EVALUATION OF PULSE ELECTRIC FIELDS TO REDUCE FOODBORNE PATHOGEN LEVELS IN SCALDER/CHILLER WATER DURING POULTRY PROCESSING

A Thesis

by

BRADLEY C. MARTIN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2008

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	Marcos X. Sánchez-Plata
Committee Members,	Jorge L. Alvarado
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ABSTRACT

Evaluation of Pulse Electric Fields to Reduce Foodborne Pathogen Levels in Scalder/Chiller Water During Poultry Processing. (December 2008)

Bradley C. Martin, B.S., Texas A&M University

Chair of Advisory Committee: Dr. M. Sánchez-Plata

Poultry slaughtering encompasses a series of processing steps with the objective of harvesting the consumable meat. The scalding process consists of the submersion of carcasses in hot water tanks to facilitate the removal of feathers during slaughter. However, the use of a common scalding tank increases the likelihood of carcass cross contamination considering that dirt, fecal material and even digestive and intestinal contents carrying pathogens and other bacteria are widely spread during this operation. Similar cross contamination occurs in the process of chilling carcasses, which also requires submersion of broilers in communal tanks filled with ice and cold water. A plausible approach to reduce contamination in scalders or chillers is the use of Pulsed Electric Fields (PEF) to decontaminate scalder/ chiller water. PEF uses electricity to kill bacteria suspended in liquid media and could be utilized in poultry scalders and chillers to reduce bacterial contamination on carcasses and reduce the potential risk of pathogens reaching the final consumer.

A pilot scale system was assembled by the use of a pulse electric field generator (Model SF-700, Simmons. Eng. Co., Dallas, GA) coupled with a commercial scalding

tank (Dunkmaster®, Knase Company Inc, MI). *C. coli* and *C. jejuni* along with marker strains of Novobiocin and Nalidixic acid resistant *S.* typhimurium and *S.* enteritidis strains were used in challenge studies evaluating the effects of the PEF on carcasses, scalder and chiller water contamination.

The system was evaluated with 0, 0.5, and 1% sodium chloride in the water with 40 volts of electric current and 0.54 of amperage. Samples were collected at 0, 40, 80, 160, 200 s of treatment with a 10 s on, 5 s off cyclical pulses. The use of PEF in regular scalder/chiller water showed little effect on *Salmonella* and *Campylobacter* reductions. However, with the addition of 0.5% NaCl caused a significant ($P<0.5$) log CFU/ml reduction of *Salmonella and Campylobacter* within the scalder/chiller water at 40, 80, and 160 seconds respectively.

“Every day is a life, being born in daybreak and dying when you go to sleep. Live every day as amazingly as possible. Learn what you can and make it worth something. For once you go to sleep, that day has died; it will never happen again, only to be remembered or forgotten. Carpe Diem." Author Unknown

This thesis is dedicated to those who pushed me beyond the limits I set for myself.

I love you all,
Brad

ACKNOWLEDGEMENTS

Major accomplishments in life are rarely achieved alone; this thesis is no exception. Without my entire family's support and love, none of this could have been possible. Thank you especially to my wonderful wife, Melissa. You have put your dreams and plans on hold to allow me to chase mine. You spent countless hours with me counting plates, making media, conducting experiments, and so much more; never hesitating to help me when needed. It's now time for me to return the favor. To my parents, Curtis and Sharon Martin, without whom, I could have never gotten as far as I have in life. Your financial and moral support can always be counted on. From gas money to escape the monotony of school, if just for the weekend; to a phone call from home to remind me just what I'm working towards and the importance of education in my life. My family's consistent love and support will always be cherished.

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CHAPTER I

INTRODUCTION

LITERATURE REVIEW

The production of chickens has long been associated with the presence of *Salmonella* spp. and *Campylobacter* spp. that can cause human enteric illnesses (Deming et al, 1987). *Salmonella* spp. and Campylobacter spp. present one of the greatest public health hazards in the United States. It has been estimated that approximately 1.4 million cases of Salmonellosis and 2.5 million cases of Campylobacteriosis occur annually due contamination of food products leading to approximate 580 and 124 deaths respectively (Smith et al, 1999). Estimates are that Salmonellosis alone results in medical costs and lost productivity ranging from ~$0.5-2.3 billion (CDC, 2001; Frenzen et al, 1999). *Salmonella* is usually the second most often isolated bacteria, after Campylobacter, associated with food-borne disease (~27.4% of total), with poultry and poultry products accounting for approximately 50% of transmission of *Salmonella* infections (CDC, 1984).

The most common vehicles of *Salmonella* and *Campylobacter* transmission in poultry products are upon the feathers of animals arriving at the processing plants or upon the carcasses that become cross-contaminated with intestinal contents during processing (Oosterom et al, 1983; USDA-FSIS, 2004). With more than 2,800 known

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This thesis follows the style of the journal of Poultry Science.

Salmonella serotypes found in different food animals and the environment, the Food Safety and Inspection Service (FSIS) published a final rule in 1995 establishing performance standards based on the prevalence of this pathogen for certain classes of recently indicated that despite an initial drop in the incidence of *Salmonella* in poultry processing establishments and raw products (FSIS/USDA, 1995). However, FSIS carcasses achieved after the implementation of these standards, the current trends for the last three years indicate a steady increase. This clearly indicates the need to implement stricter controls along the food chain process and the need to develop novel intervention strategies to reduce the presence of *Salmonella* in final processed poultry products.

Salmonella spp. and *Campylobacter* spp. originate in the digestive tract and are spread by cross contamination (Lillard, 1989). Cross contamination can occur at many points in the field or during processing where fecal matter is present. Chicken carcasses and parts are frequently contaminated; this contamination is easily spread to other carcasses from the intestinal tract or from fecal material on feet and feathers (Deming et al, 1987). Cross-contamination is a particular problem at critical steps during processing including defeathering, evisceration and chilling. Cross-contamination from the hands of workers and from equipment and utensils can easily spread the bacterium to uncontaminated carcasses. Further contamination can then occur during subsequent processing, cut-up and preparation activities. Therefore, cross-contamination during normal processing and methods to alleviate it are crucial issues to be addressed by poultry processors, regulators and consumers alike. As a result, research has focused on

effective methods to substantially decrease contamination during the final stages of processing (Thompson et al, 1979; Lillard et al, 1987; James et al, 1992) while minimal efforts have been implemented at the initial stages, where most contamination occurs.

Several methods for chemical and mechanical decontamination of carcasses have been tested and reported in the literature (CAST, 2004). Water sprays with and without bactericides have also been investigated at various pressures, temperature and concentration combinations for decontamination of poultry surfaces (Bautista et al, 1997). Cox et al, (1978) reported a 1 log reduction in total counts of surface bacteria on broiler breast skin from carcasses which had been immersed in 60°C water for 1 min, a 2 log reduction using 71°C water, and a 0.5 log reduction using water below 60°C. Carcasses receiving 60°C water treatment or higher exhibited a partially cooked appearance, thus affecting the organoleptic properties of the product. Several decontamination strategies have been proposed and researched over the years (Dincer and Baysal 2004), but the demand for better safety and quality, less energy consumption, and lower costs have compelled poultry processors and researchers to devise better techniques and approaches to address the matter.

Recently, a great amount of attention has been devoted to bacterial inactivation by electrical treatment in several food processing applications (Toepfl et al, 2007) These efforts are aimed at minimizing the use of thermal energy and chemicals as antimicrobial interventions. In the last few years, electrical treatments that rely on pulsed electrical fields (PEF) have received the greatest emphasis (Schoenbach et al, 1997; Schoenbach et al, 2000; Ravishankar et al., 2002; Abou-Ghazala et al, 2002; Beveridge et al, 2002;

Dincer and Baysal, 2004; Feng et al., 2004; Reyns et al., 2004; Wu et al., 2004; Korolczuk et al, 2006; Wesierska and Trziszka, 2007; Toepfl et al, 2007). Schoenbach et al, (1997, 2000) demonstrated that bacteria inactivation by PEF can be achieved using the appropriate pulse width (60 ns to 1 ms), amplitude (100 V/cm – 100 kV/cm), and single-shot or repetitive operation approach. Ravishankar et al, (2002) also found that temperature and pH level also play a role in PEF treatments which can reduce bacteria count by 3-log. Recent studies have focused on ways to preserve electrodes, minimize energy requirements, and utilize stacked pulses (Wu et al, 2004). In egg processing, Wesierska and Trziszka (2007) determined that PEF reduced bacteria counts by as much as 5-log.

POULTRY RELATED FOODBORNE PATHOGENS

Salmonella spp.

Theobald Smith, research-assistant to Daniel E. Salmon, discovered the first strain of *Salmonella* – *Salmonella* cholerae suis – in 1885. Since that time, the number of strains of *Salmonella* known to cause Salmonellosis has increased to in excess of 2,800. *Salmonella* spp. is a genus of the family Enterobacteriaceae. *Salmonella* spp. is a gram-negative non-spore forming rod shaped bacterium. Its optimal growth temperature is 37°C (Holt, *et al*, 1994) the growth rate is reduced at temperatures below 15°C, and prevented below 7°C. *Salmonella* strains are commonly found in aerobic conditions but

are classified as facultative anaerobic. Water activity and pH are important for the growth of *Salmonella*. The minimum water activity and pH needs for growth of *Salmonella* is 0.94 and 3.8 respectively (ICMSF, 1996). Conditions with much lower water activity can maintain *Salmonella's* viability, however no growth is sustained until water activity has been increased to preferred conditions. Most strains of *Salmonella* are mobile with the exception of only a few species. Most *Salmonella* organisms are found in the intestinal tract of man and other animals as either pathogens or commensals. This frequent characteristic of *Salmonella* causes it to be very common in all types of fresh meats and can also be found in the environment surrounding the habitat of livestock. *Salmonella* is easily transmitted through feces. Infected humans and animals can become carriers and continually shed the bacterium during defecation.

It is estimated that only about 3 percent of *Salmonella* cases are officially reported nationwide, and many milder cases are never diagnosed. The true incidence is, undoubtedly, much higher (Mead, 1999). This is often due to the short duration of the infection and the lack of patients needing to be evaluated by a medical specialist. After infection of *Salmonella*, Salmonellosis can progress through the body very quickly. Symptoms may appear in as little as 6 hours after the ingestion of *Salmonella*. Persons who become infected with *Salmonella* commonly develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection (CDC, 2001). The illness usually lasts 4 to 7 days, and most infections do not require medical treatment. However, in some cases, the diarrhea may be so severe that the patient needs to be hospitalized. In some very severe cases, Salmonellosis may spread from the intestines to the blood stream and

then to other body sites. Other prolonged effects could include arthritis in joints that last up to 3-4 weeks after the initial onset

***Campylobacter* spp.**

Campylobacter is the most common bacterial cause of human gastro-intestinal infections. *Campylobacter* is widespread within intestinal microflora of many warm-blooded animals. *C. jejuni* and *C. coli* are the main representatives of pathogenicity from the genus *Campylobacter*, and they have genomes approximately 1.7Mb in size, as determined by pulsed-field gel electrophoresis (Taylor, 1992). *Campylobacter* is extremely susceptible to the environment; however, under favorable conditions, *Campylobacter* can inflict havoc on processors and consumers alike. *Campylobacter* is a gram-negative pathogen with curved rod shape that needs a micro-aerophilic environment to grow. Campylobacters have been known to cause disease in animals since the early 1900's. *Campylobacter* is endemic in chickens at much higher levels than salmonella. It pervades all stages of chicken production, and its initial appearance in poultry by 4 weeks of age may be related to the decline in maternal antibodies (Aho, 1988). This organism has now become the leading cause of bacterial diarrhea in the United States and Britain with an estimated 2.4 million cases each year in the United States. It has since passed the widely known *Salmonella* (Atabay et al, 1998). Since Campylobacter is widespread among the intestinal micro flora of many warm-blooded animals, food processors are required to become more vigilant in their food handling processes to avoid product contamination

Recent developments for methods to isolate *Campylobacter* from samples have allowed researchers to better understand and study this organism. Many healthy chickens carry these bacteria in their intestinal tracts (Aho *et al,* 1988, Fernández *et al*., 2000). Additional known sources of these bacteria are contaminated non-chlorinated water and even home pets. The infective dose of *C. jejuni* is considered to be small. Human feeding studies suggest that about 400-500 bacteria may cause illness in some individuals, while in others, greater numbers are required.

