EFFICACY OF ELECTROLYTICALLY GENERATED HYPOCHLOROUS ACID (ELECTROLYZED WATER) ON FRESH AND PROCESSED MEATS

By

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LIST OF COMMON ACRONYMS

ml = milliliters

L = liters

ppm = parts per million

CFU = colony forming units

g = grams

FAC = free available chlorine

ORP = oxidation reduction potential

min = minute

sec = second

cm = centimeters

µg = micrograms

µl = microliters

psi = pounds per square inch

INTRODUCTION

In the United States alone there are 4.1 million cases of foodborne illness each year (Mead et al., 2000). These illnesses result in the loss of $2.9 to $6.7 billion annually from leading companies in the food industry (Powell and Attwell, 2000). This loss is mainly due to recalls from products contaminated with one of the major foodborne pathogens. The pathogens of most concern to the food industry are pathogenic strains of *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella enteriditis*, *Shigella*, toxin-producing *Staphylococcus*, and *Clostridium perfringens* (Mead et al., 2000).

L. monocytogenes is the culprit in most foodborne disease outbreaks arising from processed meats. In one example, *L. monocytogenes* was responsible for the recall of 16 million pounds of processed deli meat linked to four deaths and three miscarriages (Olsen et al., 2005). The bacterium is a Gram-positive psychrotroph and therefore, may grow at refrigeration temperatures. Listeriosis, the disease caused by *L. monocytogenes*, causes flu-like symptoms that in immune-compromised individuals can proliferate into septicemia, meningitis, and encephalitis. In pregnant women, the disease can cause miscarriages and stillbirths (FDA, 2001a).

E. coli O157:H7 is the leading cause of foodborne illness associated with fresh meats, especially ground beef. Recently, *E. coli* was attributed to contamination resulting in a recall of 21.7 million pounds of frozen ground beef

patties which were suspected of causing 32 cases of foodborne illness (Anonymous, 2007a). The disease symptoms resulting from *E. coli* O157:H7 infection include severe cramping, bloody stools, and a low grade fever. In immune-compromised individuals, the disease can result in hemolytic uremic syndrome and eventually kidney failure (FDA, 2001a).

The US Department of Agriculture (USDA) has tried to combat these organisms by using novel approaches and incentives. HACCP is one of these novel approaches. HACCP stands for hazard analysis critical control points and allows a facility to determine the point in their process where their greatest risks are in order to control them (Anonymous, 2001b). One of the novel incentives is the post-lethality reduction steps. These steps use a combination of chemical, thermal, or processing techniques that reduce the likelihood of product contamination. The chemicals that are allowed for use as part of the post-lethality reduction steps are listed as part of the code of federal regulations. The list is known as the safe and suitable ingredients list.

The safe and suitable ingredients list includes all of the commonly used antimicrobials. For fresh meats, two commonly used antimicrobials are organic acids and sodium hypochlorite. Studies have shown sodium hypochlorite to be an effective antimicrobial (Chantarapanont et al., 2004; Stopforth et al., 2004). An even better alternative are organic acids especially lactic acid (Van Netten et al., 1997; Stopforth et al., 2004). The processed meat industry commonly uses sodium lactate and sodium diacetate. Both substances have been shown to be effective antimicrobials and when used in combination can have an even greater

reduction of pathogenic bacteria (Qvist et al., 1994; Juneja et al., 2004; Serdengecti et al., 2006).

Electrolytically generated hypochlorous acid (electrolyzed water) is also listed as a safe and suitable ingredient by the USDA's Food Safety and Inspection Service (USDA-FSIS). It is allowed for use in the fresh meat industry as well as the poultry industry. Electrolyzed water uses a electrolysis in order to convert a weak brine solution into two solutions, the anolyte or the catholyte. "Anolyte solution is highly oxidized and functions as a very fast acting, anti-microbial agent that destroys bacteria and other microorganisms in a very short period of time" (Anonymous, 2005). The anolyte solution (electrolyzed water) is composed of hypochlorous acid which has been shown to be an effective antimicrobial (Albrich et al., 1986; Barrette et al., 1989; Hurst et al., 1991).

Studies have shown electrolyzed water to be an effective sanitizer in several ways. It has been shown to be effective against pure cultures of *E. coli* O157:H7, *Salmonella enteritidis*, *L. monocytogenes*, and *Salmonella typhimurium* (Venkitanarayanan et al., 1999; Fabrizio and Cutter, 2003; Park et al., 2004; Ayebah et al., 2006). When used on contact surfaces, electrolyzed water was shown to reduce biofilm formation and bacterial contamination (Ayebah et al., 2005; Ayebah et al., 2006; Park et al., 2002; Deza et al., 2007). With the recent outbreaks of foodborne illness associated with leafy green vegetables, it is important to note that electrolyzed can be used as an effective sanitizer on vegetables (Anonymous, 2006; Izumi, 1999; Chyi-Shen et al., 2005; Wang et al., 2004; Yang et al., 2003; Koseki et al., 2004a; Koseki et al., 2004c; Deza et al.,

2003). It can also be used effectively on fruits (Koseki et al., 2004b). Electrolyzed water has been tested on poultry surfaces and shell eggs as well (Russell, 2003; Park et al., 2005; Kim et al., 2005). Lastly, electrolyzed water has been shown to reduce levels of *Vibrio* species from oysters (Ren and Su, 2006).

Electrolyzed water has the potential to make an impact in the safety of the food system. The use of chlorine to eliminate bacteria has been a long standing and well accepted idea. Electrolyzed water is simply a chlorine-based solution generated by electrolysis. The technological advance of being able to generate a chlorine based solution from electrolysis of a salt solution was not widely implemented until the mid-1970's. It was only within the last 15 years that it took notice within the food industry as an automated process to produce a potential antimicrobial. In the following pages, one will find a detailed approach to determining the effectiveness of electrolyzed on the natural flora and pathogens commonly associated with meat products. Lastly, one will find the results detailing this effectiveness and a discussion of these results.

REVIEW OF LITERATURE

2.1 Background of food safety

Food preservation and food safety are not new concepts. Humans have been preserving food products since the switch from food gatherers to food producers. The most rudimentary methods of food preservation have been used for over 8000 years. History shows that the Egyptians and Sumerians of 5000 years ago salted fish and meats to extend the shelf life. These ancient cultures were also some of the first to produce butter and cheese (Jay, 2000). Butter and cheese making, which uses a method of culturing and aging, extends the shelf life of dairy products. Culturing can also be used as a means to produce lactic acid or ethanol. This type of culturing, called fermentation, uses naturally occurring microorganisms to biochemically modify a typical food product (Montarjemi, 1996). Beer, a fermented beverage, has been traced as far back as 7000 BC (Jay, 2000). Although not known at the time, salt, lactic acid, and ethanol were controlling undiscovered spoilage microorganisms. By controlling these microorganisms, the shelf life of those products was increased.

Salting and culturing have continued to be employed as food safety measures in the industry but many advances in science and technology have helped to evolve food systems. Canning, which was patented in 1810, was the first major technological advance in food preservation and safety (Jay, 2000). At the time, the microorganisms which cause spoilage and illness were not

understood but people did realize that there was a connection with heating and preservation. The next major advancement came in 1854 when Pasteur began using specific heat treatments to remove undesirable organisms (Jay, 2000). It wasn't until 1865 that artificial freezing became a possibility (Jay, 2000).

The new century brought increased food preservation through novel packaging and treatment ideas. The first controlled atmosphere packages were produced in 1928 (Jay, 2000). Nisin, sorbic acid, and chlortetracycline, which are all common food preservation treatments, weren't used on food products until the mid-1950's. Although the possibility of using irradiation on food products to eliminate bacteria became apparent in the 1940's, it was not accepted in the United States until the 1990's (Jay 2000). All preservation applications were ultimately used to reduce spoilage caused by unwanted microorganisms. For most of history though, these microorganisms were undiscovered.

At the time of Pasteur, the study of microorganisms had just begun to blossom. The idea that microorganisms caused disease, especially diseases from food, was even less understood. Justinus Kerner and Francesco Selmi had proposed that certain diseases were caused by foods but neither had understood that the disease causing agents were microorganisms. It wasn't until Gaernter first isolated *Salmonella enteriditis* from meat in 1888 that the association between food-borne disease and microorganisms was established (Jay, 2000). After Gaernter, numerous studies began isolating disease causing agents from food products. For example, within 20 years scientists had discovered *Clostridium botulinum*, *Bacillus cereus*, and had associated *Staphylococcus* with

food poisoning. The 20th century introduced the scientific world to a number of new foodborne disease causing agents. These include the major organisms of concern in today's food industry such as hemorrhagic colitis producing strains of *Escherichia coli*, *Listeria monocytogenes*, and *Campylobacter jejuni* (Jay, 2000).

2.2 Microorganisms of importance to the food industry

The organisms listed above are part of a list of commonly encountered foodborne disease causing agents. This group is made up of all pathogenic strains of *E. coli*, *L. monocytogenes*, *C. jejuni*, *S. enteriditis*, *Shigella*, toxin producing *Staphylococcus*, *Clostridium perfringens* and others. These bacteria make up the majority of the 4.1 million estimated bacterial food-borne illnesses each year. Of these bacteria, *Campylobacter* causes the greatest number of illnesses with 1.9 million cases, estimated. *Salmonella* is second causing an estimated 1.3 million illnesses each year. A major difference between these two illnesses is that of the 1.9 million cases of *Campylobacter* only 99 of the cases are estimated to result in death where as with *Salmonella* 533 cases result in death. *Salmonella* and *Campylobacter* are important food safety considerations because of the number of cases in which they are involved. Although their death-to-case ratio is low, the large number of cases still results in a considerable number of fatalities. *L. monocytogenes* and *E. coli* O157:H7 on the other hand are involved with much fewer cases but have a significantly higher death-to-case ratio. *L. monocytogenes* results in death in about 20% of all illnesses (Mead et al., 2000). These disease outbreaks cost the United States food industry an estimated $2.9 to $6.7 billion annually (Powell and Attwell, 2000).

2.3 *Escherichia coli* O157:H7

E. coli O157:H7 are members of the Enterobacteriaceae family. It is a Gram-negative rod measuring between 2 to 4 μm in length. Five types of *E. coli* cause intestinal disease derived from contaminated food products. These are enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggreative (EAEC), and enterohemorrhagic (EHEC) *E. coli*. *E. coli* O157:H7 is a type of EHEC strain which causes some of the worst types of manifestations (Ryan and Ray, 2004). The illness begins with severe abdominal cramping and diarrhea. Low grade fever and vomiting are associated with select cases. The illness then manifests into watery and bloody diarrhea. It is commonly self limiting and lasts for an average of eight days. In immune compromised individuals and young children, the infection can result in hemolytic uremic syndrome (HUS) (FDA, 2001a). Hemolytic uremic syndrome is the leading cause of kidney failure in children and is usually accompanied by microangiopathic hemolytic anemia and thrombotic thrombocytopenia (Razzaq, 2006).

E. coli O157:H7 has found a niche on a certain food product. Ryan and Ray (2004) state, "the emergence of EHEC is related to its virulence, low infecting dose, common reservoir (cattle), and changes in the modern food processing industry that provide us with fresher meat (and bacteria)" (Ryan and Ray, 2004). *E. coli* O157:H7 is commonly associated with fresh meat products specifically ground beef. The Topps Meat Company LLC. (Elizabeth, NJ) outbreak of 2007 involved the recall of 21.7 million pounds of frozen ground beef

patties (Anonymous, 2007a). The contaminated ground beef had been associated with 32 cases of *E. coli* O157:H7 food poisoning in Connecticut, Florida, Indiana, Maine, New Jersey, New York, Ohio, and Pennsylvania (Anonymous, 2007a).

2.4 *Listeria monocytogenes*

L. monocytogenes has also found a niche within the meat industry. Unlike *E. coli* O157:H7, *Listeria* proliferates on processed meats such as RTE meats. RTE meats are cooked and/or smoked during processing which eliminates the flora associated with typical fresh products. Further handling after cooking, re-introduces *L. monocytogenes* to the meat product. Some RTE meats are not reheated or not heated properly prior to consumption creating a dangerous cycle which can lead to human infection.

L. monocytogenes is a Gram-positive coccobacillus. The organisms are psychrotrophic and have been shown to grow in temperatures as low as 1°C. In humans, the bacterium is able to attach to phagocytes and survive in macrophages. Its ability to survive in macrophages allows it to transfer from cell to cell without being detected by the human immune system (Ryan and Ray, 2004). The organism produces the disease listeriosis which begins as nausea, vomiting, and persistent fever. Listeriosis has the potential to manifest into a multitude of diseases including septicemia, meningitis, encephalitis, and intrauterine or cervical infections. In pregnant women, cervical or intrauterine infections can result in spontaneous abortion or stillbirth (FDA, 2001a).

In October 2002, 46 culture confirmed cases, seven deaths, and three stillbirths were associated to *L. monocytogenes* food poisoning. The outbreak was linked to cases in New York, Pennsylvania, New Jersey, Delaware, Maryland, Connecticut, Massachusetts, and Michigan. The Pilgrim's Pride Foods' Franconio, PA processing plant was forced to recall 27.4 million pounds of fresh and frozen ready-to-eat (RTE) turkey and chicken products (Anonymous, 2002).

2.5 Measures to improve food safety

2.5.1 Government regulations

Until 1906, there were few rules or regulations governing the food industry. After being disgusted by the conditions described in Upton Sinclair's "The Jungle," President Theodore Roosevelt demanded change within the nation's meat facilities. The act that came out of this was the Federal Meat Inspection Act. In order to enforce the measures in the act, two governmental bodies had to be formed. These were the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA).

The USDA has control of the United States' meat industry. The agencies of the USDA set guidelines, rules, and regulations to protect the safety of the nation's meat supply. One of the biggest improvements in food safety came as a mandatory directive from the FDA and USDA. HACCP, which stands for hazard analysis critical control points, involves seven basic principles. These principles can be found in the table below. HACCP focuses on the prevention and reduction of bacterial pathogens on meat based products. Implementation of the

HACCP plan began in meat facilities on January 27, 1997 and was completed on January 25, 2000 (USDA-FSIS, 1996).

Table 1. The Seven Principles of HACCP

1. Analyze hazards.
2. Identify critical control points.
3. Establish preventive measures with critical limits for each control point.
4. Establish procedures to monitor the critical control points.
5. Establish corrective actions to be taken when monitoring shows that a critical limit has not been met.
6. Establish procedures to verify that the system is working properly.
7. Establish effective record keeping to document the HACCP system.

(from Anonymous, 2001b)

The Federal Meat Inspection Act gave a definition to ingredients that would be considered an adulterant. According to the act, "the term 'adulterated' shall apply to any carcass, part thereof, meat or meat food product if it bears or contains any added poisonous or added deleterious substance other than one which is a food additive" (USDA-FSIS, 1971). Ground beef is considered adulterated when it is contaminated with *E. coli* O157:H7 and all products that test positive for the organism must be recalled (USDA-FSIS, 2004). In regards to *L. monocytogenes*, United States Department of Agriculture's Food Safety Inspection Service (USDA-FSIS) has demanded a zero tolerance policy for all RTE meat products. In order to control the growth of *L. monocytogenes*, the USDA-FSIS has established a flexible program for RTE manufacturers in which they may fit into any of three processing categories or "Alternatives." Alternative 3 requires facilities producing RTE products to have a set sanitation program. For processors choosing Alternative 2, they not only require a set sanitation program but also a post lethality treatment of their product that either suppresses

the growth of microorganisms or reduces or eliminates microorganisms. For those processors choosing Alternative 1, they must employ the use of all three measures (USDA-FSIS, 2003). The incentive for implementing the additional processes required for Alternatives 1 and 2 is reduced testing by USDA-FSIS. The post lethality treatments allowed for use in or on meat products have been listed in the Safe and Suitable Ingredients document (USDA-FSIS, 2008).

2.5.2 Ingredients allowed for use in meat and poultry products

In order to eliminate *E. coli* O157:H7 from ground beef and to reduce the presence of *L. monocytogenes* from RTE meats, meat producers have begun using additives and topical spray treatments which inhibit or reduce microorganisms. Some examples of additives that are allowed include sodium and potassium lactate, sodium diacetate, and sodium citrate. Examples of topical applications are lactic acid, lauric arginate, octanoic acid and hypochlorite.

Additives used in the meat industry are typically injected into the meat product or added as part of an emulsion, as in frankfurters. Sodium and potassium lactate can be added in amounts equal to or less than 4.8% of the finished product weight. The addition of 2% sodium lactate suppressed growth of *L. monocytogenes* for 28 days in bologna type sausages (Qvist et al., 1994). *C. perfringens* was reduced by greater than 1 log CFU/ml in roast beef when the product formulation included sodium lactate (Juneja and Thippareddi, 2004). Sodium diacetate, which can not be used at levels above 0.25% based on the finished product weight, is commonly added along with sodium or potassium lactate. When sodium or potassium lactate is used in combination with sodium

diacetate, there is a greater inhibitory effect. Serdengecti et al. (2006) found that not only was the combination lethal to *S. enteriditis* but it also delayed the growth of *L. monocytogenes* (Serdengecti et al., 2006).

Topical treatments are typically added in one of three ways. The first is by a simple spray application while the second uses a spray-in-package method. The spray-in-package method utilizes a vacuum packaging system to evenly spread an antimicrobial over the surface of a product. The third application is by immersion dipping. Lactic acid, which must be used at levels below 5%, and hypochlorite, which can be used at various levels depending on the product type, are typically added by spray application or immersion. Lauric arginate, which can be used at or below 200 ppm, and octanoic acid, which is allowed at levels below 220 ppm, are added by any of the three application types.

The organic acid, lactic acid, has been shown to reduce 1.8 log CFU/ml of Gram-negative bacteria when electrostatically sprayed onto "hot" pork carcasses (Van Netten et al., 1997). Stopforth et al. (2004) showed similar findings when a greater reduction was seen with lactic acid when compared to the reductions with sodium hypochlorite (Stopforth et al., 2004). Chantarapanont et al. (2004) tested sodium hypochlorite and octanoic acid on the viability of *C. jejuni* on chicken skin. When inoculated chicken pieces were immersed in either a hypochlorite solution (pH 7.2) or an octanoic acid solution, each of which was at 100 ppm of active ingredients for 15 min, levels of C. jejuni were reduced by 1 log CFU/ml (Chantarapanont et al., 2004). Burnett et al. (2007) showed up to a 2.99 log CFU/ml reduction of *L. monocytogenes* when whole roast turkeys were

immersed in a 1% solution of octanoic acid at a pH of 4.0 (Burnett et al., 2007). Luchansky et al. (2005) spray-in-package treated hams with 4, 6, and 8 ml of a 5% solution of lauric arginate. This resulted in over a 1.48 log CFU/ml reduction in less then 24 hours (Luchansky et al., 2005).

2.5.3 Electrolyzed water as a sanitizer

USDA-FSIS considers electrolyzed to be “electrolytically generated hypochlorous acid.” It is allowed for use on red meat carcasses down to a quarter of a carcass, whole or eviscerated poultry carcasses, in water used in meat and poultry processing, in poultry chiller water, for reprocessing contaminated poultry carcasses, on giblets and salvage parts, and on beef primals. Depending on the product it can be used from 5 to 50 ppm free available chlorine (FAC) (USDA-FSIS, 2008).

2.6 Electrolyzed water composition

Electrolyzed water uses electrolysis in order to convert a weak brine solution into various oxidizing products. The brine solution itself is a saturated mixture of sodium chloride and water. This saturated mixture is introduced into a cell containing inert positively and negatively charged electrodes separated by a septum (Al-Haq et al., 2005). The anolyte solution is highly oxidized and can be used as a fast acting anti-microbial agent. The catholyte solution, which is generated at about $1/10^{th}$ the rate of anolyte, is alkaline and can act as a mild degreaser (Anonymous, 2005).

