USING CRITICAL PARAMETERS TO ENSURE EFFICACY OF SELECTED HARVEST AND FABRICATION INTERVENTION STRATEGIES USED TO CONTROL *Escherichia coli* O157:H7 AND *Salmonella*

A Thesis

by

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ABSTRACT

The objective of this study was to identify critical parameters of application for selected harvest and fabrication intervention strategies used to control *Escherichia coli* O157:H7 and *Salmonella*. Paired, boneless, beef strip loin (n=120, IMPS 180) were selected at a commercial cow harvest facility, transported to the Food Microbiology Laboratory, Texas A&M University, and inoculated with nonpathogenic, rifampicin-resistant *E. coli* organisms (ATCC #1427, 1428, 1430) to simulate harvest floor contamination. The beef strip loins were inoculated hot (~30°C) and then subjected to one of three chemical treatments (L-lactic acid, peroxyacetic acid, and acidified sodium chlorite) including subset variations for concentration and pH. Lactic acid was applied warm (~53°C) and at room temperature (~25°C), whereas the peroxyacetic acid, and acidified sodium chlorite were applied at room temperature (~25°C). Lactic acid was applied at concentrations of 2.5% and 5% using different water sources (tap and distilled), and at a common pH of ~2.2 using different water sources (tap and distilled). Peroxyacetic acid was applied at concentrations of 210 ppm and 150 ppm, and acidified sodium chlorite was applied at concentrations of 500 ppm and 1200 ppm. Half of the strip loins received the chemical interventions prior to chilling or "hot" (~25°C), whereas the other half received the interventions after a chilling for ~24 h at ~2°C. When applied to hot strip loins, only the 2.5% and 5% lactic acid treatments resulted in a greater than 1 log reduction, but for chilled strip loins all treatments achieved greater than a 1 log reduction. When tap water was used to prepare the intervention, there was a

difference between reductions for hot (0.68 CFU/cm^2) and chilled (2.02 CFU/cm^2) product, but there were no differences between hot and chilled for distilled water. Also, there were no differences in reductions between using tap and distilled water for hot products or for chilled products. The pH of the meat surface was lowest for the 5.0% lactic acid (3.07) and highest for the 150 ppm peroxyacetic acid (6.07). These data support the significance of conducting in-plant validation studies utilizing the specific parameters used in the plant.

DEDICATION

I dedicate this work to my family and friends. Without the constant love and support of my family and friends, none of my achievements would have been possible.

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NOMENCLATURE

ASC	Acidified Sodium Chlorite
ATCC	American Type Culture Collection
CDC	Centers for Disease Control
CFU	Colony Forming Unit
FSIS	Food Safety and Inspection Service
HACCP	Hazard Analysis Critical Control Point system
IMPS	Institutional Meat Purchase Specifications
PBS	Phosphate Buffered Saline
STEC	Shiga Toxin-Producing *Escherichia coli*
TSB	Tryptic Soy Broth
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION

Food safety is a dynamic situation, and the beef industry continues to be criticized for contributing to foodborne illnesses. The Centers for Disease Control reported a decline in foodborne infections related to Shiga toxin-producing *Escherichia coli* (STEC) O157:H7, but an increase in *Salmonella* infections. These have been the two primary pathogens of concern in raw beef products, and today the non-O157:H7 STECs are added to the list of concerns. Pressure continues to be placed on establishments by the United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) to improve their food safety programs which should then result in continued decreases in foodborne illnesses and product recalls.

Providing the safest possible beef products for the consumer is a primary goal for all establishments. HACCP and food safety programs are typically designed to prevent, eliminate, or reduce to an acceptable level specific pathogens that have been identified as reasonably likely to occur in the product. Research funding entities have provided significant support since the 1992-1993 outbreak of *E. coli* O157:H7 associated with Jack-in-the-Box hamburgers to help identify antimicrobial interventions. Most of the initial antimicrobial interventions focused on reducing pathogen contamination by treating the carcasses during harvest and upon entering fabrication. During the last several years additional research addressing interventions that can be applied to

subprimals prior to packaging, trimmings prior to grinding, and to finished ground beef have provided information to assist with the development of food safety programs.

Unfortunately, we continue to struggle with recalls and food safety illnesses associated with beef. Based on discussions with establishments and variation in pathogen testing results across establishments, it is apparent that establishments need additional data on the critical parameters of the available interventions. These data will allow them to improve their HACCP and food safety programs to ensure that the in-plant interventions are being applied in a manner to achieve optimal efficacy and to ensure that they are monitoring the parameters that are crucial for successfully controlling the pathogens of concern. Therefore, this project investigated variables that may impact the efficacy of interventions and aimed to identify the critical parameters and procedures for effectively monitoring them.