The most common symptoms associated with an infection of campylobacter, also known as Campylobacteriosis, is diarrhea, cramping, and abdominal pain. Recently, *C. jejuni* has been identified as the predominant cause of antecedent infection in Guillain–Barré syndrome (GBS) and Miller Fisher syndrome, two frequent forms of acute inflammatory polyneuropathy (Farnell et al, 2006, Kuroki et al, 1991). Onset of symptoms seems to occur within two to five days after being exposed to pathogens, with symptoms lasting between seven and ten days with a few extreme cases, lasting even longer. Controlling *Campylobacter* with interventions, proper handling and chilling is very effective in preventing its spread and growth in poultry products.

Campylobacter affects the gastrointestinal tract polyneuropathy (Farnell et al, 2006). Onset of Campylobacteriosis brings patients moderate discomfort and slight interruption to their normal day. Higher numbers of children under 5 years and young adults age 15 to 29 are more frequently afflicted than any other age group. Few cases (1 per 1,000) result in fatalities. Those cases that have been associated with fatalities due to *C. jejuni* were seen in cancer patients or in patients with debilitated immune systems

which could not readily fight off the bacterial infection. An important concern with expectant mothers is septic abortion caused by this food pathogen. *C. jejuni* has a long history in causing abortions with sheep and is a true concern for farmers. This has not been the case with humans as only a few reported human abortions have been found to be caused by *C. jejuni*.

Outbreaks are usually small consisting of less than 50 people, and have been associated with contaminated water sources or milk that was not pasteurized properly. Doctors have been reluctant to prescribe antibiotics with the exception of severe cases, preferring to advise increasing fluid intake and rest. When antibiotics are indicated, Erythromycin is often prescribed. In cases of septicemia, Gentamicin is most commonly prescribed. After an infection has occurred, feces can remain positive for up to 7 weeks following treatment (Fraser, 2004). It is important to wash hands frequently after bowel movements, especially for parents who change their children's diapers, in order to reduce spreading the bacteria and risking re-infection.

As indicated, *Campylobacter* is an extremely weak bacterium compared to others. The campylobacter that causes enteritis in humans cannot grow well below 30 degrees Celsius and have difficulty growing and multiplying on chilled food or on ambient stable foods stored below 30º C. The optimum growth temperature of these strains is 42º C and the maximum 47º C. Campylobacters are acid sensitive and will not grow below pH 4.9. *Campylobacter* spp. grows best in the pH range 6.5 to 7.5 and has an optimal pH range of 4.9 to 9 0. Campylobacter is very sensitive to salt; 2.0% salt is

sufficient to inhibit them, even under otherwise optimum growth conditions (Atabay et al, 1998).

Isolation of *C. jejuni* from food is difficult because the bacterium is usually present in very low numbers (unlike the case of diarrheal stools in which 10^6 bacteria/gram is not unusual). The methods require an enrichment broth containing antibiotics, special antibiotic-containing plates and generally a microaerophilic atmosphere with 5% oxygen and an elevated concentration of carbon dioxide (10%) (ICMSF, 1996). Isolation can take several days to a week.

POULTRY PROCESSING STEPS OF CONCERN

Scalding

Consumer preference for intact skin on most poultry products requires scalding to aid in feather removal. Scalding is used to relax the feather follicle to facilitate feather removal during the picking process which immediately follows scalding. There are several types of scalding techniques and temperatures which are used in the poultry industry. The most commonly used is that of submersion scalding. This process has a vast range of temperatures which are utilized to achieve the desired final product characteristics. Scalding has been classified into two categories; hard scalding (sub scald), performed at 60°C for 45 seconds; and soft scalding (semi scald), performed at 53°C for 120 seconds. Hard scalding removes the outer most layer of skin which contains the vast majority of xanthophylls found within skin and provides the yellow

pigment commonly associated with the broiler (Sams, 1990). With this pigmented skin removed, final carcasses characteristics tend to be a pale whitish color. This carcasses characteristic tends to be preferred by the majority of the consumers in the United States, especially in the southern states and by most fast food companies. Companies that coat chicken products with batter and breading, will demand to be supplied with chicken that has been hard scalded. Chicken carcasses that have been subjected to hard scalding have a tendency to allow for better batter breading pick-up due to the lack of the waxy skin that is removed during hard scalds. This waxy skin can act as a barrier and prevent much of the batter breading from attaching to chicken parts. This waxy skin can dramatically reduce a company's batter breading pick-up and can lead to reduced profits and higher cost to the final consumer. On the other hand, soft scalding leaves the outermost layer of skin attached to the carcass leaving the with a pale yellow skin tone. This is preferred in many Central and South American countries. Soft scalded broilers have a higher demand in northern states within the United States; however hard scalding is the most predominant technology.

Scalding is the first processing step carcasses encounter during processing where there is a high degree of cross-contamination potential. Flocks become heavily contaminated by feces during the grow-out and transportation processes (Bailey et al, 1987). This same feces and other organic material can harbor bacteria and common pathogens, until inactivated or transferred to another surface. These bacteria, including pathogens, can be transferred to the scalder water and contaminate the remaining birds that come behind (Lillard et al, 1971). As previously stated, immersion scalding is the

predominant method of scalding in commercial poultry processing plants. This scalding process uses large amounts of water held in extensive heated tanks. Water is often changed daily, only after the day's production quota has been met. This practice allows for bacteria accumulation (Genigeorgis et al, 1986). One common practice to reduce cross-contamination is the addition of fresh heated water and the release of contaminated water. Also, birds are allowed to travel through the scalding system into cleaner water (counter flow) allowing for some rinsing action to take place. This is accomplished by adding fresh water near the end of the scalding trough, while contaminated water is released near the beginning of the scalding trough/ process. With proper steps to reduce cross-contamination, bacterial counts on the skin of broilers will usually remain low (1 Log) and do not differ from the counts of the skin of the live bird (Bailey et al, 1987; Walker and Ayres, 1956).

While using high temperature scalding (hard scald) some bacteria and pathogens can become stressed and inactivated; however, complete disinfection of the water is never achieved during production by temperature effect only (Izat et al, 1988). Constant recontamination by dirty flocks replenishes the scalding tank water with large amounts of fresh bacteria. Also, hard scalding has been associated with a decrease in carcass shelf life. This is thought to be caused by the removal of the cuticle layer, which happens at temperatures above 58°C. Shelf-life reduction can also be due to a bacterial selection process which happens during scalding. High temperature scalding reduces bacterial competition, allowing weaker spoilage organisms to recontaminate the carcasses after

scalding. Once contaminated with spoilage bacteria, these organisms can flourish without competing for attachment space and nutrients.

Chilling

Carcass chilling is often seen as one of the most important steps for bacterial control and can exert the greatest effect on bacterial growth and the shelf-life of the poultry products. Chilling can reduce the amount of bacteria present on poultry carcasses, if the process variables such as temperature, pH, concentration of antimicrobials, etc., are controlled properly. However, the opposite can also be true if proper process controls are not implemented and followed carefully. USDA requires that broiler carcass temperature be reduced to 4.4°C (40°F) or less within 4 hours for carcasses under 4 lb (1.82 Kg); 6 hours for carcasses 4 to 8 lb (1.82- 3.63 Kg) and 8 hours for carcasses over 8lb (3.63Kg) (USDA, 1973; Houston, 1985). To achieve this, poultry companies have developed several alternatives of chilling the carcasses to properly reduce bacterial growth.

The two main ways to chill poultry carcasses are immersion chilling and air chilling. Immersion chilling is the most widely used and least expensive method to reduce carcass temperature. It is also faster than many other approaches. The immersion chilling process in broilers has been targeted by competing protein companies as a way for the poultry industry to sell excess water, while this process is not allowed for pork and beef cooling. This is due to the fact that immersion chilling increases the possibility of water retention up to 6 to 12% of the bird's weight. The poultry industry is allowed to

operate with this minimal water pickup as long as it can be shown to be an unavoidable consequence of the processes used to meet food safety requirements while clearly labeling the amount of water retained on the final product. Large troughs or paddle systems are used in stainless steel tanks and filled with chilled water usually secured from the local community closest to the processing plant. As with scalding, a major concern with immersion chilling is the common water shared by all processed poultry during the day's production. Contamination of the immersion chiller can result in continual contamination of incoming carcasses that come in contact with the water after initial contamination has occurred (Lillard, 1971; Bailey et al, 1987). Counter flow, over flow, and the addition of different antimicrobial agents in the chilling water have been utilized with some success, to reduce the possibility of cross contamination (Knoop et al, 1971; Bailey et al, 1987; May, 1974). Counter flow is similar to that which is used during the scalding process. Fresh chilled water is introduced were carcasses exit the chiller and dirty warmer water is released at the beginning of the chilling process, were hot carcasses enter the chiller to start the cooling off process. The carcasses move toward the end of the chilling process with the aid of large paddles or auger systems. These paddles or auger devices push batches of carcasses against the flow of water, allowing carcasses to move into cleaner, colder water. This process helps in a two-fold manner, considering that the process can act as a second rinse while the colder water can reduce the total bacterial counts on broiler carcasses while inhibiting bacterial growth (Houston, 1985). As previously mentioned, water is continually released from the chiller. This is due to the requirement which states that chiller water should overflow at a rate of 1.89-

2.5 litters (1/2 gallon) for each broiler that enters the chiller. This is done to minimize microbial and solids buildup (Houston, 1985; Bailey et al, 1987). Once released from the chiller, contaminated water is sent to be treated and recycled to reduce the plants environmental foot print in the community. This process is quite effective at returning the water cleaner than it was received, however it does not come cheap. While this requirement is essential for reducing bacteria build-up, wasted water increases the production costs and ultimately this cost is passed along to the consumer via higher product cost.

In addition to the many process controls which can be implemented, the use of antimicrobials has been an area of growing interest. The most commonly used antimicrobial in the chiller is chlorine. Concentrations of up to 20-50 ppm of free chlorine residual are allowed to be added to chiller water to control microbial loads (USDA, 1973). Chlorine addition tpo chillers at these concentrations represents a significant difference when comparing poultry processing practices between the US and Europe. Chlorine usage is restricted in Europe based on the potential formation of organic compounds, known as tri-halo methanes, which have been shown to cause carcinogenic effects when fed in large amounts to laboratory mice. However, these low concentrations have been considered safe in the US and are commonly used by processors.