The separate anolyte and catholyte solutions are composed of several different compounds. The theoretical sequence of chemical reactions involved in

the production of electrolyzed oxidizing water can be summarized as follows. In the electrolysis chamber, sodium chloride dissolved in water dissociates into negatively and positively charged ions. The negatively charged chloride and hydroxyl ions are adsorbed to the anode, with each ion releasing an electron to become a radical. The two ion radicals combine, forming hypochlorous acid which separates from the anode (Venkitanarayanan and Ezeike, 1999). The components which make up both solutions can be found in the table shown below. Studies have suggested that hypochlorous acid can penetrate microbial cell membranes and in turn exert antimicrobial action through the oxidation of key metabolic systems (Albrich et al., 1986; Barrette et al., 1989; Hurst et al., 1991). It is customary to refer to electrolyzed water as the acidic anolytic solution produced after electrolysis and will be referred to as such from this point further.

Table 2. Chemical Equations Associated with the Production of Anolytic and Catholytic Electrolyzed Water

Anolytic Components	Catholytic Components
1. Generation of free radicals, active oxygen, and hydrogen peroxide $H_2O = H^+ + OH + e^-$ $OH + OH = H_2O_2$ $H_2O = O + 2H^+ + 2e^-$ 2. Generation of ozone gas $3H_2O = O_3 + 6H^+ + 6e^-$ $O + O_2 + O_3$, $O + O_2 = O_3$ $O_2 + H_2O = O_3 + 2H^+ + 2e^-$ ($E^o = 2.07$ V) 3. Generation of oxygen gas $2H_2O = O_2 + 4H^+ + 4e^-$ $4OH^- = O_2 + 2H_2O + 4e^-$ $H_2O_2 = O_2 + 2H_2O + 4e^-$ $2Cl^- + 2H_2O = 4H^+ + 4Cl^- + O_2$ 4. Generation of oxygen gas and dissolved chlorine $2Cl^- + O_3 + 2H^+ = O_2 + Cl_2 + H_2$ $2Cl^- + 2O_3 = 3O_2 + Cl_2 + 2e^-$ 5. Generation of chlorine gas and dissolved chlorine $2Cl^- = Cl_2(g) + 2e^-$ ($E^o = 1.359$ V) $2HOCl + 2H^+ + 2e^- = Cl_2 + 2H_2O$ ($E^o = 1.63$ V) $Cl_2(g) = Cl_2(aq)$ 6. Generation of hypochlorous acid and hypochloric acid $Cl_2(aq) + H_2O = HCl\ (=H^+ + Cl^-) + HOCl$ $Cl^- + H_2O = HOCl + H^+ + 2e^-$ $2Cl^- + H_2O = HOCl + HCl + 2e^-$ $2Cl^- + H_2O_2 = 2HOCl + 2e^-$ $ClO^- + H_2O = HOCl + OH^-$ 7. Generation of hypochlorite ion, etc. $Cl_2(aq) + 2OH^- = ClO^- + Cl^- + H_2O$ $Cl_2(aq) + H_2O = ClO^- + Cl^- + 2H^+$ $HOCl = ClO^- + H^+$ $2HOCl\ ClO^- = ClO_3^- + 2Cl^- + 2H^+$	1. Generation of hydrogen gas $2H_2O + 2e^- = H_2 + 2OH^-$ ($E^o = -0.828$ V) $2H_2O = H_2 + 2OH$ 2. Generation of hydrogen and sodium hydrate $2Na^+ + 2H_2O + 2e^- = H_2 + 2NaOH$ $2Na + 2H_2O = H_2 + 2NaOH$ $Na^+ + OH^- = NaOH$ 3. Generation of hydroxide ion and separation of sodium $Na^+ + e^- = Na$ $2OH + 2e^- = 2OH^-$ $O_2 + 2H_2O + 4e^- = 4OH^-$

2.7 Properties of electrolyzed water

The properties of electrolyzed water contributing to a bactericidal effect include pH, oxidation reduction potential (ORP), and free available chlorine (FAC), which are various characteristics that affect the antimicrobial properties of hypochlorous acid. These properties can be altered during the generation process. The brine and water flow rates, temperatures, sodium chloride concentration and voltage can have an effect on altering the properties of the finished electrolyzed water. Ezeike and Hung (2004) showed that the pH and ORP of electrolyzed water solutions were most affected by the sodium chloride concentration followed by voltage, flow rate and temperature, respectively. In the case of FAC, however, the flow rate was relatively more important than voltage. Their study showed some obvious generalities that the higher the sodium chloride concentration and voltage, the higher the ORP and FAC of electrolyzed water and that an increased flow rate will produce electrolyzed water with lower ORP and FAC due to the shorter residence time in the electrolytic cell (Ezeike and Hung, 2004).

Once produced, electrolyzed water has a window of time in which it retains its greatest effectiveness. Agitation, light exposure, and storage conditions are all factors that may affect the length of usable time. Len et al. (2002) looked at how these factors affect an electrolyzed water solution produced through electrolysis of a 0.1% sodium chloride solution at 14 A and 7.4 V. Under open storage conditions agitation increased the rate of chlorine loss approximately 5-fold, but it was not significantly affected by diffused light. Under

closed conditions, the effect of diffused light was more significant compared to agitation but only after two months of storage in glass containers. The rate of chlorine loss was also affected by the initial pH of the solution. As the pH increased, the rate of chlorine loss decreased (Len et al., 2002).

2.8 Electrolyzed water on bacterial cultures

Although the FAC concentration may be the biggest inhibitory factor, all of the properties mentioned above go into determining the effectiveness of electrolyzed water on microorganisms. When held at an FAC range of 43 to 86 ppm, the effectiveness of electrolyzed water increased as temperatures increased. Cultures of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* were reduced by approximately 7 log CFU/ml when exposed to electrolyzed water at either 4°C or 23°C for 5 minutes. The amount of exposure time needed to achieve the same effect was reduced to 2 minutes when solution temperatures were increased to 35°C and 1 min when at 45°C (Venkitanarayanan and Ezeike, 1999).

Electrolyzed water at 100 ppm FAC showed an 8 log CFU/ml reduction in cultures of either *Salmonella typhimurium* or *L. monocytogenes*. The cultures were exposed to the 25°C solution for 5 minutes. Fabrizio and Cutter (2003) suggested the Gram-negative bacterial membrane is more fluid at 25°C compared to cooler temperatures, owing to its high phospholipid composition. They felt that as the membrane becomes more fluid, the antimicrobial agent can enter the cell more readily (Fabrizio and Cutter, 2003).

Another study looked at how the pH of the electrolyzed water affected its antimicrobial capability. Electrolyzed water was produced at either pH 3.0, 5.0 or 7.0. The two bacteria tested at each pH were *L. monocytogenes* and *E. coli* O157:H7. According to Park et al. (2004), with sufficient residual chlorine, electrolyzed water can be applied in a pH range between 2.6 and 7.0 while still achieving complete inactivation of the bacteria. This does not coincide with data presented by other researchers. Park et al (2004) chose an exposure time of only 30 seconds and an FAC range of 0.1 to 5.0 ppm showing that complete inactivation was achieved with an FAC of only 2.0 ppm while previous research showed bacterial elimination to take at the least 5 minutes with an FAC as high as 40 ppm (Park et al, 2004).

Electrolyzed water produced at two different amperages showed no difference in reduction levels. Water analysis was completed on two separate electrolyzed water solutions. One was produced at 14A while the other was at 20A. The solution at 14A contained an FAC level of 44 ppm while the other solution was at 94 ppm. Planktonic *L. monocytogenes* cells exposed to both solutions for either 1 or 5 minute(s) showed an 8 log CFU/ml reduction within 1 minute of exposure to either solution (Ayebah et al., 2006).

2.9 Effect of electrolyzed water on contaminated contact surfaces

In nature, bacteria often grow as aggregated assemblies of cells. These assemblies, called biofilms, can exclude antimcrobials while at the same time have a channel system to provide nutrients to internal cells (Ryan and Ray, 2004). Ayebah et al. (2006) looked at the effect electrolyzed water would have

on biofilms formed by *L. monocytogenes*. The biofilms were produced on stainless steel coupons by immersing them in an inoculated low nutrient broth. The coupons remained in the inoculum for 4 hours and then were incubated for an additional 48 hours. After allowing for biofilm formation, the coupons were treated for either 30 or 60 seconds in an electrolyzed water solution with an FAC concentration at 85 ppm with a pH of 2.29. Both exposure times resulted in a greater than 4 log CFU/ml reduction of viable *L. monocytogenes* (Ayebah et al., 2006). These results agree with their prior research in which an exposure time of 120 seconds resulted in an even greater reduction of *5.21 log CFU/ml of L. monocytogenes* (Ayebah et al, 2005).

Park et al. (2002) showed that the antimicrobial capability of electrolyzed water was not dependent on surface type. They looked at five commonly used food contact surfaces, glass, stainless steel, glazed ceramic tile, unglazed ceramic tile, and vitreous china. Electrolyzed water at 50 ppm of FAC was applied to the surfaces inoculated with either *Staphylcoccus aureus* or *Enterobacter aerogenes* for a total time of 5 minutes. No statistical difference in antimicrobial activity was shown between surface types but surfaces treated with agitation did show differences in lethality. Surfaces treated without agitation showed only a 2.5 log CFU/ml reduction of *E. aerogenes*, while treatments with agitation increased reduction up to 6.16 log CFU/ml. Similar reductions were seen with inoculated surfaces of *S. aureus* (Park et al., 2002).

The study performed by Park et al. (2002) may not have shown a difference between surface types but another study did show such differences.

Deza et al. (2007) used two different types of cutting boards, wood and plastic, which were inoculated with either *E. coli*, *L. monocytogenes*, *Pseudomonas aeruginosa*, or *S. aureus*. The inoculated boards were treated with electrolyzed water at approximately 60 ppm of FAC with an average pH of 7.76. An exposure of only 1 minute on the inoculated plastic cutting boards resulted in an average reduction of 5 log CFU/50 cm^2. With the inoculated wood cutting boards, the exposure time had to be increased to 5 minutes in order to reach a reduction level of 4 log CFU/50 cm^2. Deza et al. (2007) suggested that wooden boards readily absorbed the bacterial suspension during the 5 minute inoculation step. Thus, the bacteria were able to proliferate within the surface pores (Deza et al., 2007).

2.10 Effect of electrolyzed water on produce

2.10.1 Effect of electrolyzed water on leafy green vegetables

In October of 2006, the Center for Disease Control announced that 199 people had become sick from fresh spinach contaminated with *E. coli* O157:H7. The bacterium which is usually connected to outbreaks in ground beef has also been the implicated in cases involving lettuce and green onions (Anonymous, 2006).

Izumi (1999) tested the bactericidal effect of an electrolyzed water solution at 20 ppm FAC with a pH of 6.8 on the natural flora of 50 g samples of spinach. Three different types of treatments were performed on the samples. The first treatment involved a continuous flowing wash at 2 L/min with an exposure time of 3 minutes. The second used a dip method in which samples were immersed in a

500 mL solution for 3 minutes. The final treatment was also used a dip method except that air blowing at 25 L/min was injected into the solution. Each treatment was followed by a 1 min tap water rinse. The natural flora of the spinach was reduced 1.5 logs CFU/ml using the first treatment method. Both dip treatments showed a reduction of only 0.1 logs CFU/ml greater then the rinse. A second objective of this study was to look at the effect FAC concentration would have when solutions were applied for 4 minutes. When the FAC concentration was adjusted to either 15 or 30 ppm there was more than a 0.5 log CFU/ml reduction when compared to the control. At 50 ppm no detectable organisms were recovered from the sample (Izumi, 1999).

Chyi-Shen et al. (2005) performed a study that mimicked the method used above by Izumi (1999). The same volumes and ratios as described above were retained in this experiment as well, however the treatment times were changed. The first treatment involved soaking in electrolyzed water at 50 ppm FAC for either 9 or 15 minutes or soaking in the catholytic portion produced during electrolysis (AK) for either 3 or 5 minutes. The second treatment used the continuous rinse method for 9 minutes with electrolyzed water which was followed by a 3 min AK rinse. The last treatment involved continuously changing the electrolyzed water three or five times for every 3 minutes and was followed by AK water for 3 or 5 minutes. Chyi-Shen et al. (2005) not only used spinach but also included chinjon (a leafy green vegetable) and cabbage. The electrolyzed water treatment solution was at approximately 50 ppm FAC for each study. This level of chlorine produced a reduction of up to 1.7 logs CFU/ml of

natural indigenous bacteria on spinach samples in the first treatment. The other two samples were only slightly lower. For the AK solution used in treatment one, the largest reduction was seen in cabbage with a reduction of 0.5 log CFU/ml. The second treatment method resulted in a reduction of 1.6 log CFU/ml on cabbage and spinach but only a 0.7 log CFU/ml reduction on chinjon. The third treatment did show a higher reduction, of 2.2 log CFU/ml, on spinach samples but failed to produce a greater reduction on chinjon or cabbage when compared to the other two treatment methods (Chyi-Shen et al., 2005).

Wang et al. (2004) used chlorine levels between 15 and 30 ppm FAC on cilantro samples, which are similar to those used by Izumi (1999). In this study, 1.5 kg of fresh cilantro was washed in 45 L of electrolyzed water. Each sample was washed with agitation for 5 minutes. In the Izumi (1999) study, approximately 0.5 log CFU/ml of natural bacteria were removed when samples were rinsed for 4 minutes. This study showed that rinsing for an extra minute only increased the reduction by 0.16 log CFU/ml on similar leafy green vegetables (Wang et al., 2004).

In 2003, a study was completed in which lettuce samples were inoculated with several types of bacteria and then treated with an electrolyzed water solution. Heads of lettuce were cut and measured out in 25 g samples. The samples were then inoculated with either *L. monocytogenes*, *E. coli* O157:H7, or *S. typhimurium*. Each sample was treated in 800 mL of electrolyzed water at 300 ppm FAC for 5 minutes with agitation while being held at a temperature of 30°C. Several different pH levels of electrolyzed water were used on the samples.

Reductions of *S. typhimurium* and *L. monocytogenes* ranged from 1.5 to 2.0 and 1.7 to 2.1 log CFU/ml, respectively, when the treatment solutions were in a pH range from 4 to 9. These reductions were not significantly different from one pH to another. This was not the case with *E. coli* O157:H7, which was reduced in a range of 1.3 to 2.2 log CFU/ml at pH levels between 4 and 7 (Yang et al., 2003).

In several other studies examining the effect of electrolyzed water on microbial levels on lettuce, unique methods were used in an attempt to reduce inoculated bacteria. In one study, they tested the effect of a mild heat pre-treatment with the alkaline, catholytic portion (AK) on the efficacy of electrolyzed water (Koseki et al., 2004a). Lettuce samples for this experiment were inoculated with either *E. coli* O157:H7 or *Salmonella* sp. For pre-treatment, 10 pieces of lettuce at 5 cm^2 were placed into 1.5 L of solution for either 1 or 5 minutes. Subsequent treatments were also for either 1 or 5 minutes in 1.5 L of solution. The mildly heated (50°C) pretreatment with AK for 1 minute with a subsequent treatment of electrolyzed water resulted in a 2.7 log CFU/g reduction of both pathogens, regardless of the duration of the subsequent treatment with electrolyzed water. It was also noted that, extending the pre-treatment time of the mildly heated solution increased the bactericidal effect. This was regardless of the extent of exposure to subsequent treatment with electrolyzed water. (Koseki et al., 2004c).

Koseki et al. (2004a) employed the use of frozen electrolyzed water to control pathogens on lettuce. In this experiment, lettuce samples were inoculated with either *L. monocytogenes* or *E. coli* O157:H7. They examined

sample size to ice ratio and the storage time of frozen electrolyzed water with a concentration range of 20 to 200 ppm FAC and a pH of 2.6. In regards to the weight ratio, when 10 times the weight of ice was used relative to the sample size, *L. monocytogenes* was significantly reduced by 1.5 log CFU/g. *E. coli* O157:H7 was reduced by 1.0 log CFU/g when three and four times the weight of ice was used relative to the sample size. In regards to storage time, *L. monocytogenes* was reduced by 1.3 log CFU/g within 2 hours of storage time. Similar reductions were seen within only 1 hour of storage time for *E. coli* O157:H7 (Koseki et al., 2004a).

2.10.2 Effect of electrolyzed water on cucumbers

Although safety in the leafy green vegetable industry has become a priority in recent years, these vegetables are not the only ones in which electrolyzed water could be an effective sanitizer. Several studies have looked at the benefits of using electrolyzed water on cucumbers. In one study, 50 g samples of sliced cucumbers were treated with a running solution of electrolyzed water (2 L/min). Samples were treated for 4 minutes with electrolyzed water at three different FAC concentrations, 15, 30, or 50 ppm. The three concentrations were held at a constant pH of 6.8. With a reduction of 0.8 log CFU/g of the naturally occurring bacteria, the electrolyzed water solution at 50 ppm produced a significantly greater reduction than did the control or the other two FAC concentrations (Izumi, 1999).

A different study examined the effect both electrolyzed water and the alkaline, catholytic portion (AK) had on the natural flora on whole cucumbers.

The samples were dip treated in 500 mL of treatment solution. An electrolyzed water solution at 50 ppm produced a reduction of 1.0 log CFU/g when applied for 15 minutes to a 50 g sample. When the electrolyzed water treatment was followed by an AK treatment for 5 minutes, the reduction was only increased by 0.2 log CFU/g. A much greater reduction was seen when the electrolyzed water solution was changed out every 3 minutes for a total exposure time of 15 minutes. When an AK treatment of 5 minutes with ultrasonic shaking was added on to the end of the 15 minute treatment, reductions reached up to 2.2 log CFU/g (Chyi-Shen et al., 2005). Koseki et al. (2004b) showed similar reductions when cucumber sticks were dip treated in AK for 5 minutes followed by treatment in 2 L of electrolyzed at 32 ppm with a pH of 2.6 for 5 minutes. This study though showed a 0.5 log greater reduction of naturally occurring bacteria in only 10 minutes of exposure to electrolyzed water when compared to previous studies (Koseki et al., 2004b).

2.10.3 Effect of electrolyzed water on other vegetables

Two studies have tested electrolyzed water's ability to eliminate bacteria on other vegetables including radishes, potatoes, bell peppers and carrots. Izumi (1999) used 50 g samples of chopped bell peppers with electrolyzed water at 20 ppm of FAC with a pH of 6.8. The greatest reduction was seen when the samples were dip treated with agitation for 3 minutes in electrolyzed water and then followed by a rinse in tap water for 1 minute. When rinse treated for 4 minutes with electrolyzed water, there was a 0.8 log CFU/ g reduction compared to a rinse with tap water for the same amount of time (Izumi, 1999). Chyi-Shen et

al. (1999) increased the exposure time of the peppers and also included a treatment with the alkaline, catholytic solution (AK). The greatest reduction of 2.2 log CFU/g was seen when electrolyzed water at 50 ppm was replaced every 3 minutes for a total exposure time of 15 minutes followed by an ultrasonic wash in AK for 5 minutes. The reductions observed with ultrasonic treatments in water alone were 1.8 log CFU/g less then the treatments with electrolyzed water (Chyi-Shen et al., 1999).

Izumi (1999) also tested carrots, Japanese radishes, and potatoes. All three vegetables received a running rinse (2 L/min) treatment for 3 minutes, a dip treatment in 500 mL, and a dip treatment with air blowing at 25 L/min through the solution. For the running rinse treatment, dip treatment, and air blowing treatment, potatoes showed the greatest reductions with 1.7, 1.4, and 2.1 log CFU/g, respectively. When the samples were treated for 4 minutes in 500 mL, carrots showed the greatest reduction when compared to the same treatment with tap water. Of the vegetables mentioned, carrots samples were the only ones used to examine 3 different levels of FAC. The greatest log CFU/ml reduction was seen with the highest level tested which was 50 ppm (Izumi, 1999).

