technologies, Santa Clara, California) fitted with a secondary oven and cryogenic modulator. The column set used for two-dimensional GC was a SolGel-Wax^{TM} 30 m x 0.25 mm ID x 0.25 μm film thickness (SGE, Austin, Texas) polyethylene glycol 1st dimension column and an RTX 17-01 1.0 m x 0.1 mm x 0.1 μm (Restek, Bellefonte, Pennsylvania) 14% cyanopropylphenyl-86%dimethyl polysiloxane 2nd dimension column. Both columns were conditioned according to manufacturer's recommendations prior to use. The GC inlet liner was a 0.75 mm ID Siltek deactivated SPME liner (Restek, Bellefonte, Pennsylvania), set at 250ºC, and operated in pulsed splitless mode at a pulse pressure of 37 psi for 1 min. The split vent was opened 2 min after injection. The GC was operated with 1.3 mL/min helium carrier gas flow in constant flow mode. The initial oven temperature was held at 40ºC for 2 min, followed by a linear temperature increase at 5ºC/min until 140ºC, and then the temperature was increased at 10ºC/min to 250ºC and then held at 250ºC for 3 min. The secondary oven that contained the second dimension column was maintained at a temperature 10ºC higher than the main oven throughout the chromatographic run. The modulator offset was +30ºC with a 1.5 sec 2nd dimension separation time and a 0.3 sec hot pulse. Liquid nitrogen cooled nitrogen gas at 18 psi was used for cold pulses, and compressed air at 35 psi was used for hot pulses. The time-of-flight mass spectrometry detector was operated with an ion source temperature of 200ºC and -70eV. The voltage for the detector was set at 1500V. A mass range of 25-500 Daltons was scanned at a rate of 200 spectra per sec with no solvent delay employed.

Data processing and analysis. Data processing was done using ChromaTOF®

software (version 3.25, Leco Corporation, St. Joseph, Michigan). Data were processed to detect and quantify peaks based on unique masses determined by the internal deconvolution algorithm. The data processing parameters are shown in Table 2.3. The NIST/EPA/NIH Mass Spectra Library (National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, 2005) was used as the spectral library for tentative identification of peaks by ChromaTOF®. Chemical names were assigned to the identified peaks that had a minimum mass spectral similarity of $\geq$800. A unique mass (U) was assigned to each peak by the ChromaTOF® deconvolution algorithm and was then used for area calculations. A composite sample was produced by mixing equal amounts of all samples in an experiment so the composite would contain all components that were present in any of the samples. The composite sample was chromatographed to create a standard reference peak table that should then contain all components that are present in any of the samples. Through use of the compare function in the ChromaTOF® data processing method, peak tables for each sample were standardized against the composite peak table such that the area of each detected peak would be based upon the same unique mass in all samples so that relative peak areas among samples could be compared. In addition, each unidentified peak was assigned the same number in all samples where it was detected. This assignment allowed the peak areas of detected components to be compared among samples even though the component was not identified. Peak tables from all samples were exported to Excel® for analysis. Blank peak area cells were outputted for peaks in the reference sample that were not found in a particular dill pickle sample. The blanks cells were filled with a randomly selected number from 1 to 50 to reflect a possible response below detection limits for undetected components. Log

transformation of peak areas was performed on peak area values. An analysis of variance (ANOVA) of log peak areas by quantification name was conducted to detect differences between treatments (version 9.1.3 SAS® software, SAS Institute, Cary, NC). A significance level of $p<0.05$ was used after adjustment of p-values to control the false discovery rate using the method of Benjamini and Hochberg (1995). Principal component analysis (PCA) were constructed using SAS (version 9.1.3 SAS® software, SAS Institute, Cary NC) PROC PRINCOMP to characterize differences among pickle products based upon volatile component profiles.

2.4 Results and Discussion

2.4.1 Evaluation of cucumbers after acidified, bulk storage

Regardless of the acetic acid concentration or storage temperature, the firmness of cucumbers within a treatment stored for 120 days did not change ($p<0.05$) (Figure 2.5). However, cucumbers stored in 1.5% acetic acid were less firm ($p<0.05$) than cucumbers held in 3.5% acetic acid (Figure 2.5). The average firmness of cucumbers stored in 3.5% acid was 12.8 ± 0.1 N compared to a firmness of 10.9 ± 0.1 N for cucumbers stored in 1.5% acetic acid. Despite having somewhat lower firmness than cucumbers stored in 3.5% acetic acid, even at 45ºC there was not a statistically significant loss of firmness of the cucumbers stored for 120 days (Figure 2.6). Blanching cucumbers before brining did not affect texture retention during storage when held at 18ºC or 30ºC. Cucumbers that were blanched and held at 45ºC were significantly less firm from all other treatments after 30 and 120 days in storage (Figure 2.6). The average firmness for blanched cucumbers held at 45ºC was 8.5 ± 0.6 N compared to 10.4 ± 0.8 N for cucumbers that were not blanched and stored at 45ºC.

The extent of curing in the stored cucumbers was evaluated at the same times as tissue firmness. Cucumber tissue curing began in the seed cavity and over time extended into the mesocarp tissue. Higher temperatures accelerated development of the cured appearance in the acidified, stored cucumbers. Cucumbers stored in 3.5% acetic acid at 18ºC maintained a level of cure of 10% or less for 120 days (Figure 2.7), which was the lowest cure among the treatments tested. Cucumbers stored in 1.5% acetic acid at 18ºC were not significantly different in cure compared to 3.5% acetic acid for 60 days, but after 60 days cure increased to about 20%. All other treatments had significantly greater cured appearance ($p<0.05$) than the

3.5% acetic acid, 18ºC treatment from 30 days onward (Figure 2.7). In another lot of cucumbers (Figure 2.8), cucumbers put in bulk storage in 1.5% acetic acid also cured to a level of 20% after 120 storage at 18ºC. When cucumbers were blanched at 70ºC for 2 min prior to acidified, bulk storage the degree of cure was the same as non-blanched cucumbers except for an anomalously high data point after 30 days storage. At 30ºC, however, blanching substantially reduced curing ($p<0.05$) so that by 120 days cure was 40% in the blanched cucumbers, but 80% in the non-blanched cucumbers. In 45ºC storage blanching also reduced cure, but the relative effect was not as great as was observed for 30ºC storage.

Loss of firmness and cure as a result of high temperature storage (>30ºC) in shelf stable pasteurized pickles was reported by Nicholas and Pflug (1960). Blanching treatments could retard tissue curing by deactivating enzymes that are present in the fruit and alter cucumber cell walls (Fellers and Pflug, 1968; Maruvada and McFeeters, 2009). Storage temperature has been reported to have the greatest effect on retention of firmness in brined cucumbers, particularly at temperatures above 26.6°C (Fellers and Pflug, 1968; McFeeters and Fleming, 1989; McFeeters and Fleming, 1990; Nicholas and Pflug, 1960). Low storage temperature is the factor that has the greatest effect in slowing the development of a cured appearance in stored, acidified cucumbers. Higher equilibrated acetic acid concentrations also had an inhibitory effect on tissue curing.

2.4.2 Evaluation of pasteurized dill pickles prepared from cucumbers stored in 3.5% acetic acid

Pasteurized dill pickles were prepared from bulk stored cucumbers and compared to

control dill pickles made with fresh cucumbers from the same lot. The concentrations of sugar, acetic acid, anhydrous calcium chloride, sodium benzoate and salt were adjusted to be the same as in the pickles made directly from fresh cucumbers by either increasing the high fructose corn syrup or decreasing the amount of vinegar, calcium chloride, sodium benzoate or salt in the cover brine used to make the dill pickles. In the case of storage in 3.5% acetic acid, the cucumbers were equilibrated with a sufficient volume of water to reduce the acid to the correct level in the final product if no acid was put in the cover solution.

The effect of time in acidified bulk storage on pasteurized dill pickles made from the stored cucumbers was determined by storing cucumbers in 3.5% acetic acid for 30, 60, 90 and 120 days. The cucumbers were then processed into pasteurized dill pickles and held at 25ºC for 30 days to allow equilibration of the product. The dill pickles were then evaluated for the degree of cure (Figure 2.9). Sixty days after the cucumbers were initially put in storage the average cure in the pickles was 60%. As the time in bulk storage increased to 120 days the degree of cure in the pickles made from the stored cucumbers increased to about 95%. In contrast, the degree of cure in the pickles made directly from fresh cucumbers and stored for equivalent periods of time was only about 35% and finally increased to 45% 150 days after the product was pasteurized. This was the case whether pickles were kept at 25ºC or refrigerated at 4ºC. Considering the curing pattern of cucumbers stored in 3.5% acetic acid at 30ºC before they were pasteurized (Figure 2.7), the results indicated there was approximately a 50% increase in the cure when stored cucumbers are converted into pasteurized dill pickle products. The conclusion from the results in Figure 2.9 is that pasteurized pickle products made from bulk stored cucumbers will have a high degree of cure

compared to pickles made directly from fresh cucumbers

In addition to the differences in cure, most other sensory attributes were significantly different in pasteurized dill pickles prepared from cucumbers stored in 3.5% acetic acid compared to pickles prepared directly from fresh cucumbers (Table 2.4). Firmness, saltiness, and dill flavor while significantly different, differed by less than 1 point on a 15-point scale. The appearance of the pickles from bulk stored cucumbers was different from pickles made from fresh cucumbers even though panelists were trained to ignore cure in making this evaluation. Pickles made from stored cucumbers were described as gray/green in appearance while reference pickle samples made from fresh cucumbers had a yellow hue. The "other" category was intended to capture flavor differences compared to the reference sample that was provided at each tasting session. The reference sample made directly from fresh cucumbers and stored at 4ºC was included in the unknown samples and, as expected, no "other" flavors were detected. Likewise, a control sample made from fresh cucumbers and stored at 25ºC was not perceived as having "other" flavors. However, pickles made from bulk stored cucumbers had "other" flavors that panelists described as chemical, chlorine, pool, and hay. These results show that when cucumbers are stored in 3.5% acetic acid and dill pickles are prepared from those cucumbers, they have sensory differences compared to pickles made from fresh cucumbers. Other than cured appearance the differences between pickles made from the bulk stored cucumbers and pickles made directly from fresh cucumbers did not change as the time in bulk storage increased. Sensory differences after 150 days of bulk storage were very similar to the differences perceived after only 30 days in bulk storage.