The alternative to immersion chilling is air chilling. This process is fairly new to the US industry and has been used for some time in Europe (EU) (80% of market) and Canada (20% of market). Air chilling, as it sounds, consist of using cold air to chill the

carcasses to the required temperature within the time frame approved. In an air chill system, the lack of a common water bath reduces the risk of cross-contamination from carcass to carcass and selection of psychotropic bacteria may be decreased (Knoop et al, 1971). Water as a cooling medium is replaced by cold air drafts which can have less potential of transferring microorganisms to adjacent carcasses. Air chilled broilers are processed individually on an assembly line and contact between birds is minimized. The use of an air chill application could be detrimental to pathogens affected by aerobic environments like *Campylobacter* spp., which are also very sensitive to drying conditions (ICMSF, 1996). Many studies have compared air-chilling to immersion chilling and many indicated that spoilage occurs sooner with immersion-chilled broilers than those subjected to air-chilling (Knoop et al, 1971). It was also shown that psychotropic organisms predominated after immersion chilling ultimately leading to spoilage sooner than the birds that had been air-chilled.

Studies have shown unreliable results when assessing the immersion-chillers as a point of contamination in the processing plant. Studies have reported an increase in the prevalence of *Salmonella* during immersion chilling (James et al, 1992); while others (Cason et al, 1997) have reported no change in the prevalence of *Salmonella* and a decrease in the prevalence of *Campylobacter* in the immersion-chiller at a processing plant. The same study reported *Salmonella* and *Campylobacter* levels of 20 and 94%, respectively, post-immersion chill.

Pulsed Electric Fields

Reducing the prevalence of pathogenic organisms on poultry carcasses at the processing level is an important task. In order to extend shelf-life and increase safety, the poultry industry has had to constantly develop new interventions to deal with these problems. One such cutting edge technology that can be considered as an intervention is Pulsed Electric Fields (PEF). PEF is a non-thermal processing technology which uses varying amounts of electricity to kill bacteria suspended in liquid media and may not be significantly affected by organic matter contents when applied in a continuous system. Recently, a great amount of attention has been given to bacterial inactivation by electrical treatment in several food processing applications to minimize the use of thermal energy and chemicals such as nitrite and nitrate salts (Toepfl et al., 2007). However, in the last few years, more emphasis has been placed on electrical treatments that rely on PEF (Schoenbach et al, 1997; Schoenbach et al, 2000; Ravishankar et al., 2002; Beveridge et al., 2002; Dincer and Baysal, 2004; Feng et al, 2004; Reyns et al, 2004; Wu et al, 2004; Korolczuk et al, 2006; Wesierska and Trziszka, 2007; Toepfl et al, 2007). Schoenbach et al. (1997, 2000) demonstrated that bacteria inactivation by PEF can be achieved using the appropriate pulse width (60 ns to 1 ms), amplitude (100 V/cm – 100 kV/cm), and single-shot or repetitive operation approach. Ravishankar et al. (2002) also found that temperature and pH level play a role in PEF treatments which can reduce bacteria count by up to 3-log. Recent studies have focused on ways to preserve electrodes, minimize energy requirements, and make utilization of stacked pulses (Wu et al., 2004).

The lethal effects of PEF on microorganisms were first described by Doevenspeck (1961). Lethal effects of PEF on microorganisms were also described by Sakurauchi and Kondo (1980), Jacob et al. (1981), Mizuno and Hori (1988), Mizuno and Hayamizu (1989), Sato et al. (1988), Sato (1989) and Jayaram et al. (1992). All of these authors used artificial media such as salt solutions and buffer medium, in small batch treatment vessels of less than 10 ml. Further evaluations by Sale and Hamilton (1967, 1968) have systematically analyzed the effects of PEF on microorganisms. These authors have demonstrated that killing of bacteria and yeast cells by PEF depends on the electrical field strength and the treatment time. The thermal effects and electrolytic products associated with PEF could be excluded as agents that cause the killing.

There are many theories about how PEF works to inactivate bacterial cells; however, the most widely accepted theory is that when an external electric field is applied to a cell, a transmembrane potential is induced. It is believed that transmembrane potentials of between 10mV and 1 V can induce permeabilization of the cell membrane (Sale and Hamilton 1968; Zimmermann et al. 1980, 1988). The electromechanical compression of the cell membrane was proposed by Zimmermann (1986). Zimmermann theorized that “because of the attraction of opposite charges induced on the inner and outer surfaces of the cell membrane, compression pressures occur resulting in a decrease in membrane thickness. If critical electrical field strength is exceeded, i.e. if the transmembrane potential rises to about 1 V, the membrane is permeabilized by pore formation. This permeabilization can be reversible or irreversible, depending on the

electrical field strength and the treatment time of the pulses applied (Zimmermann 1986).”

CHAPTER II

EVALUATION OF PULSE ELECTRIC FIELDS TO REDUCE FOODBORNE PATHOGEN LEVELS IN SCALDER/CHILLER WATER DURING POULTRY PROCESSING

OVERVIEW

Slaughtering encompasses a series of processing steps with the objective of harvesting the consumable meat. The scalding process consists of the submersion of carcasses in hot water tanks to facilitate the removal of feathers during slaughter. However, the use of a common scalding tank increases the likelihood of carcass cross contamination considering that dirt, fecal material and even digestive and intestinal contents carrying pathogens and other bacteria are widely spread during this operation. Similar cross contamination occurs in the process of chilling carcasses, which also requires submersion of broilers in communal tanks filled with ice and cold water. A plausible approach to reduce contamination in scalders or chillers is the use of Pulsed Electric Fields (PEF) to decontaminate scalder/ chiller water. PEF uses electricity to kill bacteria suspended in liquid media and could be utilized in poultry scalders and chillers to reduce bacterial contamination on carcasses and reduce the potential risk of pathogens reaching the final consumer.

A pilot scale system was assembled by the use of a pulse electric field generator (Model SF-700, Simmons. Eng. Co., Dallas, GA) coupled with a commercial scalding

tank (Dunkmaster®, Knase Company Inc, MI). *C. coli* and *C. jejuni* along with marker strains of Novobiocin and Nalidixic acid resistant *S.* typhimurium and *S.* enteritidis strains were used in challenge studies evaluating the effects of the PEF on carcasses, scalder and chiller water contamination.

The system was evaluated with 0, 0.5, and 1% sodium chloride in the water with 40 volts of electric current and 0.54 of amperage. Samples were collected at 0, 40, 80, 160, 200 s of treatment with a 10 s on, 5 s off cyclical pulses. The use of PEF in regular scalder/chiller water showed little effect on *Salmonella* and *Campylobacter* reductions. However, with the addition of 0.5% NaCl caused a significant ($P<0.5$) log CFU/ml reduction of *Salmonella and Campylobacter* within the scalder/chiller water at 40, 80, and 160 seconds respectively.

INTRODUCTION

The impact of food-borne illness on consumers and the food industry can be devastating. According to the CDC, food-borne diseases cause approximately 76 million illnesses in the US, resulting in approximately 5,000 deaths and costing an estimated 9.2 billion dollars (Mead et al., 1999). Two food-borne pathogens often associated with poultry meat are *Salmonella* spp. and *Campylobacter* spp. (Deming et al., 1987; Jones *et al*., 1984; Juven et al., 1986). Among 5.2 million bacterial related foodborne cases in the US, *Campylobacter* spp. accounts for 2.5 million (approximately 50%) (Mead et al., 1999), being considered the leading cause of diarrhea in developed countries causing 200 to 730 deaths annually (Tauxe et al., 1988; Sivak et al., 1997; Rice et al*.,* 1996; Atabay et al*.,* 1998). Similarly, *Salmonella* non-typhoidal accounts for 1.4 to 4 million of estimated cases (approximately 20%) annually (Mead et al., 1999), resulting in 800 to 4,000 deaths yearly (USDA, 1996).

Specifically, *Salmonella* and *Campylobacter* represent human health risks when contaminated poultry is not cook properly or is cross-contaminated after cooking (Skirrow, 1982). Cross contamination can occur at any stage in the process of bringing the product to the consumer, beginning at the farm and continuing during processing (USDA, 1996). Slaughtering and processing steps such as scalding, picking and chilling are a source of cross contamination (Lillard, 1989), and meat can become contaminated with pathogens from intestinal contents, skin or feathers (Oosterom et al., 1983). Epidemiological studies have shown a strong relationship between *Campylobacter*

enteritis and handling and consuming raw or inadequately cooked poultry (Deming *et al*., 1987; Harris et al., 1986). Reports from the National Broiler Baseline Database in 1994-1995 indicated that 88.2% of raw commercial broilers tested positive for *C. jejuni/coli* (USDA, 1995; Tauxe et al., 1988). It was also estimated that between 20% (USDA, 1995) and 35% (Lillard, 1989) ready-to-cook broilers tested positive for *Salmonella*, whereas only 3-4% of those broilers entering the plant were *Salmonella* positive. Thus, cross-contamination during normal processing and ways to alleviate it are important issues to poultry processors, government officials and consumers.

Cross-contamination can happen at any time during poultry processing, however scalding and chilling are considered to be have the greatest impact in the amount of cross-contamination occurring. This is largely due to the use of communal water baths in both the scalding and chilling tanks. Water is used to efficiently transfer a different temperature to the carcass to aid in picking or chilling of the carcasses.

Being one of the initial steps in poultry processing where carcasses come into contact with one another; scalding is a very important step where microbiological controls can be exerted. Scalding aids in the removal of feathers on the carcass. This is achieved by water which has been heated to between 128-142°F in large tanks while carcasses are submerged and pulled along by shackles through the tank of heated water. While relaxing the feather follicles to aid in picking, the water also rinses the feathers of fecal or other types of organic material that may have accumulated on the broiler at the farm or during transportation to the processing plant. This organic material can then accumulate in the water and harbor bacteria. The scalder water is often utilized for an

entire day's production quota without significant draining in between processed flocks. This allows those flocks with high counts of bacteria, to contaminate the water and cross-contaminate other flocks that pass subsequently after the dirty flock. Steps to reduce cross-contamination are counter flow water and constantly adding fresh heated water to dilute or wash out potential microbial built-up.

Another major processing step of concern for potential carcass cross-contamination is chilling. Since carcasses are coming from the evisceration step and are warm after being submerged in the scalder tank, a chilling step is necessary to lower the carcass temperature and reduce bacterial growth. USDA-FSIS requires that in a HACCP environment, appropriate standards are needed for the cooling of carcasses reducing the temperature to 4.4°C (40°F) within 4 hours (USDA, 1973; USDA 1996; Houston 1985). Because of these regulatory limits of temperature, chilling is usually included as a critical control point (CCP) on HACCP plans for most broiler facilities (CFR. 381.66) (USDA, 1996). If regulatory limits are not achieved, some preventive measures include the maintenance of residual chlorine levels of 20-50ppm or proper overflow of fresh chilled water (1/2 gallon per bird entering the chiller). Because of the counter current water flow, the chicken exits the chiller coming in contact with the coldest, cleanest water. In addition to reducing carcass temperature faster than other methods, the immersion-chilling process rinses away some of the bacterial loads from the carcass and transfers them into the surrounding water, which then can be retransferred to the adjacent carcass.

Most of the interventions commercially available have been shown to be relatively effective in reducing microbial contamination on poultry carcasses, especially in controlled studies. However, when applied in commercial settings results are not always as expected. This may be due to the relatively short time of action that these agents had on the carcass surface due to the continuous nature and speed of the processing line, low concentrations allowed, and the liquid nature of the agent, which implies continuous dripping of the intervention. Therefore, alternative means to destroy bacteria in commercial settings need to be evaluated for higher success reducing pathogenic loads.

We propose to use a process known as Pulse-Electric-Fields (PEF) to inactivate bacteria, and especially pathogenic organisms in commercial poultry processing settings. PEF has been extensively used to inactivate pathogenic organisms in liquid matrices including juices, liquid foods and other semi-liquid products. PEF is a non-thermal processing technology which uses high amounts of electricity to kill bacteria suspended in liquid media and it is not significantly affected by organic matter contents in continuous systems as is the case with some chemical alternatives. Due to the liquid nature of some of the conventional interventions steps used in commercial poultry processing operations, we believe that the addition of PEF in scalding and chilling tanks will reduce bacterial loads in water and potentially on the carcass surfaces over a continuous processing scenario.