Deza et al. (2003) examined the use of electrolyzed water's antimicrobial effect on tomatoes. Whole tomatoes were inoculated with either *E. coli* O157:H7, *S. enteriditis*, or *L. monocytogenes*. Inoculated samples were placed in a bag along with 100 mL of electrolyzed water at 89 ppm FAC with an approximate pH of 8.00. The samples were then shaken for either 30 or 60 seconds. This

method produced a reduction of greater than 3.6 log CFU/ml regardless of bacterial type. There was not a significant statistical difference between treatment times at 30 or 60 seconds on *E. coli* O157:H7, *S. enteritidis*, or *L. monocytogenes* (Deza et al., 2003).

2.10.4 Effect of electrolyzed water on fruit

Research has shown that electrolyzed water has approximately the same efficacy on fruits as it does on vegetables. Koseki et al. (2004b) were able to obtain a 0.9 log CFU/strawberry reduction of naturally occurring bacteria. The strawberries were in samples of 150 g and were treated for 10 minutes in 2 L of continuously flowing electrolyzed water at 32.1 ppm of FAC with a pH of 2.6 (Koseki et al., 2004b). In another study on apples inoculated with *E. coli* O157:H7, they showed a reduction of 1.08 log CFU/cm^2. Apple samples (60 g) were dip treated in a 600 mL solution of electrolyzed water for 8 minutes. The FAC of the solution at a pH of 2.7 was higher than in the previous study at 68 ppm of FAC. This could account for the increased bacterial reduction on the apples. This same study also looked at inoculated cored cylindrical cantaloupe samples (250 g). The cantaloupes were treated for a total time of 15 minutes which resulted in only a minor change in reduction of 1.15 log CFU/cm^2 (Wang et al., 2006).

2.11 Effect of electrolyzed water on poultry and shell eggs

With 1.3 million cases of salmonellosis every year, *Salmonella* ranks second in the United States as a source of food poisoning (Mead et al., 2000). Studies have associated the presence of *Salmonella* in poultry hatcheries with

contamination among broiler flocks (Christensen et al., 1997). The organism is very versatile in its ability to spread from one source to another and can be isolated from a variety of sources within the broiler hatchery including individual eggs (Cox et al., 1990).

Several studies have shown electrolyzed water to be an effective sanitizer against *Salmonella* on shell eggs. Russell (2003) inoculated eggs with not only *Salmonella* but also *L. monocytogenes*, *S. aureus*, and *E.coli*. Each sample was electrostatically sprayed for 15 seconds each hour for 24 hours with electrolyzed water at 8 ppm FAC with a pH of 2.1. Electrolyzed water was able to, on average, completely eliminate *S. typhimurium* from 4.75 whole eggs, *S. aureus*, *L. monocytogenes*, and *E. coli* from 11.5, 11.75, and 11.5 whole eggs, respectively, out of a total of 15 whole eggs per bacterial strain (Russell, 2003).

Park et al. (2005) showed that a combination of the catholytic, alkaline solution (AK) and electrolyzed water had the potential to reduce pathogens and indigenous microbial flora on shell eggs. The study dip treated eggs inoculated with *Salmonella enteritidis* or *L. monocytogenes* in solutions of electrolyzed water at one of three FAC concentrations, 16, 41, or 77 ppm with pH values of 2.7, 2.5, and 2.5, respectively. To examine the combined effects, an inoculated egg was first prewashed with AK for 1 min and then transferred into an electrolyzed water solution at one of the above mentioned FACs. Their best post-wash reduction was obtained with the solution of 77 ppm of FAC when the sample was treated for 5 minutes. This treatment reduced *L. monocytogenes* by 4.00 log CFU/ml and *Salmonella* by 3.48 log CFU/ml when compared to controls. When the prewash

with AK was included, a reduction of 4.38 log CFU/ml was seen for *L. monocytogenes* and 3.66 log CFU/ml was seen for *Salmonella* (Park et al., 2005).

Since there is a relatively high frequency of contamination of poultry with *C. jejuni*, raw poultry products have been perceived to be responsible for a significant amount of the 1.9 million human cases (White et al., 1997; Mead et al., 2000). Kim et al. (2005) treated whole chickens inoculated with *C. jejuni* by treatment with electrolyzed water at 47 ppm of FAC and a pH of 2.5. When *C. jejuni* was spot inoculated on the dorsal area of chickens, reductions of 2.33 log CFU/ml were obtained with an electrolyzed water immersion (Kim et al., 2005).

2.12 Effect of electrolyzed water on seafood

The United States produces more than 27 million pounds of oysters each year and most of them are sold and consumed raw without further processing (Hardesty, 2001). *Vibrio parahaemolyticus* and *V. vulnificus* are two pathogenic bacteria commonly associated with shellfish and are the leading causes of foodborne infections associated with seafood consumption in the United States (Andrews, 2004). Ren and Su (2006) performed a study which allowed oysters to depurate electrolyzed water to determine its effect on *Vibrio* species.
"Depuration is a controlled process allowing shellfish to purge sand and grit from the gut into clean seawater" (Ren and Su, 2006). Significant reductions were not only seen on pure cultures of *V. parahaemolyticus* and *V. vulnificus* but also on contaminated oysters depurated in electrolyzed water (30 ppm FAC) containing 1% NaCl for 2 hours (Ren and Su, 2006).

CHAPTER I

METHODOLOGY

3.1 Bacterial strains

Eight strains of *L. monocytogenes* were used for experiments involving processed meats including four generic strains that were moderately adherent to environmental surfaces [39-2 (retail hotdog isolate), V7-2 (serotype ½ a, milk isolate), 383-2 (ground beef isolate), and Scott A-2 (serotype 4b, clinical isolate)] and four that were strongly adherent [CW 99-38-2 (ground beef isolate), CW 62-2 (retail frankfurter isolate), CW 50-2 (retail frankfurter isolate), and CW 77-2 (retail frankfurter isolate)]. Two generic *E. coli* strains (ATCC 51739 and ATCC 895) and five *E. coli* O157:H7 strains from meat isolates [55(2)-AC1, 299(2)-AB3, 237(2)-AC1, 131(2)-AC1 and 114(2)-AC1] were used for experiments involving fresh meats. Two strains of *Salmonella enteritidis* (CDC H3527 & CDC H3502) were also used. To mimic natural spoilage problems, some experiments used a strain of *Leuconostoc mesenteroides* (Canadian bacon dextran-producing isolate). All cultures were transferred from frozen stocks at -75°C into sterile Tryptic Soy Broth (TSB) tubes at a 1:100 dilution and incubated for 24 hours at 30°C. Cultures were then re-transferred before use. Strains of *L. monocytogenes* used in this study were resistant to both streptomycin (100 µg/ml; Sigma Chemical Co., St. Louis, MO) and rifamycin S/V (10 µg/ml; Sigma). *E. coli* O157:H7 strains were resistant to both novobiocin (100 µg/ml; Sigma) and

streptomycin (100 µg/ml; Sigma). Recovery of inoculated organisms from non-sterile food products on media containing two antibiotics to which they were resistant excluded the recovery of indigenous bacteria.

3.2 Electrolyzed water generation

Electrolyzed water was generated using an EcaFlo 080 electrolyzed water generator. The generator was produced by Integrated Environmental Technologies, Inc (Little River, SC) and supplied to Oklahoma State University by SanAquel LLC (Unitherm Food Systems Inc. (Bristow, OK). Electrolyzed water was generated at 5 Amps with 23% brine injection and at a pH of approximately 6.5 according to the manufacturer's instructions. On the day of the experiment, electrolyzed water was diluted to an acceptable free available chlorine (FAC) level using distilled water. Certain experiments required electrolyzed water solutions at alternate pH levels. In this instance, the pH was adjusted by modifying the generator conditions during production. Proper cleaning and maintenance (acid flush of electrolysis chamber and tubes) were performed as instructed by the manufacturer.

3.3 Water analysis

In all studies, each treatment solution was analyzed for pH, oxidation reduction potential (ORP), conductivity, total chlorine, and free available chlorine (FAC). The pH and ORP were measured using an Oakton (Vernon Hills, IL) pH 110 combination meter according to the manufacturer's directions. An Oakton Con 6 meter was used to measure the conductivity according the manufacturer's directions. Total chlorine was analyzed using the Hach Instrument (Loveland,

CO) digital titrator iodometric method (Method # 8209). FAC measurements were taken using the Hach Instrument's digital titrator DPD-FEAS method (Method # 8210).

3.4 Spray systems

Three types of spray systems were used in our experiments. The first was an Ortho Lawn and Garden manual sprayer. This type of sprayer used an air pressurized holding tank to expel solution through a single sprayer nozzle.

The second type of sprayer was a pilot plant version of a typical online sprayer commonly used in the food industry. This multi-nozzled sprayer expelled treatment solutions at 20 psi with the assistance of a pump. For all experiments, only one sprayer nozzle was used in order to control rinse wash off and splatter.

The third sprayer type was connected to the multi-nozzled sprayer. It used the same pump to supply the treatment solution to the nozzle. Unlike the multi-nozzled sprayer, this sprayer was connected to a pressurized air source which expelled the solution at 80 psi. This single nozzle spray system produced a mist-like spray over a larger surface area.

3.5 Effect of storage temperature on the shelf life of electrolyzed water

An electrolyzed water solution was diluted to 50 ppm FAC using distilled water. After being distributed evenly into plastic containers, the solutions were placed at 5°C, 21°C, or 37°C. The solutions were analyzed for pH, ORP, conductivity, total chlorine, and FAC at given time points.

3.6 Effect of electrolyzed water on bacterial cultures

Electrolyzed water solutions were diluted to approximate FAC levels of 5, 25, 50, or 100 ppm. Each solution was distributed into sterile test tubes in 9 ml aliquots. To each tube, 1 ml of an overnight cocktail of *L. monocytogenes, E. coli* O157:H7, or *Salmonella spp.* was added. The tube was vortexed for 10 to 15 seconds. Samples were immediately diluted in 0.1% BPW. After serial dilutions, each sample was spiral plated using an EddyJet (IUL Instruments, Cincinnati, OH). *L. monocytogenes* was plated on TSA with rifamycin and streptomycin. *E. coli* O157:H7 and *Salmonella spp.* were plated on TSA. Plates were incubated for 48 hours at 30°C and counted using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.7 Efficacy of electrolyzed water on contaminated slicing blades

The efficacy of electrolyzed water to reduce *L. monocytogenes* on 4 in x 4 in sections of stainless steel coupons used to mimic slicing blades was examined using clean and dirty slicing blades. “Dirty blades” had been smeared with pieces of Canadian bacon and then allowed to dry for 30 minutes. Each blade had been previously marked with a 5 x 5 cm^2 area using a sterile stainless steel frame and edible dye. Then, 100 µl of a four strain cocktail of strongly adherent strains of *L. monocytogenes* was spread throughout the marked area. The inoculum was allowed to attach for 30 minutes at 5°C before use. The blades were then placed in sterile baskets. In one trial, clean slicing blades received a 30 second spray using the air-assisted sprayer (80 psi) while in another trial dirty slicing blades received a 30 second spray using the industry sprayer (20 psi). For these sprayer types, electrolyzed water at 5 ppm FAC was used for both

blade types. A separate trial involving electrolyzed water at increased FAC levels (25 ppm and 250 ppm) utilized a manually pressurized sprayer (i.e. Ortho Lawn and Garden sprayer). After spray treatment, remaining bacteria were removed from the blades using a sponge moistened with 25 ml of 0.1% BPW. The sponge was placed back into a stomacher bag and stomached for two minutes on a medium setting. After serial dilutions in 0.1% BPW, each sample was spiral plated using an EddyJet (IUL Instruments) on TSA with rifamycin and streptomycin. Plates were incubated for 48 hours at 30°C then counted using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.8 Electrolyzed water treatment of fresh meats

3.8.1 Treatment of beef samples with electrolyzed water

For one study, a large beef roast was sliced to 1/8th inch thick pieces. An inoculation area of 5 x 5 cm^2 was marked using a sterile stainless steel frame and edible dye on each slice. Overnight cultures of *E. coli* O157:H7 were mixed into a cocktail and diluted 1:10 with 0.1% buffered peptone water (BPW). Then, 250 µl of the inoculum was spread evenly throughout the marked area. Inoculated samples were held at 5°C for 30 minutes to allow for attachment. Each slice was sprayed for 30 seconds using the industry sprayer at 20 psi. Electrolyzed water used during the experiment was diluted to an FAC level of approximately 24 ppm. During spraying, the slice was placed in a sterile basket that allowed for spray run off to be collected. After spraying, beef samples were placed in a sterile stomacher bag along with 25 ml of 0.1% BPW. The sample was stomached for two minutes on a medium setting, appropriate serial dilutions

were made using 0.1% BPW and then spiral plated using an EddyJet (IUL Instruments). All samples were plated on tryptic soy agar (TSA) with novobiocin and streptomycin. Plates were incubated for 48 hours at 30°C then counted using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.8.2 Electrolyzed water spray and dip treatments of raw beef carcass plate samples

Surface sections from raw beef carcasses (i.e. beef plates) were obtained from the meat pilot plant at Oklahoma State Unversity's Robert M. Kerr Food and Agricultural Products Center. The plates were cut to form 6 in x 6 in sections. For inoculated samples, a 5 x 5 cm^2 square was marked using a sterile stainless steel frame and edible dye on to each piece. Sample areas were inoculated with 100 µl of a four strain overnight culture cocktail of *E. coli* O157:H7. Inoculated samples were again held at 5°C for 30 minutes prior to treatment in order to allow for inoculum attachment.

Inoculated samples were subject to either a dip or spray treatment for comparison. The spray treatment was at a constant pressure of 80 psi using an air-assisted sprayer for 30 or 60 seconds. Dip treatments were in 4000 ml of treatment solution for 15, 30, or 60 seconds with agitation. Electrolyzed water used for inoculated samples was at an approximate FAC level of 53 ppm. Naturally aged samples (i.e. uninoculated samples with high microbial loads) were dip treated with agitation in 4000 ml of an electrolyzed solution at approximately 23 ppm of FAC. Spray recovery liquid was collected in a sterile container during treatment. All samples including recovered liquid samples were

diluted using 0.1% BPW. All samples were spiral plated using an EddyJet (IUL Instruments). Inoculated samples were plated on TSA with novobiocin and streptomycin. Naturally aged samples were plated on TSA with no added antibiotics. Plates were incubated for 48 hours at 30°C and read using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.8.3 Electrolyzed water for use in a carcass wash system

A carcass steam pasteurizer (Frigoscandia, Stockolm, Sweden) was modified so that electrolyzed water solutions could be sprayed from storage tanks through the steam nozzles. This modification was used to determine electrolyzed water's efficacy on reducing surface microflora on beef carcasses. Beef animals had been harvested at the Oklahoma State University Robert M. Kerr Food and Agricultural Center's pilot plant meat processing facility. On each carcass half, a 20 x 10 cm^2 area was marked using a sterile stainless steel frame and edible dye. The area was sponge-inoculated with a two-strain mixture of overnight culture of generic *E. coli* diluted 1:10 with 0.1% BPW. Carcass halves were held for 30 minutes after inoculation and prior to spraying to allow for bacterial attachment. Halves were then sprayed for 30 seconds in a carcass spray chamber. Electrolyzed water used in the experiment was diluted on the pilot plant kill floor to target an FAC level of 50 ppm. Spray run off was collected below each carcass sample into a sterile container. Sterile sponges were used to swab viable bacteria from the inoculated area on each sample. Sponges were then placed in a sterile stomacher bag with 20 ml of 0.1% BPW. The sample was stomached for 2 minutes on a medium setting, serial dilutions were made

using 0.1% BPW and then spiral plated using an EddyJet (IUL Instruments). All samples were plated on tryptic soy agar (TSA). Plates were incubated for 48 hours at 30°C and then counted using a colony counter (IUL Countermat Flash 5.0, IUL Instruments).

3.9 Electrolyzed water treatment of processed meats

3.9.1 Evaluation of electrolyzed water for reduction of *Listeria monocytogenes* or *Leuconostoc mesenteroides* inoculated onto the surface of beef chubs encased in fibrous cellulose casings

Fibrous cellulose cased beef chubs were obtained from Oklahoma State University's Robert M. Kerr Food and Agricultural Products Center. On each chub, three 1 in^2 areas were marked using a sterile stainless steel frame and edible dye. Then, 100 µl of a cocktail of *L. monocytogenes* or *L. mesenteroides* was spread evenly throughout the area. The inoculum was allowed to sit for 5 minutes at refrigerated temperatures. Electrolyzed water used as a spray treatment was diluted to approximately 20 ppm FAC. Samples were then subjected to a 20 second spray treatment using a manual Ortho Lawn and Garden sprayer. After treatment, each sample area was cut out from the chub and placed into a sterile stomacher bag along with 10 ml of 0.1% BPW. The sample was stomached for 2 minutes on a medium setting. After serial dilutions in 0.1% BPW, each sample was spiral plated using an EddyJet (IUL Instruments). *L. monocytogenes* was plated onto Brain Heart Infusion (BHI) agar with rifamycin and streptomycin. *L. mesenteroides* was plated on BHI agar.

Plates were incubated for 48 hours at 30°C and then counted using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.9.2 Reduction of *Listeria monocytogenes* and *Leuconostoc mesenteroides* on Canadian bacon using electrolyzed water

For this experiment, 3.5 in diameter logs of Canadian bacon were obtained from a local processor. The logs were cut into usable pieces (approximately 6 in length and 3.5 in diameter). Each piece was marked with a 5 x 5 cm^2 square using a sterile stainless steel frame and edible dye. An overnight inoculum (100 µl) was spread evenly throughout the area. Depending on the experiment, the inoculum was either a mixed-strain cocktail of strongly adherent *L. monocytogenes* or a dextran producing meat contaminant that has been a problem on Canadian bacon (*Leuconostoc mesenteroides*). After inoculation, the samples were placed at 5°C for 30 minutes to allow for attachment. Samples were either dip treated in 4000 ml of treatment solution or spray treated depending on the trial. For different trials, different spray systems were used (Ortho Lawn and Garden sprayer or the industry like sprayer). Samples were subjected to treatment for 15, 30, or 60 seconds. Electrolyzed water used for these experiments ranged from 26 – 32 ppm FAC. For one set of experiments electrolyzed water was compared with a solution of house hold bleach (sodium hypochlorite). The bleach was diluted using distilled water until a final concentration of 26 ppm was reached. The solution was then treated with citric acid in order to adjust the pH to 6.5, the approximate pH of the electrolyzed water

solution. During treatment each Canadian bacon piece was placed into a sterile basket. For spray treatment, the basket was placed to allow collection of the spray run off. For dip treatments, the remaining treatment solution was also sampled. Following treatment, a section of each sample area was aseptically removed and placed into a sterile stomacher bag along with 10 ml of 0.1% BPW. The sample was stomached for two minutes on a medium setting. After serial dilutions in 0.1% BPW, each sample was spiral plated using an EddyJet (IUL Instruments) on TSA plates. Samples inoculated with *L. monocytogenes* had rifamycin and streptomycin added to the TSA plates. Plates were incubated for 48 hours at 30°C and then counted using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.9.3 Effect of electrolyzed water at different pH levels on frankfurters inoculated with *L. monocytogenes.*

Frankfurters were prepared at and purchased from Oklahoma State University's Robert M. Kerr Food and Agricultural Products Center. Frankfurters were dip inoculated in a bacterial suspension of approximately 8 log CFU/ml by diluting a four strain cocktail of moderately adherent *L. monocytogenes* in 0.1% BPW. The frankfurters were removed from the inoculum after 5 minutes and placed at 5°C for 15 minutes to allow for attachment. Individual frankfurters were placed in sterile baskets that allowed for spray run off rinse off liquid to be collected. The rinse off liquid was collected in a sterile container below the basket. Each frankfurter was sprayed for 30 seconds using an air assisted sprayer. Three electrolyzed water solutions at different pH levels were used as

spray treatments. The FAC concentrations varied from 35 – 48 ppm. Remaining bacteria were removed from the frankfurters by massaging 2 ml of 0.1% BPW around each sample. After serial dilutions in 0.1% BPW, frankfurter samples and recovered solutions were spiral plated using an EddyJet (IUL Instruments) on TSA with rifamycin and streptomycin. Plates were incubated for 48 hours at 30°C then counted using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.10 Efficacy of electrolyzed water in the presence of various protein levels

In response to the results obtained in various experiments with meat products, we chose to look at the effect of organic material (i.e. protein levels) on electrolyzed water's capability to kill bacteria. Full strength electrolyzed water was diluted to an approximate FAC level of 50 ppm. The diluted solution was dispensed in 90 ml aliquots. Gelatin from cold water fish was dissolved in distilled water at the following levels, 1.0%, 0.5%, and 0.25%. The given concentrations were then added in 10 ml amounts to the 90 ml electrolyzed water samples to obtain final dilutions of 0.1%, 0.05%, and 0.025%, respectively. Following addition of the gelatin, each solution was tested for pH, ORP, conductivity, total chlorine, and FAC.