Effect of storage time on the characteristics of pasteurized dill pickles made from cucumbers bulk stored in acidified solutions for 60 days.

Pasteurized dill pickles were prepared from acidified cucumbers that were stored in 3.5% acetic acid brines for 60 days at 25ºC prior to pasteurization. The pasteurized pickles were then evaluated from 60 to 240 days after they were made into pasteurized, shelf-stable dill pickles. Dill pickles prepared directly from fresh cucumbers of the same lot and stored at 4ºC and at 25ºC served as reference and storage control samples for comparison with pickles prepared from the bulk stored cucumbers. A decrease in firmness and an increase in cure were the only significant differences ($p<0.05$) when comparing the effect of control pickles stored at 25ºC and pasteurized dill pickles made from acidified bulk stored product. Reference dill pickle samples held at 4ºC maintained a cured appearance in the 30% to 40% range from 120 days after pasteurization, when they were initially evaluated, until the storage experiment was concluded at 300 days after pasteurization (Figure 2.10). Control cucumbers stored at 25°C had a cured appearance the same as the 4ºC pickles for 180 days, but then cured appearance increased until it reached 70% after 300 days of storage. Pickles made from cucumbers that were in acidified storage for 60 days prior to pasteurization were 70% cured during at the initial sensory evaluation 120 days after the cucumbers were put into bulk storage. The pickles had a completely cured appearance after 300 days (Figure 2.10). Therefore, curing continues to occur in acidified, bulk stored cucumbers after being processed into pickles. Firmness of pasteurized dill pickles made from acidified, bulk stored cucumbers decreased from 8 N at 120 days of storage to 6 N after 300 days of storage (Figures 2.11). In contrast the firmness of the control pickles stored at 25ºC maintained an

instrumental firmness near 10 N, similar to the 4ºC reference samples, for 300 days.

Pasteurized pickles made from acidified, bulk stored cucumbers stored at 25ºC had an "other" flavor with a mean intensity of 3.5 ± 0.6. The "other" flavor in pasteurized pickles made from acidified, bulk stored cucumbers was not present in pickles made directly from fresh cucumbers whether the samples were stored at 4ºC or at 25ºC. The panelists used terms such as chlorine, pool, dirt, and barny to describe this flavor that was not perceived in pickles prepared directly from fresh cucumbers. The perceived intensity of the "other" flavor did not increase from 120 to 300 days. The "other" attribute was higher in intensity than the "other" attribute found in the pickles made from acidified, bulk stored cucumbers at various time points. Panelists had an average oxidized flavor for control pickles of 0.27 ± 0.1 after 180 days and an average of 0.83 ± 0.2 after 300 days. The oxidized intensity was not significantly different.

2.4.3 Evaluation of pasteurized dill pickles prepared from cucumbers stored in acetic acid brines of varying concentrations

It would be beneficial if cucumbers could be bulk stored in acetic acid at a concentration substantially lower than 3.5%. The 1.5% acetic acid level was investigated because the stored cucumbers could be made into pasteurized pickles without a washing step to remove acetic acid. The amounts of the brine components in cucumbers stored in 1.5% acetic acid were selected so that when put into a pasteurized product with a 60:40 cucumber:brine ratio in the jars, the stored cucumbers could be used without any adjustment. This would reduce the cost of vinegar, reduce the organic waste generated, and reduce the

time and labor required to make consumer products from bulk stored cucumbers. Since initial experiments showed that development of cured appearance was more rapid when cucumbers were stored in the lower concentration of acetic acid, two variations of brining cucumbers with 1.5% acetic acid were investigated. One lot of cucumbers were blanched at 70ºC until the center of the cucumbers were heated to 70ºC. In another treatment cucumbers were bulk stored in 1.3% acetic acid and a small portion of acetic acid was replaced by addition of 16mM fumaric acid. All cucumbers were stored for 60 days at 25ºC prior to conversion to pasteurized dill pickles.

Dill pickles made from stored cucumbers at the first evaluation point 90 days after storage had higher degrees of cure than reference and control pickles made directly from fresh cucumbers (Figure 2.12). Non-blanched cucumbers stored in 1.5% acetic were 60% cured at that point and then continued to increase in cure until it approached 90% 180 days after the cucumbers were stored. Dill pickles prepared from the other lower acid storage conditions as well as the 3.5% storage all were near 50% cured 180 days after initial storage. The cure of control pickles stored at 25ºC increased slowly from a 35% cure after 90 days until they were also about 50% cured 180 days after the fresh cucumbers were pasteurized. Reference dill pickles stored at 4ºC throughout the experiment maintained cured appearance at 35%.

Pickles made from cucumbers that were acidified and bulk stored in 1.5% acetic acid had significantly lower crunchiness scores ($p<0.05$) than all other treatments (Table 2.5). When fumaric acid was added to the cover solution of cucumbers stored in 1.3% acetic acid, the pickles maintained significantly higher crunchiness score, which was statistically not

different ($p<0.05$) from that of control pasteurized dill pickles made from fresh cucumbers and stored at 25ºC. Cucumbers that were blanched prior to storage in 1.5% acid or non-blanched cucumbers stored in 3.5% acetic acid maintained a level of crunchiness equal to that of pasteurized fresh cucumbers that were stored at 4ºC after pasteurization. Sensory scores for firmness were in a narrow range of 7.5 to 8.1 for all treatments. Control dill pickles stored at 25ºC and the pickles made from non-blanched cucumbers stored in 1.5% acetic acid were significantly less firm than the reference pickles stored at 4ºC, those made from blanched cucumbers stored in 1.5% acetic acid, and pickles made from cucumbers stored in 3.5% acetic acid. The dill pickles made from the treatment in which 16 mM fumaric acid was added to the storage brine were intermediate in firmness and not significantly different from any of the other treatments. Bell et al. (1972) studied the effects of organic acids on cucumber tissue firmness and found that cucumbers stored in acetic acid were firmer in texture than those stored in alternative organic acids. Blanching of cucumber fruit helps to inhibit softening of fruit associated with enzymatic activity (McFeeters, et al., 1985).

The intensity of dill flavor also was scored in a narrow range of 3.9 to 4.5 on the 15-point scale (Table 2.5). The non-blanched stored cucumber treatments were perceived to have a higher intensity of dill flavor than pickles made directly from fresh cucumbers. There is not an obvious explanation for this result. All pasteurized dill pickles had an "other" flavor that was not present in the reference pickle product. Control pickle samples had an increasing oxidized flavor over time. The "other" attribute was described as chlorine, chemical and metallic for all the bulk stored samples. The "other" attribute in cucumbers

blanched prior to bulk storage was described as grassy or cooked. While a descriptive panel does not provide information on a like/dislike scale, the fact that there was a consistently perceived difference in flavor in pickles made from the bulk stored cucumbers compared to pickles prepared according to current commercial practice and the selection of terms to describe this difference, suggest that a goal of future research might be to avoid this flavor difference.

2.4.4 Use of two-dimensional gas chromatograph/mass spectrometry volatile analysis to analyze the effect of different bulk storage brines on dill pickle products made from bulk stored cucumbers

Pickles made from fresh cucumbers and pickles made from cucumbers that were acidified and bulk stored in varying brines for 60 days and then held as pasteurized pickled product for 120 days were analyzed in triplicate using GCxGC-TOFMS. Pickles made from the acidified, bulk stored cucumbers and reference pickles made from fresh cucumbers and stored at 4ºC were compared to control pickles made from fresh cucumbers and stored at 25ºC. Since control pickles made from fresh cucumbers represent what consumers are currently purchasing, all comparisons were made relative to it. Samples analyzed had about 650 detectable peaks. Control and reference pickles made from fresh cucumbers were compared to show the effects of storage temperature. Control and reference pickles had 10 components that were determined to be significantly different ($p<0.05$). Di-2-propenyl trisulfide and diallyl disulphide were found to be in higher concentration in the reference pickles compared to the control pickles (Table 2.6). Furfural and unknown 127 (Table 2.6)

were found to be lower in reference samples in comparison to control pickles made from fresh cucumbers and stored at 25ºC. Further investigation into component concentration, component threshold and whether a component is flavor active need to be considered in order to differentiate differences in flavor based upon the significantly different ($p<0.05$) volatile components.

To determine the effects of making pickles from different acidified, bulk storage treatments, control pickles made from fresh cucumbers and stored at 25ºC were compared to pickles made from bulk storage treatments and stored at 25ºC. Treatments were different from control pickles made from cucumbers by 15 to 18 components for each of the four bulk storage treatments evaluated. Out of the 24 components that were significantly different between at least one treatment pair in this experiment, 10 components were found to be significantly different ($p<0.05$) from the control pickle sample (Table 2.7) for dill pickles made from all four of the bulk storage treatments. The magnitude of difference was greatest for diallyl disulphide and di-2-propenyl trisuflide which were present in much higher concentrations in dill pickles made from bulk stored cucumbers (Table 2.7).