Another objective of this project was to evaluate the effects of PEF on the development and completion of rigor mortis in processed broilers, when the electric

current is applied directly on the submersion tank. We assumed that the PEF process even at this low application levels will exercise poultry muscles (contraction-relaxation cycles) and consume significant levels of remaining adenosine tri-phosphate (ATP) present in muscular fibers. It is well know that carcasses deboned before rigor completion (4 hours after slaughtering) tend to be tougher than aged carcasses due to muscle contraction after deboning. The residual ATP present in muscle fibers causes muscle to contract if exposed to external stimulus such as cut-up, trimming practices and exposure to extremely cold environments. The conventional way to address this problem is by aging carcasses for at least 4 hours post-mortem, which in turn causes bottlenecks in commercial settings. Another alternative is the use of still expensive electrical-stimulation equipment which exercises the muscles before the scalding operation. The consumption of residual ATP by exposure to PEF cycles was expected to allow processors to debone carcasses sooner, thus reducing the need for expensive aging times and massive refrigerated storage areas in some facilities. The potential for faster deboning in commercial settings will increase throughput of these facilities by overlapping first and second processing shifts, thus reducing operational costs.

Reducing the prevalence of pathogenic organisms on poultry carcasses at the processing level is an important task. The proposed intervention modifications can also be adapted to processing operations that follow processing guidelines approved under the "organic" labeling requirements considering that no chemicals are used to destroy bacteria with these settings. Considering the limitations in applying chemical interventions under organic processing guidelines; these efforts may allow niche market

producers to process safer poultry meat under compliance. Reducing pathogen incidence in poultry products will not only benefit consumers by reducing the likelihood of foodborne outbreaks; but will also allow processors to operate with prevalence below the *Salmonella* performance standard regulation, while extending the product's shelf-life and maximizing meat tenderness for higher market competitiveness.

OBJECTIVES

1. To validate the use of Pulse Electric Fields (PEF) in scalding and chilling water as an intervention to inactivate *Salmonella* spp. and *Campylobacter* spp. during processing of broilers.

2. To evaluate the effect of the PEF-submersion tank treatment on quality parameters of broilers after commercial processing.

3. To assess the commercial application of the PEF-submersion tank in a pilot-scale processing facility during scalding and chilling by determining *Salmonella* spp. levels.

HYPOTHESIS

The main hypothesis for this experiment was the assumption that pulse electric fields will inactivate bacteria contaminating the water medium in submersion tanks at commercial operations; PEF will also reduce the risk of cross contamination and hence reduce the prevalence of common poultry-related pathogens present on broiler carcasses during and following processing. It was also assumed that PEF will exercise the muscles thus using residual ATP present that could in turn improve product tenderness after early deboning.

MATERIAL AND METHODS

Objective 1

Equipment Design. A simple experimental set up was assembled and used for all tests. The set up consisted of a 200 ml glass beaker and stir bar atop a stir plate. Electric pulses were provided by an electric stunner device (Model SF-700, Simmons. Eng. Co., Dallas, GA) (Figure 7). Copper jacketed graphite electrodes were used to apply electricity to the solutions along with pH meters, ORP meters and digital thermometer devices to facilitate data acquisition.

PEF Treatments. Our approach consisted of making use of pulsed electric fields (PEF) in combination with adjustments in salt content and temperature to treat poultry scalder and chiller submersion water. The use of NaCl was considered to facilitate the conduction of electricity in the water system to enhance lethality. Scalder experiments were run using water collected from a local commercial poultry processor by selecting the water overflow of the final section of the scalding tank to simulate organic matter loads on commercial settings. Chiller water was simulated by using local tap water, with and without the addition of up to 25 ppm of sodium hypochlorite to come as close as possible to simulating processing plant conditions.

Preparation of Inoculum. *Salmonella* Typhimurium and Enteritidis as well as *Campylobacter jejuni* and *C. coli* were obtained. Salmonella strains were made resistant to Nalidixic Acid (NA) and Novobiocin (NO) and then grown on tryptic soy agar (Difco) slants and stored under refrigeration. Three days before each experiment, cells were resuscitated by two consecutive transfers into tryptic soy broth supplemented with 20 ppm of Nalidixic Acid (NA) and 25 ppm of Novobiocin (NO) and then incubated at 37°C for 18-24 h. Immediately before conducting the PEF experiments the individual strains of Salmonella were combined in a 50ml test tube to produce a cocktail inoculum. Similarly, *Campylobacter* strains were inoculated on Bolton broth tubes supplemented with lysed horse blood plates and placed in air tight plastic bag. All air was removed from the bag and a mixture of 5%O_2, 10%CO_2 and 85% N_2 gas was added to inflate the

bag and produce a microaerobic environment. Bags were then incubated for 48 h at 42°C and then stored under refrigeration temperatures until needed. Forty eight hours before each experiment each *Campylobacter* strain was enriched in Bolton broth and incubated at 42°C under microaerophilic conditions. Immediately before conducting the PEF experiments the individual strains of *Campylobacter* were combined in a 50ml test tube to produce a cocktail inoculum.

Experimental Design. 99ml of experimental solution (either scalder water or chilling water) was added to a 200ml glass beaker along with 1ml of either the *Salmonella* or *Campylobacter* cocktail in separate experiments. The copper electrodes were then fully lowered into the solution and the stir bar was activated to provide a consistent agitation and to prevent any stagnation of the water to resemble the conditions in a commercial scalder/ chiller. The PEF generator was set to apply its maximum 40 volts, generating ~0.54 amps with intervals of 10 seconds on and 5 seconds off pulses. One ml samples of inoculated submersion water were taken at 0, 40, 80, 120, 160, and 200 seconds of PEF treatment and placed into refrigerated tubes with 9ml of sterile 0.1% buffered peptone water (BPW) for subsequent enumeration.

Microbiological Determinations. Serial dilutions were completed with tubes containing 9ml of sterile 0.1% buffered peptone water (BPW), 0.1ml was then placed onto XLT4 plates supplemented with 20 ppm NO and 25 ppm NA or Campy-Cefex plates for Salmonella and *Campylobacter* respectively. XLT4 plates were allowed to incubate for 24 hours at 37°C while Campy-Cefex plates were placed in air tight plastic bags. All air was removed from the bag and a mixture of 5%O_2, 10%CO_2 and 85% N_2 gas was added to inflate the bag and produce a microaerobic environment. Bags were then incubated for 48 h at 42°C.

Objective 2

Experimental Design. A 60 gallon capacity, jacketed scalding tank (Dunkmaster®, Knase Company Inc, MI) was installed with a shackle mechanism to facilitate the experiments (See Figure 8). The tank was assembled below a four-shackle mechanism movable by a polley system to facilitate submersion and removal of carcasses from the water media. This tank was coupled with an electric stunner device (Model SF-700, Simmons. Eng. Co., Dallas, GA) that was used with copper jacketed graphite electrodes to deliver PEF to the water medium. This system was installed at the Microbial Challenge Pilot Plant at the Poultry Science Center at Texas A&M University.

Experimental Design. Whole broilers were obtained from the Texas A&M University Poultry Farm and Processing Center in College Station, Texas. Carcasses were commercially processed in pilot plant processing facility and then subjected to PEF under scalding conditions to determine the effect of the PEF treatments on quality carcass parameters when applied directly in the tank. The PEF generator was set to apply its maximum of 40 volts that generated 0.54 amps with intervals of 10 seconds on and 5 seconds off. Carcasses were then defeathered, eviscerated and washed with tap water. Immediately after chilling (1h) in a static ice-water chiller, carcasses were manually deboned and the breast and thigh muscle samples were collected. Deboning was performed after 4 and 24 hours to simulate early deboning and aged deboning, respectively. These breast samples were evaluated for cook loss, drip loss and shear force using the Allo-Kramer method based on standard procedures. The same parameters were performed on control samples for comparative purposes using standard methodologies (Sams, 1990).

Objective 3

Equipment Design. A jacketed scalding/chilling tank (Dunkmaster®, Knase Company Inc, MI) was installed to facilitate the experiments. Two pumps were fitted to the tank and used to circulate the water solution to a remote plastic five gallon treatment container. This container was coupled with an electric stunner device (Model SF-700, Simmons. Eng. Co., Dallas, GA) that was used with copper jacketed graphite electrodes to deliver PEF to the water medium. This system was installed in our Microbial

Challenge Pilot Plant at the Poultry Science Center at Texas A&M University. This facility allowed us to work directly with pathogenic organisms on the surface of broiler carcasses and adapt a recirculation system of the scalder/ chiller water.

Preparation of Inoculum. *Salmonella* Typhimurium and Enteritidis as well as *Campylobacter jejuni* and *C. coli* were obtained from commercial poultry samples by Dr. J. Allen Byrd's laboratory at the USDA Agricultural Research Center in College Station, Texas. Salmonella strains were made resistant to Nalidixic Acid (NA) and Novobiocin (NO) and then grown on tryptic soy agar (Difco) slants and stored under refrigeration. Three days before each experiment, cells were resuscitated by two consecutive transfers into tryptic soy broth supplemented with 20 ppm of Nalidixic Acid (NA) and 25 ppm of Novobiocin (NO) and then incubated at 37°C for 18-24 h. Immediately before conducting the PEF experiments the individual strains of Salmonella were combined in a 50ml test tube to produce a cocktail inoculum.

Experimental Design. When conducting experiments the immersion tank was filled with 60 gallons of tap water, 0.5% NaCl-water solution and 1% NaCl-water solution and either heated to 45° and 57°C or cooled with ice to 0-4°C. To mimic processing plant scalding and chilling conditions, at least four previously processed broiler carcasses were added to the immersion tank before starting the experiment and allowed to release organic material for the duration of the experiment. Four liters of *Salmonella* spp. cocktail obtained as previously described weres used to inoculate the 60

gallons of water for each of the salt-solution levels and temperature conditions. Ten ml samples were taken every hour on the hour for four and 8 hours of continuous processing and PEF application with treatment pulses of 10s on and 5s off. The PEF generator was set to apply its current maximum of 40 volts.

CHAPTER III

SUMMARY

RESULTS

Objective 1

Salmonella spp. and *Campylobacter* spp. cocktails were subjected to PEF at varying temperatures to simulate chilling, sub scalding, and semi scalding conditions. Bacterial counts were converted to $\log_{10}$ counts per ml and analyzed using SPSS (SPSS Inc., 2006). Reductions in tap water/scalder water using PEF for both *Salmonella* and *Campylobacter* cocktails were constant throughout all time intervals evaluated, but not always significant ($P > 0.05$). However, the most significant reductions ($P < 0.05$) were usually recorded at 200 seconds and was chosen as the target exposure time for these experiments. Results explained below were compared to controls samples that were not subjected to PEF, to treatments that receved the PEF application and the treatments that contained varying percentages (0, 0.5 and 1%) of NaCl and/or 25ppm of sodium hypochlorite. All data was statistically analyzed using SPSS (SPSS Inc., 2006).

***Salmonella* Spp.**

Scalder. Two scalder temperatures were evaluated with S*almonella* spp. cocktails, 45ºC and 55ºC. Significant differences ($P<0.05$) were noticed with the use of 0.5% and 1% NaCl at 200 s with a temperature of 55ºC. A 2.9 log reduction was achieved with 0.5% NaCl. The greatest reductions, 3.2 logs was achieved with 1% NaCl in the water. With a lower temperature of 45ºC, significant differences ($P<0.05$) were also noted at 200 s with reductions of 4.4 and 4.3 logs on the 0.5% and 1% NaCl solutions, respectively. The largest reductions were achieved without the use of NaCl, 6.1 logs. This result was significantly different ($P<0.05$) when compared to the control, but were not significantly different ($P>0.05$) when compared to treatments containing NaCl. These variations may be attributed to the experimental conditions, and how the electric current is delivered to the cells in the container.