The second part of this experiment involved the addition of bacteria to the system. After the gelatin was added to the electrolyzed water aliquots, the solution was allowed to sit for 2 minutes. The solutions were then dispensed in 9 ml portions into test tubes. An *L. monocytogenes* cocktail (1000 µl) was then added to each test tube. The test tube was vortexed for 30 seconds and then

immediately diluted with 0.1% BPW. After serial dilutions, solutions were spiral plated using an EddyJet (IUL Instruments) on TSA with rifamycin and streptomycin. Plates were incubated for 48 hours at 30°C then counted using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

3.11 Statistics

For most experiments, the results were analyzed using a one way analysis of variance (ANOVA). A pairwise multiple comparison was then completed for each using the Holm-Sidak method. The free available chlorine in the electrolyzed water shelf life experiment did not pass the normality test. Hence ANOVA was not an appropriate method. Instead a Friedman repeated measures analysis of variance was performed on the test results. A pairwise multiple comparison was then completed using the Tukey Test. All statistical analysis was performed using SigmaPlot at a P value of 0.05.

RESULTS AND DISCUSSION

We examined the effectiveness of electrolyzed water (EW) as an antimicrobial for both raw and ready-to-eat (RTE) meats. Although EW is allowed for use on raw beef carcasses (50 ppm Cl^-) and smaller cuts (20 ppm Cl^-) as a 'safe and suitable ingredient', it is not currently allowed on RTE meats until a GRAS or direct food additive petition is submitted and accepted by the FDA. The sponsors of this research project were intending to use our data for such a submission.

4.1 Antimicrobial activity in solutions and on surfaces

We first examined the effect of storage temperature and time on the active ingredient in EW. The active agent in EW is considered to be free available chlorine (FAC) that is present as hypochlorous acid when the solution is below pH 7.0 or hypochlorite when it is above pH 7.0. Many non-technical representatives of commercial companies like to use oxidation reduction potential (ORP) of solutions to measure EW effectiveness because of the ease of using pocket ORP analyzers (similar to pH meters) to measure ORP whereas a comparable portable FAC analyzer requires a chemical titration. However, both USDA and FDA stipulate that measurement of the FAC is the only factor that determines regulatory compliance. It has been our experience that you can obtain the same ORP reading on 10-fold dilutions of solutions of EW, whereby the active agent (FAC) is diluted. When examining FAC vs total chlorine (Fig. 1)

as well as ORP, pH, and conductivity (Fig. 2) during storage at 5°C, 21°C, and 37°C, we observed that FAC levels in EW are more stable at 5°C than at higher temperatures (Fig. 1A). The graphical representation of electrolyzed water held at 37°C appears to have less FAC but it is not significantly different then the solutions held at 21°C (Fig. 1A). This statistical anomaly is due to a large standard deviation observed in our trials (perhaps more replications/samples would have demonstrated a significant difference). The decreasing trend for FAC during the storage interval at 37°C (Fig. 1A) follows a similar decline as that observed for total chlorine (Fig. 1B). It is not clear why this occurred, but it could be the result of decomposition of hypochlorous acid radicals whereby chlorine gas is given off and lost from the solution, resulting in reduced free and total chlorine. This may also explain why the pH also decreased for the solution held at higher temperature (Fig. 2A). Since hypochlorous acid is an oxidant, it can oxidize any residual organic material that may be present and not be available for analysis. The instability over time at higher temperatures may affect intended storage conditions by commercial processors who may either consider storage of EW for long periods of time or have storage tanks that are subject to high temperatures. Since we are using an automated electrical generator to produce EW, there is no pressing need to store EW solutions for long periods, considering that EW can be made at higher than intended use levels (500 ppm FAC) and diluted for intended application levels (20-200 ppm FAC). Little change was noticed in ORP (Fig. 2B) or conductivity (Fig. 2C) of either solution during the extended storage trials.

Electrolyzed water was tested on various pathogens of importance to the food industry, demonstrating that EW at higher FAC levels can effectively eliminate pathogens in solution. At 250 ppm FAC, no viable cells were recovered from trials with either *E. coli* O157:H7, *Salmonella* spp., or *L. monocytogenes*, generating >6-log reduction of these pathogens in solution (Fig. 3). We further examined the effectiveness of lower FAC levels on *L. monocytogenes*. Electrolyzed water at 25, 50, and 100 ppm was effective in reducing *L. monocytogenes* by 1.67, 3.72, and 7.36 logs (CFU/ml), respectively, when compared to a buffered peptone water (BPW) control (Fig. 4). However, EW at 5 ppm FAC was no more effective than tap water (i.e., < 1 log reduction) which is allowed to have up to 5 ppm of FAC. Although FAC levels as high as 200 ppm are allowed as sanitizers on food contact surfaces, there would have to be sufficient time for drainage of residual liquid or a post-water rinse before food contact is allowed as transfer to the food would constitute EW as a 'direct food additive' on RTE meats where it is not currently allowed. Applications that would utilize 5 ppm FAC would not have to worry about food additive issues because 5 ppm FAC is allowed in tap water. However, the argument would ensue that if 5 ppm EW is effective, then why not use tap water? The level in tap water is allowed at up to 5 ppm, but it doesn't mean that the level out of the tap is at that level whereas an EW generator can provide exactly 5 ppm.

L. monocytogenes can form biofilms on equipment surfaces in food processing facilities, develop harborages, and become a source of contamination in food processing plants. In the meat industry, *L. monocytogenes* is a common

contaminant of RTE meat products and sanitation vigilance is paramount for processors of RTE meat products. One of the primary concerns for sanitation is not only elimination of pathogens from obvious food contact surfaces, but also from tight junctions that are hard to sanitize. A common food contact surface that can readily be involved in spread of microbial contamination are stainless steel slicing blades for the manufacture of pre-sliced luncheon meats and/or spiral-sliced hams. We examined the effect of using EW as a sanitizing rinse for stainless steel slicing blades comparing the efficacy of water vs. electrolyzed water at 5, 25, and 250 ppm FAC on both clean and 'dirty' blades. On clean blades, the impact of the spray rinse washed off a considerable level of our *L. monocytogenes* inoculum that was allowed to adhere for 30 min (Fig. 5). However, for EW rinse treatments, we obtained a 1.36-, 3.6-, and <5.7 log reduction, respectively, for EW of 5, 25, and 250 ppm FAC over and above that which was reduced by water displacement (Fig. 5). However, on 'dirty' blades pre-greased before inoculation by slicing motions with a RTE ham product, the reduction of *L. monocytogenes* were reduced to 0-, 0.64-, and 3.3 logs, respectively, with EW of 5, 25, and 250 ppm FAC (Fig. 5). The data suggests that organic material significantly reduces the effectiveness of the hypochlorous acid in EW, especially at lower FAC levels and this may be a detrimental factor on applications with actual meat products (i.e., high organic load). The organic material is likely getting oxidized by the hypochlorous acid before it has a chance to interact with the bacteria that might be present.

4.2 Antimicrobial activity on raw beef carcass and raw red beef sections

Beef roast sections inoculated with *E. coli* O157:H7 were sprayed for 30 sec with either tap water or electrolyzed water (24 ppm) using an industrial sprayer (20 psi). In addition to sampling the sprayed product, we recovered the rinse solutions as well to examine if spray treatment would lend itself to dispersal of surface contaminants (Fig. 6). There was no significant difference between the level of cells recovered from tap water vs. EW treated beef sections (Fig. 6). However, during examination of the recovered rinses we did not obtain any viable counts from the EW recovered rinse solutions, suggesting that EW rinses could eliminate the spread of bacteria in beef processing plants where such solutions may be used.

We further examined surface sections from naturally-aged beef carcass sectons (i.e., 6x6 inch beef plates) by dip treatment with agitation in 4000 ml of either tap water or EW (53 ppm FAC) to see if complete immersion in EW solution would render better results. We obtained no significant difference with EW as compared to tap water, regardless of treatment time (15, 30, or 60 sec), suggesting that the organic load on the carcass sections is rendering the EW ineffective (Table 3). However, we again observed little or no recovery of viable cells from recovered EW rinse solutions whereas tap water rinses had considerable levels or organisms (Table 3). Additional beef carcass sections were inoculated with *E. coli* O157:H7 and sprayed with an air-assisted spray system (80 psi) for 30 or 60 sec, resulting in similar situation whereby little or no

reduction was observed with EW compared to water whereas recovered rinse solutions demonstrated significant reductions in the washed off flora (Table 3).

A Frigoscandia carcass pasteurizer was modified to allow the application of electrolyzed water spray through 8 spray nozzles surrounding hanging half-carcasses that would otherwise deliver steam for surface pasteurization of beef carcasses. Using half-sides of beef carcasses inoculated with a mixture of generic *E. coli* strains (non-pathogenic), spray treatments using electrolyzed water (50 ppm FAC) at the maximum level of FAC were compared to spray treatments with tap water. The treatment solutions, tap water and electrolyzed water (50 ppm of FAC), were still not effective at removing inoculated generic *E. coli* from the carcasses even with the aid of the multi-nozzled steam pasteurizer (Fig. 7). It was most noticeable that rinse treatments in the carcass pasteurizer on half-carcasses did not reduce inoculated levels as was observed in laboratory trials with an industry sprayer (Fig. 7). This could be due to the misalignment of the inoculated sections with the direct spray stream in the modified Frigoscandia carcass pasteurizer and inoculations performed on fatty carcass surfaces. In this study the rinse solutions also represented drippings from the entire carcass which is overwhelmingly greater than the smaller, inoculated area. Although the levels of *E. coli* were significantly lower in the recovered EW solutions than that of tap water, they were not completely eliminated as in prior studies. This may represent the impact of the excess organic material being washed off of the entire carcass relative to rinse treatments with smaller beef sections used previously.

4.3 Antimicrobial treatments with RTE meats

We also examined the potential use of EW as an anticmicrobial for RTE meats (should a company wish to pursue obtaining FDA food additive approval for use on RTE meats). Currently, EW (hypochlorous acid or hypochlorite) is not allowed as a rinse treatment on RTE meats and it would be considered a 'new food application'. EW can be used on plastic-encased RTE products but not on permeable (cellulose) encased products in which it would still be considered to be subject to 'food contact'. Some of our data herein is an effort to see if EW could be effective on such products, considering that 'effectiveness' is a criteria for USDA-FSIS allowance on meat products whereas 'safety' is a consideration for FDA allowance of substances applied to foods. That is, USDA-FSIS would first require that FDA approve a substance for use on foods and only then would the USDA-FSIS be in a position to make an approval based on the effectiveness of the substance for the intended impact. In previous experiments, we used FAC concentrations that were allowed by the USDA-FSIS on raw meats. At the time of these experiments, electrolyzed water was not recognized as a safe and suitable ingredient by the USDA-FSIS on RTE meats. We therefore based our FAC concentrations for the following experiments on those allowed for fresh meats.

As a pathogen of concern on RTE meats, we examined the effect of electrolyzed water as a sanitizer to reduce potential levels of *L. monocytogenes* on beef chubs as a model RTE product since many RTE items are often manufactured and shipped as encased products. Fibrous cellulose was chosen

as a common casing which presents a challenge because of potential leakage of organic material on the outer surface. When electrolyzed water at 20 ppm FAC was applied for 15, 30, or 60 sec, it was effective at significantly reducing *L. monocytogenes* counts compared to control treatments at all processing times and water at 15-sec, but not significantly greater than reductions obtained with water rinse treatments for 30- or 60- sec treatment times (Fig. 8A). As with other experiments, analysis of recovered rinse solutions demonstrated a siginificant reduction of more than 5-logs of *L. monocytogenes* in the 'rinse off' solution using EW than when compared to water (Fig. 8B).

We also examined RTE Canadian bacon since a local company was having problems with a slime-producing contaminant (*Leuconostoc mesenteroides*) on 'logs' of fibrous cellulose-encased Canadian bacon, presumed to be carried on the surface of the logs from the manufacturer. We therefore examined the impact of EW on reducing both *L. monocytogenes* and *L. mesenteroides* with the same process. Both double distilled water and electrolyzed water at 32 ppm FAC significantly reduced the numbers of *L. monocytogenes* compared to the control when each solution was sprayed at 20 psi. Electrolyzed water did not, however, reduce a greater number of *L. monocytogenes* when compared to the double distilled water spray (Table 4). For *L. mesenteroides,* electrolyzed water at 32 ppm, when compared to the control and double distilled water, gave a significantly greater reduction, but one which may not be considered of great practical significance (Table 4). Again, analysis of both organisms in the recovered rinse solutions demonstrate that EW

is very effective in eliminating organisms rinse off the surface of products, giving greater than 5-log reduction compared to that obtained with water treatments (Table 4). This can be an important consideration where 'displacement of organisms' from one area to another could be a problem in processing plants.

We further examined dip treatment of Canadian bacon using both double distilled water and electrolyzed water (31 ppm FAC) as the processor we worked with indicated that they would consider a rinse deluge system if it were to be effective in removing either organism. In dip treatments for 15-, 30-, and 60 sec, we did not obtain any appreciable reduction of *L. mesenteroides* with EW compared to water controls (Fig. 10). This may be due to the dipping of a large organic load into a fixed volume of hypochlorous acid (EW) whereby the organic material is quickly reducing the FAC before it has a chance to inhibit the microbes. However, the residual FAC remaining in the dip tanks were sufficient to eliminate the organisms rinsed off into solution as none were detected from the EW rinse solutions even at the longest rinse time whereas ~2.5 cfu/ml were obtained from the 4 liters of rinse solution with water as the rinse agent (Table 5).

Many smaller meat producers do not have access to large spray systems and therefore we also examined the use of manual spray canisters (i.e., garden sprayer) in our study to simulate what a small meat processor may use to treat samples. As with other spray treatments, we found that all solutions (water, bleach, and electrolyzed water) at both 30 and 60 sec significantly reduced the level of *L. monocytogenes* inoculated onto Canadian bacon compared to the controls. Although statistically different (greater), the results with distilled water

and electrolyzed water (30 & 60 sec) were slightly better in reducing *L. monocytogenes* compared to treatments with bleach. In terms of reduction of *Listeria* on product, the bleach/EW trials were no better than the water rinse treatments, whereas they were significantly better in regard to eliminated the organisms that washed off the product as they did not survive in the recovered solutions (Fig. 11).

In all of the previous experiments, electrolyzed water was used at approximately pH 6.5-6.7 whereby it is supposedly at its highest degree of hypochlorous acid (above pH 7.0, it should be in the hypochlorite form). We chose to examine EW solutions at several lower pH values in order to examine if the efficacy of electrolyzed water could be improved at lower pH values. Electrolyzed water at pH 6, pH 5, and pH 4 and double distilled water all significantly reduced the number *L. monocytogenes* when compared to the control frankfurters, presumably due to rinse displacement of the inoculum from the inoculated frankfurters. However, there was no difference in efficacy between the double distilled water and the electrolyzed water solutions (Fig. 12). However, we did not recover any *Listeria* from the recovered EW rinse solutions whereas the water rinses still had very high counts of bacteria (Fig. 12).

Of the 10 studies, four were on fresh meats while the other six were on RTE meat products. Electrolyzed water was found to be ineffective in all four studies on fresh meats. Of the six studies on RTE meat products, five showed electrolyzed water was an effective antimicrobial compared to the controls. Two studies showed that electrolyzed water was more effective then tap water or

double distilled water for at least one treatment time. The remaining four studies on RTE meat products showed that tap water or double distilled water and electrolyzed water were equally as effective. These results lead us to believe that the reduction observed between treated samples and control samples is simply that unattached bacteria were rinsed off during treatment. At least with the EW solutions, if they were not killed by the treatment solution while on the meat product, they most certainly were killed when they were rinsed off into the solutions.

Of the ten trials where recovered rinse solutions were studied, electrolyzed water significantly reduced bacterial levels for one or more treatment times in nine of the studies when compared to water rinse treatments, but more significantly, reduced bacterial levels to undetectable in nearly all of them. We believe that the organic material present in meat products was rendering electrolyzed water ineffective. We used a protein solution to examine the effect of organic material on the residual FAC concentration of electrolyzed water after a brief exposure. Protein levels as low as 0.025% had a significant lowering of FAC levels in electrolyzed water and resulted in a 25-fold reduction at 0.05% and complete elimination of FAC at 0.1% protein (Fig. 13). When *L. monocytogenes* was added to these electrolyzed water/protein solutions, the antimicrobial capability of electrolyzed water dramatically reduced, correlating to the reduction in FAC. At dissolved protein levels of 0.1% and 0.05%, electrolyzed water could not reduce bacterial levels by a significant amount (Fig. 14).

Contamination can occur from a variety of sources and it is important that bacteria are not cross-contaminated from one product to another or from one production site to another. The high organic load that EW is exposed to on meat products (most on raw beef, somewhat less on permeable casing, and likely less on impermeable casing) exhausts the oxidizing capacity of hypochlorous acid in EW. However, we have demonstrated that it has potential application as a surface sanitizer in meat facilities where bacteria when rinsed from a meat product or contaminated surface will not survive if the the organism is carried into the electrolyzed water rinse solution. By eliminating bacteria in rinse liquids, one cross-contamination may be reduced and prevented.

Current regulations do not permit the use of electrolyzed water on RTE meats. However, it is allowed for use on fresh meat at levels up to 50 ppm and poultry up to 30 ppm FAC (USDA-FSIS, 2008). The research presented above suggests that although electrolyzed water is allowed on fresh meats it is not an effective antimicrobial for the fresh meat industry. Fresh meat and poultry contain organic matter loads with which electrolyzed water can not compete. The current regulations also do not allow more than 20 ppm of FAC on retail cuts (USDA-FSIS, 2008). Increasing the allowable levels of FAC would increase the effectiveness of electrolyzed water on fresh meat products. Hypochlorous acid, however, can still be used as a surface sanitizer at higher levels than those used in our study (up to 200 ppm), but would have to either allow a period for it to drip off or rinsed with potable water before food samples are applied to the surfaces (i.e., conveyor belts). Electrolyzed water was also not effective when sprayed

directly onto RTE meats, although encased product may reduce the exposure of organic material to the hypochlorous acid. Currently, hypochlorous acid is allowed for use as a surface sanitizer only on RTE meats that are encased in impermeable casing.

