THERMAL DESTRUCTION OF *LISTERIA MONOCYTOGENES* IN A PARTIALLY-FERMENTED DILL PICKLE INTENDED FOR REFRIGERATOR STORAGE

by

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(Under the direction of Elizabeth L. Andress)

ABSTRACT

Listeria monocytogenes can be found on fresh produce and the bacteria can survive and grow in slightly acid refrigerated foods. One homemade refrigerator dill pickle procedure calls for partial fermentation of cucumbers in a salt brine at room temperature; pickles are then refrigerated up to 3 months. This study examined heat treatment procedures to ensure safety from *L. monocytogenes* for this procedure. Cucumbers were inoculated with a five-strain cocktail of *L. monocytogenes* and fermented for 7 days. Pickles were then heated at 71.1˚C, 82.2˚C, and 100˚C, and samples taken of the brine, core and skin during heating time. Total populations of *L. monocytogenes* were measured and log reductions in *L. monocytogenes* were calculated. Results revealed variability in reductions within a treatment, but the population generally decreased with increased heating time. Findings also suggest that heating at 100˚C is most practical but for additional time than what was studied.

INDEX WORDS: *Listeria monocytogenes, L. monocytogenes,* refrigerator dill pickles, heat-treatment, partially-fermented, cucumber, refrigerator storage, listeriosis

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DEDICATION

To my husband, my daughter and my mother.

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CHAPTER 1

INTRODUCTION

Refrigerator dill pickles are a popular type of partially-fermented cucumbers that consumers can make in their home. Many different types of procedures exist on the internet and in recipe books. The United States Department of Agriculture (USDA) has published and regularly updates a publication, Complete Guide to Home Canning, in which scientifically sound procedures are given to ensure consumer access to information regarding safe food preservation practice (USDA, 2009). In 1989, the USDA procedure for refrigerator dill pickles (USDA, 1988) was withdrawn due to concerns about the ability of *Listeria monocytogenes* to survive in the finished product (Andress, 2008).

The organism was first isolated in 1926 by Murray et al. and was labeled under the classification *Listerella monocytogenes*, but then later reclassified *Listeria monocytogenes*, which today it is known. This organism produces monocytosis in infected organisms, which is where the word "*monocytogenes*" comes from in the classification. *Listeria* contains six species: *L. seeligeri*, *L. ivanovii*, *L. inoccua*, *L. grayi*, *L. welshimeri*, and *L. monocytogenes. Listeria monocytogenes* is the only species that is known to be a human pathogen (Muriana and Kushwaha, 2006).

Consumption of contaminated lettuce, radishes and carrots caused the first known outbreak in 1979 in Boston, Massachusetts (Muriana and Kushwaha, 2006). Since the early 1980's when *Listeria monocytogenes* was labeled as a harmful foodborne pathogen, concern has arisen over its ability to survive and even multiply in more acidic environments and cooler temperatures than most pathogens (Cataldo et al., 2007). In 2005, a study was done to test the

survival of *Listeria monocytogenes* after partial fermentation using the former USDA refrigerator dill procedure (Kim et al., 2005). In addition, after one week partial fermentation, these pickles are stored in the refrigerator at temperatures that *Listeria monocytogenes* has been known to survive and grow in. The results of this study showed significant survival of the pathogen, which consumers could possibly consume.

The current study was undertaken to determine if a heat treatment could be applied to a certain partially-fermented dill pickle between the fermentation period and refrigerator storage to ensure safety from *Listeria monocytogenes* while maintaining quality. The pickles were inoculated with a 5-strain cocktail of *Listeria monocytogenes* and then subjected to three different heat treatments, at 71.1˚C, 82.2˚C and 100˚C. Samples were taken of the skin, core and brine and were then enumerated for *Listeria monocytogenes* and aerobic bacteria. Simple texture analysis was performed during these steps to compare textures of raw cucumbers and heat-treated pickles to help inform the next experimental steps to be taken.

CHAPTER 2

LITERATURE REVIEW

Overview of Vegetable Pickling and Fermenting at Home

Vegetable Fermentation

Preserving vegetables is a common household activity and the results of some methods are various products all known as pickles. Although pickled cucumbers are commonly known as "pickles," many other vegetable products are known by the term "pickles" as well. Pickled vegetables may be fermented, acidified, or treated with a combination of fermentation and acidification.

Fermenting, a type of food preservation uses salt brine and naturally occurring lactic acid bacteria to ferment and preserve foods. Fermented food products, such as sauerkraut and traditional dill pickles, may be soaked in the brine for 3 days to 3 weeks during the fermentation process. During the process, colors and flavors change and acidity increases. The increase in acidity is important to the safety and the preservation of the finished product as many spoilage microorganisms cannot tolerate acidic environments. Pasteurization and refrigeration are commonly applied to these products to increase stability and quality, although their use often depends on the length of time of fermentation (Fleming, 1992).

As mentioned earlier, fermentation utilizes the naturally occurring flora to ferment the vegetable. This process can be broken into four stages: initiation, primary fermentation, secondary fermentation, and post-fermentation (Fleming 1992). In the initiation step, heavy salt concentration is used to make the environment conducive to lactic acid bacteria growth. Eventually, the lactic acid bacteria will sufficiently lower the pH so that the other bacteria cannot

survive. This step completes the primary fermentation, where different types of lactic acid bacteria are active. In secondary fermentation, other microorganisms begin to become active and give rise to other desirable characteristics of the finished product. In commercial processes, post-fermentation may give rise to additional oxidative yeasts and molds that may grow on the surface of the brine, if not exposed to ultraviolet light after fermentation (Fleming, 1992).

Pickled Cucumbers

Pickled cucumbers, or pickles, are a commonly consumed fermented food item. During the 1990's, there was an increase in the consumption of packaged foods, including pickles. While previous trends saw pickle consumption decreasing and fresh fruit and vegetable consumption increasing, after the 1990's, there was a 7-fold increase in purchase of finished pickle products. Lower salt refrigerator pickle purchase increased as well (Estes and Cates, 2001).

Pickles have also been popular as far back as biblical times; the Bible mentions pickles twice and the use of pickles goes as far back as 3,000 years ago. Pickles have been used to treat ailments, enhance beauty, and are a staple condiment. They have only 15 calories for a large dill pickle, no cholesterol, caffeine, or saturated fat. They are after all, a vegetable. The nutrient content of pickled cucumbers changes according to the method of pickling, as the base is the raw cucumber. Even the juice is used as a relief aid for muscle cramps in many sports teams (Mt. Olive, 2008).

There are several types of pickles, with many flavors and styles achieved through various recipes. Some pickles receive a heat through canning after filling of jars, and some do not. In fact, three classifications of pickles have traditionally been documented in the research literature: unfermented, partially fermented and fully fermented. In unfermented products, acid

is added in the form of a brine that may contain vinegar and/or fruit juices, such as lemon or lime. However, pasteurization is still needed for long-term storage to kill organisms that could ferment or spoil the brine. In partially fermented products, pasteurization can be utilized to stop the fermentation process in order to achieve a desired characteristic of the final product. By-products of the incomplete fermentation that could spoil the product also must be inactivated or killed. In fully fermented products, further action must still be taken after completion of fermentation to ensure safety and lack of spoilage from microorganisms during long-term room temperature storage (Etchells and Jones, 1942).

Just as there are many types of pickles, there are many different recipes. Since at least the early 1900's, USDA has been known as a reliable source of pickling recommendations for home use. Beginning in the 1930's, the USDA undertook major research projects, partially at the U.S. Food Fermentation Laboratory in North Carolina and in cooperation with state Agriculture Experimental Stations to develop research-based recommendations for home pickling and fermenting (Nummer and Andress, unpublished). However, many more recipes can be found on the Internet, in cookbooks, or simply passed down from generation to generation. With the multitude of non-research tested recipes and procedures available comes the risk of foodborne illness and contamination. Safety of foods preserved at home has been a concern even since the 1930's. In an article entitled, "Home Canning and Public Health," Fred Tanner documents the need for reliable information to help those who process food at home keep their food safe from contamination (Tanner, 1935).

One type of pickle is called a refrigerator dill pickle, also called half-sours in many sources. They are so named because they are the product of partial fermentation, and in many procedures, they are allowed to sit in a salt brine for 1 week before refrigeration. Just as with all

types of homemade pickles, there are various recipes and procedures for what consumers make as half-sours or refrigerator dill pickles (Morash, 1982; Rowley, 2010; USDA, 1988; Ziedrich, 1998). They are usually characterized, however, by short room-temperature fermentation periods of several days to a week. Some recipes call for only a salt brine fermentation; others put a little vinegar in the fermentation brine.

Due to the fact that they are not fully fermented, there is more potential concern for foodborne illness than with fully fermented products. These pickles traditionally have not been canned by consumers. Without full fermentation, the pH may not get low enough to guarantee total safety from harmful microorganisms (Sapers et al., 1979). Fermentation and refrigeration both reduce the chances of pathogen survival and growth, but risk is not eliminated. Refrigeration was the recommended method of storage when USDA last offered a home method for making refrigerator dill pickles (USDA, 1988). Due to concerns about whether the risk of Listeriosis was eliminated sufficiently, USDA withdrew the refrigerator dill recommendation in 1989 (Andress, 2008; USDA, 1989)

Texture attributes may be different for refrigerator dill pickles than for canned pickles. Because salt concentrations are much lower in the half-sours than in other types of pickles, particularly salt-stock pickles, softening is more likely. Other microorganisms involved in fermentation and components of common spices, such as garlic, also contribute to the softening that occurs over time with refrigerator pickles. Another common problem in this type of pickle is gas formation due to some microbial activity involved in fermentation. This gas production often results in “bloaters” or pickles that have gas bubble formation on the inside (Fleming, 1992).

It is worth mentioning again that some types of cucumber pickles are not fermented at all. Fresh-pack or quick-pack process pickles are pickles that are not fermented. In this process, the whole or sliced cucumbers are placed in jars and then a hot brine of salt, spices and vinegar (or other acid) is poured over them, and then they are canned. Canning is done to kill pathogens that can cause infection and spoilage organisms like molds and yeasts that can remain active in acid foods. However, even canned foods have their own risk of potentially causing illness. Home canners may use inappropriate or unapproved procedures, which leave open the potential for the food to cause harm.

Fermented Vegetables and Pathogenic Microorganisms

Certain microorganisms may be suited to the environment created in fermented or pickled products. A pathogen, *Listeria monocytogenes*, can survive and grow in some acid environments, unless there is intervention to stop the growth and survival. *L. monocytogenes* can cause illness and in high-risk populations can cause serious complications (Cataldo et al., 2007). If vegetables are improperly pickled or fermented, then the acidity will be lower and allow other pathogens to be of concern (Sapers et al., 1979). At high enough pH, *Clostridium botulinum* spores can germinate into vegetative cells and these can multiply and produce toxin if stored at room temperature in an anaerobic moist environment such as inside a sealed jar of food. Botulism toxin causes a potentially fatal poisoning if ingested. Even if improperly acidified foods are canned, heat treatment for a typical pickled product will not be sufficient enough to kill spores of *C. botulinum*; proper acidity levels are required to ensure safety of the pickled food from the hazard of botulism.

Overview of Home Canning

Home canning can be a safe, fun and affordable way to prepare vegetables, fruits, and even meats. Commonly, people can at home to preserve foods from their home garden or locally available seasonal produce. Foods may be preserved in a boiling water canner or pressure canner, depending on acidity of the food; low-acid foods must be canned in a pressure canner to ensure that spores of pathogens have been killed. In a boiling water canner, jars filled with food are submerged and then boiled for a specified amount of time. The time for boiling the jars in the canner is researched to ensure that sufficient heat is delivered to the food to kill pathogens and other spoilage microorganisms. Only acid foods with pH less than 4.6, like fruits, are recommended for processing in a boiling water canner, in addition to some normally low-acid foods that are treated with enough acid or may become acidified to pH less than 4.6, such as pickles. Low acid foods, including vegetables, need higher temperatures to kill possible pathogens as the combination a boiling temperature and practical processing time is not effective to eliminate harmful bacterial spores at the higher pH levels. Pressure canners are used for low acid foods since the pressure allows the canner to reach higher temperatures than boiling water canners.

When one preserves food in a canner at home, scientific principles must be followed in order to prevent contaminants from ending up in the final product and to ensure safety from activity of microbial contamination of the food. Scientific principles lead to procedures that include standardized weights and measures. These procedures for home preservation should be properly tested so that the consumer finishes with a quality, safe end product. Sound advice for canning acidified foods at home is to use only properly tested recipes and procedures, since the typical home canner cannot do needed food analyses in his or her kitchen. USDA publishes such

recommended tested procedures in a publication entitled Complete Guide to Home Canning, to alleviate use of dangerous recipes that have not been tested (USDA, 2009). Still, there are many resources available to consumers that do not give safe information.

Pathogens of greatest concern for low-acid canned foods include spore-forming pathogens that produce vegetative cells and/or toxins in the absence of oxygen, such as *Clostridium botulinum*. Depending on the treatment of the food, many other common foodborne pathogens are of concern in under-processed low-acid or slightly acidic foods, including, but not limited to *Listeria monocytogenes, Salmonella,* and *Escherichia coli* O157:H7*, Staphylococcus aureus*, and *Campylobacter jejuni*. Canned foods can be contaminated before the process due to naturally occurring microflora as well as poor sanitation practices in the kitchen. Some toxins produced by bacteria in temperature-abused foods can survive canning heat.

Listeria monocytogenes

Listeria monocytogenes is a gram-positive rod-shaped bacterium that can grow and multiply at refrigeration temperatures and is resistant to heat and freezing. The microorganism is considered a psychrotroph, meaning that the optimum temperature range for growth is 5˚C to 30˚C. However, *L. monocytogenes* can grow and multiply in temperatures ranging from 1˚C to 45˚C. This pathogen is also able to survive considerable levels of salt and can survive in 10% to 12% sodium. Survival is also seen under various forms of refrigeration, freezing, heating and drying. According to some researchers, *L. monocytogenes* is one of the most heat-resistant types of bacterial cells (Muriana and Kushwana, 2006).

L. monocytogenes has been isolated from the feces of many animals, including humans. Because the bacteria are present in feces, it is common to find the microorganism in land occupied by animals. It is also found in soil and silage and plants that are grown near or on land

occupied by animals. The incidence of *L. monocytogenes* isolated from sewage from different countries is numerous. In some instances, the incidence of *L. monocytogenes* was even higher than other common microorganisms including *Salmonella*. In some cases, *L. monocytogenes* was isolated when *Salmonella* was not. Vegetables can harbor *L. monocytogenes*. They pick up the microorganism from the surrounding environment as is common in plants (Beuchat, 1996).