Chiller. Treatments with *Salmonella* spp. cocktails in chilling conditions showed that the colder medium reduced PEF's performance as compared with the water temperatures in the scalder trials. However, with the use of 1% NaCl or 0.5% NaCl and 25ppm NaClO the performance could be increased. *Salmonella* was significantly ($P<0.05$), but not greatly reduced by PEF under conditions without NaCl or with the use of only 0.5% NaCl. Reductions of 0.8 logs for PEF only treatments and 0.9 logs for treatments with 0.5% NaCl were observed. With treatments of 1% NaCl or 0.5% NaCl and 25ppm NaClO reductions were 1.8 and 4.6 logs respectively.

***Campylobacter* Spp.**

Scalder. Two scalder temperatures were also evaluated with *Campylobacter* spp. cocktails, 45ºC and 55ºC. Significant differences ($P<0.05$) were noticed at 200 s with both temperatures. At 55ºC *Campylobacter* was reduced below detection levels on all treatments, which was greater than 5.0 logs, The greatest reductions (6.1 logs) was achieved with 0.5% NaCl. This may be due to the fact that we were able to plate dilution 0 on this treatment as compared to dilution 1 on the remaining treatments; therefore we could not claim a more significant inactivation on the other two treatments. With the lower temperature of 45ºC, significant differences ($P<0.05$) were also noted at 200 s. A 5.8 log reduction was achieved with PEF only and PEF combined with 0.5% NaCl. These results were below the detection limits of dilution 1. A reduction of 4.6 logs was reached with 1% NaCl in the medium.

Chiller. Treatments with *Campylobacter* spp. cocktails in chilling conditions showed that the colder medium reduced PEF's performance. However, with the use of 0.5% NaCl and/or 25ppm NaClO the inactivation performance was increased. *Campylobacter* was significantly ($P<0.05$) reduced on all treatments when compared to the control. PEF showed to reduce *Campylobacter* the best with 0.5% NaCl. A reduction of 5.7 logs was detected with 0.5% NaCl. PEF only and 1% NaCl with PEF did not show as good of results as 0.5% NaCl did. A decrease of 2.3 logs for PEF only and a decrease of 2.7 logs with the 1% NaCl and PEF combination were recorded. Treatments with 0.5% NaCl and 25ppm NaClO show remarkable results. *Campylobacter* was eliminated below detectible limits on all dilutions with the combination of 0.5% NaCl and 25ppm NaClO. Results were not statistically reported due to the lack of detectable colony forming units at all six time intervals. Further research is needed to confirm these results; however, it can be concluded that the PEF treatment is more lethal against *Campylobacter* as compared with *Salmonella* cultures under the conditions tested.

Objective 2

Chicken carcasses processed in our pilot plant were subjected to either two or four minutes of PEF treatment under scalding conditions at 55^{O}-57^{O}C. Carcasses were then processed under commercial conditions and then deboned immediately after 1 hour of chilling (early), after 24 hours (late) or after 4 hours (control). Three replications were conducted for all treatments. Breast meat samples were cooked to an internal temperature of either 74^{O}C or cooked at 100 ^{O}C for 15mins. Temperatures were only compared between themselves. All data was statistically analyzed using SPSS (SPSS Inc., 2006).

Texture Analysis

Shear Force. No significant differences ($P<0.05$) were noted when treatments were compared to the control treatment for both cooking methods (Tables 7-10). Early deboning treatments that were cooked at 100^{O} C for 15mins had an average shear force value of 0.156 g/Kg, 0.157 g/Kg, and 0.162 g/Kg for control, 2min, and 4min respectively. Early deboning treatments that were cooked to an internal temperature of 74^{O} C had an average shear force value of 0.159 g/Kg, 0.146 g/Kg, and 0.156 g/Kg for control, 2min, and 4min respectively. Late deboning treatments that were cooked at 100^{O} C for 15mins had an average shear force value of 0.185 g/Kg, 0.164 g/Kg, and 0.179 g/Kg for control, 2min, and 4min respectively. Late deboning treatments that

were cooked to an internal temperature of 74^{O} C had an average shear force value of 0.146 g/Kg, 0.151 g/Kg, and 0.181 g/Kg for control, 2min, and 4min respectively.

Cook Loss. No significant differences (*P<0.05*) were noted when treatments were compared to the control treatment for both cooking methods (Tables 11-14). Early deboning treatments that were cooked at 100^{O} C for 15mins had an average cook loss value of 24.01%, 23.79%, and 23.33% for control, 2min, and 4min respectively. Early deboning treatments that were cooked to an internal temperature of 74^{O} C had an average cook loss value of 22.97%, 22.96%, and 21.29% for control, 2min, and 4min respectively. Late deboning treatments that were cooked at 100^{O} C for 15mins had an average cook loss value of 23.19%, 24.63%, and 25.14% for control, 2min, and 4min respectively. Late deboning treatments that were cooked to an internal temperature of 74^{O} C had an average cook loss value of 18.63%, 23.10%, and 20.64% for control, 2min, and 4min respectively.

Drip Loss. No significant differences (*P<0.05*) were noted when treatments were compared to the control treatment (Tables 15, 16). Early deboning treatments had an average drip loss value of 3.54%, 3.58%, and 3.48% for control, 2min, and 4min respectively. Late deboning treatments had an average drip loss value of 4.03%, 3.67%, and 3.04% for control, 2min, and 4min respectively.

Objective 3

To test the feasibility of PEF in a commercial setting, we attempted to use the same low current PEF generator coupled with a larger 60 gallon commercial scalder/chiller system (Dunkmaster®, Knase Company Inc, MI) furnished with a remote treatment station where recirculated water was treated. We also used the same copper jacketed graphite electrodes to deliver PEF shocks to the water medium as before. To enhance the PEF distribution in the treatment cell 1% NaCl was added to the water medium for all treatments.

Salmonella spp. *Salmonella* spp. cocktails were not noticeably affected in anyway by the use of PEF at any of the two temperature ranges (4ºC and 45ºC) when the treatment was applied in the bucket cell. Salmonella was unrecoverable within 14 seconds after adding the bacterium to the scalder at 55ºC. These results seemed to indicate our PEF generator would be inadequate to treat the larger amount of water medium that was transferred to the side bucket cell. Current efforts are being focused on ways to increase the electrical output of a PEF generator, which will be able to treat larger tanks so that we could resemble the results observed in the small scale application unit.

DISCUSSION

Salmonella Spp.

Scalder. *Salmonella* spp. survival was found to show some variability during PEF treatments under scalding conditions at 45ºC. With the use of as little as 0.5% NaCl during the administration of PEF, we were able to drastically reduce this variation and achieve a constant reduction over the entire 200 seconds of PEF treatment (Table 2). Many poultry processing plants are exploring the possibility of reducing the scalding temperatures. This reduction in temperature will reduce their production cost, but may potentially increase the incidence of foodborne pathogens contaminating consumer's products. With the use of PEF in those scalder tanks set to lower temperatures by treating the scalder water on a separated cell, processing plants can reduce their production cost without increasing the possibility of foodborne pathogens building in the scalder. Scalders running at the traditional higher temperatures will also benefit from this new technology. Our results show that we could consistently obtain a 2.4 log reduction of *Salmonella* spp. in the scalder water after 200 seconds of PEF (Table 3). We also used NaCl to help administer PEF, and were able to increase our reduction by 1.6 logs over the use of PEF alone; giving us a final reduction of an estimated 4 logs in the 55ºC scalder. NaClO was not tested in the scalder with PEF, due to its tendency to be drastically affected by high amounts of organic material rendering it ineffective at killing pathogens and because at high temperature chlorine solutions tend to be gassed off, thus minimizing the antimicrobial effects of chlorine.

Experiments with the PEF generator in the larger scalding/chilling tank system showed no effect on bacterial counts after treatment when compared to control samples. PEF was found to be very efficient when coupled properly to a treatment container of appropriate size for the generator. All of our current experiments were limited to the maximum 40 volts and 0.54 amps of our small scale PEF generator. Larger PEF generators will be needed to reduce pathogens in larger tank systems. Future research will be needed to evaluate the proper ratio of electrical current to treatment tank size. Obtaining this proper ratio will allow processing plants to treat their liquid medium and achieve desired results without exposing surrounding workers to higher than needed electrical currents. Poultry processing companies have a safe track record of utilizing electrical currents in processing plant environments. Many of the needed safety precautions are already implemented into poultry processing plants. Additionally, the PEF process can be configured to pump the contaminated water away from worker's environments into a safer treatment area. Once the water has been treated, the cleaner water can be reintroduced back into the heating/chilling system and pumped into the scalder/chiller tanks. This method will prevent any accidental contact of electricity to workers.

Chiller. *Salmonella* spp. reductions with PEF did not perform as well under chilling conditions as it did under scalding conditions. Nevertheless, significant ($P<0.05$) reductions were obtained (Table 1). Using PEF with 1% NaCl provided a 1.8 log reduction in counts when compared to controls using 1% salt and no PEF. As mentioned before, many poultry processing companies utilize NaClO in chiller tanks as an antimicrobial agent. We also coupled NaCl and PEF with NaClO and did achieve even greater reductions, proving the synergistic effect the combination can have. With the synergistic effect of chlorine, salt and PEF we could observe up to 2.31 log reduction on *Salmonella* spp.

***Campylobacter* Spp.**

Scalder. PEF's effects on *Campylobacter* spp. in scalders were magnified compared to those effects documented with *Salmonella* spp., at both temperatures tested. *Campylobacter* spp. was affected greatest by 55ºC scalder temperatures. Additionally, significant variation was experienced between *Campylobacter* replications. These experiences can partially be attributed to the temperature, being so close to the lethal temperature for *Campylobacter*; and to its lack of survivability in environments that are less than ideal for its survival. This being said; PEF affected *Campylobacter* greatest with 0.5% NaCl, (Table 6) reducing its presents by 6.3 logs within 200 seconds compared to controls containing only scalder water.

Scalders set to run at lower temperatures will also benefit from the use of PEF to reduce *Campylobacter*. Reductions were less than those seen with the higher

temperature scalders, but still significant. *Campylobacter* spp. was reduced by 5.8 logs (Table 5). These results were obtained without the use of 1% NaCl, both 0.5 and 0% NaCl with PEF killed the bacteria better than with the use of 1% NaCl. More research is needed to understand this effect. Standard deviations on all treatments between replications were below 0.59, suggesting no outliers were recorded. Control experiments performed on *Campylobacter* spp. with 1% NaCl showed insignificant reductions over 200 seconds. This phenomenon could not be immediately rationalized and will require additional investigations.

Chiller. Poultry processing companies are at higher risk to *Campylobacter* spp. contamination during chilling of the eviscerated carcasses then at any other step during processing, and in need of effective interventions to control it (Stern, et al, 2001). PEF results indicated that it too could be a possible alternative. *Campylobacter* was reduced 5.7 logs with the use of 0.5% NaCl in the chiller water and no NaClO was used to obtain these numbers. These results were also obtained without the use of 1% NaCl, both 0.5 and 0% NaCl with PEF killed the bacteria better than with the use of 1% NaCl. Standard deviations on all treatments between replications were below 0.61, suggesting no outliers were recorded. Control experiments performed on *Campylobacter* spp. with 1% NaCl showed insignificant ($P>0.05$) reductions over 200 seconds.