Our research suggests that electrolyzed water will not be an effective directly applied antimicrobial for the meat and poultry industry, but rather, a sanitizer for processing environments, food contact surfaces, and product wrapped in impermeable cases. In poultry dip tanks, it is recognized that although microbial flora on the processed poultry carcasses are not significantly diminished, there are no detectable bacteria in the chill tanks that have otherwise been described as 'bacterial soup'. There is still potential for use of electrolyzed water though as a food contact surface sanitizer, perhaps in lieu or in rotation with quaternary ammonium compounds. This avenue could prove electrolyzed water to be an effective sanitizer for sanitation operations. Given microbial problems in the produce industry, electrolyzed water may find better application on fruits and vegetables that expose the hypochlorous acid to less organic material than would be obtained from direct application on meat products. We look forward to other applications that show how electrolyzed water may be used as an effective antimicrobial.

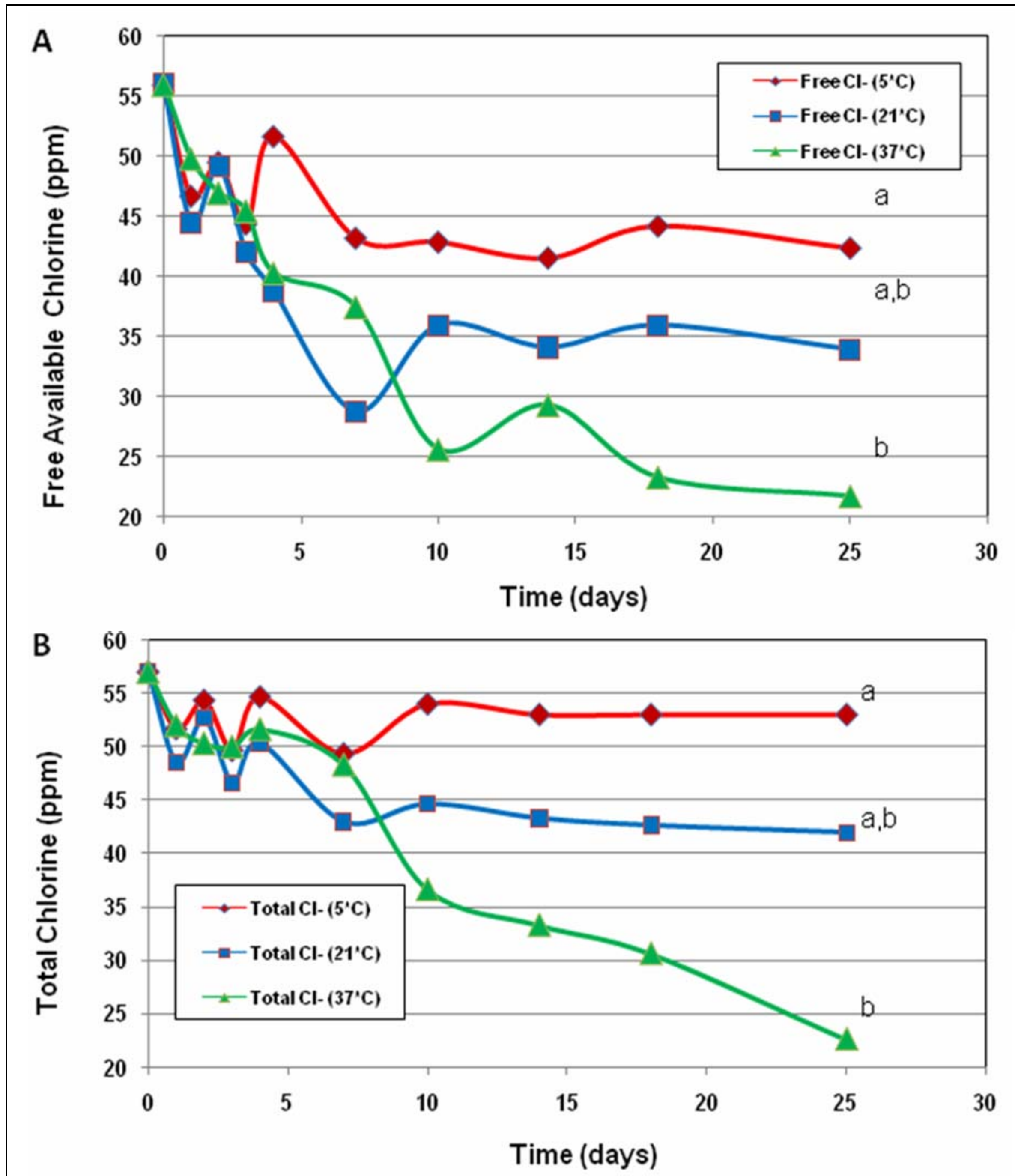

Figure 1. Measurement of free (panel A) and total chlorine (panel B) of EW solutions stored for 25 days at 5°C, 21°C, or 37°C. All trials were performed in triplicate replication of paired samples and data points represent the means (standard deviations are not shown to prevent clutter). Treatments that share the same lower case letters are not significantly different ($P > 0.05$); treatments with different lower case letters are significantly different ($P < 0.05$).

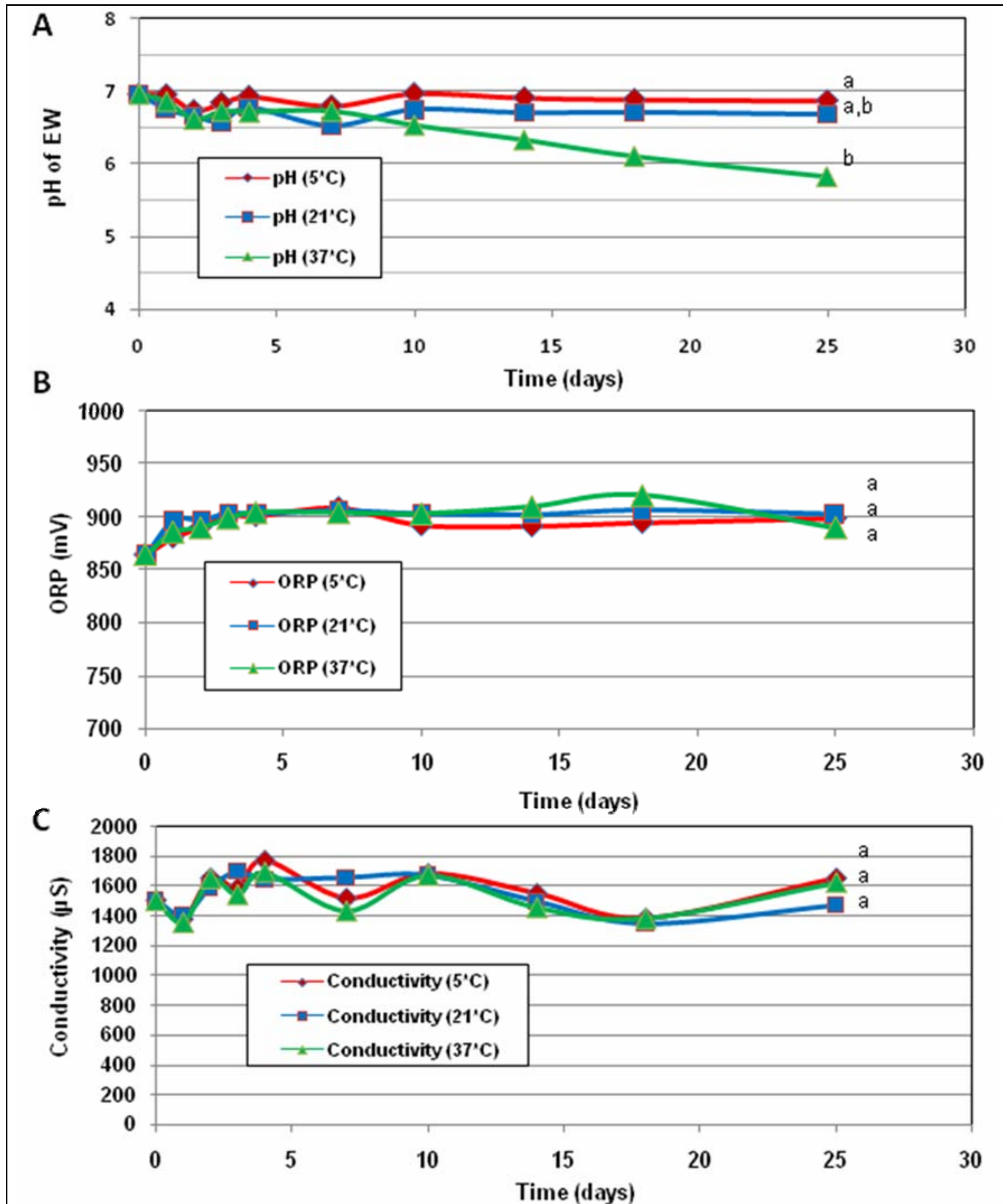

Figure 2. Measurement of pH (panel A), ORP (panel B), and Conductivity (panel C) in EW solutions stored for 25 days at 5°C, 21°C, or 37°C. All trials were performed in triplicate replication of paired samples and data points represent the means (standard deviations are not shown to prevent clutter). Treatments that share the same lower case letters are not significantly different ($P > 0.05$); treatments with different lower case letters are significantly different ($P < 0.05$).

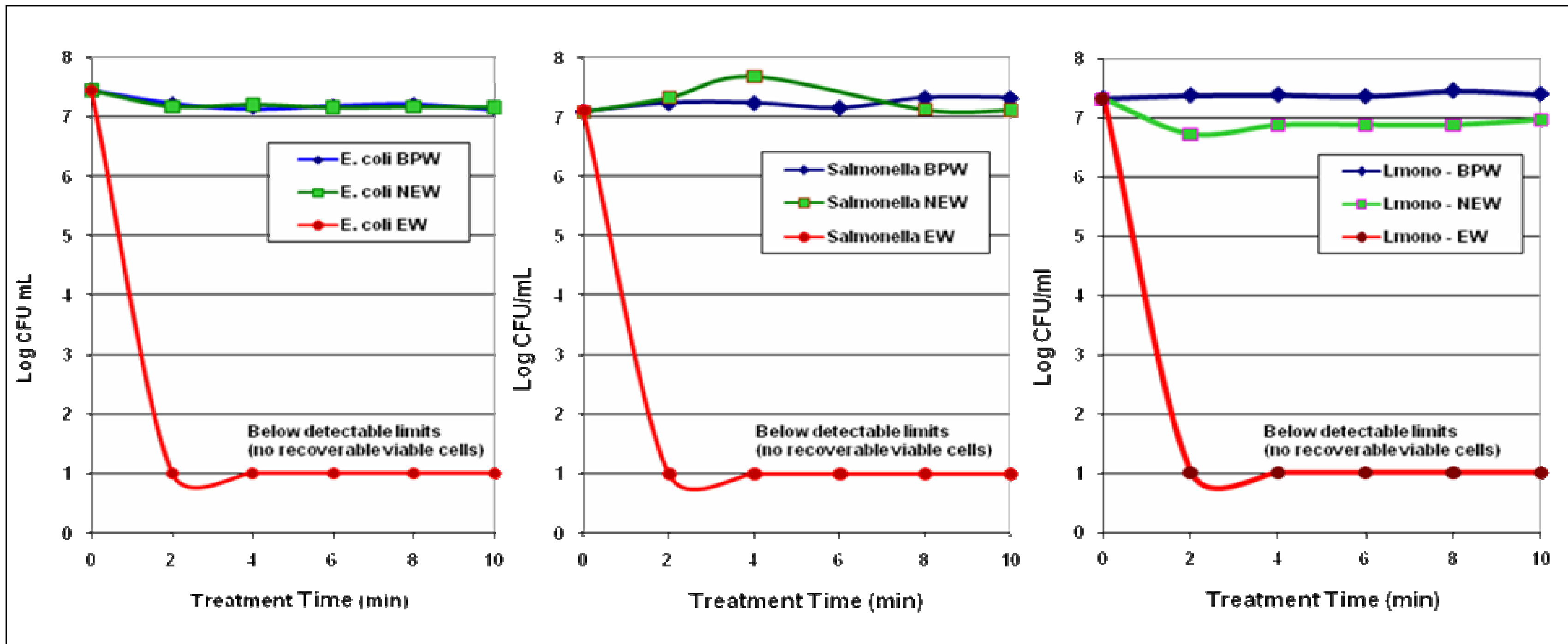

Figure 3. Effect of electrolyzed water (hypochlorous acid; 250 ppm FAC; 820 ORP) on mixtures of strains of *E. coli* O157:H7, *Salmonella* spp., or *Listeria monocytogenes* for 0, 2, 4, 6, 8, and 10 min at room temperature. The data points are the means of duplicate trials (error bars were not shown to prevent clutter).

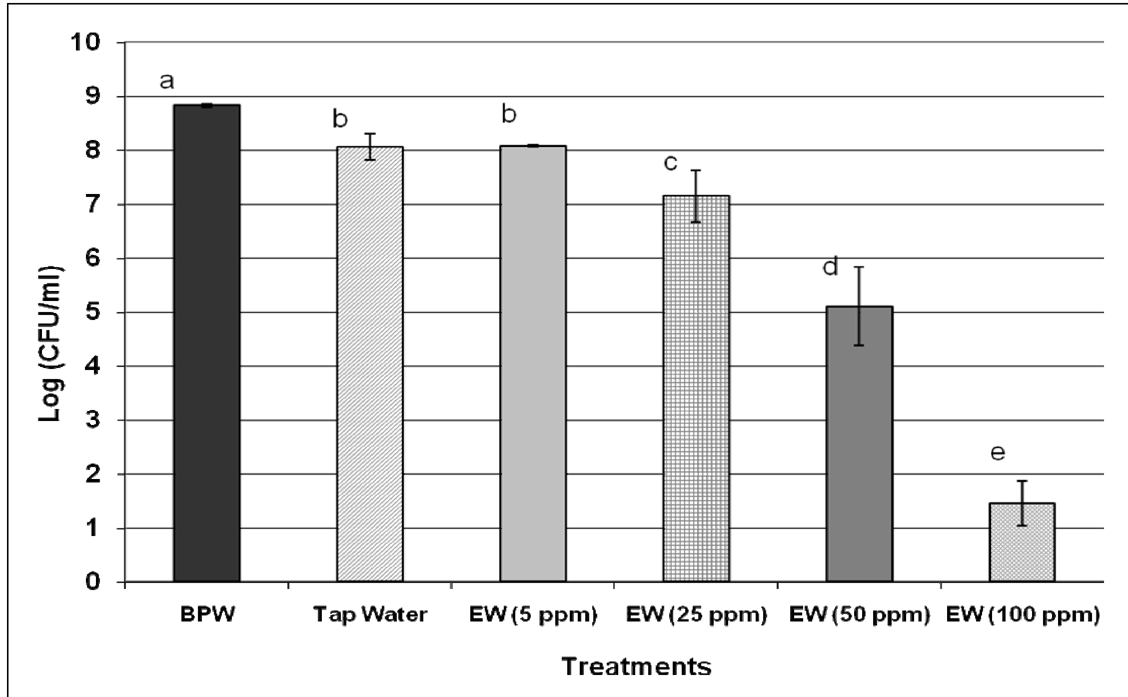

Figure 4. A mixture of 4 strains of *L. monocytogenes* was subjected to a short treatment time (10-15 sec) with buffered peptone water (BPW), tap water, and electrolyzed water (5-, 25-, 50-, and 100 ppm FAC). All trials were performed in triplicate replication of paired samples and data points represent the means. Treatments with the same lower case letter are not significantly different ($P > 0.05$); treatments with different lower case letters are significantly different ($P < 0.05$).

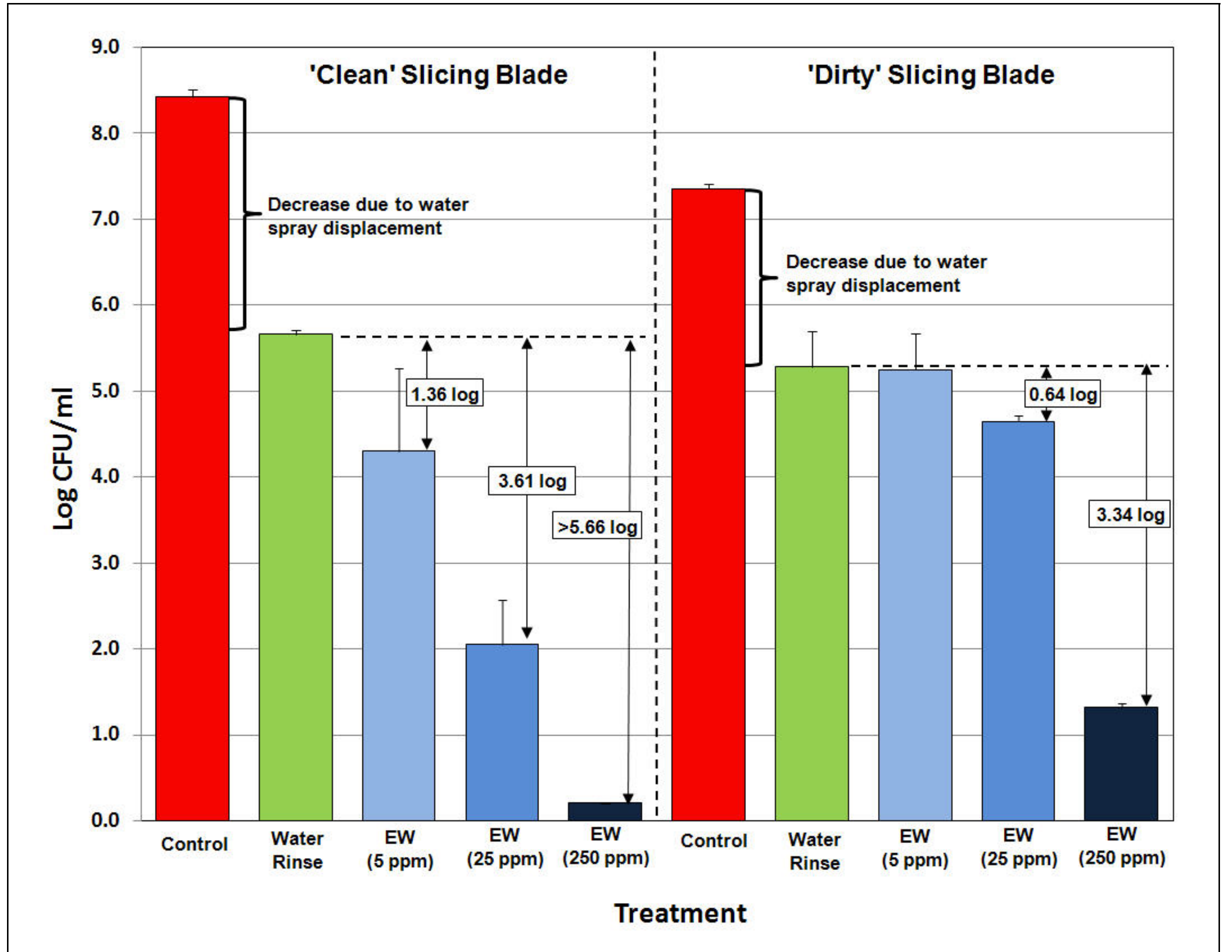

Figure 5. Clean or dirty sections (5x5 cm^2) of stainless steel slicing blades were inoculated with *L. monocytogenes* and spray treated with water, 5-, 25-, or 250 ppm FAC of electrolyzed water. The 'dirty' slicing blades were used to make several cuts through RTE deli turkey breast to condition the blade with an organic load.