The way that *L. monocytogenes* behaves on vegetables is important to determine the method needed to make the food safe. Pathogens can grow in a very complex environment on the vegetable. Many different types of molds, spoilage microorganisms, viruses and bacteria often survive and grow in interaction with one another. As spoilage and non-spoilage microorganisms grow together, they can produce characteristics in the environment that are protective to pathogens. For example, *L. monocytogenes* is known to exist in multispecies biofilms. A biofilm is a congregation of microorganisms, including bacteria, yeasts and molds in a matrix structure. This matrix structure is formed from exopolysaccharides that the bacteria secrete. Biofilms allow the bacteria to survive in harsher environments, as the matrix structure prevents certain antimicrobial treatments from contacting the microorganism. *Listeria monocytogenes* in a multispecies biofilm has been known to survive treatment with 500 ppm of free chlorine applied over 20 minutes (Beuchat, 2002; Norwood and Gilmour, 2000).

In addition to the way the bacteria behave, one must remember that the environment of the vegetable itself is also important. The microorganism can inhabit crevices, cracks, and intercellular spaces in the produce. In these places, it can grow to high enough numbers that the produce may become unsafe for people to eat. The number of *L. monocytogenes* cells needed to infect a person depends on many factors. Healthy individuals can consume more of the pathogen than children, the elderly, pregnant women, and those with compromised immune systems.

Pathogens, including *L. monocytogenes* can infiltrate to the vegetable interior when the water pressure on the surface of the vegetable overcomes the hydrophobic nature of the vegetable surface as well as the internal gas pressure. Also, if the temperature of the water suspension of the cells is lower than that of the vegetable, infiltration can be enhanced (Beuchat, 2002).

Foods commonly associated with *L. monocytogenes* include ready-to-eat foods, unpasteurized and pasteurized cheeses and milk, vegetables, and meats. Since a major outbreak of listeriosis from coleslaw in 1981, this bacterium has been a concern for ready-to-eat and processed foods. Some outbreaks are the result of post-process contamination in a food plant, because the microorganism survives well on surfaces in the environment (CDC, 2008; Doyle et al., 2001; Bueuchat, 1996).

Acidity and *Listeria monocytogenes*

Listeria monocytogenes has been known to withstand considerable ranges of pH. The pathogen is best suited in an environment that has a pH of 6-8. However, it has been documented that L. *monocytogenes* can grow and survive in environments with pH levels ranging from 4.1 to 9.6. It is important to note that growth of *L. monocytogenes* for minimum pH is a function of incubation temperature, presence of salts or inhibitors, and the substrate or nutrients on which the bacterium is growing (Jay, 1996).

Many studies have focused on the combined effect of pH and sodium chloride (NaCl). The effects on growth of *L. monocytogenes* are additive, but have not been found to be synergistic. For example, the time to visible growth (the point at which one colony forming unit of bacteria becomes visible upon observation) at 30˚C with no NaCl added has been found to be 5 days. At the same temperature, it took 8 days with NaCl at 4% and 13 days for NaCl levels at 6%. All of these findings were at the same pH level of 4.66. When the temperature was lowered

to 5˚C, a pH of 7.0 was required to see any growth. In this environment, it took 9 days to see any growth with no added NaCl. With NaCl at 4%, it took 15 days to see growth and with NaCl at 6%, it took 28 days to see growth at 5˚C and pH 7.0 (Jay, 1996).

Acid Tolerance of *Listeria monocytogenes*

Studies have shown the *L. monocytogenes* can become acid-tolerant in some environments because the cells may adapt physiologically to help the microorganism survive in a hostile environment. *Listeria monocytogenes* can undergo induced acid-tolerance when subjected to sublethal pH levels. This finding is important, because during food processing, acids may be added to foods as a means of protection to prevent the growth and survival of spoilage microorganisms and pathogens (O'Driscoll et al., 1996).

Acid tolerance response is a defense mechanism by which bacteria can adapt to endure lower pH levels. When certain bacteria are subjected to sublethal acidity, changes occur in the bacteria that allow further survival in lower pH levels (Baik et al., 1996). In 1996, a study was performed by Davis et al. to evaluate the acid tolerance response of *L. monocytogenes*. Many studies have been performed with other types of pathogens, including *Escherichia coli* and *Salmonella typhimurium.* However, less research had been done in the past regarding Gram-positive bacteria, such as *L. monocytogenes*, regarding the acid tolerance response. Davis et al. (1996) found that after a one-hour challenge in a sublethal pH of 5.8, the survival of *L. monocytogenes* cells increased 1,000 fold when subsequently exposed to pH 3.0 after 30 minutes over an identical sample subjected only to the acid pH level 3.0. After 2 hours in the low pH level, the sample that was subjected first to the pH 5.8 still showed survivors. According to the researchers, this proves that *L. monocytogenes* displays acid tolerance when subjected first to sublethal pH. This is significant when examining *L. monocytogenes* survival in foods where the

acidity level increases over time, such as fermented products. During the fermentation process, acid levels may be mild at first. However, over time the acidity level increases gradually until the final product reaches the desired level of acidity. Potentially, if the food contains cells of *L. monocytogenes*, the fermentation process, having started at a mild acidity may induce the acid tolerance response, allowing the pathogen to have increased survival once the product reaches the desired acidity level. Furthermore, refrigerating or storing such products at low temperatures will not provide protection against *L. monocytogenes* as the pathogen is known to survive and grow at refrigeration temperatures.

In another study, *L. monocytogenes* cells were subject to a pH level of 5.5 for one hour. This exposure to a mildly acidic environment produced an acid tolerance response (ATR) that protected the microorganism, allowing it to endure more acidic conditions (pH 3.5) (O'Driscoll et al., 1996). This ATR also allowed for protection against other environmental stresses, like thermal stress, osmotic stress, and the addition of other compounds like crystal violet and ethanol. With prolonged exposure to the low acid environment, the study found that the *L. monocytogenes* can mutate. The mutants that were isolated showed resistance to lower pH. This may suggest that exposure to mild pH levels may produce acid-tolerance that allow the pathogen to withstand longer exposure to even lower pHs with the potential to produce mutant cells that allow for selection towards cells that are naturally more acid-tolerant (O'Driscoll et al., 1996). In a subsequent study aimed at determining the significance of the acid tolerance response, *L. monocytogenes* displayed acid tolerance in acidified dairy products, including yogurt, cottage cheese and cheddar cheese. Also, the pathogen displayed acid tolerance during active fermentation of milk with lactic acid (Gahan et al., 1996). These findings are important in regard

to the production of partially-fermented pickle products which may contain *L. monocytogenes,* especially if conditions may not be adequate to prevent the growth and survival of the pathogen.

***Listeria monocytogenes* in Foods**

Research has confirmed the ability of *L. monocytogenes* to survive and grow in foods for long periods of time. Jay (1996) cites several examples of studies that document the hazard. At a temperature of 3˚C, strains Scott A. and V7 can survive for 28 days at inoculation levels of 10^4 -10^5/g. Another case in camembert cheese showed that Scott A. and V7 along with two other strains, when inoculated at 10^4 -10^5/g, survived 18 days, with some of the strains increasing to 10^6-10^7/g even after 65 days of ripening (Jay, 1996).

In cold-pack cheeses with 0.3% ascorbic acid added, *L. monocytogenes* has been shown to survive a mean of 130 days at 4˚C. In lettuce, both the vegetable and its juice showed growth of *L. monocytogenes* under refrigeration of 5˚C for 14 days. In the same study, *L. monocytogenes* was recovered from two samples that were uninoculated (Jay, 1996). According to Jay, the aforementioned studies are typical and represent the overall resistance of the microorganism in foods. He also states these findings are consistent with studies in non-food environments as well.

As of 1995, the United States placed stringent restrictions on the amount of *L. monocytogenes* that can legally be in a ready-to-eat food product. *L. monocytogenes* is considered an adulterant, which means that any product containing the pathogen is considered adulterated and can be recalled or taken off the market. There must be an absence of the organism in a 50 gram sample of the food (Jay, 1996).

Listeriosis

L. monocytogenes was first confirmed in isolation from human samples in 1929 (Farber and Peterkin, 1991). However, only since the early 1980's has this pathogen gained serious recognition. During this time, several serious outbreaks with fatalities occurred. Listeriosis is the term for the illnesses caused by *L. monocytogenes*. It is possible that 1 to 10% of humans may carry the organism in their intestines asymptomatically (FDA, 2009). According to the FDA (2009) and CDC (2008), listeriosis is clinically defined when the organism is isolated from the blood, cerebrospinal fluid, or other normally sterile site such as the placenta or fetus. Most victims of listeriosis do not realize they have the infection until clinical diagnosis (CDC, 2008; Doyle et al., 2001).

The infectious dose is not known, although it is believed the dose may vary by strain and characteristics of the infected person. Fewer than 1,000 organisms are believed to be enough to cause symptoms in susceptible persons, at least from cases contracted through raw milk (FDA, 2009). Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may be the only symptoms expressed while other complications can be severe and even life-threatening.

Some populations, including the elderly, young and immune-compromised, are at greatest risk for these complications. Pregnant women are at risk with complications in pregnancy including stillbirth and spontaneous abortion. Other symptoms in high-risk audiences may include septicemia, meningitis (or meningoencephalitis) and, intrauterine or cervical infections in pregnant women. When meningitis occurs, the overall mortality may be 70%; with septicemia, mortality may be 50%, and from perinatal/neonatal infections, the mortality rate may be greater than 80% (CDC, 2008; FDA, 2009).

Research on Refrigerator Dills and *Listeria monocytogenes* Survival

Pickles have been prepared in homes for hundreds of years and it was thought that the acidity created in the brine during the fermentation process was enough to ensure safety of the finished product. Cole et al. (2008) found that the effects of acidity and salt concentration on survival and growth of *L. monocytogenes* were merely additive and greatly influenced by temperature. Also, low salt levels (4-8%) had a protective effect in the presence of low pH against inactivation of the microorganism. This is important to consider with refrigerator dill procedures because fermentation occurs at room temperature with a salt concentration typically of about 3%. So, according to the research of Cole et al., it is possible that *L. monocytogenes* could grow at room temperature with salt concentrations up to 6% and pH of 4.66, conditions not unreasonable for the fermentation step. This is concerning because it is well established that *L. monocytogenes* can grow and survive at refrigerator temperatures and possible at relatively low pH. Putting the dills into the refrigerator would not necessarily ensure safety of the product (Cole et al., 2008). Other studies have found that *L. monocytogenes* in cabbage juice can multiply to significant levels in pH levels as high as 5.0-6.1 with NaCl concentrations at 0-1.5%. At greater than 2% NaCl, levels of *L. monocytogenes* was shown to decrease (Beuchat, 1996). It is interesting to note that contact with carrot juice has been shown to decrease the amount of *L. monocytogenes* cells (Nguyen-the and Lund, 1990). Cooked carrots have not proven to have the same effect on the microorganism (Beuchat and Brackett, 1990).

In 2005, Kim et al. performed a research study in response to the earlier concern surrounding *L. monocytogenes* in the previous USDA refrigerator pickle procedure that was published in 1988 and then withdrawn. This study examined the survival of the bacteria in brines of three different salt concentrations. For the procedure, cucumbers were rinsed and then

the blossom-end was cut off. The cucumbers were then submerged in a 5-strain *L. monocytogenes* inoculum for 15 minutes and allowed to drain for the same amount of time. Once inoculated, the brine was prepared and the cucumbers were allowed to ferment at room temperature for 1 week and then refrigerated to up to 3 months; this is a common practice in the preparation of refrigerator dills. Each salt concentration had 3 reps. The results showed that for all 3 salt concentrations, there was significant survival of *L. monocytogenes* on the surface and interior of the pickles, even after 2 months of refrigeration. This is a major concern for those who ferment pickles at home. If *L. monocytogenes* can survive in brine fermented for one week with concentrations of salt below, above and at those used in recipes for home-processed pickles, then there is a possibility that the organism could potentially be in this type of pickles prepared at home. This organism can survive and grow at refrigerator temperatures, and possibly cause listeriosis (Kim et al., 2005).

Also in 2005, Breidt and colleagues inoculated batches of fresh-pack pickles and then used heat treatment to determine what it would take to kill at least 5 log of *L. monocytogenes* in the brine. After 5 strains of *L. monocytogenes* were cultured and then added to the pickle batch, heat treatment was applied and samples were taken at specified time intervals. Enumeration of the samples was completed to determine the number of bacteria that survived. It was found that the time needed to obtain such a kill is 1.7 seconds at 65˚C. This type of fresh-pack pickles normally would undergo heat treatment of at least 5 to 10 minutes in a boiling water canner for long-term room temperature storage. Therefore, the pickles should be safe, even if they contain a pathogen that may be acid-resistant in the brine (Breidt et al., 2005). In the case of typical refrigerator dills, such as the previous USDA recipe, typically no heat treatment is given and may leave the opportunity for *L. monocytogenes* to survive and grow.

Objective, Hypotheses and Specific Aims

The objective was to evaluate possible heat treatment procedures that consumers can perform at home after the partial fermentation process in the USDA refrigerator dill recipe to ensure that there is no risk from *L. monocytogenes* when consuming the pickles.

Hypotheses

1. A heat treatment of 100°C for 15 seconds will produce at least a 5 $\log_{10}$cfu reduction of *L. monocytogenes* in the tissue of refrigerator dill pickles.
2. If there is at least a 5 $\log_{10}$cfu reduction of *L. monocytogenes* in the tissue, then there will be at least a 5 $\log_{10}$cfu reduction in the brine and skin.

Specific Aims

The specific aims of this project were to:

1. Determine if heating this style partially-fermented dill pickles in the fermentation brine can produce a product safe from *L. monocytogenes* for refrigerator storage.
2. Determine the fate of *L. monocytogenes* in the brine, on the pickle surface, and in the interior of the pickle after post-fermentation heat treatments.
3. Determine if a practical consumer recommendation can be made for producing a safe refrigerator dill pickle using the former USDA procedure.

CHAPTER 3

METHODS

Pickling cucumbers were obtained and rinsed lightly and then partially-fermented at room temperature in a seasoned salt brine according to a standard recipe. After inoculation with a 5-strain cocktail of *Listeria monocytogenes*, the batch of pickles was subject to heat treatments with the potential to kill the *L. monocytogenes*. Three replications of each treatment were attempted. Texture analysis was performed on both raw cucumbers and pickles heated at three different temperatures to determine if changes in texture integrity varied by heat treatment. Results of the texture analysis were also used to decide whether or not heating could be recommended for consumers that would not cause significant differences in the texture of the heated product versus pickles without heating.