As mentioned before, a widely accepted intervention in western poultry processing is in the use of NaClO in the chiller water at varying concentrations. We also chose to test PEF effectiveness with 25ppm of NaClO and 0.5% NaCl. When compared

to controls only containing NaClO, counts were reduced by 4.5 logs; which was below detectable limits under the experimental conditions tested. *Campylobacter* spp. counts were drastically reduced with this combination; however, we were unable to report a significant ($P<0.05$) difference due to the reduction beyond detectable limits. Nevertheless, these results hold true promise for campylobacter reduction in chiller tanks with PEF.

Texture Analysis

Efforts to examine PEF's role in muscle tenderization were unsuccessful (Table 7-16). Depletion of ATP by electrical stimulation requires higher electrical pulses than our PEF generator was able to produce. Previous studies indicate electrical currents of at least 350 mA to see an effect on muscle tenderization (Sams, 1999). No significant differences ($P>0.05$) were noticed between Allo-Kramer shears, cook loss, and drip loss data in both treatments, when compared to controls where PEF was not used.

This research demonstrated the ability of Pulsed Electric Fields to be a very powerful and efficient tool at reducing microbial loads in chiller and scalder tanks. It also validates that the use of a properly sized PEF generator is essential for PEF to be effective as a lethal alternative to other microbial interventions. PEF has endless possibilities for use in food processing plants, or any other application where sanitation of liquids is needed. With further validation, PEF can be used to meet the USDA organic certification criteria needed to allow food products to be labeled as "Organic", since the interventions does not require the use of chemicals which may not be approved. . Once

certification is achieved, organic food processing plants can make use of PEF to assist them on reducing bacterial loads and control pathogens, while still meeting the many demanding federal and state regulations concerning safe food processing methods.

Final Conclusion

This research provides evidence that PEF canbe effective at reducing *Salmonella* spp. and *Campylobacter* spp. in poultry processing settings. In many of the treatments we used to validate this intervention, *Salmonella* spp. was reduced on an average of approximately 2 logs from the control samples; while *Campylobacter* spp. was reduced by an average of approximately 3 logs when compared to control samples. Although many treatments out performed these reductions, averages were affected by variation between replications. This variability could be attributed to many factors including, cell viability, shaking conditions, electrode density and capacity, etc.. One such theory is the possibility that the organic material could shelter bacterial cells from receiving the lethal dose of electrical shock. Feathers, shavings, and fecal material could all reduce the transmission of electrical shocks through the water medium. With this data, PEF appears to perform at its best as a part of a synergistic approach to eliminating pathogens in liquids. With the addition of either 0.5% or 1% NaCl PEF could be transferred from the electrodes to the liquid medium with increased efficiency. As well as NaCl, NaClO appeared to help electrical pulses reduce bacteria counts in chiller tanks. This can be attributed to the increased stress placed upon the cells to deal with the harsh environment we induce. Sodium hypochlorite is already a common addition to most poultry chiller

tanks and PEF could easily be integrated into these chiller tanks with little effort to reduce recirculated water contamination. All of our treatments were only conducted to a maximum of 200 seconds. Once placed in a commercial setting, PEF would be ideally used continually throughout the production cycle only being stopped to allow workers to drain and clean the scalding or chilling tanks. This approach will allow PEF to continually treat the water as more contamination is introduced.

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APPENDIX A

TABLES

Table 1

PEF results for *Salmonella* spp. under chilling conditions

	0 sec	40 sec	80 sec	120 sec	160 sec	200 sec
Control	7.0ax	6.9ax	6.9ax	7.0ax	6.9ax	6.9ax
	0.11	*0.13*	*0.09*	*0.08*	*0.08*	*0.08*
PEF Only	6.8ax	6.7ax	6.7axy	6.6abxy	6.3abyz	6.1bz
	0.05	*0.11*	*0.04*	*0.04*	*0.09*	*0.26*
PEF + 0.5% NaCl	7.0ax	6.8ax	6.7ax	6.6abxy	6.2abyz	6.0bz
	0.01	*0.08*	*0.10*	*0.14*	*0.14*	*0.24*
PEF + 1% NaCl	7.0aw	7.0aw	6.8aw	6.4abx	5.5by	5.1cz
	0.07	*0.05*	*0.00*	*0.05*	*0.10*	*0.21*
PEF + 0.5% NaCl + 25ppm NaClO	5.9bx	4.8by	4.5by	3.8cy	2.3cz	2.3dz
	0.20	*0.17*	*0.20*	*0.23*	*0.58*	*0.36*

Means followed by different letter (a, b, c and d) within the column are significantly different ($P < 0.05$); Means followed by different letter (w, x, y and z) within the row are significantly different ($P < 0.05$). Italicized numbers represent the standard mean error.

Table 2

PEF results for *Salmonella* spp. under scalding conditions at 45ºC

	0 sec	40 sec	80 sec	120 sec	160 sec	200 sec
Control	6.8bx	6.9ax	6.8ax	6.8ax	6.8ax	6.8ax
	0.08	*0.08*	*0.09*	*0.07*	*0.08*	*0.09*
PEF Only	6.7bw	6.8aw	6.5bw	5.2cx	2.7by	0.7bz
	0.06	*0.06*	*0.07*	*0.27*	*0.96*	*0.00*
PEF + 0.5% NaCl	6.8bw	6.6abw	6.4bw	5.8bw	4.7abw	2.4bx
	0.04	*0.09*	*0.05*	*0.21*	*0.90*	*1.13*
PEF + 1% NaCl	7.1aw	6.9aw	7.0aw	6.5aw	5.2ax	2.5by
	0.06	*0.06*	*0.11*	*0.03*	*0.20*	*0.89*

Means followed by different letter (a, b, and c) within the column are significantly different ($P < 0.05$); Means followed by different letter (w, x, y and z) within the row are significantly different ($P < 0.05$). Italicized numbers represent the standard mean error.

Table 3

PEF results for *Salmonella* spp. under scalding conditions at 55ºC

	0 sec	40 sec	80 sec	120 sec	160 sec	200 sec
Control	6.0ax	5.9ax	6.0ax	6.0ax	5.8ax	6.0ax
	0.06	*0.08*	*0.07*	*0.12*	*0.11*	*0.18*
PEF Only	5.9aw	5.8abwx	5.6abwx	5.5abwx	4.9awx	4.4abx
	0.07	*0.05*	*0.20*	*0.30*	*0.52*	*0.82*
PEF + 0.5% NaCl	5.7aw	5.7bw	5.2bw	4.8cwx	3.9axy	3.1by
	0.03	*0.04*	*0.20*	*0.47*	*0.60*	*0.20*
PEF + 1% NaCl	5.8aw	5.6bw	5.4bwx	5.1abwx	3.9axy	2.8by
	0.14	*0.11*	*0.15*	*0.33*	*1.11*	*0.64*

Means followed by different letter (a, b, and c) within the column are significantly different ($P < 0.05$); Means followed by different letter (w, x, y and z) within the row are significantly different ($P < 0.05$). Italicized numbers represent the standard mean error.

Table 4

PEF results for *Campylobacter* spp. under chilling conditions

	0 sec	40 sec	80 sec	120 sec	160 sec	200 sec
Control	6.6ax	6.5ax	6.5ax	6.6ax	6.6ax	6.4ax
	0.03	*0.03*	*0.04*	*0.04*	*0.05*	*0.05*
PEF Only	6.1cx	6.1abx	5.9bx	5.4by	5.4by	4.1bz
	0.03	*0.04*	*0.10*	*0.07*	*0.02*	*0.11*
PEF + 0.5% NaCl	6.5bv	5.6bw	2.4cx	1.5dy	0.7dz	0.7dz
	0.03	*0.33*	*0.08*	*0.38*	*0.00*	*0.00*
PEF + 1% NaCl	6.4bw	6.3aw	5.8bx	3.8cy	3.8cyz	3.7cz
	0.08	*0.02*	*0.08*	*0.02*	*0.06*	*0.03*

Means followed by different letter (a, b, c and d) within the column are significantly different ($P < 0.05$); Means followed by different letter (v, w, x, y and z) within the row are significantly different ($P < 0.05$). Italicized numbers represent the standard mean error.

Table 5

PEF results for *Campylobacter* spp. under scalding conditions at 45ºC

	0 sec	40 sec	80 sec	120 sec	160 sec	200 sec
Control	6.4ax	6.5ax	6.4ax	6.4ax	6.4ax	6.5ax
	0.03	*0.03*	*0.04*	*0.04*	*0.05*	*0.05*
PEF Only	6.5av	6.3avw	6.2bw	5.1bx	1.4cy	0.7cz
	0.02	*0.05*	*0.02*	*0.13*	*0.06*	*0.00*
PEF + 0.5% NaCl	6.2bw	5.9bx	5.1cy	0.7dz	0.7dz	0.7cz
	0.02	*0.18*	*0.05*	*0.00*	*0.00*	*0.00*
PEF + 1% NaCl	6.4aw	6.3aw	6.1bw	4.2cx	3.6by	1.9bz
	0.07	*0.08*	*0.04*	*0.02*	*0.07*	*0.34*

Means followed by different letter (a, b, c and d) within the column are significantly different ($P < 0.05$); Means followed by different letter (v, w, x, y and z) within the row are significantly different ($P < 0.05$). Italicized numbers represent the standard mean error.

Table 6

PEF results for *Campylobacter* spp. under scalding conditions at 55ºC

	0 sec	40 sec	80 sec	120 sec	160 sec	200 sec
Control	6.3ax	6.4ax	6.3ax	6.3ax	6.2ax	6.1ax
	0.02	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*
PEF Only	6.0bw	5.2abx	2.7cy	0.7cz	0.7bz	0.7bz
	0.04	*0.03*	*0.04*	*0.00*	*0.00*	*0.00*
PEF + 0.5% NaCl	6.1bx	4.3by	0.7dz	0dz	0cz	0cz
	0.07	*0.92*	*0.41*	*0.00*	*0.00*	*0.00*
PEF + 1% NaCl	6.5av	6.1aw	5.4bx	2.6by	0.7bz	0.7bz
	0.04	*0.04*	*0.10*	*0.11*	*0.00*	*0.00*

Means followed by different letter (a, b, c and d) within the column are significantly different ($P < 0.05$); Means followed by different letter (v, w, x, y and z) within the row are significantly different ($P < 0.05$). Italicized numbers represent the standard mean error.

TEXTURE ANALYSIS

Table 7

Early shear force results after PEF treatment and cooked to 100ºC

Treatment	Shear force (early)
control	0.156a
2 min	0.157a
4 min	0.162a

Table 8

Late shear force results after PEF treatment and cooked to 100ºC

Treatment	Shear force
control	0.185a
2 min	0.164a
4 min	0.179a

Table 9

Early shear force results after PEF treatment and cooked to 74ºC

Treatment	Shear force (early)
control	0.159a
2 min	0.146a
4 min	0.156a

Table 10

Late shear force results after PEF treatment and cooked to 74ºC

Treatment	Shear force
control	0.146a
2 min	0.151a
4 min	0.181a

Table 11

Early cook loss results after PEF treatment and cooked to 100ºC

Treatment	Cook loss (early)
control	24.01a
2 min	23.79a
4 min	23.33a

Table 12

Late cook Loss results after PEF treatment and cooked to 100ºC

Treatment	Cook loss
control	23.19a
2 min	24.63a
4 min	25.14a

Table 13

Early cook loss results after PEF treatment and cooked to 74ºC

Treatment	Cook loss (early)
control	22.97a
2 min	22.96a
4 min	21.29a

Table 14

Late cook loss results after PEF treatment and cooked to 74ºC

Treatment	Cook loss
control	18.63a
2 min	23.1a
4 min	20.64a

Table 15

Early drip loss results after PEF treatment

Treatment	Drip loss
control	3.54a
2 min	3.58a
4 min	3.48a

Table 16

Late drip loss results after PEF treatment

Treatment	Drip loss
control	4.03a
2 min	3.67a
4 min	3.04a

APPENDIX B

FIGURES

SALMONELLA SPP.