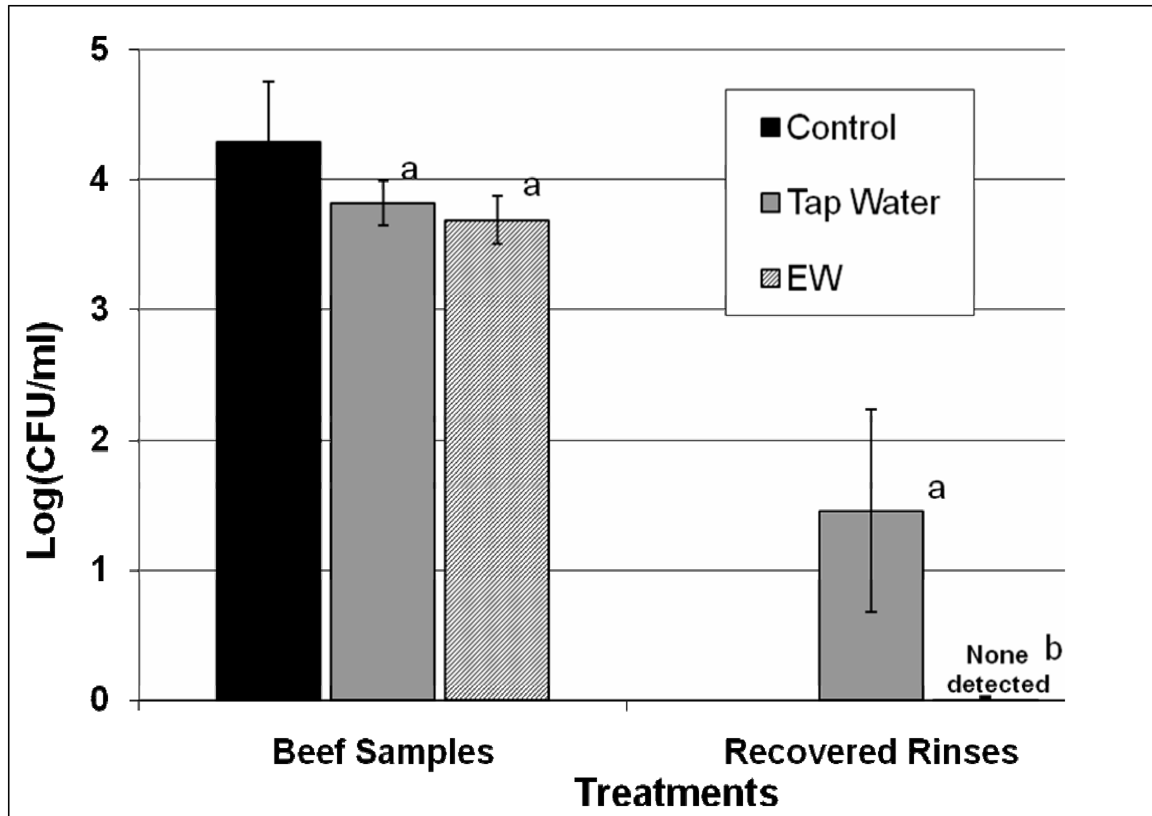

Figure 6. Beef roast slices were inoculated (5x5 cm^2 area) with a five strain cocktail of *E. coli* O157:H7. Each slice was sprayed for 30 seconds with electrolyzed water (24 ppm) or tap water using an industry sprayer (20 psi). Liquid spray rinse was collected and plated. Within each treatment, means with the same lower case letters are not significantly different ($P > 0.05$); means with different lower case letters are significantly different ($P < 0.05$).

Table 3. Surface sections from raw beef carcasses (i.e. beef plates) were cut into sections (6 x 6 in). The sections were dip treated with agitation in 4000 ml of treatment solution (15, 30, or 60 sec) or sprayed with an air-assisted sprayer at 80 psi (30, 60 sec). Treatment solutions were electrolyzed water (53 ppm FAC) or tap water. Samples were tested for aerobic plate counts of naturally aged beef for dip treatments or inoculated with *E. coli* O157:H7 for spray treatments. The remaining, or recovered, treatment solutions were also sampled. Within each treatment type, means of the same lowercase letter are not significantly different ($P > 0.05$); means with different lowercase letters are significantly different ($P < 0.05$).

Inoculated Organism	Treatment Time (sec)	Treatment				
		Beef Plate Samples Log CFU/ml			Recovery Liquid Log CFU/ml	
		Control	Tap Water	Electrolyzed Water	Tap Water	Electrolyzed Water
Natural Flora	Dip:	8.4 ± 0.6 a				
	15		7.7 ± 0.9 a	7.6 ± 0.6 a	2.6 ± 2.2 a	0.4 ± 0.7 b
	30		7.9 ± 0.6 a	7.7 ± 0.4 a	3.7 ± 2.2 a	0.3 ± 0.3 b
	60		7.5 ± 0.6 a	7.9 ± 0.5 a	3.0 ± 2.0 a	0.01 ± 0.0 b
E. coli O157:H7	Spray:	6.4 ± 0.6 a				
	30		6.1 ± 0.6 a	6.2 ± 0.6 a	4.6 ± 0.5 a	< 0.01 b
	60		6.3 ± 0.9 a	6.3 ± 0.6 a	4.0 ± 0.8 a	<0.01 b

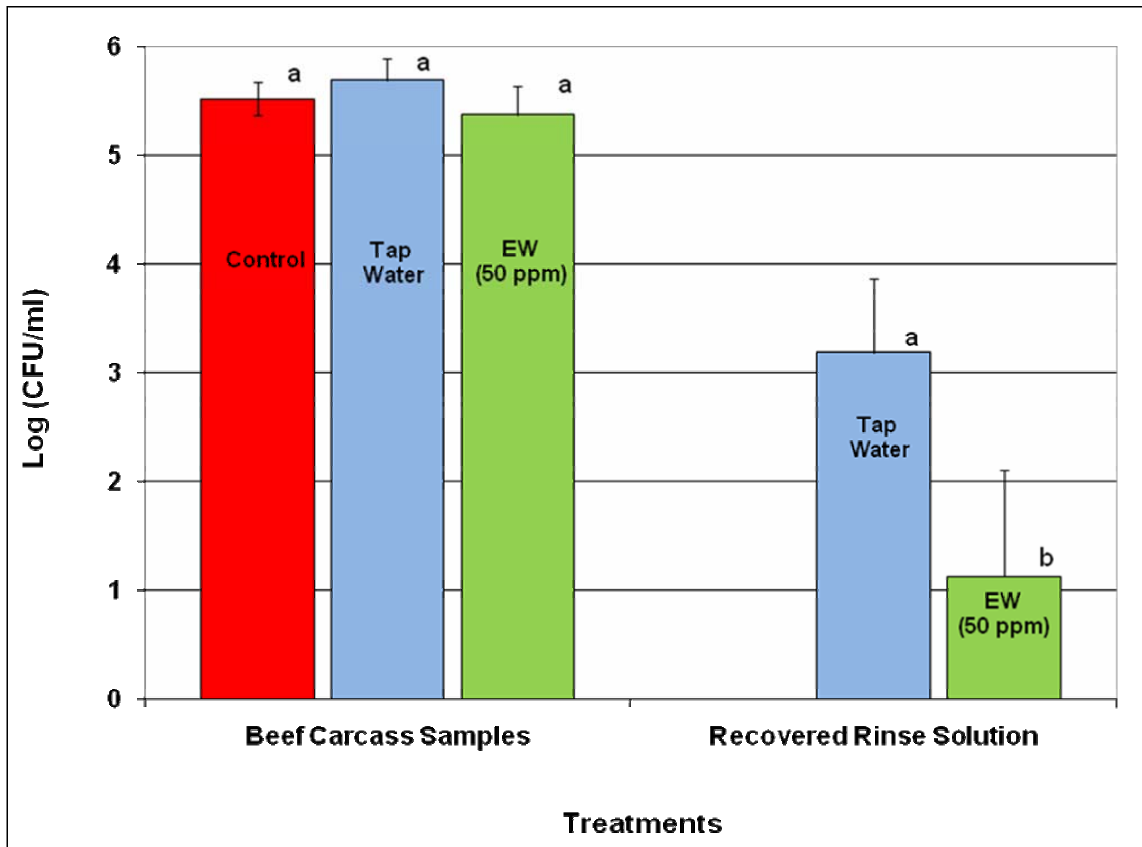

Figure 7. Electrolyzed water (50 ppm) or tap water was sprayed for 30 sec using a modified Frigoscandia Carcass Steam Pasteurizer onto carcass halves inoculated with a two strain cocktail of generic *E. coli.* Recovered spray solutions were also collected. Within each treatment type, means of the same lowercase letter are not significantly different ($P > 0.05$); means with different lowercase letters are significantly different ($P < 0.05$).

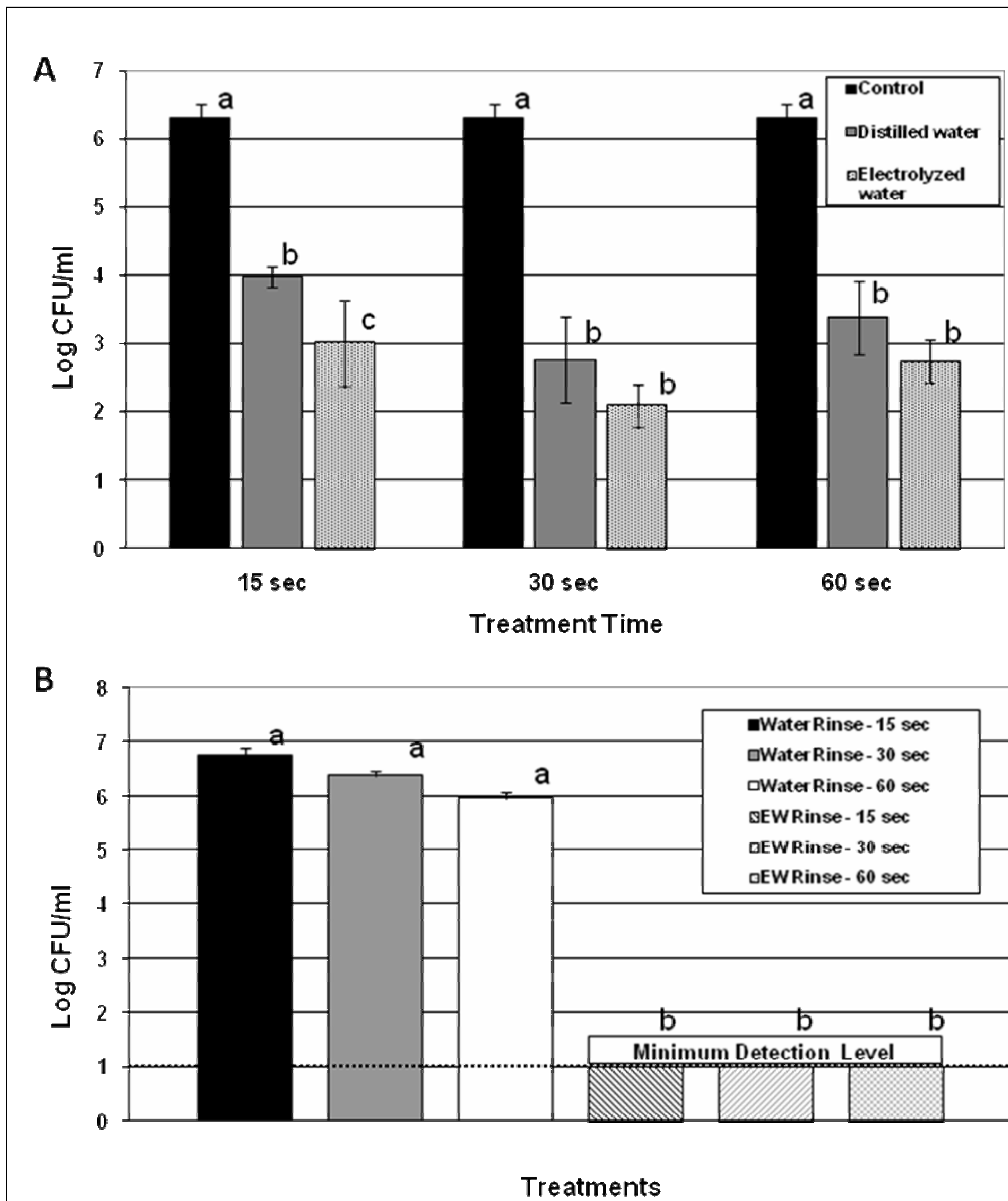

Figure 8. Spray treatment of *L. monocytogenes*-inoculated RTE beef chubs with electrolyzed water. Panel A, inoculated beef chubs sprayed for 15-, 30-, or 60-sec with water or EW (20 ppm FAC). Panel B, levels of *L. monocytogenes* in recovered rinse solutions from spray treatments above. Within a treatment, means with the same lowercase letter are not significantly different ($P > 0.05$); means with different lowercase letters are significantly different ($P < 0.05$).

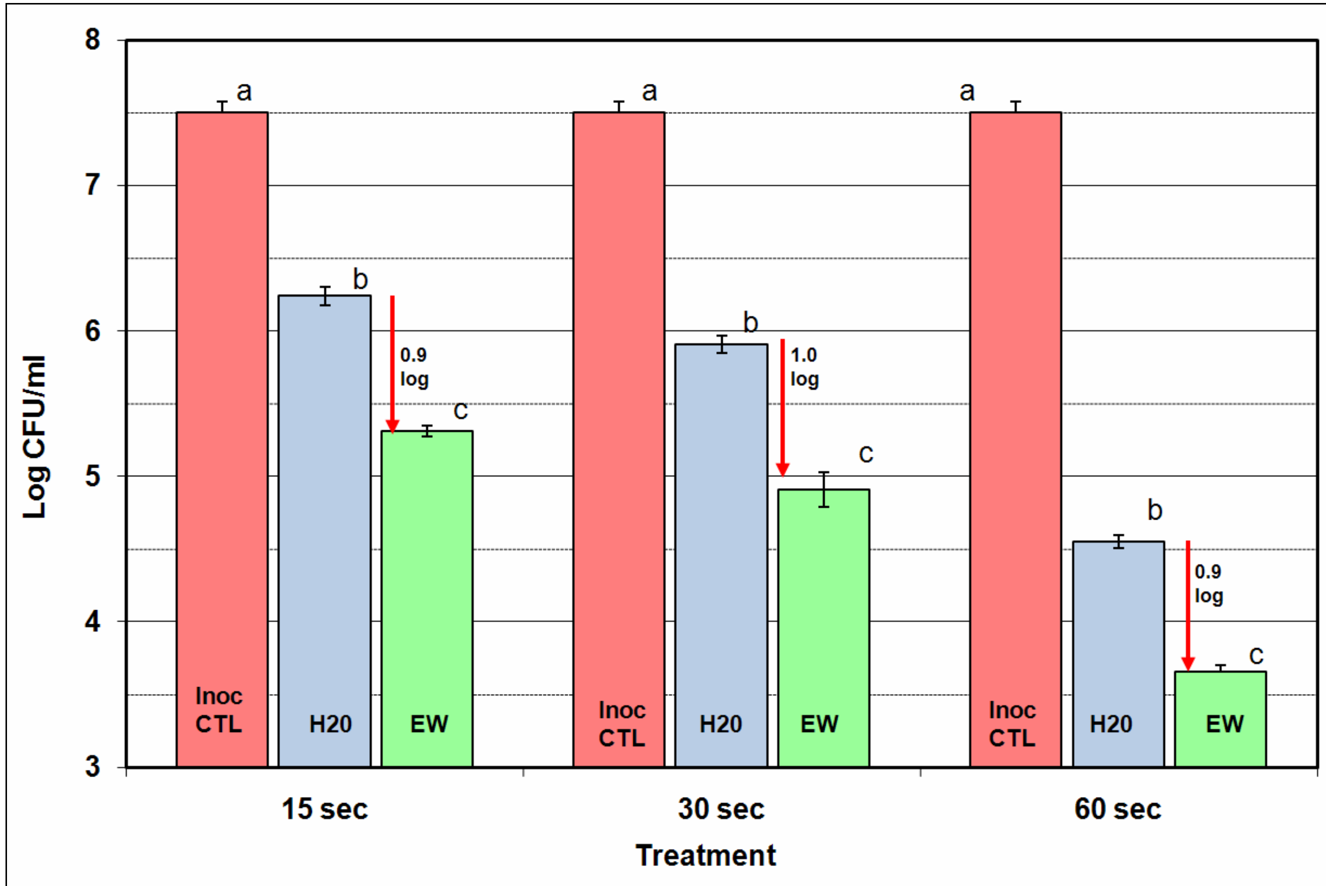

Figure 9. Fibrous cellulose-encased beef chubs, surface-inoculated with *Leuconostoc mesenteroides* were sprayed with a manually-pressurized sprayer with deionized water or electrolyzed water (~34 ppm Cl^-, pH 6.4) for 15-, 30-, or 60 sec. Within a timed treatment, means with the same lowercase letter are not significantly different ($P > 0.05$); means with different lowercase letters are significantly different ($P < 0.05$).

Table 4. Canadian bacon sections (6-in x 3.5-in) inoculated (5x5 cm^2 area) with a 4-strain cocktail of *L. monocytogenes* or *Leuconostoc mesenteroides* were sprayed (30 sec) with an industrial sprayer (20 psi) using electrolyzed water (32 ppm) or double distilled water. Spray rinses were recovered for microbial testing. Means of the same sample type (within a trial) with the same lowercase letters are not significantly different ($P > 0.05$); means of the same sample type with different lowercase letters are significantly different ($P < 0.05$).