Fermentation Procedure

Unblemished pickling cucumbers of close to uniform size (9-12 cm long and 3-4 cm wide) were purchased from an Atlanta international farmer's market. The former USDA recipe from the Complete Guide to Home Canning (USDA, 1988), also found in So Easy to Preserve, 2nd edition (Harrison, 1988) was used to make the batches of refrigerator dill pickles.

The exact original recipe reads as follows:

6 pounds of 3- to 4-inch pickling cucumbers

10 to 24 large heads of fresh dill weed or 3/4 cup dill seed

1½ gallons water

3/4 cup canning salt

2 to 3 cloves garlic, peeled and sliced

6 tablespoons mixed pickling spices

Wash cucumbers. Cut 1/16-inch slice from blossom end and discard. Leave 1/4-inch of stem attached. Place cucumbers in a suitable 3-gallon container. Add dill. Combine water, salt, garlic and pickling spices. Bring to a boil. Cool and pour over cucumbers in container. Add a suitable weight. Keep at room temperature for 1 week. Then, fill jars with cucumbers and brine. Seal and store in a refrigerator. Pickles may be eaten after 3 days and should be consumed within 2 months (Harrison, 1988).

The recipe with changes made for laboratory measurements can be summarized as follows: Make a brine with 6 L of water, 181 g of canning salt, and 50 g of mixed pickling spices and bring the brine to a boil. Remove 0.2 cm of the blossom end from the cucumbers. In a 10 L food-grade plastic container, place 2.72 kg of trimmed cucumbers along with 114 g of dill seed. Pour the brine over the cucumbers; place a weight over the top and leave at room temperature for one week. The rest of the original procedure is then substituted with the experimental methods of inoculation with *L. monocytogenes* as described below and subsequent heat treatment interventions. None of the measurement changes significantly change the ingredient proportions.

Inoculum Preparation

Five strains of *L. monocytogenes*, including 301, V7, LCDC, Brie and Scott A. were initially obtained from sources including cheeses, coleslaw, milk, and clinical subjects (Food Microbiology Lab, University of Georgia, 2008). Each of the original strains were transferred twice into 10 mL of tryptic soy broth (TSB, Becton Dickinson, Franklin Lakes, NJ) and incubated for 48 h each time. Then, a subsequent transfer was made to 4 L of TSB followed by incubation for 48 hours. The inocula were centrifuged (Allegra™ X-22R, Beckman Coulter™,

Brea, CA) at 4,550 X g for 30 min and the concentrated inocula resuspended in sterile de-ionized water immediately prior to inoculation of the cucumbers.

Inoculation Procedure

Each batch of pickles was inoculated with a 5-strain cocktail of *L. monocytogenes* that was made from equal ratios of the 5 strains grown in the tryptic soy broth. The approximate population of the final inoculums was 8 log. Six pounds of prepared cucumbers were submerged in the inoculum for 15 min and then drained on a sterile rack for 15 min. These inoculated cucumbers were used to prepare the recipe as written. Then, the batch was held for 7 d in a separate lab that was locked and away from general traffic; they were not moved. The temperature fluctuated between 20 ºC and 22.2ºC while the pickles were allowed to partially-ferment before heat treatment.

Heat Treatment Procedure

Heat treatments of the partially fermented cucumbers and brine were conducted at temperatures including 71.1, and 83.3, and 100ºC. Complete batches of post-fermentation pickles and brine were placed in a stainless steel 16-quart stockpot. Heating was done on an electric coil burner, 8-inches diameter and 1750 watts. This represents a typical electric coil large-diameter stove burner that would be found in a consumer's home. During the heating, model# RD160B Data Logger probes (Omega Engineering, Stamford, CT) were placed in the brine, core and skin of 6 randomly chosen pickles (2 probes in each of three positions). Once the target brine temperature was reached, one pickle was pulled every 15 sec up to 2 min, then every 30 sec until 5 min, 8 min, and 12 min. Some batches also included a 10-min sample and other batches had less samples removed. The lowest temperature treatment had fewer sampling intervals and higher temperature treatments had more frequent time intervals as the experimental

methods were refined. Each sample was removed and placed into a sterile stomacher bag and then laid in an ice bath to stop further heating within the pickle.

Sampling Procedure

Samples of 2.5 cm X 2.5 cm of the skin and 10 g of the core were taken from uninoculated, pre-fermentation cucumbers; inoculated, pre-fermentation cucumbers; post-brine pre-fermentation cucumbers; as well as heat-treated, post-fermentation pickles. Also, the pH of the brine was recorded on days 0, 3, 5, and 7 of the fermentation process with a pH meter (Hanna Instruments, Woonsocket, RI). For sampling, the skin and core of each sample was placed in its own Stomacher® bag with 0.1% peptone and stomached for 1 min. Serial dilutions were prepared and plated using Autoplate 4000 (Spiral Biotech, Norwood, MA) on modified oxford listeria selective media (MOX, Becton Dickinson, Franklin Lakes, NJ) plates to enumerate for *L. monocytogenes.* Also, samples were plated on plate count agar (PCA, Becton Dickinson, Franklin Lakes, NJ).

Colony forming units were determined. The data was transformed into $\log_{10}$ values, entered into a spread sheet, and trend-line analysis was conducted. The logarithm values were then used to calculate the log reductions by subtracting the logarithm value at a given sample time from the initial (pre-heat treatment) logarithm value. The reason for calculating the logarithm (log) reduction was to control for the fact that initial loads of the *L. monocytogenes* could not be standardized. That is, even though each batch was inoculated in the same manner, samples taken right before heating showed that the retention of the pathogen was not the same between batches. The detection level was calculated using 1 colony-forming unit (CFU) on a given plate and then the calculation was carried out considering the dilution and amount plated

on each plate. On plates that had 50 µL plated, a detection level of <20 CFUs (<1.30 $\log_{10}$ CFU) was used. On plates with 250 µL plated, a detection level of <4 CFUs (<0.60) was used.

In addition to plating on the MOX (Becton Dickinson, Franklin Lakes, NJ) plates, a positive/negative UVM enrichment broth was performed to detect any *L. monocytogenes* cells in samples that were recorded as undetectable by plating. One (mL) of each sample of brine, core and skin was placed in 9 mL of sterile UVM broth, vortexed and incubated for 48 h. After incubations, a sterile loop was used to streak each UVM sample on to MOX media. Subsequently, the MOX plates were incubated 48 h and growth (positive) or no growth (negative) was noted. UVM enrichment was not performed on samples from temperature treatment 71.1˚C.

Rapid Identification of *Listeria monocytogenes*

Isolates from 9 different treatments were randomly chosen to confirm the identity of suspected *L. monocytogenes.* A pure culture was obtained by streaking the primary sample onto tryptic soy agar with yeast extract (TSA-YE) and incubating at 32°C for 24 h. Next, gram-stain, oxidase and calalase tests were performed before continuing to the next step in the MicroID (Lenexa, KS). Colonies from the sample were suspended in 4.6 mL of sterile saline until a turbidity level equal to the #1 McFarland standard was obtained. The strips were inoculated and incubated as per manufacturer's instructions. After 4 h, the rhamnose and esculin reactions were checked and upon positive confirmation, the unit was incubated for an additional 20 h. After incubation was completed, 2 drops of 20% KOH was placed in VP test well. The unit was rotated as instructed in the product information pamphlet and the results were read and documented.

Texture Analysis

Texture analysis using a puncture test was performed on raw cucumbers and pickles with three heat treatments. All heat treated pickles were placed in an ice bath and held cold to simulate as closely as possible a refrigerator dill pickle. The heat treatment consisted of three different temperatures: 82.2°C for 3 min, 93.3°C for 15 sec, and 100°C for 15 sec. These exposure times were based on analysis of preliminary data available at that time. This data was then used to assist in deciding whether or not to try heat treatment at 100°C based on the integrity of the texture of the pickle. At each temperature, there were three pickles tested at three different sites on the pickle. Puncture analysis was performed using a TI-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) and the peak force (N) was notated.

Statistical Analysis

The results of the three replications across the brine, core and skin sample sources between the temperature treatments were analyzed. Then, to control for the variation in starting loads of the *L. monocytogenes*, log reductions were calculated. The log reduction for a given sample time is determined by subtracting the $\log_{10}$CFU at that time from the preheat $\log_{10}$CFU value. The means and standard deviations were then put into tables; standard deviations were not calculated in cases where there was only one usable data point. A log reduction of 5 log was determined to be significant for this study based on prior research and the hazard analysis critical control point (HACCP) system processing procedure found in 21 CFR 120 (Breidt et al., 2005).

For the log reductions in survivors of *L. monocytogenes*, a PROC GLM was also performed due to the unbalanced data set. The variables "sample source" and "time" and the interaction between the two were examined for all temperature treatments separately. This method reduced power. However, due to the fact that there were different sample times between

treatments, they had to be analyzed separately. Tukey's test was performed in cases where the interaction between variables was found to be significant.

A PROC GLM was used (SAS Version 9.2, SAS Institute Inc., Cary, NC) to analyze the time to 5 log reduction ("goal time") data as the data set was unbalanced. In all analyses, goal time is defined as the amount of time in minutes it takes to see a reliable 5 log reduction in cfu/ml of *L. monocytogenes* in the brine, cfu/g of *L. monocytogenes* in the core, and cfu/cm^2 of *L. monocytogenes* in the skin. The variable "temperature" and "sample source" and their interaction were examined for goal time. Tukey's test was performed in cases where the interaction between variables was found to be significant.

An ANOVA (SAS Version 9.2, SAS Institute Inc., Cary, NC) was performed on the puncture analysis data to compare the force (N) in three sample sources on the pickles between the different temperature treatments. The variables "skin," "time," and "sample source" were examined. Tukey's test was performed in cases where the interaction between variables was found to be significant.

CHAPTER 4

RESULTS

Mean pH

Noting the acidity levels of each batch was very important as the pH level of the brine of the pickles can effect growth of bacteria, including pathogens. Acidity also plays a role in producing desired flavors in the pickles. Final pH values lower than 4.6 in the brine recommended in the final pickle product to sufficiently inhibit the growth of *Clostridium botulinum* (Sapers et al., 1979). Accordingly, addition of an acid, commonly vinegar, is recommended in refrigerator dill pickle or half-sour recipes to lower the pH enough to ensure satisfactory preservation and safety from pathogens (Etchells et al., 1976).

The mean pH level of the pickle batches at 71.1°C started at 5.26 ± 0.92. The final reading in this batch was 3.29 ± 0.01 (Table 4.1.). The batch at 82.2°C started at 5.45 ± 0.23 and the batch at 100°C started at 5.18 ± 0.17. The batch at 82.2°C and the batch at 100°C ended with mean pH values of 4.42 ± 0.45 and 4.38 ± 0.42, respectively.

Table 4.1. Mean Brine pH During *Fermentation at 20-22.2˚C for Three Different Pickle Batches Intended for Heat Treatment at 71.1°C, 82.2°C, and 100°C.

Day	71.1°C	82.2°C	100°C
0	5.26 ± 0.92	5.45 ± 0.23	5.18 ± 0.17
3	4.23 ± 0.66	5.12 ± 0.16	4.76 ±0.33
5	3.45 ± 0.24	4.57 ± 0.44	4.50 ± 0.39
7	3.29 ± 0.01	4.42 ± 0.45	4.38 ± 0.42

*These column headings refer to the heat treatment procedure to which the batches were subjected after 7 days of fermentation.

Listeria monocytogenes **Populations By Temperature Treatment**

Enumeration of the initial loads (preheat) of *Listeria monocytogenes* shows that the level varied across temperature treatments and across the brine, core and skin. Overall between all treatments, there was decrease in the numbers of microorganisms. However, the decrease is not totally uniform or consistent in all treatments.

At temperature treatment of 71.1˚C, the mean initial load (preheat) in the brines was 6.31 cfu/ml with a standard deviation of 0.54 (Table 4.2). The core and skin initial loads (preheat) were less than the brine load. At 71.1˚C, the mean core preheat load was 4.28 cfu/g with a standard deviation of 3.19, and the mean skin load was 4.07 cfu/cm² with a standard deviation of 3.02.

The number of colony-forming units between the brine, core and skin samples was eliminated at different rates. Also, between the samples, the levels of colony-forming units decreased below detection levels at different times. In the brine, the amount of *L. monocytogenes* decreased below the level of detection by 2.5 min. In the core and skin, the amounts fell below the level of detection by 4.5 min. At this temperature treatment, inactivation did not occur uniformly across the samples. For example, in the skin, at time 0.25 min, the level of *L. monocytogenes* was found to be below the detection level, but at 1 min of treatment, the levels were found to be 1.03 ± 0.75 cfu/cm². This occurs at various times across all samples in this treatment.

Table 4.2. Mean population of *Listeria monocytogenes* by Sample Source Prior to and During Heat Treatment at 71.1˚C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
Preheat	6.31±0.54	4.28±3.19	4.07±3.02
0.00	3.30±3.82	<0.60±0*	1.29±0.98
0.25	1.19±0.83	2.78±3.08	<0.60±0*
0.50	1.25±0.92	<0.60±0*	<0.60±0*
0.75	<0.60±0*	1.10±0.71	<0.60±0*
1.00	4.36±5.42	1.71±1.92	1.03±0.75
1.25	<0.60±0*	1.90±1.84	1.05±0.64
1.50	<0.60±0*	<0.60±0*	<0.60±0*
1.75	<0.60±0*	<0.60±0*	<0.60±0*
2.00	1.27±1.15	<0.60±0*	<0.60±0*
2.50	<0.60±0*	<0.60±0*	<0.60±0*
3.00	<0.60±0*	<0.60±0*	1.19±1.02
3.50	<0.60±0*	<0.60±0*	<0.60±0*
4.00	<0.60±0*	1.33±1.26	1.06±0.80
4.50	<0.60±0*	<0.60±0*	<0.60±0*
5.00	<0.60±0*	<0.60±0*	<0.60±0*

*When numbers were below the detection level (<0.60), presumptive positive samples in UVM were not done for this heat treatment. UVM results will be indicated for other temperature treatments.

At 82.2˚C, the mean initial *L. monocytogenes* population for the brine was higher than the core and skin at 6.5 ± 0.15 cfu/ml (Table 4.3). The mean initial populations (preheat) for core and skin were 4.94 ± 0.56 cfu/g and 5.41 ± 1.34 cfu/cm², respectively. The levels of colony-forming units never fell below detection levels in the brine. However, in the core and the skin, levels fell below levels of detection at 5 min.