To further characterize differences among pickles made from bulk stored cucumbers in comparison to control and reference pickles made from fresh cucumbers the 24 components that were found to be significantly different ($p<0.05$) were used to construct a principal component biplot (Figure 2.13). The 24 components used to construct the biplot differentiated the different processing and storage treatments reasonably well (Figure 2.13). A majority of the variability, 54.3%, was characterized in PC1. Pickles made from fresh cucumbers and stored at 25ºC or 4ºC (control and reference samples respectively) loaded

closely on PC1. Pickles made from cucumbers that were acidified, and bulk stored in brines containing 3.5% acetic acid, 1.5% acetic acid, 1.3% acetic acid and 0.2% fumarate and 1.5% acetic acid and blanched cucumbers loaded closely on PC1 (Figure 2.13). Pickles made from bulk stored cucumbers were all described by the sensory panel as having an "other" flavor that was characterized as chlorine, pool, metallic, and barny. PC2 explained 15.3% of the variability (Figure 2.13). PC2 explained more of the variability between the control pickles made from fresh cucumbers stored at 25ºC and reference pickles made from fresh cucumbers and stored at 4ºC (Figure 2.13). Pickles made from cucumbers stored in 1.5% acetic acid and blanched were described by the sensory panel as having a cooked, green, or fermented flavor, which were not used as descriptors for other pickle samples and loaded the furthest from the other treatments in both PC1 and PC2 (Figure 2.13). Pickles made from blanched cucumbers had higher concentrations of 2,6,6-trimethyl-, (ñ)-bicyclo[3.1.1]hept-2-ene and 3,5,5-trimethyl-cyclohexanone and were found to be significantly different in comparison to control pickles made from fresh cucumbers (Table 2.7).

Conclusion 2.5

Storing acidified, bulk stored cucumbers at 18ºC helped maintain a fresh opaque, non-cured appearance and firm texture. Cucumbers stored with 3.5% acetic acid maintained the fresh opaque, non-cured appearance of stored cucumbers better than storage in 1.5% acetic acid. Pasteurized dill pickles made from acidified, bulk stored cucumbers maintained a firm texture, but increased in the degree of cured appearance by 40% within 30 days after pasteurization. Sour, sweet, bitter and vinegar intensities were similar for dill pickles prepared from either bulk stored or fresh cucumbers. Firmness, saltiness, and dill flavor while significantly different, differed by less than 1 point on a 15-point scale. However, pickles made from stored cucumbers had an "other" flavor that was described as chlorine, pool, barny and metallic and was not detected in product made from the fresh cucumbers. The intensity of this flavor was similar regardless of the brine composition or storage time of the cucumbers prior to pasteurization. GCxGC-TOFMS headspace volatile analysis of dill pickles prepared from bulk stored cucumbers and dill pickles made from fresh cucumbers had 24 components that were determined to be significantly different ($p<0.05$) between at least one treatment pair. Individual volatile components do not provide a clear differentiation among dill pickles made from bulk stored cucumbers and dill pickles made from fresh cucumbers. Principal component analysis (PCA) using the 24 volatile components that were determined to be significantly different was able to differentiate most treatments.

REFERENCES

Bell, T.A.; Turney, L.J.; Etchells, J.L. Influences of different organic acids on the firmness of fresh-pack pickles. *J. Food Sci.* 1972, 37, 446-449.

Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate—apractical and powerful approach to multiple testing. *J. R. Stat.: Series B-Methadolog.* 1995, 57, 289-300.

Breidt, F. Safety of minimally processed, acidified, and fermented vegetable products. In *Microbiology of Fruits and Vegetables*; Sapers, G.M.; Gorny, J.R.; Yousef, A.E., Eds.; CRC Press, Inc: Boca Raton, Florida, 2006; pp. 313-335.

Breidt, F; McFeeters, R.F.; Díaz-Muñiz, I. Fermented vegetables. In *Food Microbiology: Fundamentals and Frontiers*; Doyle, M.P.; Beuchat, L.R., Eds.; ASM Press: Washington, D.C., 2007; pp 783-793.

Corey, K.A.; Pharr, D.M.; Fleming, H.P. Pressure changes in oxygen-exchanged, brined cucumbers. *J. Am. Soc. Hort. Sci.* 1983, 108, 61-65.

da Conceicao Neta, E.R.; Johanningsmeier, S.D.; Drake, M.A.; McFeeters, R.F. A chemical basis for sour taste perception of acid solutions and fresh-pack dill pickles. *J. Food Sci.* 2007, 76, 352-359.

Fellers, P.J.; Pflug, I.J. Loss of whiteness from fresh cucumber pickles. *Food Technol.* 1968, 22, 105-108.

Fleming, H.P.; Humphries, E.G.; Fasina, O.O.; McFeeters, R.F.; Thompson, R.L.; Breidt, F. Bag-in-box technology: pilot system for process-ready, fermented cucumbers. *Pickle Pak. Sci.* 2002, 8, 1-8.

Howard, L.R. *Factors Affecting Cell Wall Components of Mesocarp Tissue in Fresh Pack Cucumber pickles*. PhD Dissertation; University of Arkansas, Fayetteville, Arkansas. UMI: Ann Arbor, Michigan; 1989.

Hutkins, R.W. Fermented vegetables. In *Microbiology and Technology of Fermented Foods.* Hutkins, R.W. Eds.; Blackwell Publishing: Ames, Iowa, 2006; pp. 246-249.

Maruvada, R.; McFeeters, R.F. Evaluation of enzymatic and non-enzymatic softening in low salt cucumber fermentation. *Int. J. Food Sci. Technol.* 2009, 44, 1108-1117.

McFeeters, R.F.; Lovdal, J.A. Sugar composition of cucumber cell walls during fruit development. *J. Food Sci.* 1987, 54, 996-1001.

McFeeters, R.F.; Fleming, H.P.; Fasina, O.O.; Papageorge, L.M. BAG-IN-BOX TECHNOLOGY: Preservation of brined vegetables without fermentation. *Pickle Pak. Sci.* 2002, VII, 34-37.

McFeeters, R.F.; Fleming, H.P.; Thompson, R.L. Pectinesterase activity, pectin methylation and texture changes during storage of blanched cucumber slices. *J. Food Sci.* 1985, 50, 201-205.

McFeeters, R.F.; Fleming, H.P. Inhibition of cucumber tissue softening in acid brines by multivalent cations: Inadequacy of the pectin "egg box" model to explain textural effects. *J. Agric. Food. Chem.* 1989, 37, 1053-1059.

McFeeters, R.F.; Fleming, H.P. Effect of calcium ions on the thermodynamics of cucumber tissue softening. *J. Food Sci.* 1990, 55, 446-449.

McFeeters, R.F.; Fleming H.P. pH effect on calcium inhibition of softening of cucumber mesocarp tissue. *J. Food Sci*. 1990, 56, 730-732, 735.

Meilgaard, M.C.; Civille, G.V.; Carr, B.T. *Sensory Evaluation Techniques,* 2nd Edition; CRC Press: Boca Raton, Florida, 1991.

Mok, Y.N. *Curing of Cucumber Pickle Mesocarp Tissue Affected by Alterations in Protein and Cell Walls*. PhD Dissertation; University of Arkansas, Fayetteville, AR. AR. UMI: Ann Arbor, Michigan; 1992.

Nicholas, R.C. Pflug, I.J. Effect of high temperature storage on the quality of fresh cucumber pickles. *Glass Pack.* 1960, 39, 35.

Reiter, W.D. *Arabidopsis thaliana* as a model system to study synthesis, structure and function of the plant cell wall. *Plant Physiol. Biochem.* 1998, 36, 1-2, 167-176.

Reiter, W.D.; Chapple, C.; Sommerville, C.R. Mutants of *Arabidopsis thaliana* with altered cell wall polysaccharide composition. *Plant J.* 1997, 12, 335-345.

Thompson, R.L.; Fleming, H.P.; Hamann, D.D.; Monroe, R.J. Method for determination of firmness in cucumbers slices. *J. Text. Stud.* 1982, 13, 311-324.

United States Code of Federal Regulations. 2006. *Acidified Foods*. 21 CFR Part 114. U.S. Department of Health and Human Services, Washington, D.C.

USDA. U.S. Standards for grades of pickles. Fed. Reg 31:102231-10305. Office of Federal Registry. National Archives and Records Service, General Services Admin. Washington, D.C.; 1966.

Zablackis, E.; Huang, J.; Muller, B.; Darvill, A.G.; Albersheim, P. Characterization of the cell-wall polysaccharides of *Arabidopsis thaliana* leaves. *Plant Physiol.* 1995, 107, 1129-1138.

Tables

Table 2.1 Cover brine concentration and final equilibrated concentrations for cucumbers that were acidified and bulk stored.

Storage Treatment	Acetic acid, %		NaCl, %		Anhydrous $CaCl_2$, %	
	Cover brine*	Equilibrated	Cover brine*	Equilibrated	Cover brine*	Equilibrated
3.5% Acetic	7.78	3.5	8.89	4	1.11	0.5
1.5% Acetic	3.33	1.5	4.45	2	0.55	0.25
1.5% Acetic, Blanched	3.33	1.5	4.45	2	0.55	0.25

* Bulk stored cucumbers are packed at 55:45 packout ratio (cucumbers:cover brine).
Na benzoate was added at a 12 mM equilibrated concentration.

Table 2.2 Cover brine concentration and final equilibrated concentration for cucumbers that were acidified and bulk stored at 25ºC prior to being processed into pasteurized, dill pickles.

Storage Treatment	Acetic acid, %		NaCl, %		Anhydrous $CaCl_2$, %		Calcium fumarate, %†	
	Cover Brine*	Equlilibrated	Cover Brine*	Equlilibrated	Cover Brine*	Equlilibrated	Cover Brine*	Equlilibrated
3.5% Acetic	7.78	3.5	8.89	4	1.11	0.5	NA	NA
1.5% Acetic	3.33	1.5	8.89	4	0.89	0.4	NA	NA
1.3% Acetic, 0.2% Fumaric†	2.85	1.3	8.89	4	NA	NA	0.25	0.13
1.5% Acetic, Blanched	3.33	1.5	8.89	4	0.89	0.4	NA	NA

* Bulk stored cucumbers are packed at 55:45 packout ratio (cucumbers:cover brine).
†Concentrated HCl acid was added to 1.3% acetic, 0.2% fumarate bulk stored cucumbers to adjust storage pH to 3.5.
Na benzoate was added at a 12 mM equilibrated concentration with exception to storage brines containing calcium fumarate.