Organism	Treatments				
	Canadian Bacon Samples (Log CFU/ml)			Recovered Rinse Samples (Log CFU/ml)	
	Control	Double Distilled Water	Electrolyzed Water	Double Distilled Water	Electrolyzed Water
Listeria monocytogenes	6.6 ± 0 a	5.1 ± 0.3 b	4.6 ± 0.1 c	5.2 ± 0.2 a	< 0.01 ± 0 b
Leuconostoc mesenteroides	6.5 ± 0 a	5.8 ± 0.2 b	4.8 ± 0.2 c	5.5 ± 0.4 a	< 2.00 ± 0 b

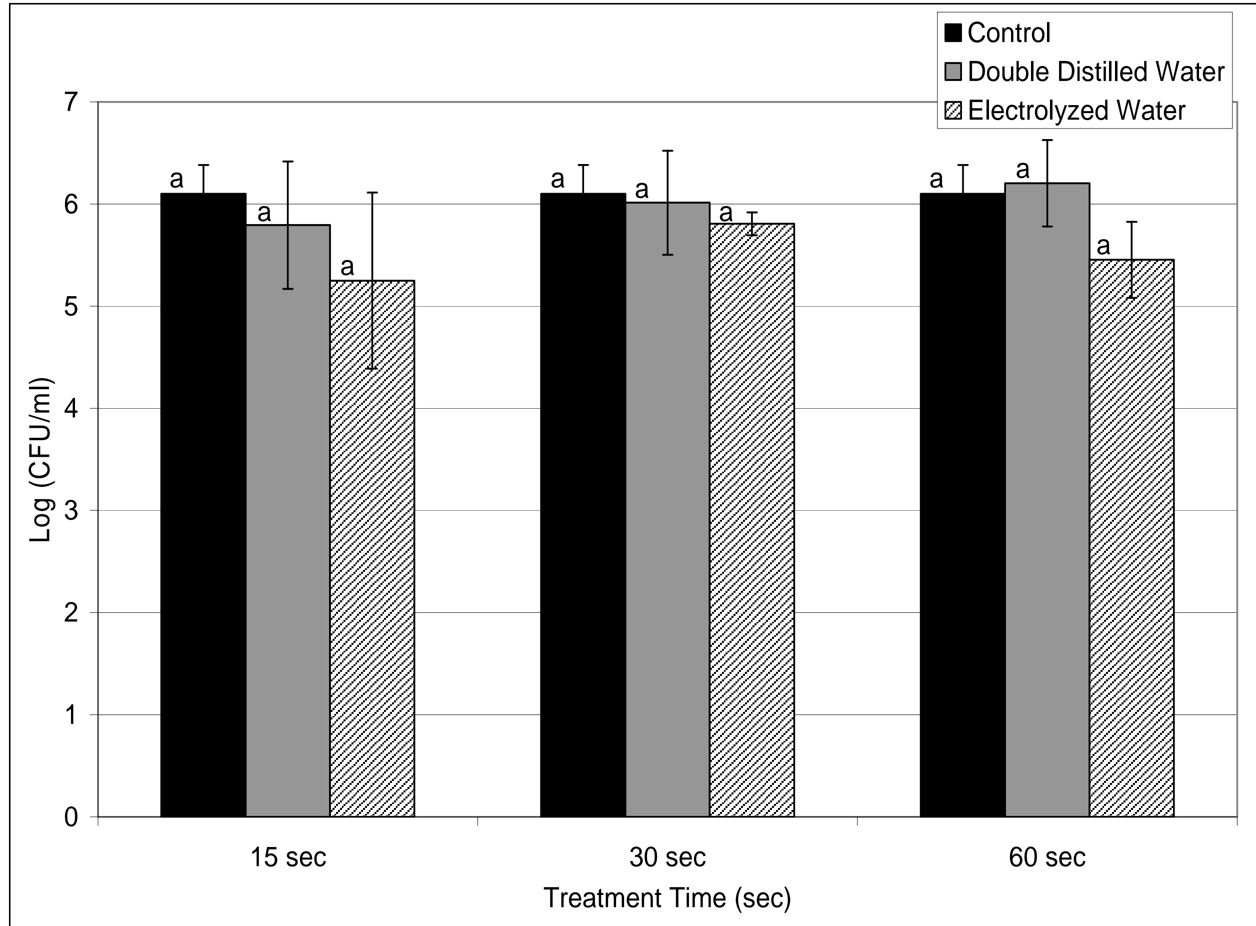

Figure 10. Canadian bacon sections (6 in x 3.5 in) were inoculated (5x5 cm^2 area) with a meat contaminant (*Leuconostoc mesenteroides*). Samples were dipped with agitation in 4000 ml of electrolyzed water (31 ppm) for 15, 30, or 60 seconds. Means with the same letter are not significantly different ($P > 0.05$).

Table 5. Recovery liquid of electrolyzed water (31 ppm) or double distilled water was collected from dip treated (4000 ml) Canadian bacon inoculated with *Leuconostoc mesenteroides*. Means of the same sample type with the same letters are not significantly different (P > 0.05). Means of the same sample type with different letters are significantly different (P > 0.05).

Dip Time (with agitation)	Residual Microorganisms in the Dip Rinse Solutions (Log CFU/ml)	
	Double Distilled Water	Electrolyzed Water
15 sec	2.45 ± 0.09 a	< 1 ± 0 b
30 sec	2.47 ± 0.07 a	< 1 ± 0 b
60 sec	2.49 ± 0.06 a	< 1 ± 0 b

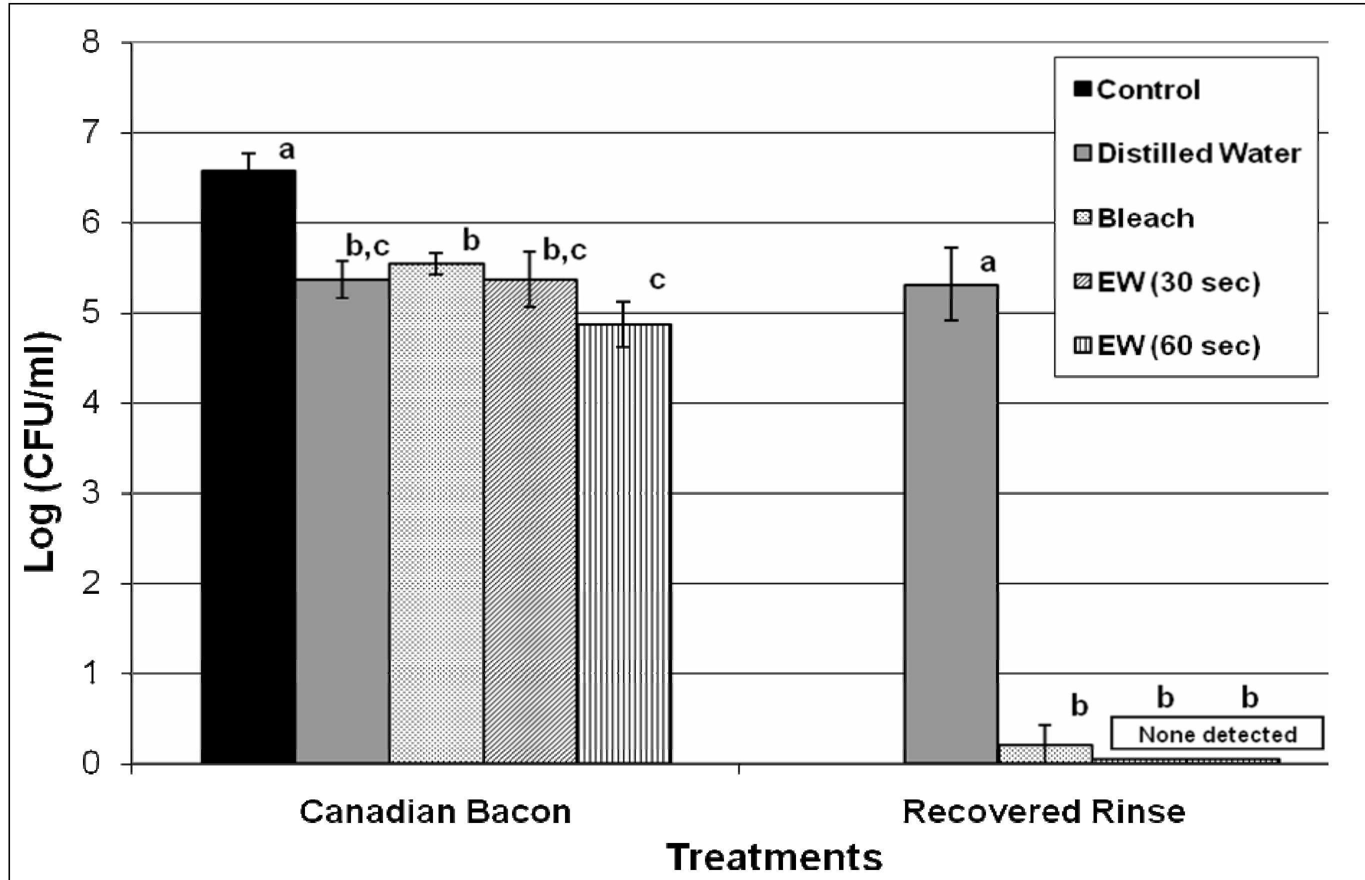

Figure 11. Canadian bacon sections (6 in x 3.5 in) were inoculated (5x5 cm^2 area) with a 4-strain cocktail of *L. monocytogenes*. Samples were spray treated for 30 sec (an extra EW sample for 60 sec) using a manually-pressurized spray canister with double distilled water, bleach (sodium hypochlorite, 26 ppm), or electrolyzed water (20 ppm). Recovered rinse solutions were also collected and plated. Means of the same sample type which share the same lowercase letters are not significantly different ($P > 0.05$). Means of the same sample type with different lowercase letters are significantly different ($P < 0.05$).

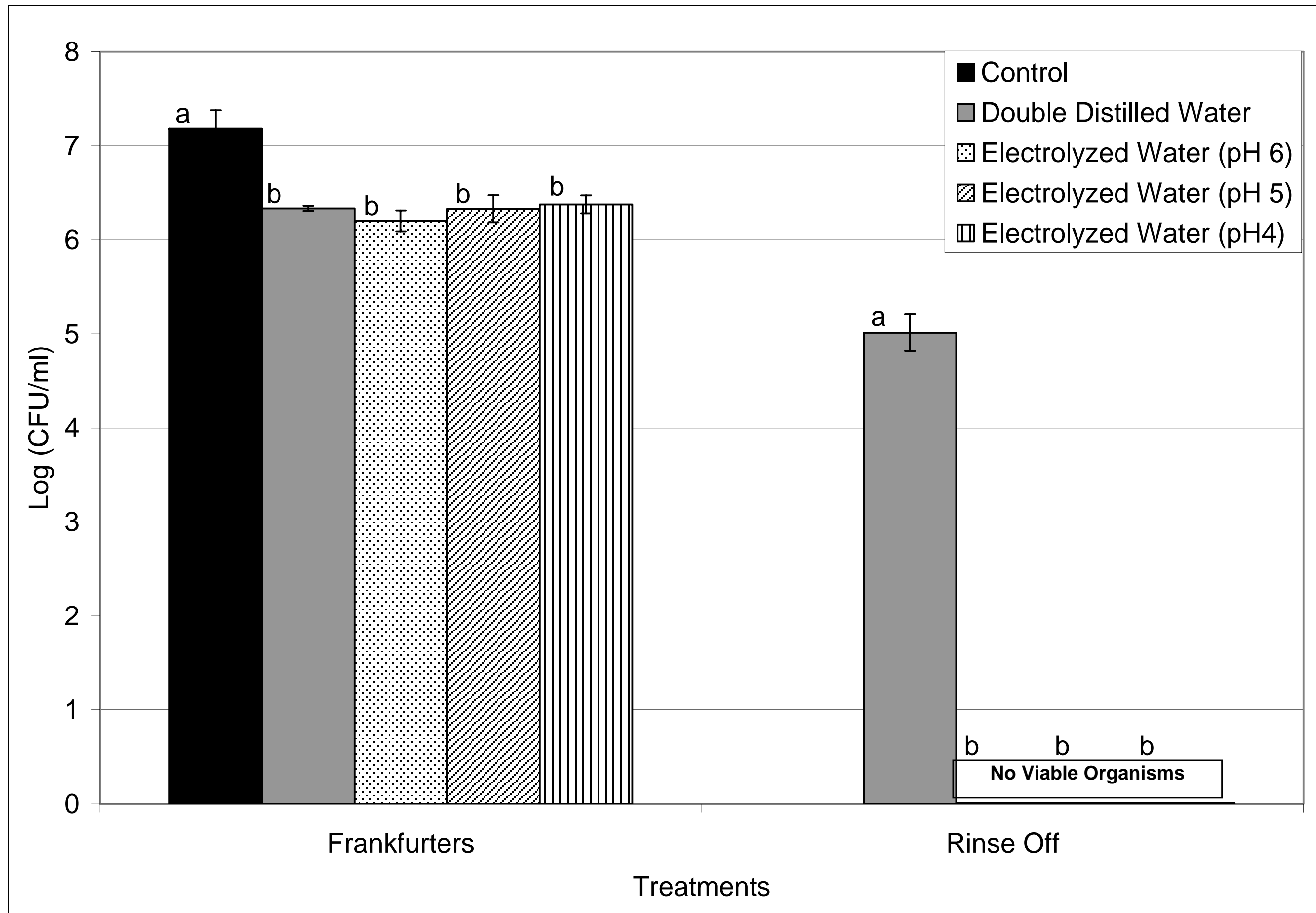

Figure 12. Frankfurters, inoculated with a four strain cocktail of *L. monocytogenes,* were spray treated using an air assisted sprayer (80 psi) for 30 seconds. Treatment solutions were electrolyzed water pH 4 (30 ppm), electrolyzed water pH 5 (27 ppm), electrolyzed water pH 6 (39 ppm), or double distilled water. Liquid spray rinse off was collected. Means of the same sample type with the same letters are not significantly different (P > 0.05). Means of the same sample type with different letters are significantly different (P > 0.05).

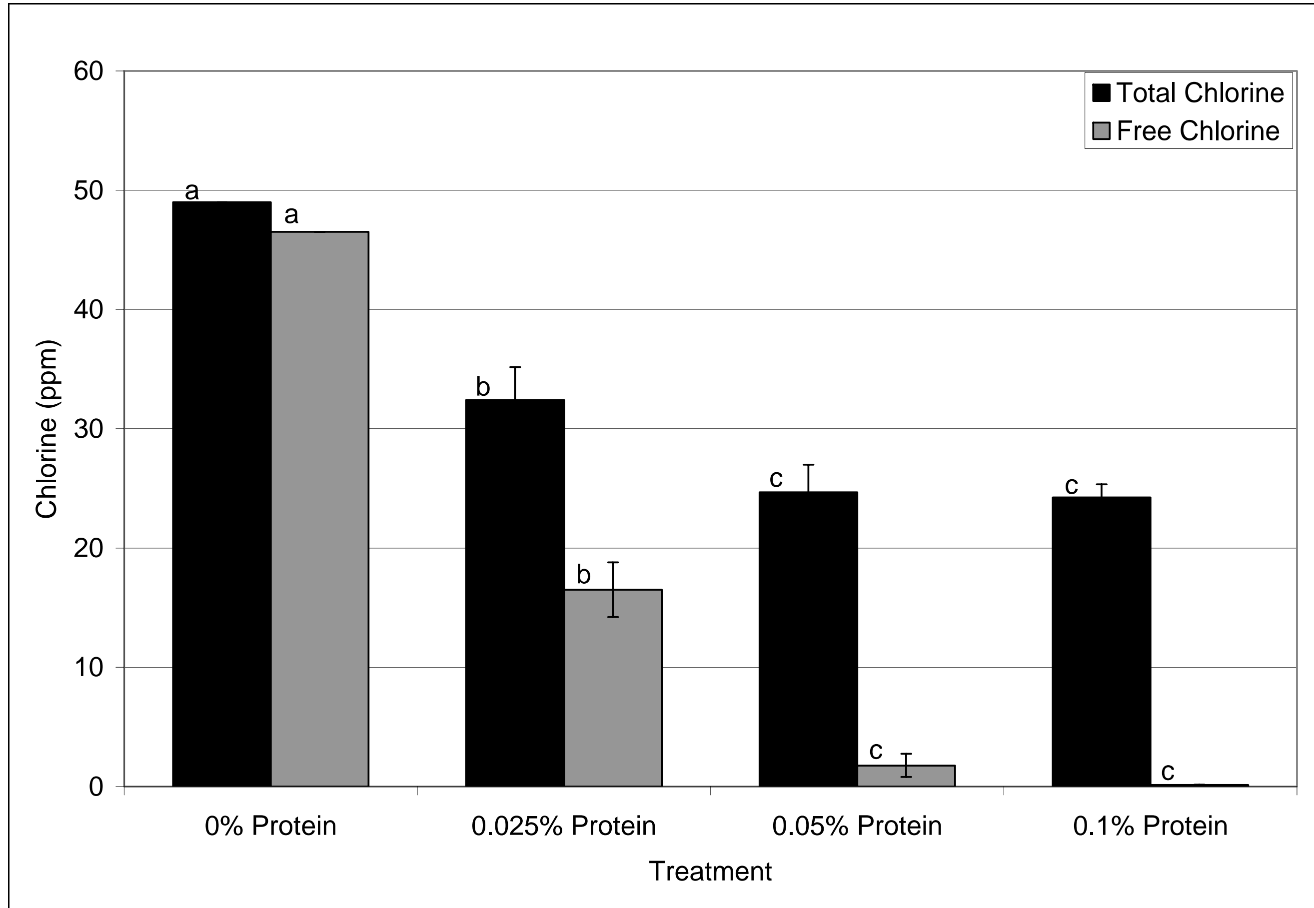

Figure 13. Electrolyzed water (50 ppm) was mixed with gelatin from cold water fish for final protein concentrations of 0.1%, 0.05%, and 0.025% and then free and total chlorine was determined. Means of the same chlorine type with the same lowercase letters are not significantly different ($P > 0.05$). Means of the same chlorine type with different lowercase letters are significantly different ($P < 0.05$).

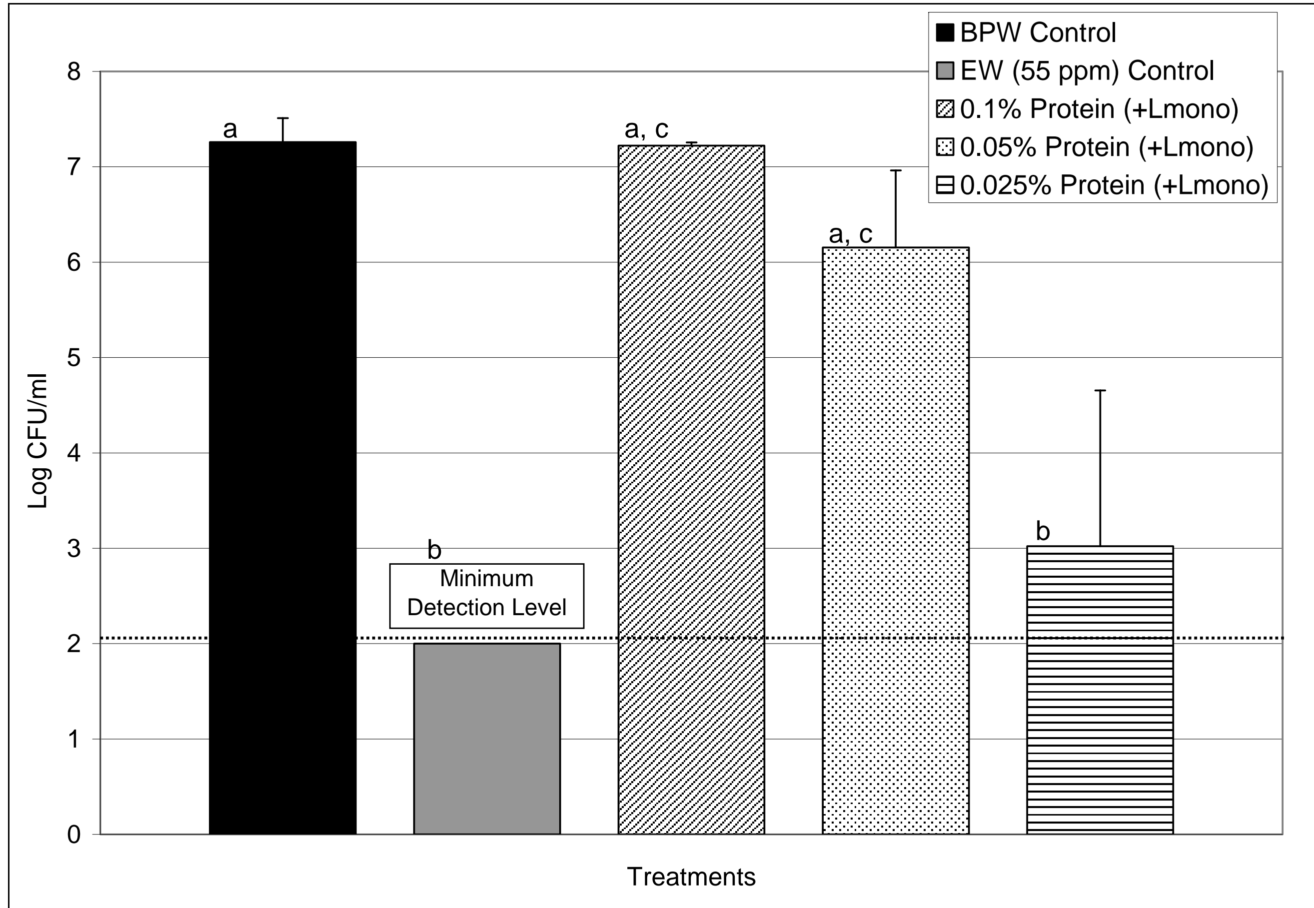

Figure 14. Electrolyzed water (50 ppm) was mixed with gelatin from cold water fish for final protein concentrations of 0.1%, 0.05%, and 0.025%. A 4-strain cocktail of *L. monocytogenes* was then added, vortexted (30 sec), and then plated. Means with the same lowercase letters are not significantly different ($P > 0.05$). Means with different lowercase letters are significantly different ($P < 0.05$).