Inactivation of *L. monocytogenes* cells did not occur uniformly at 82.2˚C. On the skin and in the core, numbers of *L. monocytogenes* colony-forming units fell below detection levels by 5 min. of heating time. Levels were still at 1.27 ± 1.15 log_{10}cfu /ml after 5 min in the brine.

There was one reading below detection level at 2 min on the skin, but subsequent readings were higher again until 5 min. Even though inactivation of *L. monocytogenes* did not occur uniformly, the overall trend for brine, core and skin was a reduction in colony-forming units. In situations where *L. monocytogenes* populations were undetectable by plating, the organism was not recoverable by enrichment.

Table 4.3. Mean population of *Listeria monocytogenes* by Sample Source Prior to and During Heat Treatment at 82.2˚C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
Preheat	6.5±0.15	4.94±0.56	5.41±1.34
0.00	1.86±1.35	2.57±1.76	2.11±1.58
0.25	1.38±0.68	1.87±1.11	2.01±2.45
0.50	1.47±0.79	1.61±1.74	3.37±1.87
0.75	1.47±0.75	2.21±2.79	1.00±0.70
1.00	1.42±0.72	1.35±1.29	2.03±2.47
1.50	0.99±0.39	0.96±0.62	1.72±1.94
2.00	1.31±0.62	1.42±1.43	<0.60±0 (0/3)
2.50	0.94±0.58	2.03±1.39	1.26±0.84
3.00	1.04±0.75	2.56±3.39	0.89±0.49
3.50	1.09±0.85	1.03±0.75	1.55±1.47
5.00	1.27±1.15	<0.60±0 (0/3)*	<0.60±0 (0/3)*

*When numbers were below the detection level (<0.60), the number in parentheses represent the number of samples containing presumptive *Listeria monocytogenes* cells after enrichment/the number of samples tested in UVM.

At 100˚C, brine and core had similar mean initial loads (preheat) of *L. monocytogenes* colony-forming units (Table 4.4.). In the brine, the initial load (preheat) was 6.89 ± 0.11 cfu/ml. In the core, the initial load (preheat) was 6.56 ± 0.30 cfu/g and in the skin, the initial load (preheat) was 4.46 ± 0.71 cfu/cm².

The overall trend for inactivation at 100˚C was a decrease, which was mostly uniform, with the exception of one value in the core at 0.25 min. The number of colony-forming units fell below detection levels in the brine at 0.25 min. In the core and skin, the number of colony-forming units fell below levels of detection at 0.75 minutes. In situations where *L. monocytogenes* populations were undetectable by plating, the organism was not recoverable by enrichment.

Table 4.4. Mean population of *Listeria monocytogenes* by Sample Source Prior to and During Heat Treatment at 82.2˚C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
Preheat	6.89±0.11	6.56±0.30	4.46±0.71
0.00	1.06±0.40	1.86±1.6	2.20±1.39
0.25	<0.84±0.40 (0/3)*	2.27±2.3	1.76±1.30
0.50	<0.84±0.40 (0/3)*	1.7±1.31	1.70±1.35
0.75	<0.84±0.40 (0/3)*	<0.84±0.40 (1/3)*	<0.84±0.40 (0/3)*
1.00	<0.84±0.40 (0/3)*	<0.84±0.40 (0/3)*	<0.84±0.40 (0/3)*

*When numbers were below the detection level (<0.84), the number in parentheses represent the number of samples containing presumptive *Listeria monocytogenes* cells after enrichment/the number of samples tested in UVM.

Reductions of *Listeria monocytogenes* by Temperature Treatment

To analyze data obtained in this study, it was necessary to control for the initial populations of *L. monocytogenes* in each replication and at each temperature treatment. Although the same procedure for inoculating the pickles was used in every treatment and replication, the numbers of cells of bacteria that the pickles and brine retained varied. To control for the variations in the starting loads between treatments and replications, the differences between the logarithm of colony forming units (log_{10} CFUs) at the sample times and the number

of $\log_{10}$ CFUs in the initial populations (preheat) were calculated. For the purposes of this study and analysis of data, this difference was called "log reduction." In each temperature treatment of 71.1˚C, 82.2˚C, and 100˚C, the analysis was completed with a model that contained two variables, time and sample source, where time was discrete. The time limits chosen for analysis of reductions were based on the point at which no detectable growth was observed in the brine. Sampling intervals also varied by temperature treatment. In the analysis, time was considered discrete instead of continuous. Significance level for this analysis is $P<0.05$.

At temperature treatment 71.1°C, there were no statistically significant differences due to time or sample source. This means that the temperature of the treatment does not depend on the sample source and the sample source does not depend on the temperature. The log reduction at time 0 min in the brine and the core are the same at 2.70 ± 3.82 (Table 4.5). The skin log reduction at time 0 min was 2.01 ± 2.84. The population decreased over time with a 5 log reduction noted after 0.5 minutes. At 1 min, the mean reduction was less, but this mean is based on replications with considerable variability. In the core and on the skin, the population reduction was initially the same as that for the brine, but did not decrease as much over time. On the skin, there was variability in the log reduction of *L. monocytogenes.* However, overall, decrease or increase was small.

Table 4.5. Mean Reduction in Population of *Listeria monocytogenes* by Sample Source Prior to and During Heat Treatment at 71.1°C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
0.00	2.70 ± 3.82	2.70 ± 3.82	2.01 ± 2.84
0.25	4.16 ± 0.09	2.70 ± 3.82	2.70 ± 3.82
0.50	5.40 ± 0	2.70 ± 3.82	2.70 ± 3.82
0.75	5.40 ± 0	2.20 ± 4.52	2.70 ± 3.82
1.00	1.95 ± 4.89	2.56 ± 2.71	3.05 ± 2.76
1.25	5.40 ± 0	1.40 ± 5.66	2.25 ± 4.46
1.50	5.40 ± 0	2.70 ± 3.82	2.70 ± 3.82
1.75	5.40 ± 0	1.11 ± 1.57	2.70 ± 3.82
2.00	5.04 ± 0.62	3.68 ± 3.19	3.48 ± 3.02
2.50	5.40 ± 0	2.70 ± 3.82	2.70 ± 3.82
3.00	5.71 ± 0.54	3.68 ± 3.19	2.89 ± 4.03
3.50	5.40 ± 0	2.70 ± 3.82	2.70 ± 3.82
4.00	5.71 ± 0.54	2.95 ± 2.73	3.02 ± 2.75
4.50	5.40 ± 0	2.70 ± 3.82	2.70 ± 3.82
5.00	5.71 ± 0.54	3.68 ± 3.19	3.48 ± 3.02

*At the temperature treatment of 71.1ºC, there are no statistically significant differences due to time or sample source.

At 82.2°C, in the brine, there is a greater reduction in population of *L. monocytogenes* as time increased, where at time 0 minutes, it is 5.31 ± 2.26 and at 5 minutes, it is 5.90 ± 1.85 (Table 4.6.). Some samples in between indicated even greater population reductions; the overall trend was still a decreasein bacteria over time. In the core, there was an overall trend of increased reduction in *L. monocytogenes* as time progressed, even though it was not a straight linear trend line. In the skin, the same trends were observed.

In the treatment at 82.2˚C, the interaction between the time and the sample source was not found to be significant. However, the sample source was very significant with a $P<0.0001$ (Tale 4.6.). The skin and core were similar to each other. At this temperature treatment, the

brine had the largest reduction in *L. monocytogenes.* When controlling for time, the population reduction in brine was significantly higher than the core and skin.

Table 4.6. Mean Population Reduction of *Listeria monocytogenes* by Sample Source Prior to and During Heat Treatment at 82.2°C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
0.00	5.31 ± 2.26 A	2.38 ± 2.31 B	3.30 ± 2.86 B
0.25	5.79 ± 1.69 A	3.08 ± 1.22 B	3.40 ± 3.16 B
0.50	5.70 ± 1.78 A	3.34 ± 1.80 B	2.04 ± 1.53 B
0.75	5.70 ± 1.76 A	2.74 ± 2.81 B	4.41 ± 1.87 B
1.00	5.74 ± 1.00 A	3.60 ± 1.37 B	3.39 ± 3.49 B
1.50	6.18 ± 1.07 A	3.99 ± 0.81 B	3.69 ± 3.00 B
2.00	5.86 ± 1.63 A	3.52 ± 1.50 B	4.81 ± 1.34 B
2.50	6.23 ± 1.38 A	2.92 ± 1.95 B	4.15 ± 1.92 B
3.00	6.13 ± 1.51 A	2.40 ± 3.40 B	4.53 ± 1.71 B
3.50	6.07 ± 1.60 A	3.91 ± 0.91 B	3.87 ± 2.60 B
5.00	5.90 ± 1.85 A	4.35 ± 0.56 B	4.81 ± 1.34 B

*****The interaction between time and sample source was not found to be significant. Within a row, means with different letters are significantly different ($P<0.0001$).

In the 100°C treatment, the log reduction of the population in the brine at 0 min started at 5.83 ± 0.43 and increased to 6.06 ± 0.32 by 1 min of heating (Table 4.7.). In the core, the log reduction started at 3.38 ± 1.52 at 0 minutes time and increased to 5.26 ± 0.45 by 1 min. In the skin, the log reduction at time 0 minutes was 2.26 ± 2.04 and it increased to 3.62 ± 1.06 by 1 min. All of the sample sources in the 100°C followed a trend of increased log reduction from time 0 min to 1 min of heating.

In the 100˚C treatment, the interaction between time and sample source is not significant. However, when analyzed separately, the sample source was found to be significant (Table 4.7.).

Table 4.7. Mean Population Reduction of *Listeria monocytogenes* by Sample Source Prior to and During Heat Treatment at 100°C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
0	5.83 ± 0.43 A	3.38 ± 1.52 B	2.26 ± 2.04 C
0.25	6.06 ± 0.31 A	3.82 ± 2.04 B	2.70 ± 1.99 C
0.5	6.06 ± 0.32 A	4.41 ± 1.04 B	3.02 ± 1.41 C
0.75	6.06 ± 0.32 A	5.26 ± 0.45 B	3.62 ± 1.06 C
1	6.06 ± 0.32 A	5.26 ± 0.45 B	3.62 ± 1.06 C

*The interaction between time and sample source is not significant. Within a row, means with different letters are significantly different ($P<0.0001$).

Time to Reach 5 Log Reductions

For the purposes of this study, it was important to know how long it takes to see a significant reduction in *L. monocytogenes*. A reduction of 5 log is commonly used in food processing standards to ensure safety of foods. Based on precedence set by a long history of food processing standards, a 5 log reduction was considered significant for this study. This experiment was designed to determine the amount of time needed to heat treat the pickles so that they achieve a 5log reduction.

The mean times in min to reach the 5 log reduction in the brine, core and skin were determined. When analyzing the time to 5 log reduction ("goal time"), the interaction between the temperature and sample source was found to be insignificant at a significance level of 0.05. Therefore, the comparison between the temperature levels does not depend on the sample source and the sample source does not depend on the temperature. When the interaction between temperature and sample source was then dropped out of the analysis, there is a significant difference ($P<0.0093$) in goal time across the different temperature treatments (Table 4.8.). There is not, however, a significant difference among sample sources when temperature is

dropped out. (LS means analysis was used due to unbalanced data as this test corrects as much as possible the bias caused by the unbalanced data.)

Table 4.8. Mean Time to 5log Reduction in *Listeria monocytogenes* in the Brine of Pickle Batches During Heat Treatment at Three Different Temperatures.

Temperature (°C)	Mean Goal Time (min)
71.1	2.39 AB
82.2	3.46 A
100	0.40 B

*Within a column, means with different letters are significantly different (P<0.0093).

Next, temperatures were compared within each sample source separately; then, the sample sources were compared within each temperature (Table 4.9). Temperature was only significant when the sample source was the core. This seems to contradict the finding that comparison of temperatures does not depend on the sample source; however, power was reduced in this analysis as the number of data points was small. There was no significant difference at significance level 0.05 across sample sources within each temperature treatment.

Table 4.9. Mean Time to 5 log Reduction in *Listeria monocytogenes* in the Brine and Core and on the Skin in Pickles Heated at Three Different Temperatures.

Temperature (°C)	Brine	Core	Skin
71.1	1.53 ± 1.28	3.00 ± 1.41 AB	2.63 ± 3.36
82.2	2.50 ± 2.50	5.00 ± 0 A	3.00 ± 2.83
100	0 ± 0	0.58 ± 0.52 B	1.00

*The interaction between temperature and sample source was not significant. Within a column, means with different letters are significantly different (P<0.0092).

Isolate Confirmation

L. monocytogenes was confirmed to be the microorganism found on the 9 randomly selected samples.

Texture Analysis

As stated previously, one of the objectives of this study was to determine a heat treatment method to apply to refrigerator dill pickles that would ensure safety from *L. monocytogenes*. Refrigerator dill pickles are not a canned product and do not receive heat treatment normally. Also, they are stored in the refrigerator. Therefore, this type of pickle can be more firm than other types of canned pickles that receive heat treatment. For this reason, it is important to know if the firmness of the pickle is affected by the heat treatment. This study examined the firmness of the pickles that were heated to 82.2, 93.3, and 100˚C as compared to raw cucumbers, which would represent the ultimate firmness possible. The test was performed with the skin on and off and in 3 sample sources on each pickle. The sample source of sampling on each cucumber was found not to be significant. The interaction between the skin type and temperature was found to be significant. This meant that the comparison between the skin type depended on the temperature and the temperature depended on the skin type. With the skin off, there were no differences among temperature treatments (Table 4.10). With the skin on, the raw cucumber required significantly less force than only the pickle heated at 100ºC. However, there were no significant differences among any of the heated pickles. Within a given heat treatment, there was a significant difference between skin types, except in the raw cucumber.

Table 4.10. Mean Puncture Force by Skin Type for Raw Cucumbers and Pickles Heated at Three Different Temperatures.

Temperature (˚C)	Skin On Force (Newtons)	Skin Off Force (Newtons)
Raw Cucumber (No Treatment)	11.65 B	11.22
82.2	16.03 ABa	8.48 b
93.3	15.98 ABa	9.14 b
100	16.86 Aa	8.74 b

*Within a column, means with different uppercase letters are significantly different ($P<0.05$). Within a row, means with different lowercase letters are significantly different ($P<0.0005$).

Aerobic Bacterial Counts By Temperature Treatment

Samples were also taken and plated on Plate Count Agar (PCA) to enumerate the numbers of aerobic bacteria. The brine and core samples for this heat treatment started with similar initial populations (preheat). The number of microorganisms in the brine was 6.29 ± 0.49 $\log_{10}$cfu (Table 4.11). The initial number of microorganisms in the core was 6.23 ± 0 $\log_{10}$cfu. The skin started with initial populations below the detection level.