Table 2.3 Data processing parameters used for analysis of GCxGC-TOFMS chromatograms of volatile components of dill pickles prepared from cucumbers stored in different brine conditions.

Data Step	Parameter*	Setpoint
Peak Detection	Baesline Offeset	0.8
	Number of Points Average for Smoothing	3
	Peak Width	0.1 sec
	Signal to Noise (S/N)	250
	Number of Apexing Masses	2
GC x GC Parameters	Match Required to Combine	500
	Override the allowed retention time shift for combine (early and late)	0.1 sec
Library Identification	First dimension peak width	15 sec
	Search Mode	Normal, Forward
	Number of library hits to return	10
	Molecular weight range	40-1000
	Mass Threshold	10
	Minimum similarity match before name is assigned	800
	Library selected	NIST mainlib
Quantification	Mass to use for are/height calculation	U (Unique mass)
Reference (Compare criteria)	Name, 1st dimension retention time (s), 2nd dimension retention time (s), and masses (unique mass in this case)	Fields populated from peak table of the composite sample
	R. T. deviation (s)	4.5
	Quantitate	Area
	Match Threshold	500
	S/N Threshold	5.0

*Parameter names and conditions are for ChromaTOF® software version 3.25, Leco Corporation, St. Joseph, Michigan.

Table 2.4 Sensory attributes of dill pickles made from cucumbers acidified and bulk stored in 3.5% acetic acid compared to dill pickles made from fresh cucumbers.

Treatment	Appearance	Firmness	Saltiness	Dill	Astringency	Other
Pickles from fresh cucumbers, 4ºC	1.5^{A}(0.7)	7.8^{A}(0.6)	7.9^{A}(0.3)	3.7^{A}(0.4)	2.1^{A}(0.3)	ND
Pickles from fresh cucumbers, 25ºC	1.7^{A}(1.6)	7.5^{A}(0.5)	7.9^{A}(0.4)	3.6^{A}(0.5)	2.0^{A}(0.3)	ND
Pickles from acidified 3.5% acetic acid cucumbers, 25ºC	3.8^{B}(1.1)	7.2^{B}(0.5)	7.4^{B}(0.4)	4.2^{B}(0.4)	2.3^{B}(0.2)	2.8^{A}(0.8)

Means in the same column not sharing a common super script are different ($p<0.05$)
Intensities were scored on a 0 to 15-point universal scale where 0=none and 15=very high intensity (Meilgaard et al., 1991).
Values in parentheses are standard deviations.
ND=Not detected
Attributes not listed were not significantly different among treatments.

Table 2.5 Sensory attributes of dill pickles made from cucumbers acidified and bulk stored in varying bulk storage conditions (Table 2.2) compared to dill pickles made from fresh cucumbers.

Treatment	Appearance	Firmness	Crunchiness	Dill	Other
Pickles from fresh cucumbers, 4ºC	ND	7.9^{A}(0.3)	9.8^{A}(0.3)	3.9^{A}(0.2)	ND
Pickles from fresh cucumbers, 25ºC	ND	7.5^{BC}(0.4)	9.1^{B}(0.3)	3.9^{A}(0.5)	ND
Pickles from 3.5% acetic,acidified cucumbers, 25ºC	2.3^{B}(0.8)	8.0^{A}(0.2)	9.7^{AB}(0.3)	4.4^{B}(0.3)	2.1^{B}(0.6)
Pickles from 1.5% acetic,acidified cucumbers, 25ºC	2.8^{AB}(1.0)	7.5^{BC}(0.4)	8.2^{C}(0.4)	4.5^{B}(0.3)	3.3^{A}(0.6)
Pickles from 1.3% acetic, 0.2% fumarate acidified cucumbers, 25ºC	2.6^{B}(0.8)	7.8^{ABC}(0.5)	8.8^{B}(0.4)	4.5^{B}(0.3)	2.8^{A}(0.8)
Pickles from 1.5% acetic, blanched acidified cucumbers, 25ºC	3.4^{A}(1.0)	8.1^{A}(0.6)	9.9^{A}(0.4)	4.2^{AB}(0.6)	3.1^{A}(1.3)

Means in the same column not sharing a common super script are different ($P<0.05$)
Intensities were scored on a 0 to 15-point universal scale where 0=none and 15=very high intensity (Meilgaard et al., 1991).
Values in parentheses are standard deviations.
ND=Not detected
Attributes not listed were not significantly different among treatments.

Table 2.6 Components that were significantly different between control pickles made from fresh cucumbers and stored at 25ºC compared to reference pickles stored at 4ºC.

Component	Fold Change*	prob t
di-2-propenyl trisulfide	7100	1.90E-07
diallyl disulphide	360	1.02E-06
Unknown 136	9	8.56E-04
furfural	-8	1.07E-03
4-(1-methylethyl)-2-cyclohexen-1-one	4	7.14E-03
4-(1-methyethyl)-phenol	4	0.0112
2-methyl-phenol	2	0.0127
1-Octanol	3	0.0198
Unknown 137	7	0.0210
Unknown 134	13	0.0282
1-Nonanol	2	0.0352
Unknown 127	-20	0.0456

*Positive numbers indicate component concentration was higher in the reference pickles. Negative numbers indicate the concentration of the component was higher in the control pickles made from fresh cucumbers.

Table 2.7 Components that were significantly different ($p<0.05$) from control pickles made from fresh cucumbers.

Component	CAS Number	3.5% Acid Fold Change†	1.5% Acid Fold Change†	1.3% Acid Fumaric Fold Change†	1.5% Acid Blanch Fold Change†
1-Pentanol	71-41-0	-3 *	-2 *	-2 *	-8 *
2-ethyl-1-hexanol	104-76-7	3 *	3 *	2 *	3 *
2-methyl-1-penten-3-one	25044-01-3	-19 *	-8 *	-9 *	-21 *
4-(1-methyethyl)-phenol	99-89-8	-180 *	-5 *	-6 *	-204 *
di-2-propenyl trisulfide	2050-87-5	5,400,000 *	3,500,000 *	8,700,000 *	21,000,000 *
diallyl disulphide	2179-57-9	8,000 *	6,300 *	11,000 *	15,000 *
Unknown 134	NA	17 *	32 *	269 *	780 *
Unknown 148	NA	8 *	11 *	53 *	91 *
Unknown 149	NA	15 *	21 *	83 *	150 *
Unknown 154	NA	4 *	3 *	2 *	10 *
1,5-Hexadien-3-ol	924-41-4	-6 *	-7 *	-4	-11 *
3,5,5-trimethyl-2-cyclohexen-1-one	78-59-1	-5 *	-7 *	-3	-5 *
furfural	98-01-1	-3	-6 *	-8 *	-11 *
Unknown 127	NA	5	19 *	111 *	870 *
Unknown 136	NA	-1	7 *	8 *	21 *
Unknown 137	NA	1	15 *	38 *	440 *
1-Octanol	111-87-5	-2	-2 *	-4 *	-1
4-(1-methylethyl)-2-cyclohexen-1-one	500-02-7	8 *	3 *	2	-1
3-methyl-3-buten-1-ol	763-32-6	-3 *	-2	-2	-6 *
3-methyl-butanal	590-86-3	6 *	-3	9 *	-2
1-Nonanol	143-08-8	-2	-1	-2 *	-1
2,6,6-trimethyl-, (ñ)-bicyclo[3.1.1]hept-2-ene	3387-41-5	1	6	13	9 *
3,5,5-trimethyl-cyclohexanone	78-59-1	-12	-10	-11	-23 *
2-methyl-phenol	95-48-7	2	-1	-2 *	1

* Components that were significantly different ($p<0.05$) from control pickles made from fresh cucumbers.
†Positive number indicates component peak area was higher in the pickles made from bulk stored cucumbers. Negative (-) numbers indicate the peak area was greater in the control pickles made from fresh cucumbers.

Figures

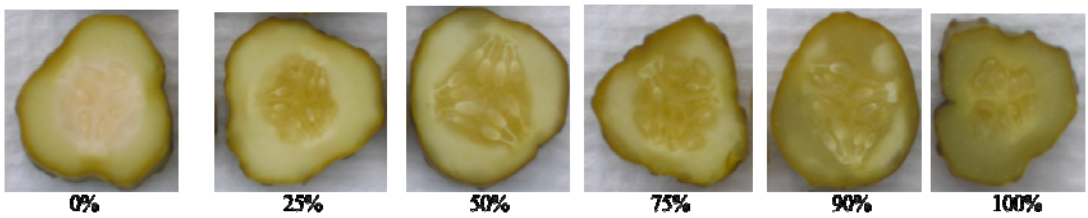

Figure 2.1 Cucumber cure chart used by sensory panels to evaluate the degree of cucumber tissue curing. Cucumbers with 0% tissue curing maintain a fresh, opaque appearance while 100% cured cucumber slices are translucent.