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APPENDIX

Introduction:

Cantaloupe has been involved in several foodborne disease outbreaks. In 1990, 245 cases of salmonellosis were reported in 30 states. All 245 cases were traced back to cantaloupe contaminated with *Salmonella* spp. Chester. In 1991, a *Salmonella* outbreak occurred which resulted in greater than 400 cases in 23 states. Again all cases were attributed to contaminated cantaloupe (Tauxe, 1997). Cantaloupe was connected to an outbreak in Oregon in 1993 in which *E. coli* O157:H7 was cross-contaminated onto sliced cantaloupe (Feng, 1995).

These outbreaks lead authorities to design a melon safety plan. The plan outlines steps from farm-to-table which are aimed at reducing the incidence of foodborne disease on melons. The plan notes that special considerations should be given to melons with netted rinds, most notably cantaloupes. One important suggestion made by the plan is to ensure that appropriate disinfectants are used during the cooling process and prior to production of fresh-cut melons (Anonymous, 2005).

Previous research has shown electrolyzed water to be an effective disinfectant on fruits such as melons. Koseki et al. (2004 b) showed electrolyzed water to be effective against the natural microflora of strawberries (Koseki et al., 2004b). Wang et al. (2006) used electrolyzed water on *E. coli* O157:H7 inoculated apples. They showed a 1.08 log (CFU/cm^2) reduction of the bacteria. During this same study, cantaloupe samples were treated for 15 min in an electrolyzed water solution with a resulting reduction of 1.15 log (CFU/ cm^2) of *E.coli* O157:H7 (Wang et al., 2006).

The research presented in the body of this thesis lead us to believe that the large organic load of fresh and processed meat inhibited the antimicrobial behavior of electrolyzed water. Cantaloupe are less likely to inhibit the antimicrobial capability of electrolyzed water. The objective of the research below is to determine the effect of electrolyzed water on the natural microflora of cantaloupe, on inoculated cantaloupe, and on the overall shelf life of fresh-cut cantaloupe.

Methodology:

Ripe cantaloupes were purchased from a local grocer. The cantaloupes were used for three separate experiments. In one experiment, we tested the efficacy of EW in reduction of the natural indigenous microflora on whole intact cantaloupes. In another set of trials, we tested whole (intact) inoculated cantaloupes. These cantaloupes were sponge inoculated with an overnight lawn of a five strain cocktail of *E. coli* O157:H7. In a third set of trials, we examined the shelf life of fresh cut cantaloupe treated with EW.

Reduction of surface microflora of cantaloupes treated with EW. Whole uninoculated cantaloupes were dip treated in 4000 ml of treatment solution for 30 seconds with agitation. The treatment solutions consisted of electrolyzed water (210 ppm FAC), bleach (201 ppm FAC), or tap water. Immediately following treatment, cantaloupes were placed in sterile bags with 25 ml of 0.1% BPW. The cantaloupes were massaged vigorously for a minimum of two minutes. The rinse liquid was then removed and serially diluted with 0.1% BPW. The dip treatment liquid was also collected for sampling. All samples were spiral plated using an EddyJet (IUL Instruments) on TSA. Plates were incubated for 48 hours at 30°C and read using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

Reduction of *E. coli* O157:H7 on the surface of cantaloupes processed with EW. *E. coli* O157:H7-inoculated cantaloupes were held at 5°C for 30 minutes prior to treatment in order to allow for inoculum attachment. The cantaloupes were then dipped in 4000 ml of treatment solution for 30 seconds with agitation. The treatment solutions were electrolyzed water (202 ppm FAC), bleach (204 ppm FAC), or tap water. Immediately following treatment, cantaloupes were placed in sterile bags with 25 ml of 0.1% BPW. The cantaloupes were massaged for a minimum of two minutes. The rinse liquid was then removed and serially diluted with 0.1% BPW. The dip treatment solution was also collected for sampling. All samples were spiral plated using an EddyJet (IUL Instruments) on TSA with novobiocin and streptomycin (to which the *E. coli* O157:H7 bacteria were resistant to). Plates were incubated for 48 hours at 30°C and read using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

Treatment of cut mellon pieces with EW. Whole uninoculated cantaloupes were dip treated in 4000 ml of treatment solution for 30 seconds with agitation. The treatment solutions were electrolyzed water (197 ppm FAC) or tap water. Immediately following treatment, cantaloupes were sliced using a sterilized knife on a sterilized cutting board. The cantaloupes were first halved and the seeds were removed. Following rind removal, the cantaloupes were cut into sixteenths and then into 1.5-inch pieces. The pieces were then placed into a sterile basket and dip treated in 4000 ml of treatment solution for 30 seconds with agitation. The treatment solutions were electrolyzed water (197 ppm FAC) or tap water. Following treatment, the pieces were evenly divided and placed into sterile containers. Samples were designated for sampling at day 3, day 6, and day 9 were placed at 5°C. Day 0 samples were immediately tested. At the time of testing all samples were diluted at a 1:1 weight ratio with 0.1% BPW. They were then stomached for two minutes on a normal setting. Following stomaching, samples were serially diluted with 0.1% BPW. They were then spiral plated using an EddyJet (IUL Instruments) on TSA with no added antibiotics. Plates were incubated for 48 hours at 30°C and read using a colony counter (IUL Countermat Flash 4.2, IUL Instruments).

Findings:

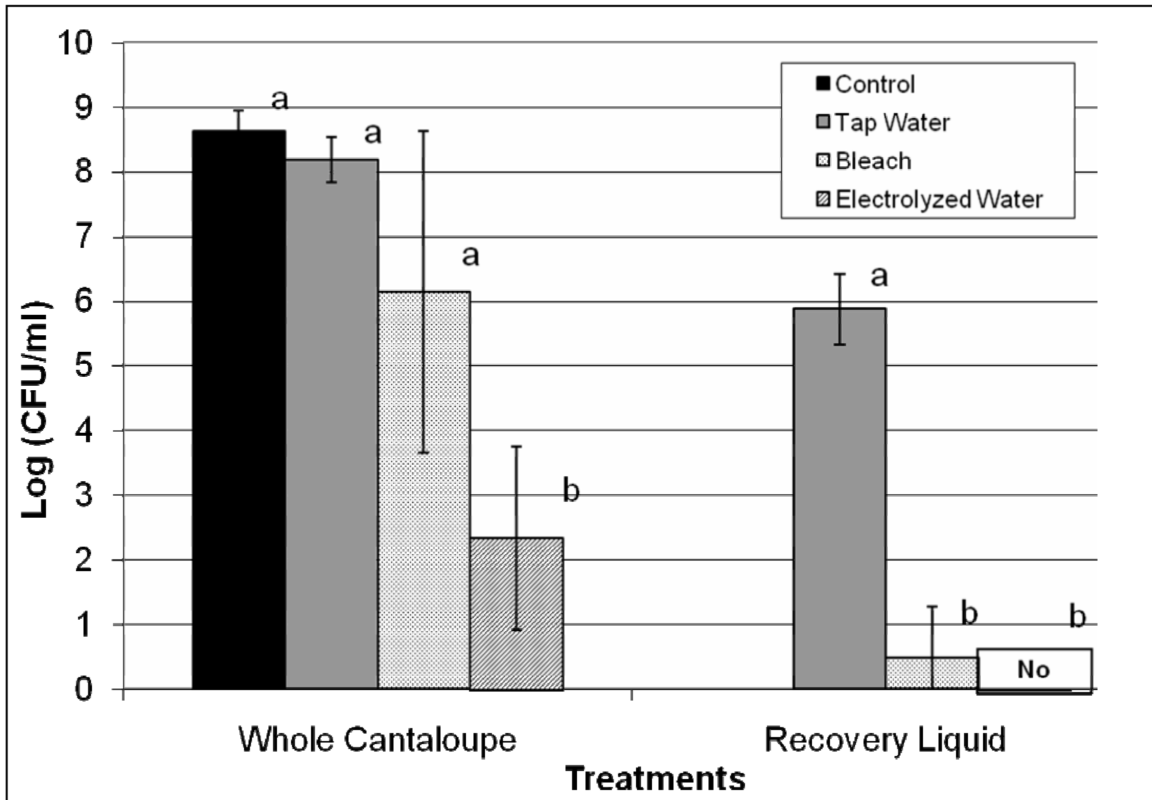

Figure 15. Cantaloupes inoculated with a five strain cocktail of *E. coli* O157:H7 were dip treated (4000 ml) for 30 sec in tap water, bleach/hypochlorite (204 ppm), or electrolyzed water (202 ppm). Recovered rinse solutions were also sampled. Means of the same sample treatment type with the same lowercase letters are not significantly different ($P > 0.05$). Means of the same treatment with different lowercase letters are significantly different ($P < 0.05$).

Levels of *E. coli* O157:H7 were significantly reduced on whole, inoculated cantaloupes relative to the control samples by more than 6-logs by electrolyzed water, but not by tap water or bleach rinses (Fig. 15). Both bleach and EW rinse solutions drastically eliminated levels of *E. coli* O157:H7 that were otherwise displaced by the rinse treatments into water as potential sources of cross-/re-contamination. The data demonstrates that EW may provide a convenient means of sanitizing the surfaces of whole cantaloupe melons before they are cut into pre-cut melon pieces.

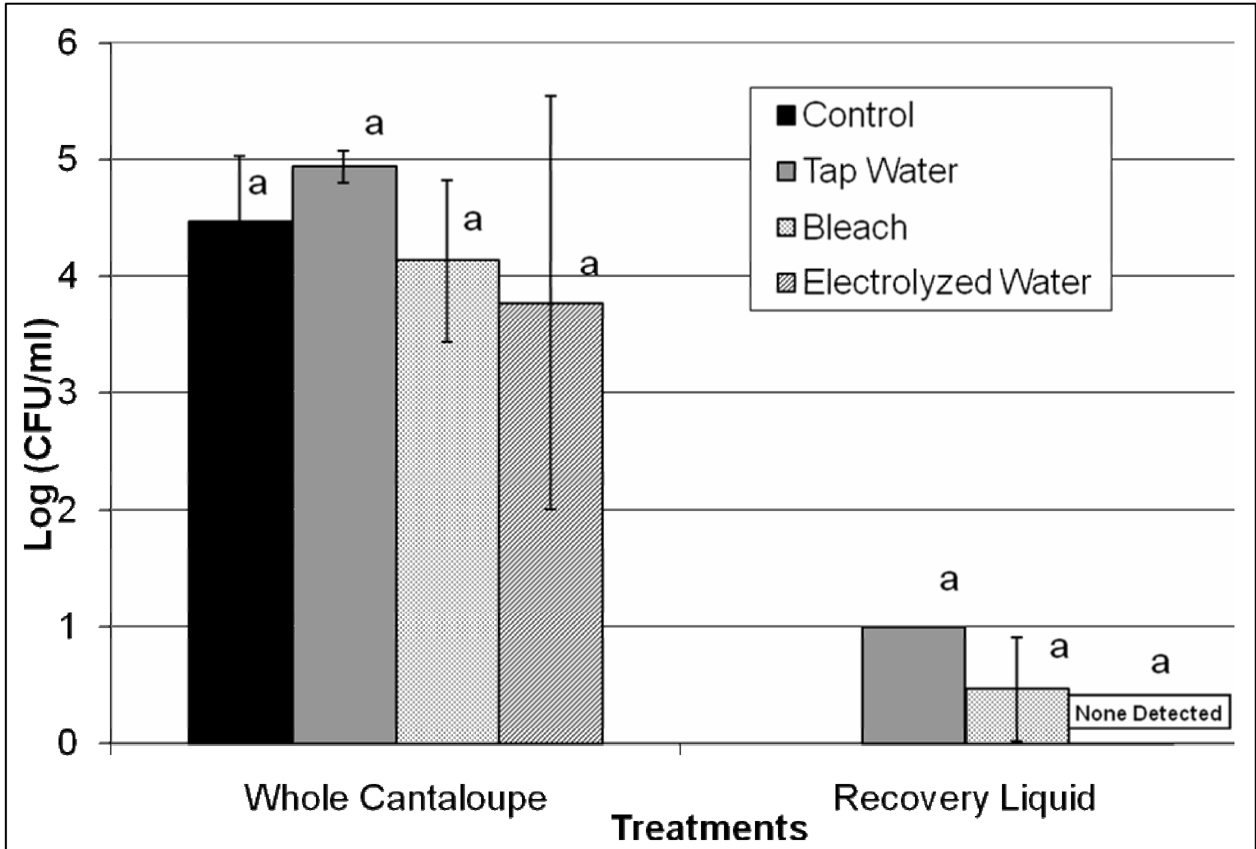

Figure 16. Uninoculated cantaloupes were dip treated (4000 ml) for 30 seconds in electrolyzed water (210 ppm), bleach (201 ppm), or tap water. Recovery liquid was also collected for sampling. Means of the same sample type with the same letters are not significantly different ($P > 0.05$). Means of the same sample type with different letters are significantly different ($P < 0.05$).

Although EW demonstrated significant reduction of bacteria on inoculated cantaloupes (Fig. 15), the use of EW to reduce indigenous bacteria on cantaloupes was not as effective (Fig. 16). This could be due to the indigenous bacteria being hidden away in difficult to reach crevices whereas the inoculated bacteria may be more available to sanitizers.

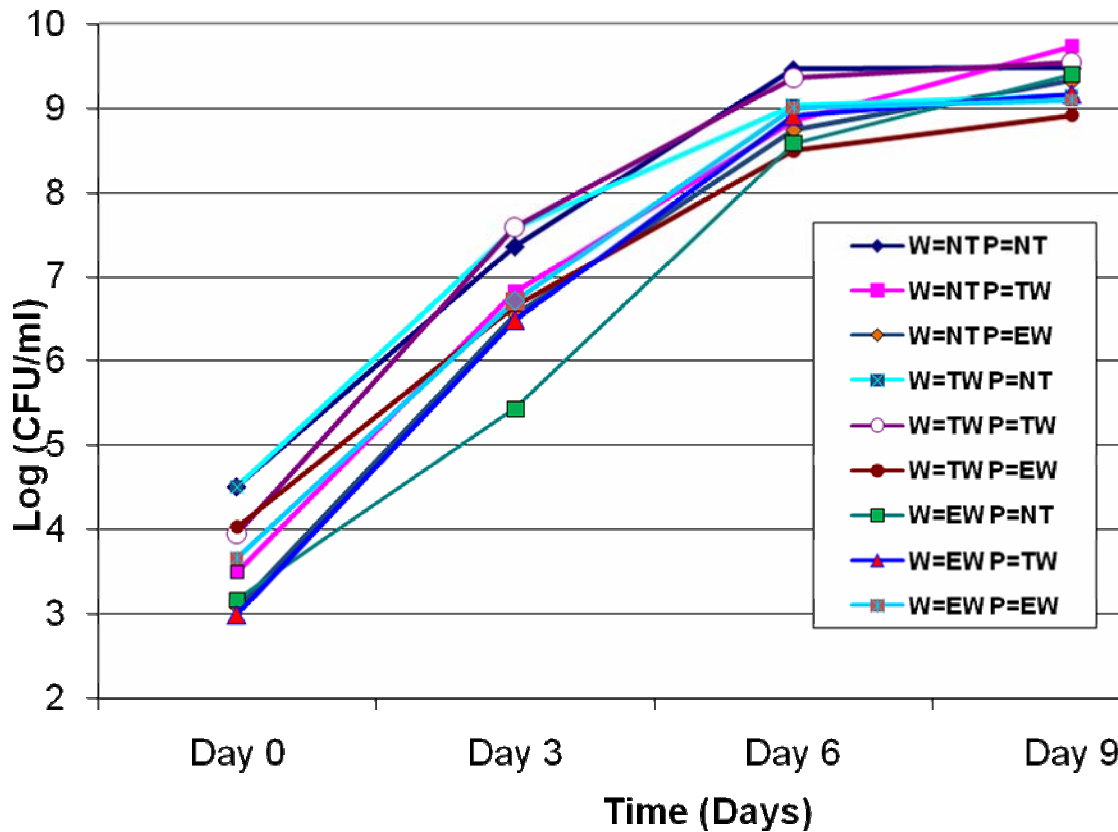

Figure 17. Uninoculated cantaloupes were dip treated (4000 ml) for 30 seconds in electrolyzed water (197 ppm) or tap water follow. Cantaloupes were sliced, halved, cut into sixteenths and then into 1.5 in pieces. Pieces were then dip treated (4000 ml) for 30 s seconds in electrolyzed water (197 ppm) or tap water. Samples were measured at day 0, day 3, day 6, and day 9. Legend: W, whole cantaloupe; P, pieces; NT, no treatment; TW, tap water; EW, electrolyzed water.

Shelf life studies in which intact cantaloupes were untreated or rinsed with water or EW, cut into pieces which were either untreated or treated with water or EW, showed almost no significant differences between the various treatments. The high contamination levels of initial pieces were based on cutting being performed on the same cutting boards that the whole melons were on.

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VITA

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Master of Science

Thesis: EFFICACY OF ELECTROLYTICALLY GENERATED HYPOCHLOROUS ACID (ELECTROLYZED WATER) ON FRESH AND PROCESSED MEATS

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Personal Data: Born in Omaha, Nebraska on March 13, 1983, the daughter of Thomas and Nancy Veasey.

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Experience: Employed in Dr. Harshavardhan Thippareddi's Food Microbiology laboratory from Septermber 2002 till October 2005 at the University of Nebraska in Lincoln, NE; Employed as Dr. Peter Muriana's graduate research assistant for Oklahoma State University from January 2006 till December 2007; Currently employed by PURAC America in Lincolnshire, Illinois

Professional Memberships:
Institute of Food Technologists; American Meat Science Association

Name: Shawnna R. Veasey Date of Degree: December, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFICACY OF ELECTROLYTICALLY GENERATED HYPOCHLOROUS ACID (ELECTROLYZED WATER) ON FRESH AND PROCESSED MEATS

Pages in Study: 84 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study:

Thorough research has been completed on the efficacy of electrolyzed water, electrolytically generated hypochlorous acid, against bacterial cultures and fresh produce. The objective of this research was to extend the body of knowledge to include fresh and ready-to-eat (RTE) meat. The efficacy of electrolyzed water against food borne pathogens of importance to the meat industry was examined using multiple types of fresh and RTE meat products. Samples were dip or sprayed treated with an electrolyzed water solution ranging from 20 to 50 ppm of free available chlorine (FAC) at a pH range of 4 to 6. Rinse off or recovery liquids were collected from each sample and evaluated for viable bacteria as well.

Findings and Conclusions:

Electrolyzed water was ineffective at reducing viable bacteria in all four fresh meat types. Of the six RTE meat sample types, electrolyzed water was effective in reducing bacterial levels when compared to the control on five of the products. When compared to a tap water or double distilled water rinse, electrolyzed water was more effective in only two of the products. In rinse off or recovery samples electrolyzed water signifcantly reduced a greater amount of viable bacteria then tap water or double distilled water in nine out of eleven studies. Of these nine studies, electrolyzed water was able to reduce the level below a detectable range in seven of them. The research suggests that electrolyzed is ineffective as an antimicrobial in the meat industry on actual product but would be effective at reducing or eliminating cross contamination due to splatter or worker mishandling.

ADVISER'S APPROVAL: Dr. Peter Muriana