In the brine, overall, the numbers of microorganisms decreased as time increased. At 2.5 minutes in the brine, the numbers of bacteria were below the detection level, and remained so as heating increased to 5 minutes. In the core, the numbers of microorganisms decreased to below the detection level at 15 minutes and remained so except for two samplings. On the skin, the numbers of microorganisms were below the detection level at preheat and time 0, although some intervals showed higher populations. The sampling times showing higher populations also had greater variability among replications. This trend was noticed in the data for all sample sources.

Table 4.11. Mean Population of Aerobic Bacteria in the Brine and Core and on the Skin of Pickles Prior to and During Heat Treatment at 71.1°C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
Preheat	6.29±0.49	6.23±0*	0.60±0*
0.00	6.95±1.34	0.60±0*	0.60±0*
0.25	1.19±0.83	0.60±0*	0.60±0*
0.50	0.60±0*	0.60±0*	0.60±0*
0.75	0.60±0*	1.45±1.20	1.63±1.44
1.00	2.7±3.63	2.48±3.25	2.83±2.69
1.25	1.24±0.90	3.65±0.07	1.52±1.30
1.50	0.99±0.55	0.60±0*	1.10±0.71
1.75	0.60±0*	0.60±0*	2.25±2.33
2.00	1.32±1.26	2.89±2.22	1.41±1.40
2.50	0.60±0*	0.60±0*	1.75±1.62
3.00	0.60±0*	0.60±0*	0.60±0*
3.50	0.60±0*	0.60±0*	0.60±0*
4.00	0.60±0*	0.60±0*	0.60±0*
4.50	0.60±0*	0.60±0*	0.60±0*
5	0.60±0*	1.80±2.08	2.27±2.89

*Numbers were below the detection level (<0.60)

Treatment at 82.2˚C produced an overall trend of decrease in the surviving numbers of microorganisms among the brine, core and skin (Table 4.12). In the brine, the initial number of bacteria was 6.23 ± 0.27 log_{10}cfu. Although there were minor increases at 2 minutes and 5 minutes, the general trend was a reduction in overall numbers of bacteria in the brine. In the core, there was also a population decrease from 5.50 ± 0.72 to below the detection level at 5 min, with several deviations from the overall trend. The initial load in the skin was 6.06 ± 1.51. The overall trend in numbers of bacteria on the skin was also a decrease. There were no detectable survivors at 5 minutes on the skin, or in the core.

Table 4.12. Mean Population of Aerobic Bacteria in the Brine and Core and on the Skin of Pickles Prior to and During Heat Treatment at 82.2°C.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
Preheat	6.23±0.27	5.50±0.72	6.06±1.51
0	3.44±1.54	3.43±2.45	3.52±2.88
0.25	2.85±1.10	2.82±1.92	3.00±2.81
0.5	2.69±0.80	4.02±2.41	4.53±3.45
0.75	2.52±0.49	3.47±2.78	2.32±2.98
1	2.32±0.43	2.50±3.29	2.80±3.80
1.5	2.14±0.46	1.99±2.41	2.64±2.95
2	2.72±0.57	3.98±1.61	0.99±0.68
2.5	2.34±0.12	3.12±2.58	2.86±1.96
3	2.28±0.14	5.34±3.20	1.17±0.98
3.5	2.20±0.10	1.75±1.09	3.34±2.42
5	2.40±0.11	0.60±0*	0.60±0*

*Numbers were below the detection level (<0.60).

During heat treatment at 100˚C, the brine initial population of bacteria in the brine was 6.67 ± 0.29 $log_{10}CFU$ and decreased to below detection levels at 0 min through 5 min of heating (Table 4.13). The initial load of microorganisms in the core was 5.61 ± 1.32 $log_{10}CFU$. The trend during heating in the core was decrease to 1.07 ± 0.40 at 5 minutes. The initial population on the skin was 4.94 ± 0.30 and decreased to 1.30 ± 0 at 5 min of heating. Overall, heating at 100˚C produced more consistent decreases in numbers of surviving bacteria over time.

Table 4.13. Mean Population of Aerobic Bacteria in Brine and Core and On Skin of Pickles Prior to and During Heat Treatment at Three Temperatures.

Time (min)	Brine $Log_{10}CFU$	Core $Log_{10}CFU$	Skin $Log_{10}CFU$
Preheat	6.67±0.29	5.61±1.32	4.94±0.30
0	0.60±0*	2.71±1.25	2.96±1.45
0.25	0.60±0*	2.50±2.08	2.52±1.34
0.5	0.60±0*	1.92±1.07	1.94±1.10
0.75	0.60±0*	1.07±0.40	1.30±0*
1	0.60±0*	1.07±0.40	1.30±0*

*Numbers were below the detection level (<1.30 in skin and <0.60 in brine and core).

CHAPTER 5

DISCUSSION

The results of this study showed that the reduction in *Listeria monocytogenes* was not consistent across all three temperature treatments and sampling sources of brine, skin and core. A linear reduction in bacteria was not demonstrated as a function of time or as a function of temperature. There was inconsistency in numbers of recovered bacteria at times, wherein at some time points the population of bacteria was more than the initial sample level. During heat treatment, it is expected that the number of colony forming units on the media plates will decrease over time. However, as the heat treatment progressed in some replications, there was a fluctuation of higher and lower numbers over time and variability was noticeable in the results. Nevertheless, the overall trends from beginning to end of each heat treatment showed decrease in numbers.

Goal Time

For time to 5 log reduction of *Listeria monocytogenes* ("goal time"), temperature did not depend on sampling source and vice versa. There were, however, significant differences among the mean goal time across the different temperature treatments. Tukey's test found that the mean goal time when processed at 82.2°C was a longer than that processed at 71.1°C treatment. At 71.1°C and 82.2°C, it took a mean of 2.39 and 3.46 min respectively to see a 5log reduction in *L. monocytogenes.* While it might have been expected that higher temperatures should reduce the amount of bacteria faster than lower temperatures a linear trend by temperature treatment was not observed. However, at 100°C, a much shorter mean of 0.40 min was needed to reach the

goal of 5 log reduction than at the lower temperatures. It is possible that by the time 100°C is reached and boiling is maintained, there is a more even distribution of heat throughout the mixture as well as to the interior of the cucumbers.

Collective factors affecting bacterial survival in the pickles may explain why there was greater reduction of *L. monocytogenes* at a 71.1°C versus 82.2°C. Past research has found that acid shock and exposure to mild acidity can produce acid tolerance response (ATR) in *L. monocytogenes* (Baik et al., 1996; Davis et al., 1996; O'Driscoll et al., 1996). The fermentation process in this study started with a pH range of 5.18-5.45 among the heat treatments. Over the course of a week, the pH slowly decreased to a range of about 3.29-4.42 among the different treatment temperatures. The pattern of exposure to acidity levels in this study is similar to the past research on acid tolerance response (ATR). Based on previous research regarding ATR, it is possible that the *L. monocytogenes* cells, being exposed to a mild acidity at first developed acid tolerance that enabled them to further survive lower acidity levels. O'Driscoll et al. (1996) found that when exposing *L. monocytogenes* to a pH of 5.5 for one hour, the bacteria were able to better withstand a subsequent pH of 3.3. Also, the exposure to the sublethal acidity was followed by increased survival in other conditions, such as increased thermal stress. The pattern in the acidic conditions in the refrigerator dill pickles in this study are similar to O'Driscoll's study and could indicate ATR and increased resistance to thermal stress. This would be one explanation for the differences in the mean goal time across the two lower temperature treatments. Perhaps the *L. monocytogenes* cells in the 82.2°C treatment may have been more resistant to stresses than the other temperature treatments. However, it is important to remember that there was reduced statistical power as the temperature treatments were examined separate from one another.

Reduction in *Listeria monocytogenes* Populations

When the time to reach a consistent 5 log reduction within all temperature treatments was statistically analyzed, the interaction between sampling source and time was found to be insignificant. In the 71.1˚C treatment, neither time nor sampling source was found to have significant differences. At both 82.2˚C and 100˚C, however, there were significant differences in sampling source, but not in time. The brine population was significantly different from that of the skin and the core at 82.2˚C. At 100˚C, the populations at all sampling source were different from one another (Figures 5.1, 5.2, 5.3).

Variability was evident in the results of the population reduction of *L. monocytogenes* in all heat treatments. This study was performed over the span of a year; thus, pickling cucumbers obtained for this project were subject to seasonal differences. This could be a contributing factor to the observed variability.

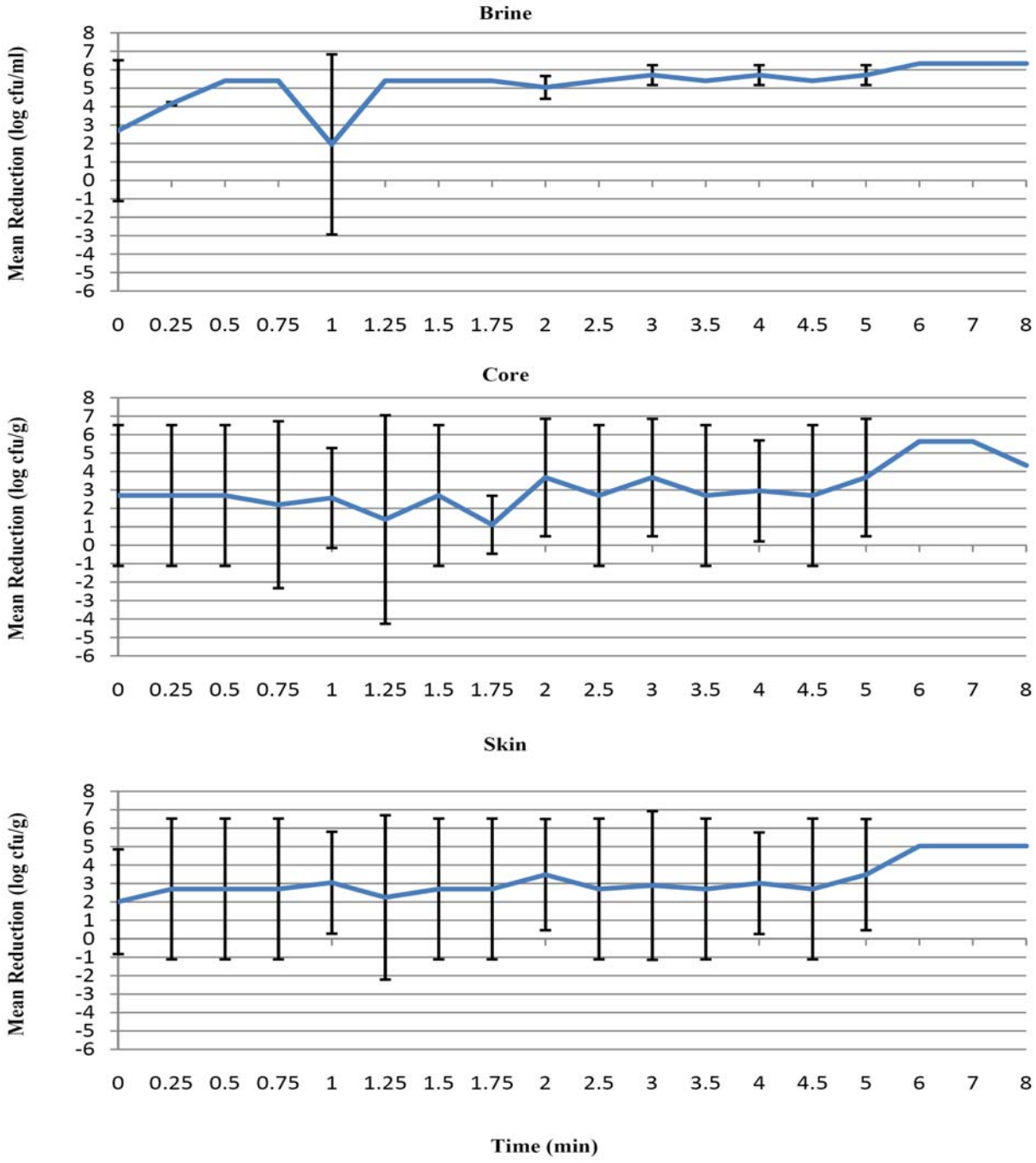

Figure 5.1. Mean Population Reduction in *Listeria monocytogenes* Over Time for Pickles Heated at 71.1°C.

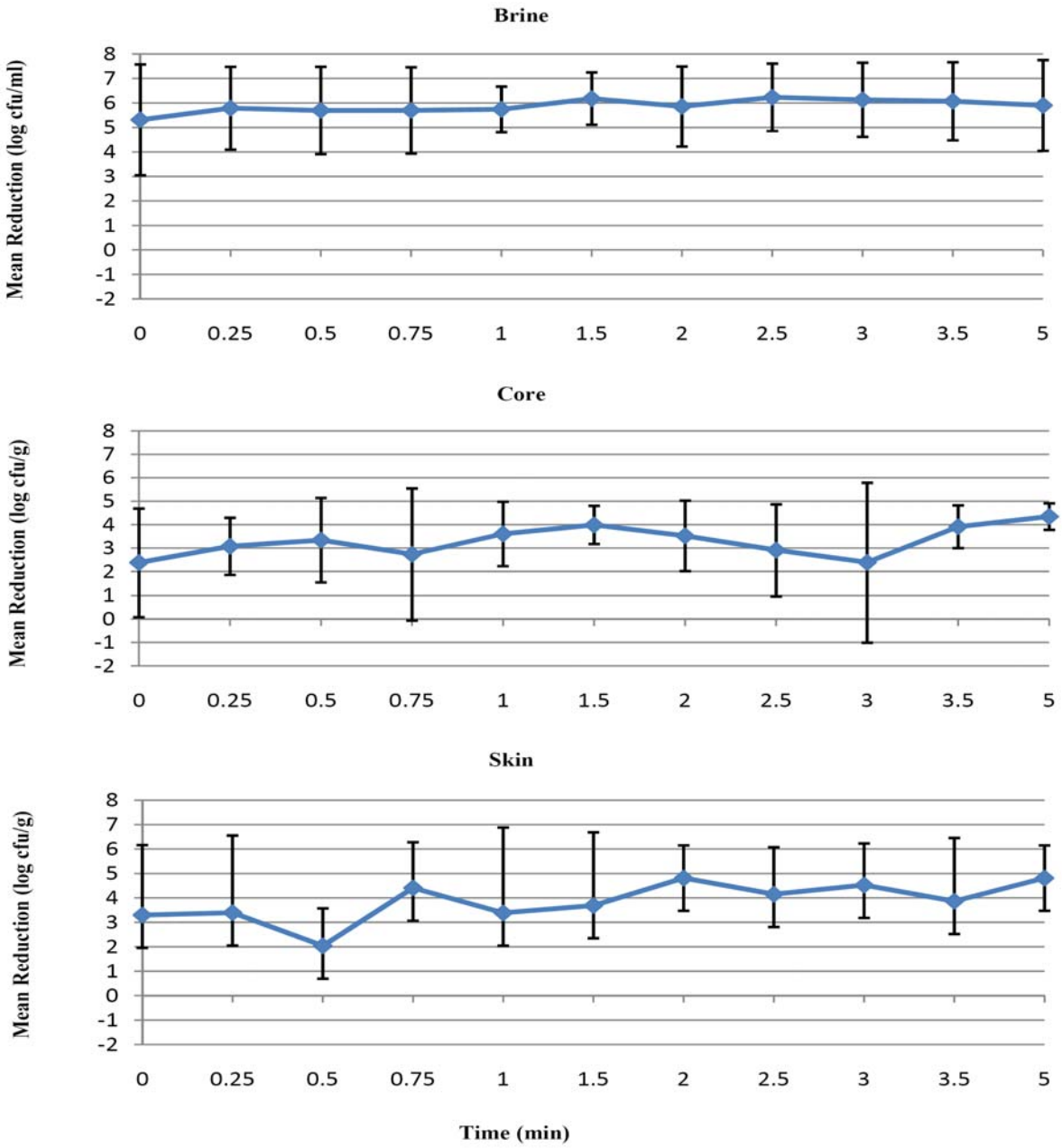

Figure 5.2. Mean Population Reduction in *Listeria monocytogenes* Over Time for Pickles Heated at 82.2°C.