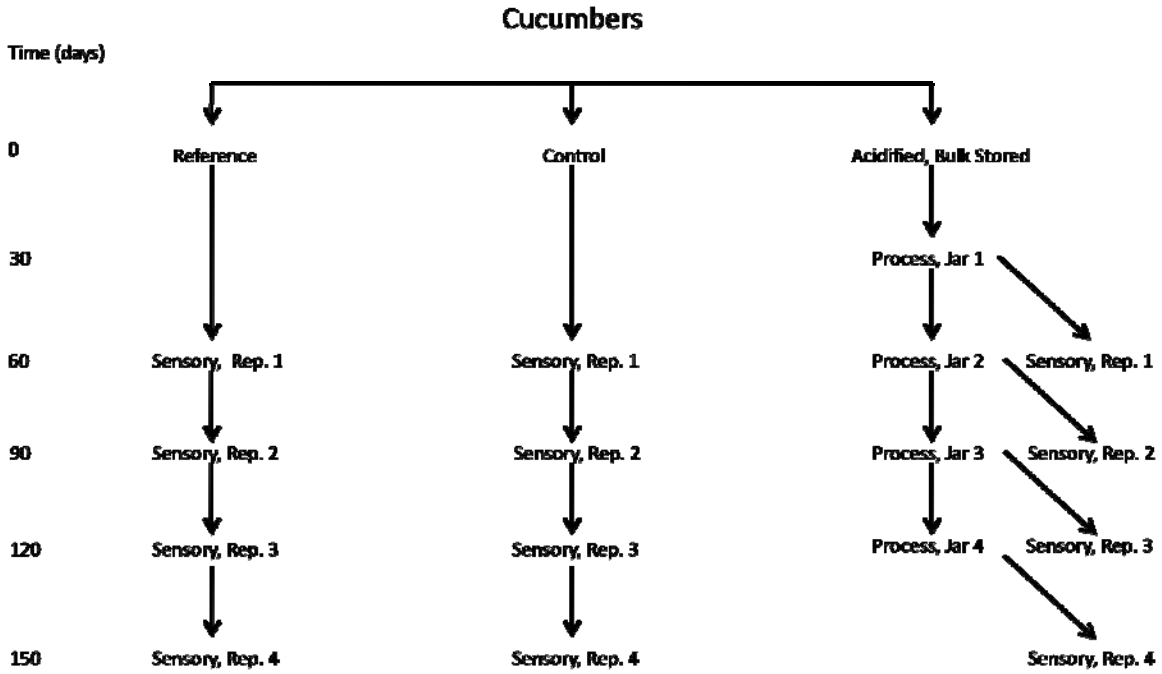

Figure 2.2 Sensory analysis sampling diagram of acidified, bulk stored cucumbers that were stored at 25ºC in 3.5% acetic acid storage brines and processed at 30, 60, 90 and 120 days into pasteurized dill pickles with the same flavorings and concentrations of acetic acid, salt, sodium benzoate, calcium chloride, and sugars dill pickles made from fresh cucumbers and stored at 4ºC (reference) and 25ºC (control). Dill pickles made from bulk stored cucumbers were pasteurized and equilibrated for 30 days prior to tasting.

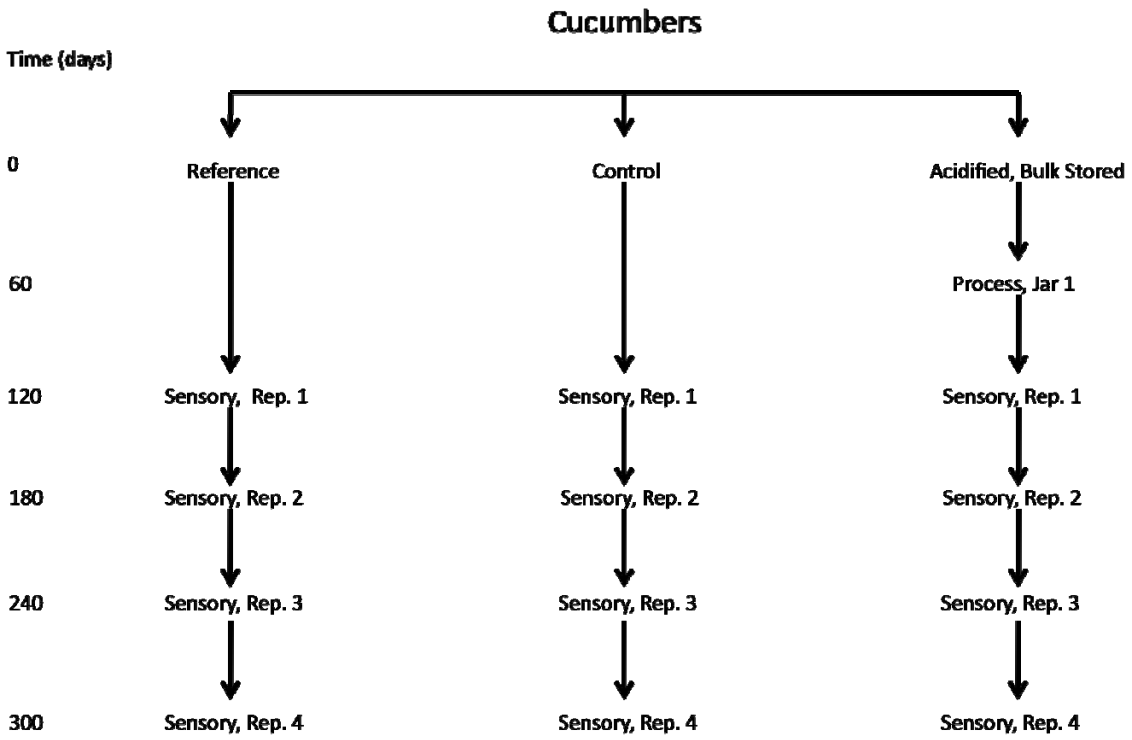

Figure 2.3 Sensory analysis sampling diagram of acidified, bulk stored cucumbers that were stored at 25ºC in 3.5% acetic acid storage brines after 60 days into pasteurized dill pickles with same flavorings and concentrations of acetic acid, salt, sodium benzoate, calcium chloride, and sugars as dill pickles made from fresh cucumbers and stored at 4ºC (reference) and 25ºC (control). Dill pickles made from bulk stored cucumbers were pasteurized and stored at 25ºC for 60, 120, 180 or 240 days prior to tasting.

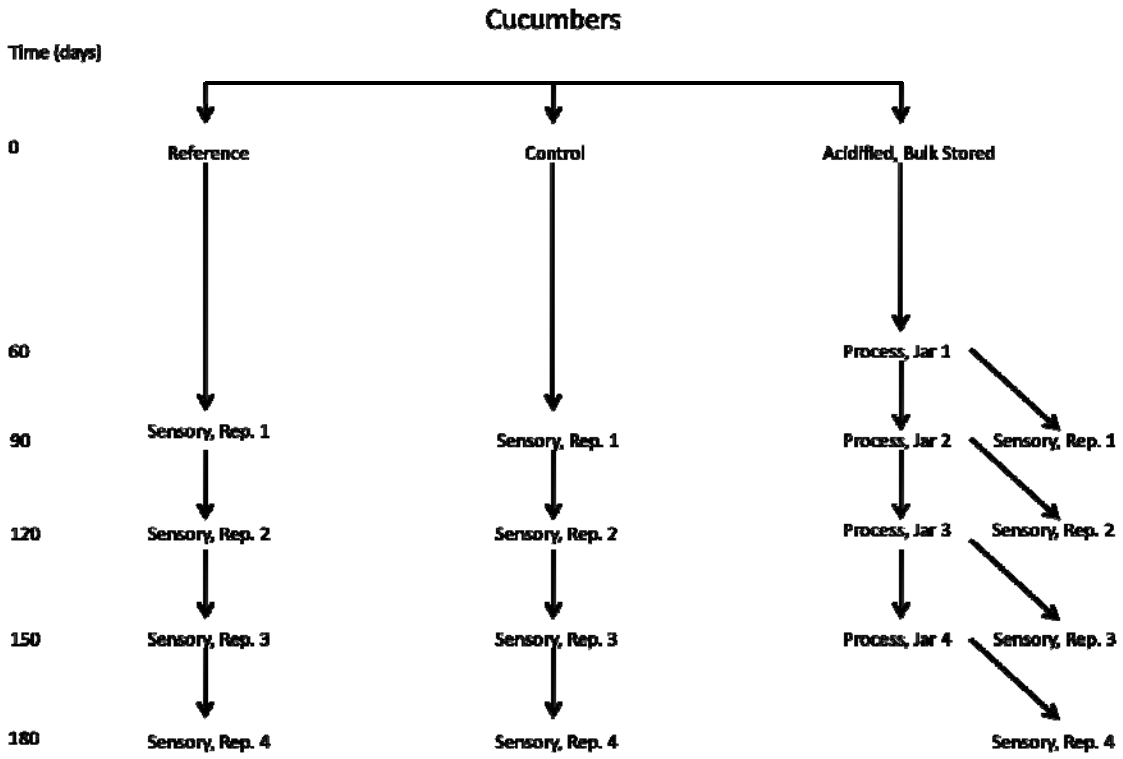

Figure 2.4 Sensory analysis sampling diagram of acidified, bulk stored cucumbers that were stored at 25ºC in different acidified, bulk storage brines for 30, 60, 90 or 120 prior to being processed into pasteurized dill pickles with the same flavorings and concentrations of acetic acid, salt, sodium benzoate, calcium chloride, and sugars as dill pickles made from fresh cucumbers and stored at 4ºC (reference) and 25ºC (control). Dill pickles made from bulk stored cucumbers were pasteurized and stored at 25ºC 30, 60, 90 or 120 days.