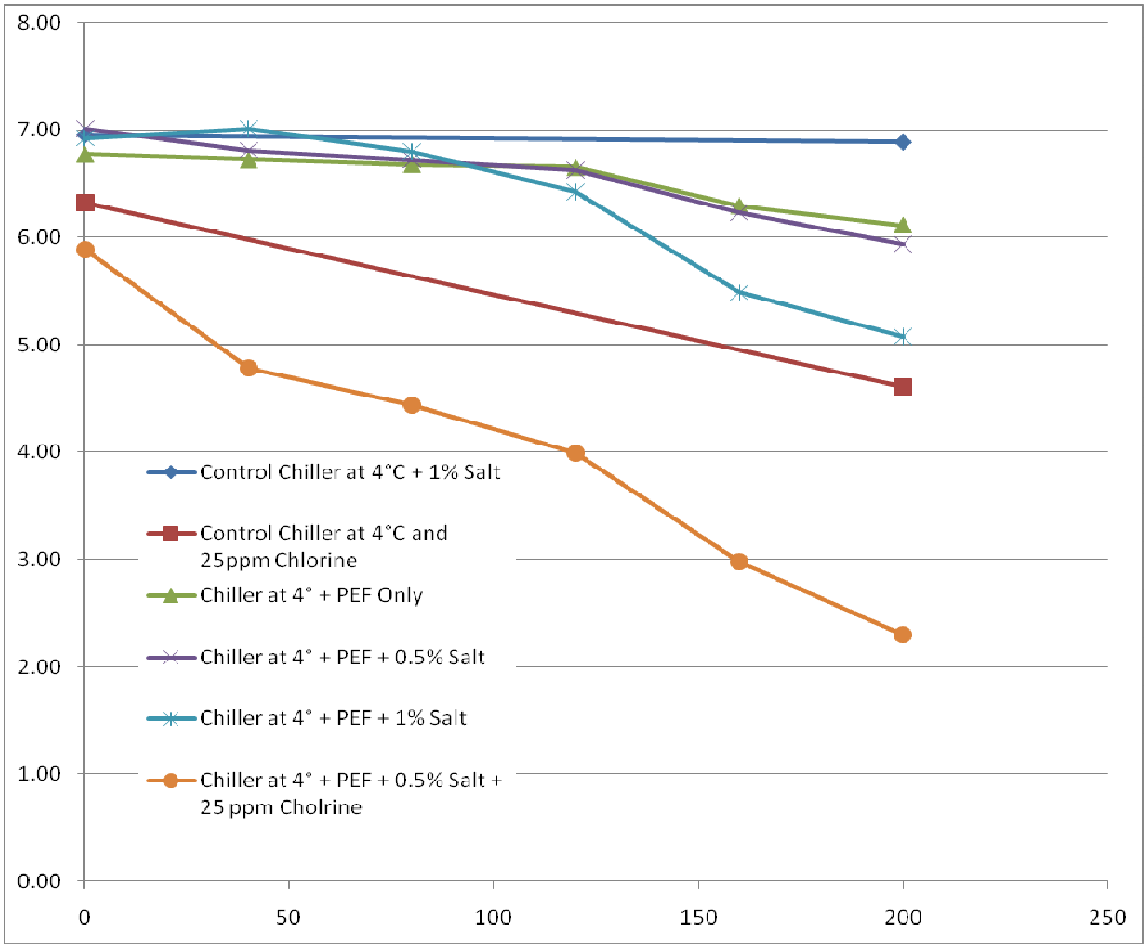

Figure 1

PEF results for *Salmonella* spp. under chilling conditions

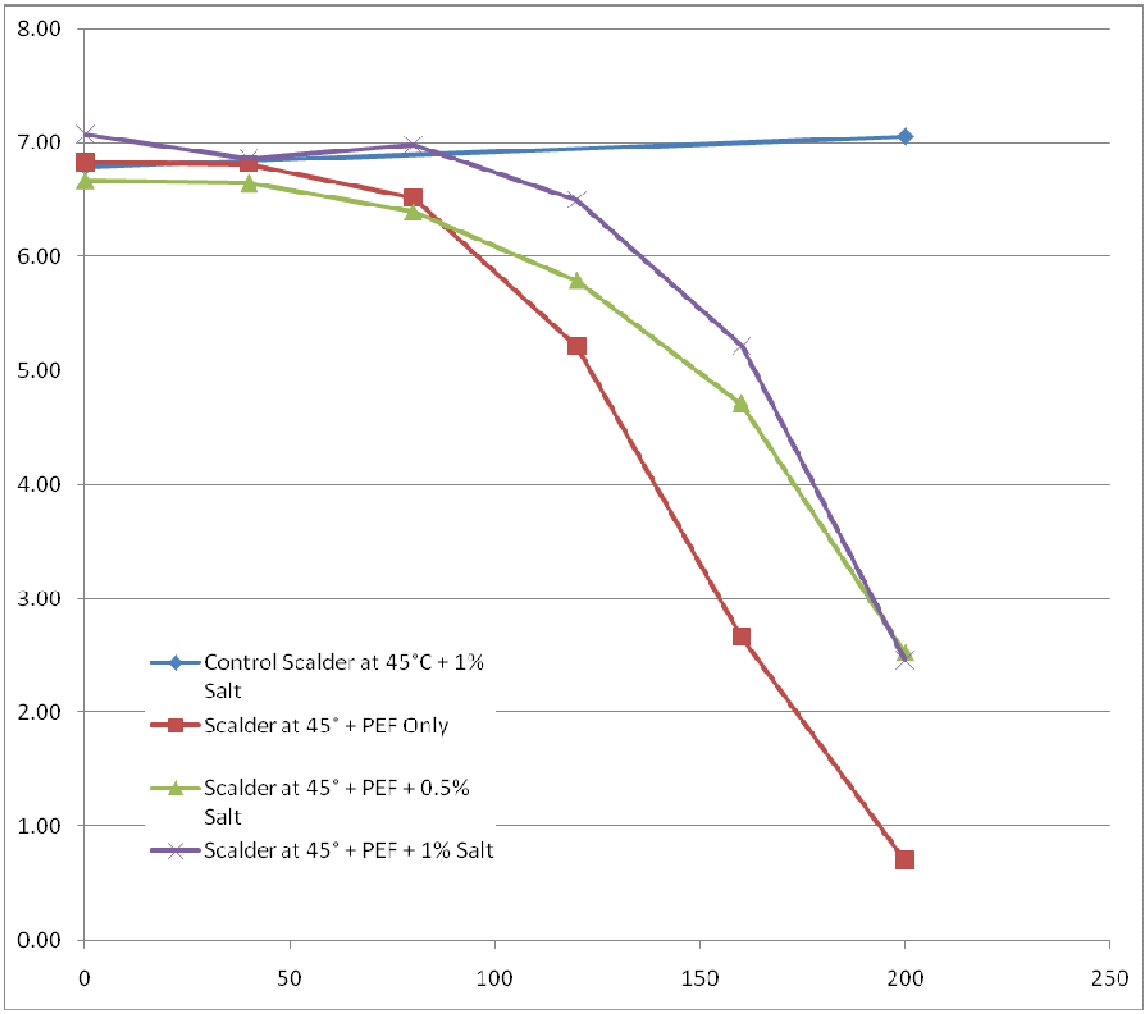

Figure 2

PEF results for *Salmonella* spp. under scalding conditions at 45°C

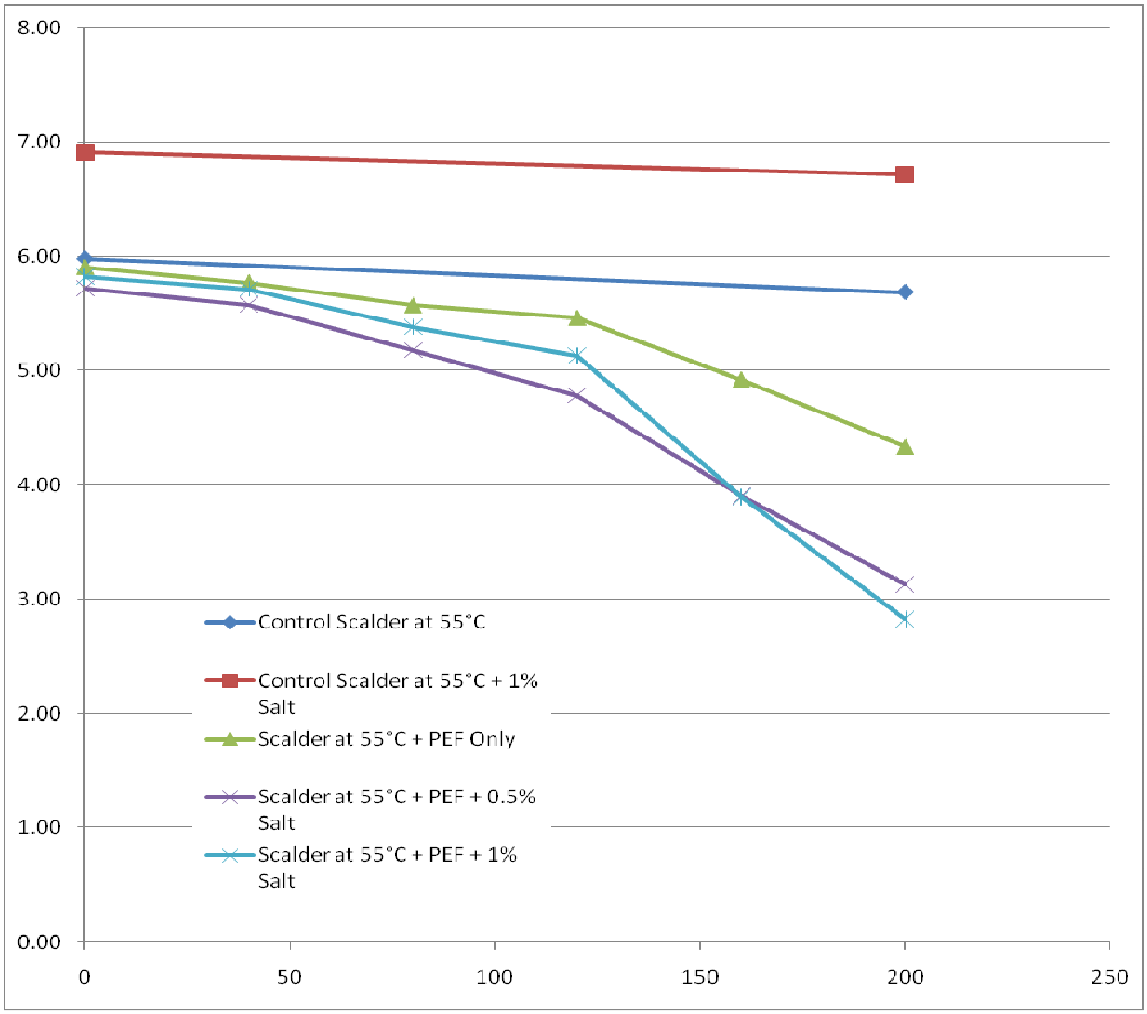

Figure 3

PEF results for *Salmonella* spp. under scalding conditions at 55ºC

CAMPYLOBACTER SPP.