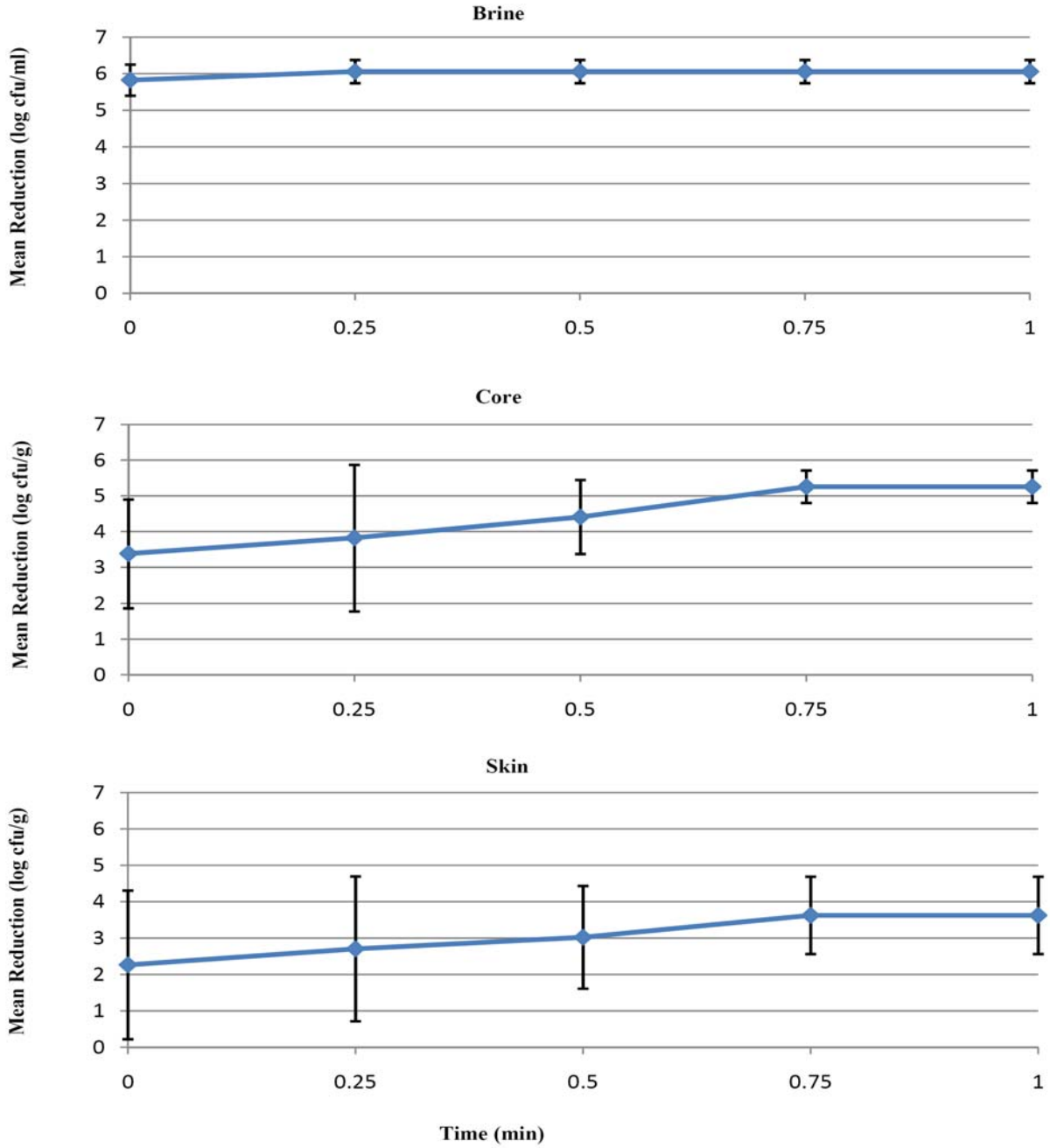

Figure 5.3. Mean Population Reduction in *Listeria monocytogenes* Over Time for Pickles Heated at 100°C.

It is not surprising that the results for reduction in the brine were significantly different from those for the core as liquid heats faster and more uniformly than solids. The skin, being a part of the pickle, as well as having its own surface characteristics, would be expected to behave more like the core than the brine. This trend was observed in this project.

It is difficult to know exactly why the populations reductions with respect to sample source occurred the way they did. It is important to remember that, although every effort was made to choose cucumbers that were very similar in size and shape, they were not precisely uniform in size or density. Pickles that were even slightly larger or denser might have heated more slowly than pickles that were smaller or less dense. Pickles that reached a higher internal temperature or that reached the goal temperature quicker might have had a larger reduction in *L. monocytogenes* populations.

Only a 2.5 cm x 2.5 cm section of skin was removed from the pickles and sampled. It is not known if the distribution of *L. monocytogenes* cells skin of the pickles was uniform. Also, it has been documented that cracks and crevices can hold and harbor microorganisms, making it harder to remove those cells (Beuchat, 2002). It is possible that certain sample sources or areas of the skin may have greater concentrations of *L. monocytogenes* than others. In this case, the sample area may or may not have been a good representation of the total number of microorganisms on the skin of the pickle. This may explain some of the variation in the counts of *L. monocytogenes* amongst the skin samples and may have contributed to the variation in the differences among all sample sources.

Similarly to the skin, the sample area alone from the cores of the pickles may not have been true representations of the total numbers of *L. monocytogenes* in the flesh of the pickles. During fermentation, pickles may not pick up bacterial cells uniformly, as solutes are found to be

taken up and distributed unevenly in previous research (Fasina et al., 2002). Bloater formation may have affected the results as there was little flesh to be sampled. The flesh that was then sampled in those pickles may have been a close representation to the skin than in pickles with more flesh.

Given the differences in the pickles that could not be helped, it may be expected that there would be inconsistent results. Some of the statistical analyses seem to be contradictory, especially in regards to the sample source. However, given the characteristics of the pickles and the behavior of *L. monocytogenes,* it is not surprising that there would be contradictions in the analysis with the number of samples available.

Texture Analysis

The puncture analysis was performed to determine whether it was worth pursuing certain heat treatments from a quality standpoint. Given the observed texture of some of the pickles at 71.1˚C and 82.2˚C treatments, the concern was that heating at 100˚C, even for a short amount of time, might cause such loss in the integrity of the texture that doing so would be pointless. It was decided to compare a brief treatment at 82.2˚C to 93.3˚C and 100˚C. One of the objectives of the study was to find a heat treatment process that would allow safe consumption of the pickles without risk from *L. monocytogenes.* Severe loss of integrity in the texture of the pickles may discourage consumers from adhering to recommendations. Although texture analysis was not the main purpose of this study, it was important to know how similar the textures of the pickles were between treatments compared to raw cucumbers, which was a representation of ultimate firmness. It is important to note that the purpose of this texture analysis was not to determine the quality of the pickle textures or to assume acceptability, but to simply compare the textures between the treatments.

Puncture tests were conducted on cucumbers and pickles with both the skin on and skin off (skin type). Measurement with the skin on would represent the peak force to break the skin and not the firmness of the flesh. The skin-off measurement would more closely approximate the firmness of the interior flesh. Statistical analysis showed that the interaction between skin type and temperature was significant. No significant differences were detected in the samples across temperatures without skin. However, within the skin-on samples, there were significant differences among the temperature treatments. The pickle heated at 100˚C required a significantly higher force than the raw cucumber; the differences between the raw cucumber and pickles heated at 82.2˚C and 93.3˚C were not significant. It makes sense that the raw cucumbers would be significantly different from the pickles heated for 15 seconds at 100˚C. It is also logical that all of the heated pickles would be similar to one another. However, it seems counterintuitive that raw cucumbers would be similar to pickles that have been heated.

The explanation of these findings relies on how the machine measures the force it takes to puncture through the pickle. When the probe contacts the samples with skin on, it encounters resistance from the skin. The force needed to puncture through the skin depends on the texture of the skin. The texture of the skin depends on the extent to which it was heated. When the skin is heated, it softens and becomes pliable. So, at higher temperatures, the skin stretches and takes more time and greater force, and the probe has to travel a greater distance to break through the skin as compared to pickles heated less. This is why the mean force is significantly lower in the raw cucumbers than the pickles heated to 100˚C. One would reason then, that the skin was what determined the force needed to puncture with the probe. This may explain why there were no significant differences in puncture force in samples with the skin removed. As there were no significant differences in the flesh among all temperature treatments, it was expected that heating

at 100˚C for 15 sec should not cause any more loss of integrity in the interior pickle texture than the lower temperature heat treatments and this treatment temperature was included in further experiments. It was expected, however, that the skin on the pickles heated at 100˚C would be more elastic in texture than the other treated pickles.

Limitations

The reason for the inconsistency in the results is unclear. However, there are possible reasons for this occurrence. First, heating was applied to batches of whole pickles. During sampling, random pickles were pulled from random spots in the stockpot at set time intervals. When heating liquids in a stockpot around large pieces of food, cold spots can occur. It is possible that some pickles were pulled from spots that were colder or hotter than others. Thus, all pickles may not have received the same degree of treatment, causing some pickles to show greater survival of *L. monocytogenes* cells.

Secondly, all efforts were put forth to use cucumbers of uniform size and consistency to make the batches of pickles. However, some cucumbers were larger, thicker or denser than others even if the differences were slight. This is important, as bigger or denser cucumbers would take longer to heat up and may not get heated as thoroughly as smaller or less dense cucumbers. Again, not having uniform heating in all pickles may cause some to have greater or lesser numbers of surviving *L. monocytogenes* cells.

Another possible reason that some cucumbers had more viable cells of *L. monocytogenes* lies in how the bacteria get to the interior of the cucumber. During brining, solutes move into and out of the tissue and the interior of the cucumber. When this happens, the bacterial cells come into the tissue with the brine. There is no way to know whether the amount of bacterial cells that travel into the tissue do so in a uniform manner in every cucumber. In previous

research, it has been found that the rate of solute exchange is dependent on the size of the cucumber. In the same study, the amount of sugar in the cucumber was also significantly dependent on cucumber size. Because of the interaction between the lactic acid bacteria with the fermentable sugars inside the cucumber, fermentation occurs in the interior of the cucumber, as well as in the brine. When fermentation occurs in the tissue, acid is produced (Fasina et al., 2002). The variation in acid levels and rate of solute exchange depending on the size and composition of the cucumber could affect the numbers of bacteria that are allowed to survive and grow in the interior of the pickles.

Sampling methods may also be the reason for the inconsistency in results. Because we desired to look at survival both on and inside the pickle, samples were taken from the skin and the core of the same pickle. Every precaution possible was taken to prevent cross-contamination of the skin and core samples of the pickle. However, the fermented and heated pickles were very juicy and often soft. Given the relatively small size of the pickles, the skin and interior flesh are very close in proximity. Some pickles had gas pockets, referred to as bloating, in them. In those cases, the interior flesh had to be scraped from the skin, thus the results of the core might resemble the results in the skin.

Another limitation of this study has to do with the number of data points used in statistical analyses. Sampling time intervals were not consistent across all temperature treatments as larger increments were at first used for the lower temperatures. As procedures were refined, sampling frequency increased for higher temperature treatments. More replications may have been useful once final procedures were decided upon; however, each replication in this type of experiment requires significant investment of time and multiple research assistants for data collection.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Many consumers process foods at home. Fermenting is a type of home food preservation that involves no canning or heating, although canning may be used post-fermentation to allow for room temperature storage of the finish products. Refrigerator dill pickles are partially-fermented and there are many recipes in existence for consumers. Some recipes are published in cookbooks or on websites or blogs. Many recipes may not be published; some consumers may modify or make their own recipes or may have a family recipe not documented or tested. Procedures may not be standardized or scientifically sound.

USDA provides safe, scientifically-sound recommendations for consumers who wish to process foods at home (USDA, 2009). However, recommendations in a previous USDA book (USDA, 1988) for refrigerator dill pickles were withdrawn over food safety concerns. The concern was that the procedure may not prevent growth and survival of *L. monocytogenes,* causing the partially-fermented pickles to be potentially unsafe for consumption (Andress, 2008; Kim et al., 2005). The purpose of this study was to test a heat treatment process that could be applied to refrigerator dill pickles after the partial-fermentation process was ended. The heat treatments applied were at 71.1°C, 82.2°C, and 100°C and the goal reduction in *L. monocytogenes* was 5 log. Another goal of the study was to see if a heat treatment recommendation could be developed for consumers who make the pickles at home.

Based on the findings and analyses of data in this study, it is not recommended that the procedure for this partially-fermented pickle include a heat treatment between the fermentation

process and storage in the refrigerator. Although there were no significant differences in the flesh of the pickles heated at 100°C for 15 seconds as compared to the raw cucumbers and the pickles heated at 93.3°C or 82.2°C, the time needed to see at least a 5 log reduction at 100°C in *L. monocytogenes* is greater than 15 sec; that is, heat treatment at 100˚ for 15 sec does not produce a 5 log reduction in *L. monocytogenes.* Therefore, a specific recommendation could be made at this time for how consumers should heat their refrigerator dill pickles.