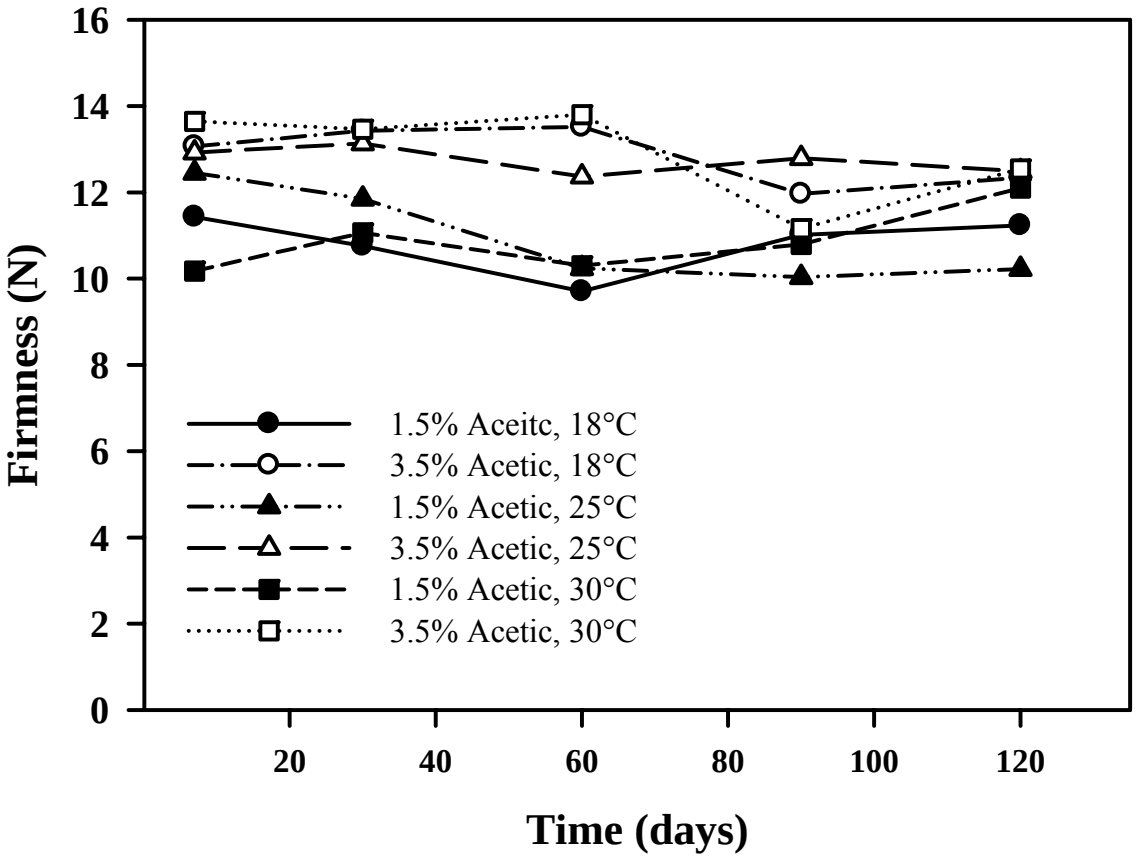

Figure 2.5 Firmness of bulk stored cucumbers stored in 1.5% or 3.5% acetic acid at 18ºC, 25ºC or 30ºC for 7, 30, 60, 90 or 120 days.

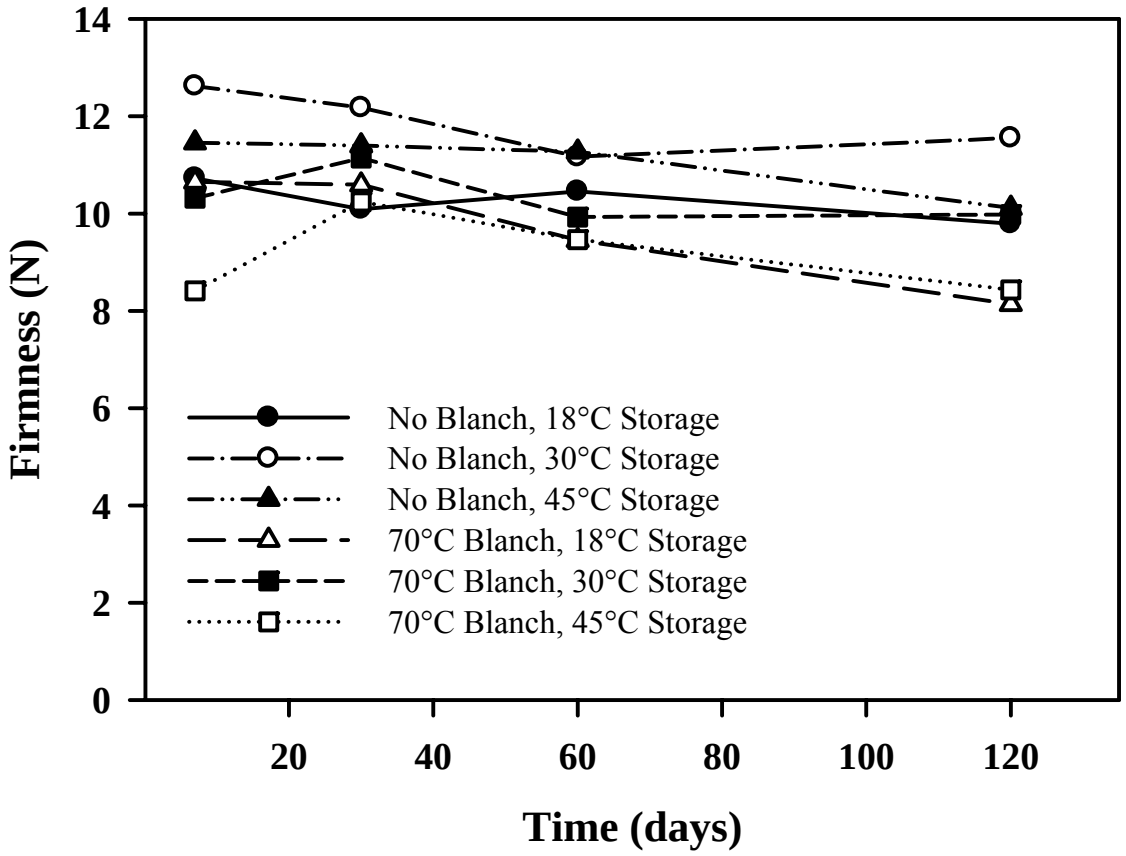

Figure 2.6 Firmness of bulk stored blanched or non-blanched cucumbers stored in 1.5% acetic acid at 18ºC, 30ºC or 45ºC for 7, 30, 60, 90 or 120 days.

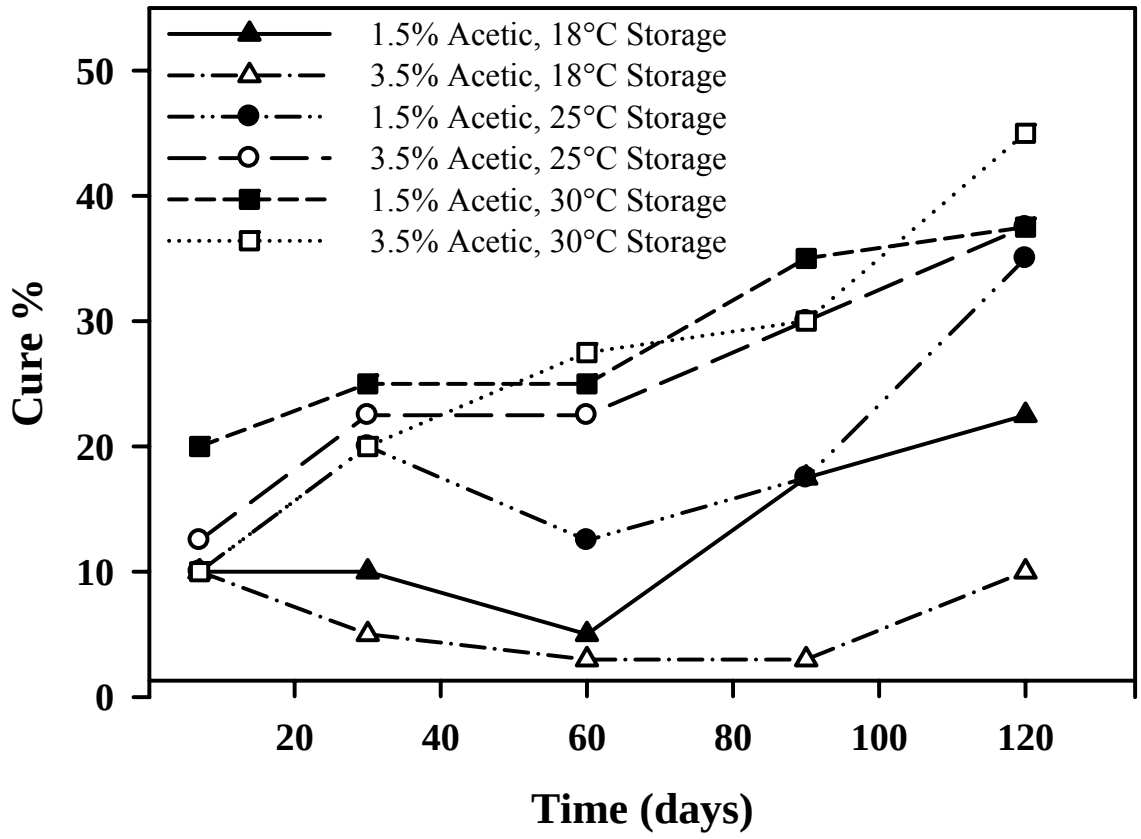

Figure 2.7 Cured appearance of bulk stored cucumbers stored in 1.5% or 3.5% acetic acid, at 18ºC, 25ºC or 30ºC for 7, 30, 60, 90 or 120 days.