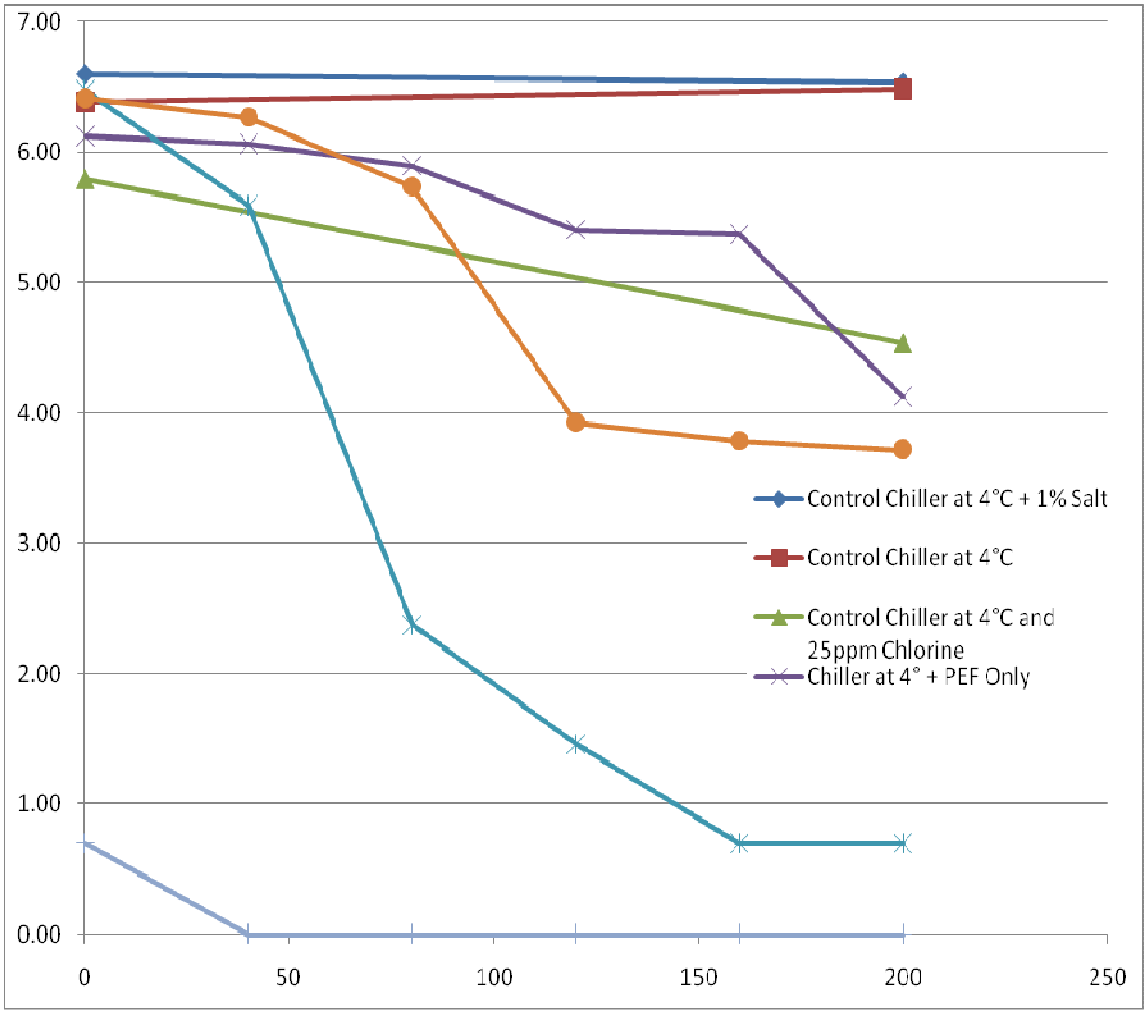

Figure 4

PEF results for *Campylobacter* spp. under chilling conditions

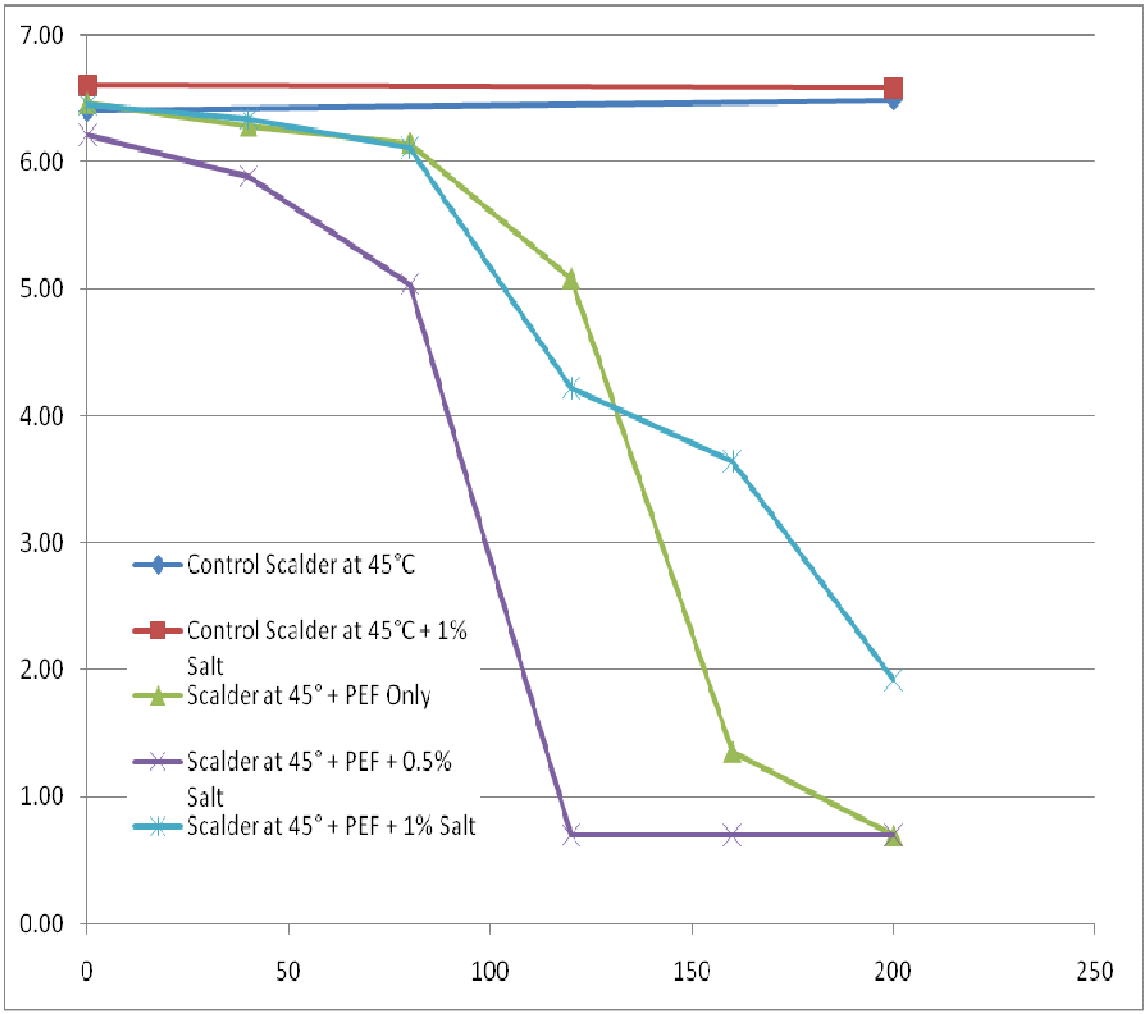

Figure 5

PEF results for *Campylobacter* spp. under scalding conditions at 45ºC

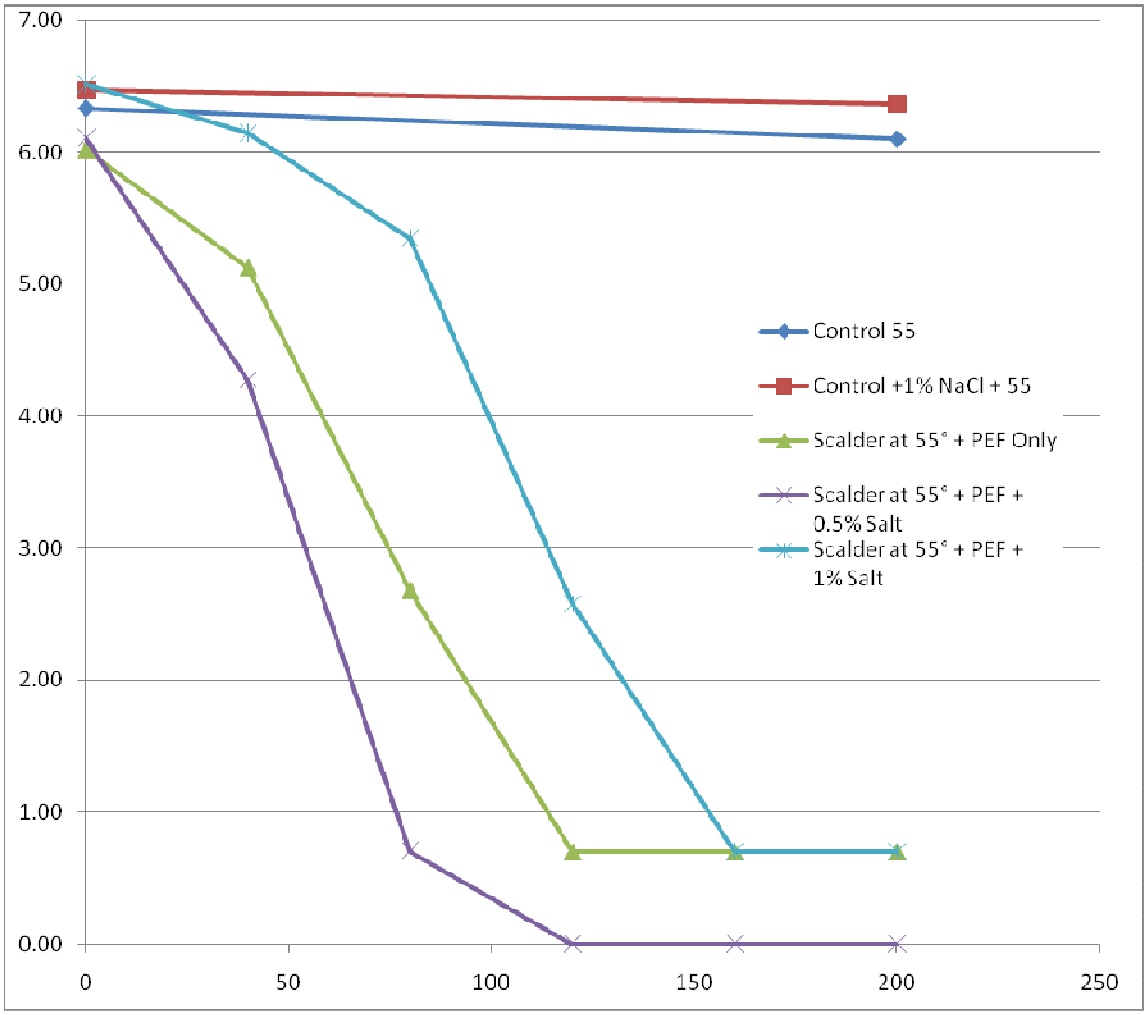

Figure 6

PEF results for *Campylobacter* spp. under scalding conditions at 55ºC

Figure 7

Electric stunner

Figure 8

Jacketed scalding tank & pump system

VITA

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