Since the recommended heating time is most likely longer than what was tested in this study, it is not known if the treatment time would result in an acceptable pickle texture. These findings, as well as other published research suggest that the role of acid tolerance response in *L. monocytogenes* needs further exploration for this type of pickle. Even the texture analyses in this study did not determine consumer acceptability.

One of the aims of this study was to examine that if there was a 5 log reduction in the core, there would also be a 5 log reduction in the skin and brine. The analysis of the time to 5 log reduction data showed that the core had the longest mean goal time across all temperatures. This means that if the pickles were heated long enough to achieve a 5log reduction in the core, then the brine and skin would also have achieved the same reduction. This is true for all temperature treatments.

Suggestions for Future Research

Because of the inconsistencies in the results of the heat treatment, I conclude that future research needs to examine alternate methods for making these refrigerator dills safe for consumption. One alternative is to lower the pH of the brines. I suggest adding an acid, like vinegar to the brines at some point during the assembly of the pickle batch or during fermentation. This would decrease the pH which would make it harder for the *L. monocytogenes*

to survive and grow in the pickles and brines (Flemming, 1992). Caution must be exercised, however, when this method is attempted. Acidity level and pH are important in the fermentation process. Acidity will affect the growth of beneficial bacteria, specifically lactic acid bacteria. During primary fermentation, lactic acid bacteria grow and lower the pH. The experimentation would need to determine if there is an amount of acid that could reduce the growth of *Listeria monocytogenes* while still allowing the lactic acid fermentation.

One alternate heating method might involve heating the brine before the pickles are added. Then, once the brine reaches the desired temperature, the pickles could be added. The advantage to this method is that one could achieve exposure to a high temperature for a longer period of time before the pickle texture deteriorates as the pickles would not have to endure the heat during a come-up time needed to get the brine up to temperature. When heating the pickles in the brine to a high temperature, like 100°C, the pickles are exposed to higher temperatures for a long period of time. This causes loss of integrity of desired texture in some parts the pickles. Also, this method may be advantageous as it is suggested that long exposure of *L. monocytogenes* to certain combinations of salt, different pH levels and temperatures could affect heat-resistance in the organism (Cole, 2008). One must be careful when coming to this conclusion as most research in heat resistance of *L. monocytogenes* has been conducted in meats and dairy products. There is little research on the subject in vegetables and produce (Mackey et al., 1989). Although a degree of resistance may have been possible during this study, it is more likely that the high-temperature treatments were substantial enough to kill the bacteria.

It may be possible to can the pickles. Although refrigerator dill pickles are subjected to a short fermentation period and then purposefully not canned to achieve certain quality characteristics, it may be necessary to can the pickles after the partial fermentation for

microbiological reasons. One would assume that the integrity of the pickles would endure similar deterioration that was observed in this study. However, further studies should be performed to determine if heat treatment under standard canning procedures would produce a safe, acceptable half-sour dill pickle. Characteristics under both refrigerator and room temperature storage of the canned product could be compared through sensory analyses.

The puncture test in this study was limited in scope and only included to aid in deciding whether or not to continue sampling at a higher heat treatment. One must remember that machines can only measure the characteristics of quality attributes in foods. Only food panels and human subjects can determine overall sensory quality and acceptability of foods (Abbot, 1999). Further sensory evaluation with a sensory panel is needed to conclude acceptability and quality of refrigerator dill pickles pasteurized at 100˚C.

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APPENDICES

Appendix A. Log Reduction in Pickles Heated at 71.1°C, 82.2°C, and 100°C.

Temperature (°C)	Time (min)	Sample source	NObs	N	Mean	Std Dev
71.1		brine	2	2	2.69897	3.81692
	0	core	2	2	2.69897	3.81692
		skin	2	2	2.00886	2.84096
		brine	2	2	4.15938	0.08835
	0.25	core	2	2	2.69897	3.81692
		skin	2	2	2.69897	3.81692
		brine	2	2	5.39794	0
	0.5	core	2	2	2.69897	3.81692
		skin	2	2	2.69897	3.81692
		brine	2	2	5.39794	0
	0.75	core	2	2	2.19897	4.52403
		skin	2	2	2.69897	3.81692
		brine	3	3	1.95332	4.88305
	1	core	3	3	2.56413	2.70906
		skin	3	3	3.04529	2.76483
		brine	2	2	5.39794	0
	1.25	core	2	2	1.39794	5.65685
		skin	2	2	2.24743	4.4555
		brine	2	2	5.39794	0
	1.5	core	2	2	2.69897	3.81692
		skin	2	2	2.69897	3.81692
		brine	2	2	5.39794	0
	1.75	core	2	2	1.11092	1.57108
		skin	2	2	2.69897	3.81692
		brine	3	3	5.04277	0.61517
	2	core	3	3	3.67544	3.18511
		skin	3	3	3.47604	3.01595
		brine	2	2	5.39794	0
	2.5	core	2	2	2.69897	3.81692
		skin	2	2	2.69897	3.81692
		brine	3	3	5.70944	0.53953
	3	core	3	3	3.67544	3.18511
		skin	3	3	2.88924	4.03092
		brine	2	2	5.39794	0
	3.5	core	2	2	2.69897	3.81692

Temperature (°C)	Time (min)	Sample source	NObs	N	Mean	Std Dev
		skin	2	2	2.69897	3.81692
	4	brine	3	3	5.70944	0.53953
		core	3	3	2.95008	2.73379
		skin	3	3	3.01597	2.75425
	4.5	brine	2	2	5.39794	0
		core	2	2	2.69897	3.81692
		skin	2	2	2.69897	3.81692
	5	brine	3	3	5.70944	0.53953
		core	3	3	3.67544	3.18511
		skin	3	3	3.47604	3.01595
	6	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	7	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	8	brine	1	1	6.33244	.
		core	1	1	4.32736	.
		skin	1	1	5.03019	.
	9	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	10	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	11	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	12	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	13	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	14	brine	1	1	6.33244	.
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
	15	brine	1	1	6.33244	.

Temperature (°C)	Time (min)	Sample source	NObs	N	Mean	Std Dev
		core	1	1	5.62839	.
		skin	1	1	5.03019	.
82.2		brine	3	3	5.31162	2.26183
	0	core	3	3	2.38356	2.30672
		skin	3	3	3.29921	2.86485
		brine	3	3	5.78769	1.68955
	0.25	core	3	3	3.07707	1.21755
		skin	3	3	3.39526	3.16148
		brine	3	3	5.69563	1.78489
	0.5	core	3	3	3.34053	1.79792
		skin	3	3	2.03918	1.53395
		brine	3	3	5.69849	1.76203
	0.75	core	3	3	2.73783	2.80843
		skin	3	3	4.41069	1.87134
		brine	3	3	5.74014	0.92954
	1	core	3	3	3.60411	1.37181
		skin	3	3	3.38735	3.49471
		brine	3	3	6.17972	1.07151
	1.5	core	3	3	3.98787	0.8128
		skin	3	3	3.69049	2.99626
		brine	3	3	5.85573	1.63287
	2	core	3	3	3.52189	1.50282
		skin	3	3	4.81207	1.34195
		brine	3	3	6.23251	1.37572
	2.5	core	3	3	2.91537	1.95238
		skin	3	3	4.15131	1.92477
		brine	3	3	6.13217	1.51162
	3	core	3	3	2.39118	3.39878
		skin	3	3	4.52629	1.70672
		brine	3	3	6.07347	1.59453
	3.5	core	3	3	3.91392	0.90953
		skin	3	3	3.86553	2.59237
		brine	3	3	5.89918	1.85178
	5	core	3	3	4.34759	0.56414
		skin	3	3	4.81207	1.34195
	8	brine	1	1	7.73239	.
100	0	brine	3	3	5.82571	0.42633
		core	3	3	3.38053	1.51913

Temperature (°C)	Time (min)	Sample source	NObs	N	Mean	Std Dev
		skin	3	3	2.26426	2.04178
		brine	3	3	6.0587	0.31773
	0.25	core	3	3	3.82315	2.04782
		skin	3	3	2.70423	1.98973
		brine	3	3	6.0587	0.31773
	0.5	core	3	3	4.40881	1.03672
		skin	3	3	3.02232	1.41182
		brine	3	3	6.0587	0.31773
	0.75	core	3	3	5.25683	0.45407
		skin	3	3	3.62438	1.06274
		brine	3	3	6.0587	0.31773
	1	core	3	3	5.25683	0.45407
		skin	3	3	3.62438	1.06274

(.) signifies missing data. Standard Deviation was not calculated when there was only one data point (N = 1)

Appendix B. Mean Goal Time for Treatments 71.1°C, 82.2°C, and 100°C.

Temperature (°C)	Sample source	N Obs	N	Mean	Std Dev
71.1	brine	3	3	1.5833333	1.2829
	core	3	2	3	1.41421
	skin	3	2	2.625	3.35876
82.2	brine	3	3	2.5	2.5
	core	3	2	5	0
	skin	3	2	3	2.82843
100	brine	3	3	0	0
	core	3	3	0.5833333	0.52042
	skin	3	1	1	.

(.) signifies missing data. Standard Deviation was not calculated when there was only one data point (N = 1)

Appendix C. Mean Puncture Force (N) by Skin Type and Sample source in Pickles Heated at 71.1°C, 82.2°C, and 100°C.

Temperature (°C)	Skin Type	Sample source	N Obs	N	Mean	Std Dev
Raw	off	1	3	3	9.10567	5.08173
		2	3	3	12.235	3.68844
		3	3	3	12.32	2.97368
	on	1	3	3	11.7747	4.65077
		2	3	3	11.929	4.84017
		3	3	3	11.2417	5.06201
82.2	off	1	3	3	7.29133	3.75573
		2	3	3	10.2763	1.18514
		3	3	3	7.87467	0.8993
	on	1	3	3	17.0037	5.25792
		2	3	3	16.0487	2.85058
		3	3	3	15.031	5.66385
93.3	off	1	3	3	7.686	0.57519
		2	3	3	9.79233	2.07882
		3	3	3	9.932	4.95854
	on	1	3	3	15.6327	3.76675
		2	3	3	18.1233	1.38475
		3	3	3	14.1843	1.83508
100	off	1	3	3	8.38433	3.12049
		2	3	3	9.27067	4.05367
		3	3	3	8.576	1.26169
	on	1	3	3	16.5287	5.07727
		2	3	3	15.7183	3.5095
		3	3	3	18.3173	4.01021

Appendix D. Log Reduction PROC GLM and Tukey's Test Analysis of Heat Treatment at 71.1°C.

PROC GLM Analysis of Log Reduction by Sample source and Time

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sample source	2	23.66402	11.8320121	1.71	0.1925
Time	5	27.27012	5.45402409	0.79	0.5632

Tukey's Test of Sample source

Sample source	diff LSMEAN	Standard Error	LSMEAN Number
brine	4.51094075	0.639989	1
core	3.17260864	0.639989	2
skin	2.97884219	0.639989	3

Tukey's Test of Time

Time	diff	Standard	LSMEAN
0	2.4689348	1.07313	1
1	2.5209146	0.876207	2
2	4.06475358	0.876207	3
3	4.0913733	0.876207	4
4	3.89183108	0.876207	5
5	4.2869758	0.876207	6

Appendix E. Log Reduction PROC GLM and Tukey's Test Analysis of Heat Treatment at 82.2°C.

PROC GLM Analysis of Log Reduction by Sample source and Time

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sample source	2	93.89154	46.94576777	13.92	<.0001
Time	7	9.669329	1.38133278	0.41	0.8929

Tukey's Test of Sample source

Tukey Grouping	Sample	diff	Standard	LSMEAN
A	brine	5.812693	0.37487869	1
B	skin	3.648194	0.37487869	3
B	core	3.196028	0.37487869	2

Tukey's Test of Time

Time	diff	Standard	LSMEAN
0	3.66479686	0.612174	1
0.25	4.08667443	0.612174	2
0.5	3.69177833	0.612174	3
0.75	4.28233721	0.612174	4
1	4.24386563	0.612174	5
1.5	4.6193596	0.612174	6
2	4.72989596	0.612174	7
2.5	4.43306469	0.612174	8

Appendix F. Log Reduction PROC GLM and Tukey's Test Analysis of Heat Treatment at 100°C.

PROC GLM of Log Reduction by Time and Sample source

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sample source	2	66.00778	33.00388794	27.48	<.0001
Time	4	9.099764	2.27494094	1.89	0.1315

Tukey's Test of Sample source

Tukey grouping	Sample source	diff LSMEAN	Standard Error	LSMEAN Number
A	brine	6.012099	0.28297558	1
B	core	4.425231	0.28297558	2
C	skin	3.047913	0.28297558	3

Tukey's Test of Time

Time	diff LSMEAN	Standard Error	LSMEAN Number
0	3.82350036	0.36532	1
0.25	4.19536202	0.36532	2
0.5	4.49660696	0.36532	3
0.75	4.97996785	0.36532	4
1	4.97996785	0.36532	5

Appendix G. PROC GLM and Tukey's Test Analysis of Puncture Force (N) in Pickles with Skin-On.

PROC GLM of Force by Temperature and Sample source

Source	DF	Type III	Mean	F	Pr > F
Temperature	3	149.6229	49.87428	3.24	0.0358
Sample source	2	3.683627	1.841813	0.12	0.8876

Tukey's Test of Temperature

Tukey Grouping		Mean	N	Temperature (°C)
	A	16.855	9	212
B	A	16.028	9	180
B	A	15.98	9	200
B		11.648	9	Raw

Tukey's Test of Sample source

Tukey Grouping	Mean	N	Sample source
A	15.455	12	2
A	15.235	12	1
A	14.694	12	3

Appendix H. PROC GLM and Tukey's Test Analysis of Puncture Force (N) in Pickles Without Skin.

PROC GLM of Force (N) by Temperature and Sample source

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Temperature	3	41.92337	13.97446	1.63	0.2024
Sample source	2	32.51583	16.25791	1.9	0.167

Tukey's Test of Temperature

Tukey Grouping	Mean	N	Temperature (°C)
A	11.22	9	Raw
A	9.137	9	200
A	8.744	9	212
A	8.481	9	180

Tukey's Test of Sample source

Tukey Grouping	Mean	N	Sample source
A	10.394	12	2
A	9.676	12	3
A	8.117	12	1

Appendix I. PROC GLM and Tukey's Test Analysis of Puncture Force (N) in Raw Cucumbers.