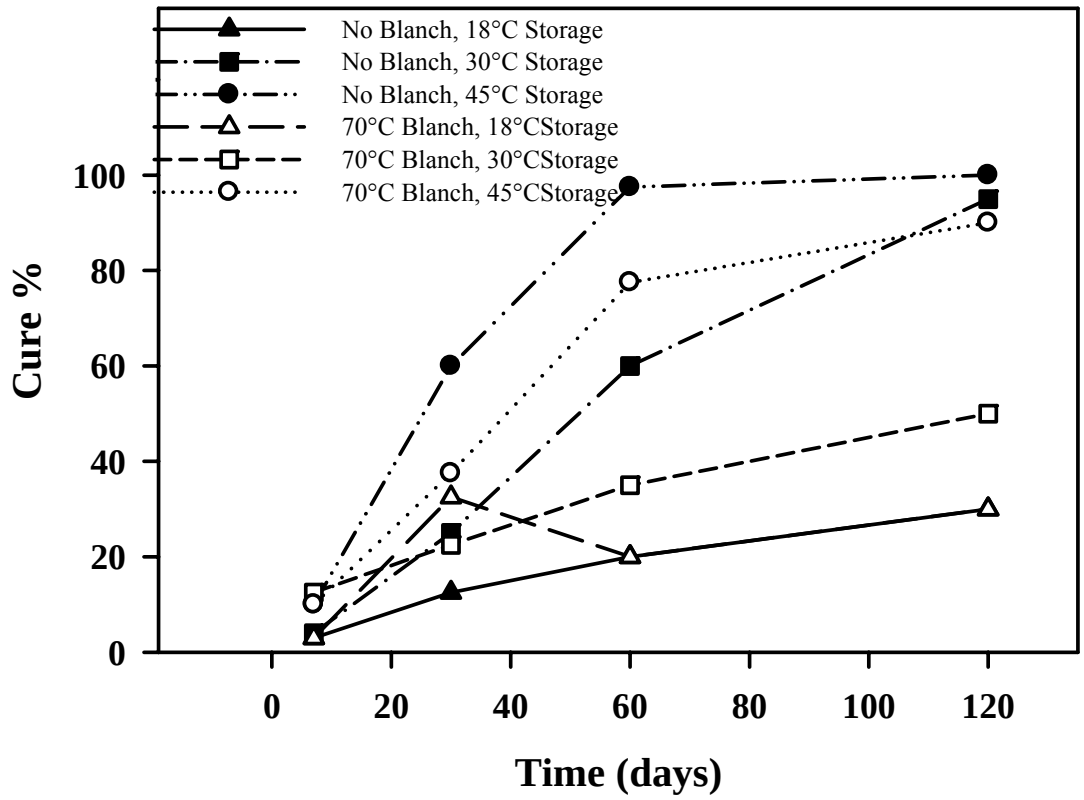

Figure 2.8 Cured appearance of acidified, bulk stored cucumbers stored in 1.5% acetic acid, at storage temperatures of 18ºC, 30ºC and 45ºC and blanched or non-blanched cucumbers after 7, 30, 60, 90 and 120 days of storage.

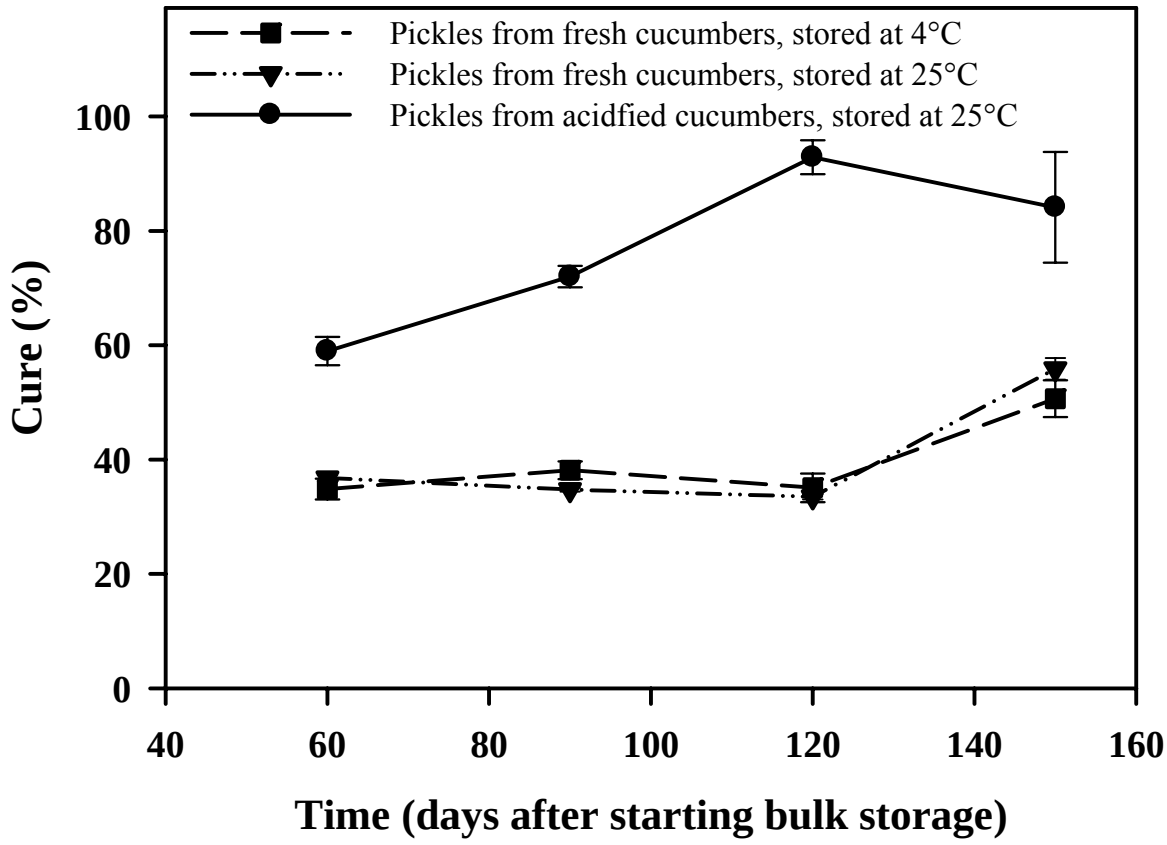

Figure 2.9 Cured appearance of pasteurized dill pickles made from cucumbers stored in 3.5% acetic acid at 25ºC (●) for 30, 60, 90 or 120 followed by 30 days storage at 25ºC as pasteurized dill pickles. Reference (■) and control (▼) pasteurized dill pickles were prepared directly from fresh cucumbers and then stored at 4ºC and 25ºC, respectively, until evaluation.

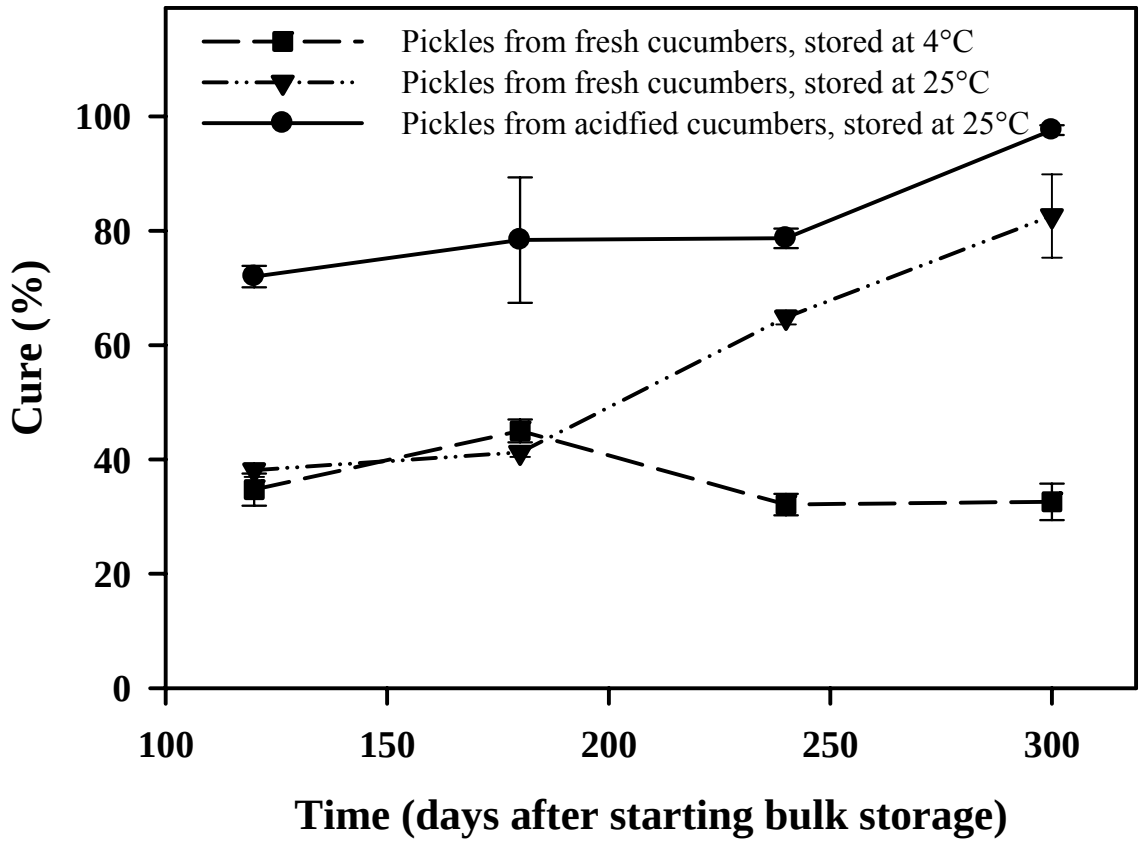

Figure 2.10 Cured appearance of pasteurized dill pickles made from acidified bulk stored cucumbers (●) that were stored for 60 days in 3.5% acetic acid at 25ºC prior to pasteurization and storage at 25ºC for 240 days as dill pickles. Reference (■) and control (▼) pasteurized dill pickles were made directly from fresh cucumbers and then stored at 4ºC and 25ºC, respectively, until evaluation.

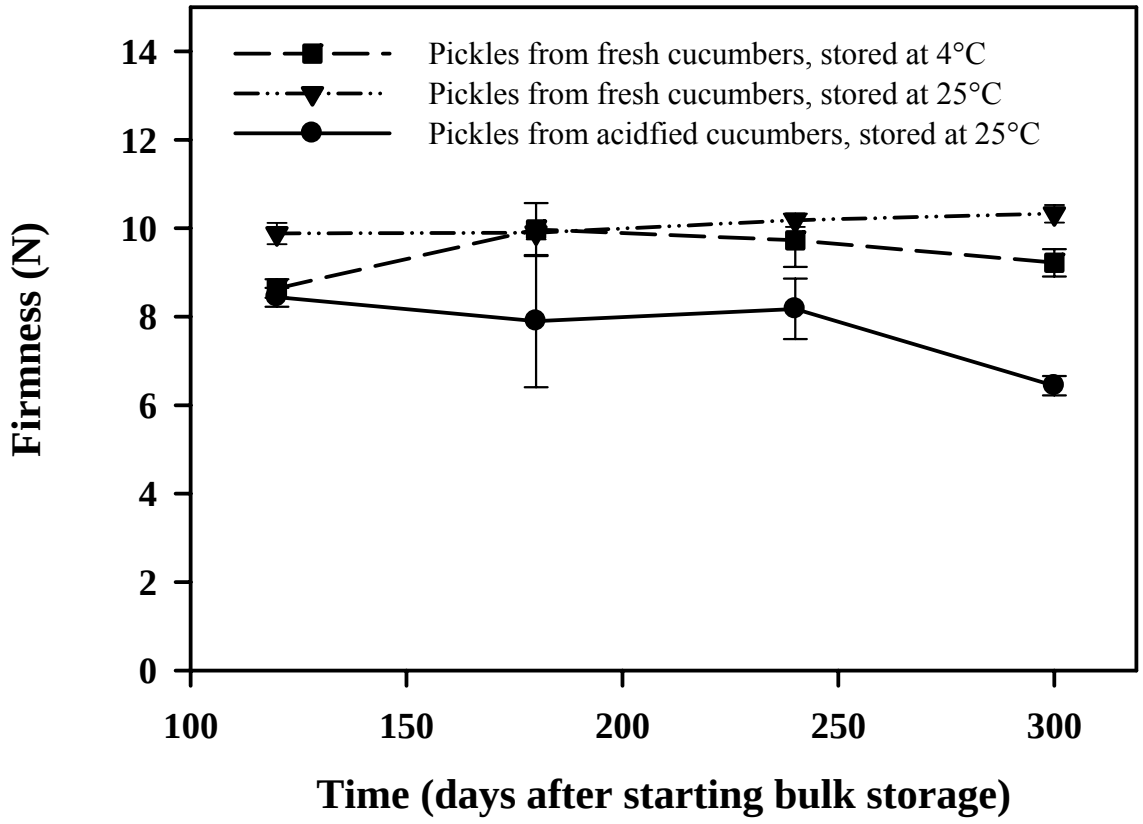

Figure 2.11 Firmness of pasteurized dill pickles made from acidified bulk stored cucumbers (●) that were stored for 60 days in 3.5% acetic acid at 25ºC prior to pasteurization and storage at 25ºC for 60, 120, 180 or 240 days as dill pickles. Reference (■) and control (▼) pasteurized pickles were made directly from fresh cucumbers and then stored at 4ºC and 25ºC respectively until evaluation.

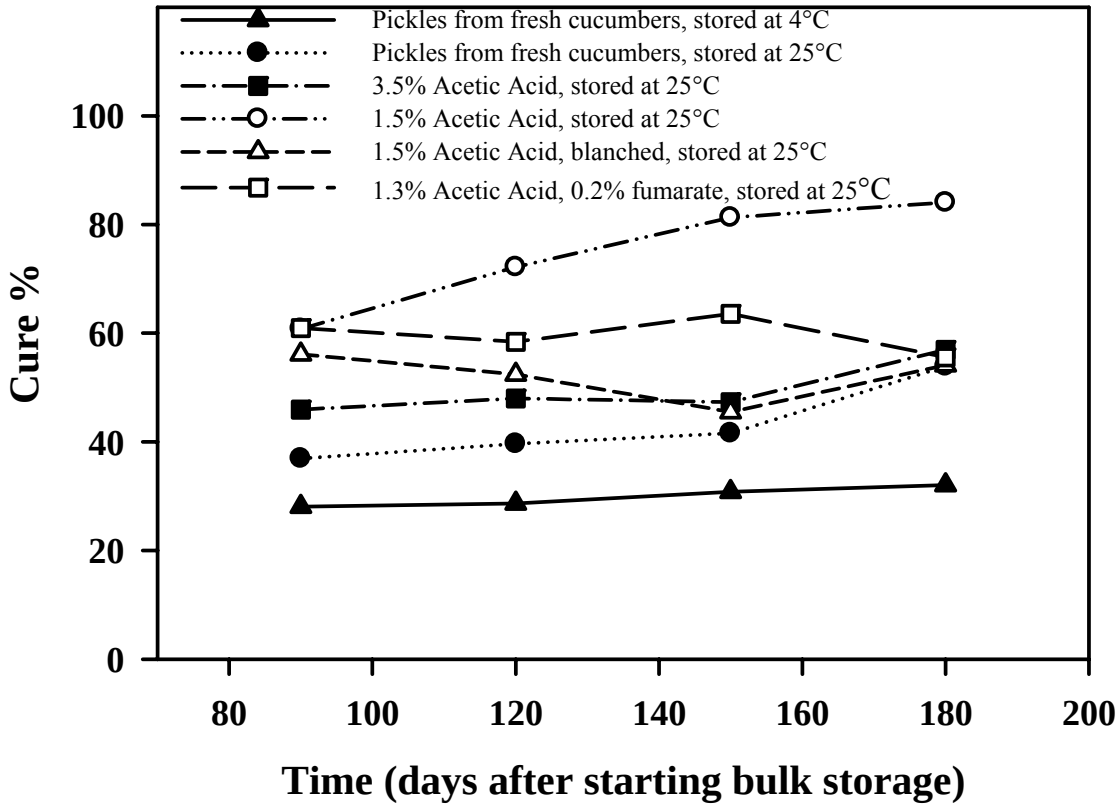

Figure 2.12 Curing of pasteurized dill pickles made from cucumbers that were acidified and bulk stored in different acidified storage conditions for 60 days at 25ºC followed by 30, 60, 90 and 120 days storage as pasteurized dill pickles at 25ºC. Reference and control pasteurized pickles were made directly from fresh cucumbers and then stored at 4ºC and 25ºC respectively until evaluation.

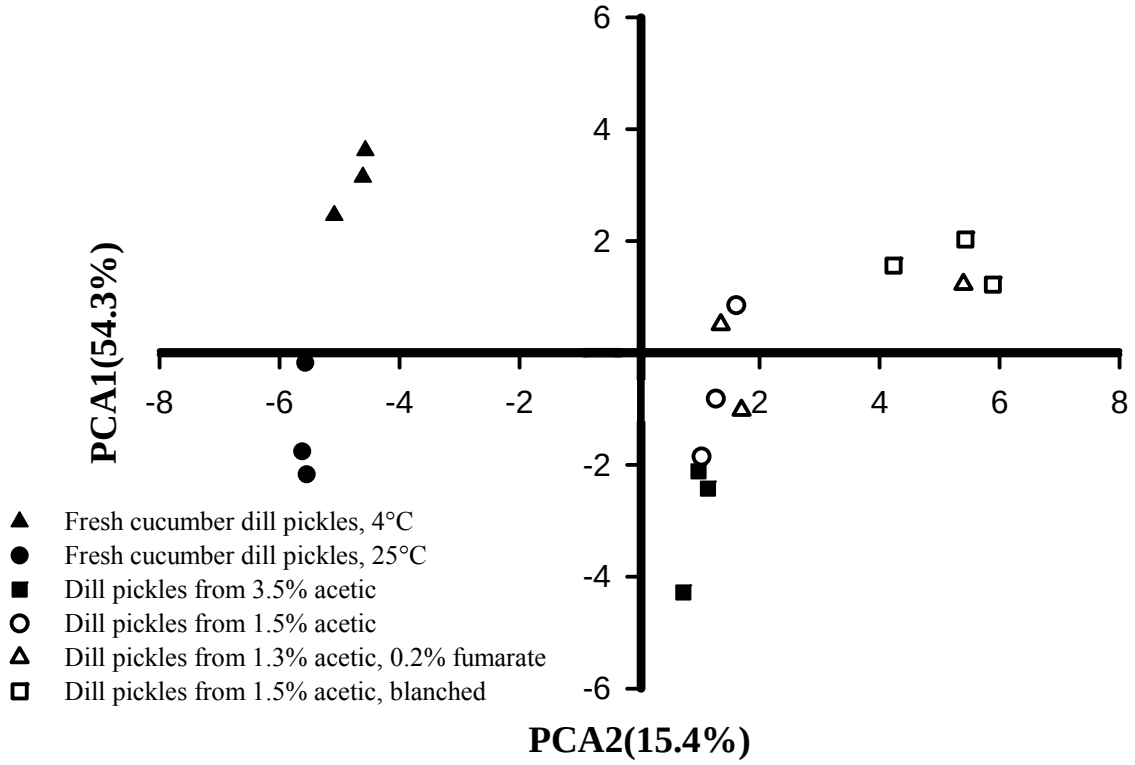

Figure 2.13 Principal component biplot based upon the log(peak areas) of 24 volatile components detected by GCxGC-TOFMS analysis that were significantly different ($p \leq 0.05$) between at least one treatment pair of dill pickles made from cucumbers acidified and bulk stored at 25ºC for 2 months then made into pasteurized dill pickles and dill pickles made from fresh cucumbers were stored at 4ºC and 25ºC that served and reference and control samples, respectively.