PROC GLM of Force (N) by Skin Type and Sample source

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Skin Type	1	0.825184	0.825184	0.05	0.8328
Sample source	2	9.16741	4.583705	0.26	0.7769

Tukey's Test of Skin Type

Tukey Grouping	Mean	N	Skin Type
A	11.648	9	on
A	11.22	9	off

Tukey's Test of Sample source

Tukey Grouping	Mean	N	Sample source
A	12.082	6	2
A	11.781	6	3
A	10.44	6	1

Appendix J. PROC GLM and Tukey's Test Analysis of Puncture Force (N) by Skin Type and Sample source in Pickles Heated at 82.2°C.

PROC GLM of Force (N) by Skin Type and Sample source

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sample source	2	8.871494	4.435747	0.34	0.7145
Skin Type	1	256.3074	256.3074	19.9	0.0005

Tukey's Test of Skin Type

Tukey Grouping	Mean	N	Skin Type
A	16.028	9	on
B	8.481	9	off

Tukey's Test of Sample source

Tukey Grouping	Mean	N	Sample source
A	13.163	6	2
A	12.148	6	1
A	11.453	6	3

Appendix K. PROC GLM and Tukey's Test Analysis of Puncture Force (N) by Skin Type and Sample source in Pickles Heated at 93.3°C.

PROC GLM of Force (N) by Skin Type and Sample source

Source	DF	Type III	Mean	F	Pr > F
Sample source	2	18.10181	9.050904	1.12	0.3523
Skin Type	1	210.7405	210.7405	26.19	0.0002

Tukey's Test of Skin Type

Tukey Grouping	Mean	N	Skin Type
A	15.98	9	on
B	9.137	9	off

Tukey's Test of Sample source

Tukey Grouping	Mean	N	Sample source
A	13.958	6	2
A	12.058	6	3
A	11.659	6	1

Appendix L. PROC GLM and Tukey's Test Analysis of Puncture Force (N) by Skin Type and Sample source in Pickles Heated at 212˚F.

PROC GLM of Force (N) by Skin Type and Sample source

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sample source	2	3.77699	1.8885	0.15	0.859
Skin Type	1	296.056	296.056	24.1	0.0002

Tukey's Test of Skin Type

Tukey Grouping	Mean	N	Skin Type
A	16.855	9	on
B	8.744	9	off

Tukey's Test of Sample source

Tukey Grouping	Mean	N	Sample source
A	13.447	6	3
A	12.495	6	2
A	12.457	6	1

Appendix M. Temperature (°C) in the Brine, Core, and Skin at Different Times For All Treatments.

Treatment, Rep	Time (min:sec)	Brine 1	Brine 2	Core 1	Core 2	Skin 1	Skin 2
71.1°C, 1	Preheat	20.7	20.7	20.7	20.8	20.3	20.9
	1:00	20.7	20.7	20.6	20.7	20.3	20.9
	2:00	21.3	22.7	20.6	20.7	20.4	21.3
	3:00	24.1	27.1	20.7	20.8	20.7	22.3
	4:00	27.9	28.6	20.7	20.9	21.3	24.0
	5:00	31.8	32.1	20.8	21.1	22.1	25.9
	6:00	35.6	36.3	21.0	21.3	23.2	27.9
	7:00	39.4	39.6	21.4	21.8	24.5	29.7
	8:00	42.9	43.5	21.9	22.6	25.9	31.4
	9:00	46.5	46.7	22.7	23.6	27.4	33.1
	10:00	49.8	50.2	23.8	24.8	29.2	35.0
	11:00	52.9	53.5	25.0	26.2	31.0	37.0
	12:00	56.3	56.6	26.6	27.9	33.0	39.2
	13:00	59.2	59.9	28.3	29.6	35.0	42.3
	14:00	62.0	64.0	30.2	31.4	36.7	45.1
	15:00	64.6	67.5	32.3	33.8	38.2	47.8
	16:00	67.6	68.3	35.0	36.0	40.3	50.8
	17:00	69.2	71.4	36.9	37.8	41.5	52.6
	18:00	71.8	72.3	39.5	40.1	42.8	54.7
	19:00	71.7	72.2	41.1	41.7	43.7	56.1
	20:00	71.7	72.0	44.6	45.0	46.8	57.0
	21:00	71.4	71.6	47.7	47.7	49.2	56.9
	22:00	70.8	71.1	50.1	49.9	50.7	56.0
	23:00	69.9	70.3	52.8	52.4	52.4	55.8
	24:00	69.1	69.5	55.5	54.9	54.9	55.2
	25:00	68.8	70.4	57.2	56.5	56.1	54.8
	26:00	69.9	71.7	59.2	58.2	57.6	54.2
	27:00	.	.	.	.	.	.
	28:00	70.9	72.3	60.3	59.4	57.7	72.7
71.1°C, 2/3	Preheat 1:00	18.3	20.3	18.3	18.5	18.3	18.1
	Preheat 2:00	24.5	23.8	18.5	18.5	18.9	18.2
	Preheat 3:00	31.1	31.8	18.9	18.5	21.6	19.0
	Preheat 4:00	30.1	32.3	21.5	19.0	22.5	21.9
	Preheat 5:00	33.3	39.4	24.4	19.6	26.0	24.9
	Preheat 6:00	38.7	43.1	26.4	20.3	29.3	27.4
	Preheat 7:00	42.3	46.9	29.8	21.6	33.2	30.5
	Preheat 8:00	45.9	48.8	31.9	23.1	36.9	33.6
	Preheat 9:00	50.8	56.9	34.6	25.0	40.5	37.1
	Preheat 10:00	54.4	59.0	37.7	27.0	44.4	40.7
	Preheat 11:00	58.0	62.6	40.0	30.0	48.4	44.8
	Preheat 12:00	59.2	61.1	44.4	32.2	50.9	47.9

Treatment, Rep	Time (min:sec)	Brine 1	Brine 2	Core 1	Core 2	Skin 1	Skin 2
	Preheat 13:00	60.0	61.6	46.4	35.1	52.0	49.7
	Preheat 14:00	61.5	61.9	48.0	39.6	53.7	51.9
	Preheat 15:00	62.9	63.7	51.0	41.1	54.4	53.1
	Preheat 16:00	64.9	64.2	53.4	43.2	55.6	54.4
	Preheat 17:00	65.8	65.5	55.4	45.6	56.8	55.6
	Preheat 18:00	66.5	67.1	57.2	47.9	58.4	56.9
	Preheat 19:00	68.2	69.9	58.0	50.9	60.3	58.5
	Preheat 20:00	69.7	69.3	60.3	52.9	61.7	59.9
	0:00	70.0	71.3	.	.	.	.
	0:15	70.0	69.0	62.0	.	.	.
	0:30	70.0	69.0	.	.	.	.
	0:45	70.0	70.0	.	55.8	.	61.0
	1:00	69.7	69.7	.	56.0	54.0	62.0
	1:15	69.0	69.9	.	57.0	.	.
	1:30	.	.	.	57.0	69.0	.
	1:45	70.4	70.1	.	.	.	62.0
	2:00	69.6	70.3	64.9	.	.	62.0
	2:30	69.6	69.8	65.1	58.0	52.0	62.0
	3:00	69.8	71.0	65.1	59.7	51.0	67.6
	3:30	69.7	70.1	65.6	60.0	50.1	62.6
	4:00	.	70.5	.	.	.	.
	4:30	69.2	70.2	65.4	61.4	48.0	62.9
	5:00	69.3	70.3	65.4	61.9	47.9	62.5
	7:00	69.0	69.2	65.2	63.5	47.3	62.1
	9:00	68.3	68.6	64.4	64.5	46.3	62.5
	11:00	67.1	67.7	63.1	65.2	45.9	63.0
	13:00	66.2	66.6	62.1	65.5	45.1	63.3
	15:00	65.3	65.5	61.3	65.4	44.3	63.0
	17:00	64.0	64.3	60.5	65.2	44.1	62.2
	19:00	62.6	62.8	59.8	64.6	45.0	61.4
	21:00	61.2	61.7	59.2	64.0	45.6	60.5
	23:00	60.3	60.5	58.3	63.3	45.5	59.4
	25:00	59.3	59.4	57.7	62.5	48.2	58.6
180°, 1	Preheat 0:00	16.7	17.0	17.0	16.9	16.9	16.9
	Preheat 5:00	27.6	27.9	17.9	19.4	24.1	26.6
	Preheat 10:00	47.3	46.0	24.3	30.4	39.3	41.2
	Preheat 15:00	61.8	61.7	36.7	45.6	55.2	58.0
	Preheat 20:00	76.9	77.1	51.9	61.7	70.6	72.5
	0:00	80.6	82.1	58.6	68.2	.	.
	0:15	80.9	83.4	59.3	68.6	76.8	79.2
	0:30	81.1	83.9	60.0	69.4	77.5	79.7
	0:45	81.9	84.0	60.9	70.2	78.0	80.0
	1:00	82.5	83.9	61.9	71.1	78.4	80.2
	1:30	81.9	83.9	63.3	72.4	78.9	80.7
	2:00	82.1	83.8	64.8	73.5	79.3	81.1

Treatment, Rep	Time (min:sec)	Brine 1	Brine 2	Core 1	Core 2	Skin 1	Skin 2
	2:30	82.4	83.7	66.1	74.5	79.9	81.0
	3:00	82.5	83.5	67.5	75.2	80.2	81.1
	3:30	83.0	83.0	68.7	76.2	80.2	81.4
	5:00	83.4	83.7	72.2	78.4	80.4	82.0
180°, 2	Preheat 0:00	17.9	18.0	18.0	18.0	18.0	17.7
	Preheat 5:00	30.9	30.1	20.3	18.5	30.2	21.5
	Preheat 10:00	47.7	48.6	30.3	27.0	44.9	27.3
	Preheat 15:00	63.4	64.9	45.9	42.7	61.8	37.5
	Preheat 20:00	78.4	78.7	62.1	59.3	76.9	54.5
	0:00	81.9	82.4	66.5	63.8	.	.
	0:15	82.5	83.0	67.2	64.5	81.5	.
	0:30	83.3	83.0	67.9	65.3	81.5	36.6
	0:45	82.8	83.4	68.6	66.1	81.4	.
	1:00	82.9	82.9	69.4	67.4	81.5	62.2
	1:30	83.1	79.6	70.1	68.3	81.5	61.0
	2:00	87.6	79.4	71.3	69.7	81.3	58.6
	2:30	82.5	78.8	72.6	71.4	81.5	56.7
	3:00	82.5	77.3	73.5	72.5	81.7	55.6
	3:30	82.2	78.0	74.3	73.8	81.8	54.3
	5:00	82.1	77.0	78.5	76.2	82.1	53.3
180°, 3	Preheat 0:00	17.4	17.3	17.3	17.5	17.3	17.4
	Preheat 5:00	29.1	28.6	20.9	18.9	25.3	24.2
	Preheat 10:00	42.2	42.1	32.9	28.3	39.8	38.7
	Preheat 15:00	58.0	56.8	48.7	42.6	54.7	54.4
	Preheat 20:00	72.4	71.4	64.4	58.0	71.4	68.9
	0:00	81.9	82.6	74.2	68.4	78.9	.
	0:15	81.7	81.4	75.0	69.2	79.0	.
	0:30	81.0	83.0	75.5	.	.	.
	0:45	80.9	81.4	75.5	70.0		.
	1:00	64.0	81.2	76.7	.	.	.
	1:30	84.4	81.9	77.2	71.9	80.8	78.8
	2:00	84.2	81.6	78.1	73.0	81.6	79.0
	2:30	83.9	81.2	78.8	74.0	82.3	79.6
	3:00	80.7	80.5	79.7	75.0	81.7	80.3
	3:30	73.3	84.4	80.1	75.7	81.7	81.1
	5:00	85.3	84.9	81.1	77.4	81.0	82.2
	8:00	84.9	84.1	80.4	79.4	80.5	83.2
100°C, 1	Preheat 0:00	18.2	18.4	18.1	18.1	18.4	18.1
	Preheat 5:00	30.7	21.4	32.9	23.9	34.2	19.3
	Preheat 10:00	47.7	30.0	49.7	34.7	50.7	19.4
	Preheat 15:00	66.7	68.0	66.1	50.3	66.9	21.2
	Preheat 20:00	83.4	83.3	82.0	67.3	81.6	25.8
	Preheat 25:00	96.7	97.3	96.8	84.3	97.6	30.7
	0:00	99.1	99.3	99.2	80.9	99.4	51.0
	0:15	98.9	99.2	98.8	81.9	98.6	49.4

Treatment, Rep	Time (min:sec)	Brine 1	Brine 2	Core 1	Core 2	Skin 1	Skin 2
	0:30	99.2	99.3	98.6	82.8	98.3	52.8
	0:45	99.2	99.2	98.7	83.8	98.3	58.3
	1:00	98.8	74.4	98.3	87.9	96.9	55.4
100°C, 2	Preheat 0:00	18.0	18.1	18.1	18.0	18.2	18.1
	Preheat 5:00	30.5	31.4	18.8	27.8	18.7	18.9
	Preheat 10:00	50.3	48.2	25.3	48.5	22.1	24.8
	Preheat 15:00	68.1	66.1	37.4	66.1	21.4	36.1
	Preheat 20:00	84.1	83.5	51.6	83.2	23.7	52.5
	Preheat 25:00	98.5	98.8	66.4	97.9	36.6	68.6
	0:00	99.3	98.9	68.3	98.6	49.7	68.6
	0:15	99.4	99.2	63.8	98.8	58.8	68.3
	0:30	99.6	98.8	77.1	98.9	49.3	69.1
	0:45	99.5	98.9	82.5	98.9	47.7	69.8
	1:00	99.2	98.9	84.9	99.0	45.5	70.4
100°C, 3	Preheat 0:00	19.2	18.8	19.1	19.2	19.4	19.3
	Preheat 5:00	31.5	31.6	22.4	19.4	21.9	22.3
	Preheat 10:00	50.2	51.3	35.5	21.7	31.1	22.8
	Preheat 15:00	61.5	68.2	51.7	26.7	50.2	23.8
	Preheat 20:00	79.5	83.4	66.9	33.3	64.9	31.1
	Preheat 25:00	96.6	97.3	80.5	45.2	72.3	96.6
	0:00	98.1	98.6	83.2	54.7	82.5	96.3
	0:15	98.5	98.9	83.0	58.3	83.1	80.0
	0:30	98.8	98.7	81.2	59.1	83.8	90.9
	0:45	98.9	98.7	93.2	63.4	84.6	56.9
	1:00	98.8	98.7	78.5	70.5	86.0	60.0

(.) signifies missing data.