CHAPTER II

REVIEW OF LITERATURE

Food borne illnesses occur in the United States every year due to cross contamination during harvest, fabrication, food handling, and in-home preparation by consumers. There are an estimated 47.8 million cases of food borne illness in the United States annually, with 127,839 hospitalizations and 3,037 deaths (Centers for Disease Control, 2011). Known food borne pathogens account for 9.4 million cases of illness, while 38.4 million cases are the result of unspecified agents (Centers for Disease Control, 2011). The most common pathogens causing illness, hospitalization, and death include Norovirus, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Staphylococcus aureus*, *Toxoplasma gondii*, *E. coli* O157:H7, and *Listeria monocytogenes* (Centers for Disease Control, 2011). Raw meat and poultry are common sources of food borne illness due to the presence of pathogenic organisms. From 1998 to 2008, beef is estimated to be the source of more *E. coli* O157:H7 related illnesses (39.4%) than any other commodity, including shellfish, poultry, and produce, while it is also estimated to be the source of 7.3% of all *Salmonella* related illnesses during that time (Painter et al., 2013). Although the incidence of laboratory confirmed *E. coli* O157:H7 infections have decreased 44% compared to 1996-1998, laboratory confirmed *Salmonella* infections have increased 3% during this time (Centers for Disease Control, 2013). This decrease in the number of laboratory confirmed *E. coli* O157:H7 illnesses can be attributed to improved harvest and fabrication interventions as well as improved microbiological testing and lotting procedures in the beef industry.

Harvest Interventions

There are several interventions used during the slaughter of beef that have been proven effective at reducing contamination. These include trimming (Castillo et al., 2002; Castillo et al., 1998a; Hardin et al., 1995; Marquez-Gonzalez et al., 2010), water washing (Castillo et al., 2002; Castillo et al., 1998a; Hardin et al., 1995; Marquez-Gonzalez et al., 2010), hot water rinsing (Castillo et al., 2002; Castillo et al., 1998a, b; Marquez-Gonzalez et al., 2010), steam vacuuming (Castillo et al., 2002; Dorsa et al., 1996; Gill and Bryant, 1997; Marquez-Gonzalez et al., 2010), steam pasteurizing (Castillo et al., 2002; Gill and Bryant, 1997; Marquez-Gonzalez et al., 2010), and organic acid spraying (Castillo et al., 2002; Castillo et al., 1998a, 1999a; Castillo et al., 2001a; Hardin et al., 1995; Marquez-Gonzalez et al., 2010).

Lactic Acid. Lactic acid treatments are often used during beef slaughter in combination with other interventions. Castillo et al. (1999a) evaluated the effect of steam vacuuming alone, and in combination with either a hot water (95°C) rinse, a warm 2% lactic acid (55°C) treatment, or both to reduce microbial numbers on hot beef carcass surfaces. All treatments showed significant reduction, however, when steam vacuuming was combined with either the hot water (95°C) rinse or the warm lactic acid (55°C) treatment, greater reduction was achieved than just by steam vacuuming alone. Another study conducted by Castillo et al. (1998a) compared reductions on three different regions of hot beef carcass surfaces using cleaning treatments of high pressure water wash or trimming, alone and combined with sanitizing treatments of hot water (95°C), a warm

(55°C) 2% lactic acid spray, or both. Larger reductions were shown when both the water wash and trimming were combined with either the lactic acid spray or the hot water treatment, with the lactic acid spray showing slightly larger reductions than the hot water on almost all areas. However, the largest microbial reduction occurred when the lactic acid spray or the hot water treatment combined with the hot water followed by the lactic acid spray.

Acidified Sodium Chlorite. Acidified sodium chlorite is approved by the Food and Drug Administration as a direct food additive to be used for decontamination of red meat carcasses. Castillo et al. (1999b) conducted a study to determine the effectiveness of acidified sodium chlorite in reducing *E. coli* O157:H7 and *Salmonella* Typhimurium on beef carcasses. Both phosphoric acid-activated acidified sodium chlorite and citric acid-activated acidified sodium chlorite were applied at room temperature to hot beef carcasses in addition to a water wash. Both acidified sodium chlorite solutions were applied for 10 s, resulting in 140 ml of solution per carcass. Citric acid-activated acidified sodium chlorite consistently produced greater reductions than phosphoric acid-activated acidified sodium chlorite, and both acidified sodium chlorite solutions showed greater reductions than water alone. With reductions up to 4.6 log CFU/cm^2 for citric acid-activated acidified sodium chlorite, and 3.9 log CFU/cm^2 for phosphoric acid-activated acidified sodium chlorite, this study shows that acidified sodium chlorite can be an effective anti microbial for decontaminating beef carcasses.

Peroxyacetic Acid. A study by King et al. (2005) evaluating the effects of peroxyacetic acid on counts of *E. coli* O157:H7 and *Salmonella* Typhimurium on chilled

beef carcass surfaces also conducted an experiment on hot carcass surfaces. In the experiment, 200 ppm peroxyacetic acid was applied at 43° C to inoculated hot beef carcass surfaces. This treatment resulted in a statistically significant reduction of 0.7 log CFU/cm^2 in both *E. coli* O157:H7 and *Salmonella* Typhimurium.

Fabrication Interventions

Many of the same chemical interventions used in slaughter of beef can also be used during its fabrication. However, fabrication interventions are not as widely used and generally have more variation in their results.

Lactic Acid. Lactic acid sprays can also be used as a chilled beef antimicrobial, although with more variable results. Castillo et al. (2001b) applied a lactic acid spray to chilled beef carcasses that had previously received interventions during the slaughter process in order to simulate industry processing conditions. Carcasses either received a water wash alone or a water wash and 250 ml of 55°C 2% lactic acid for 15 s prechilling. After chilling, carcasses received an additional treatment of 500 ml of 55°C 4% lactic acid sprayed for 30 s. In both scenarios, the post-chill lactic acid treatment resulted in additional reductions of 2.0 to 2.4 log CFU/cm^2 for *E. coli* O157:H7 and 1.6 to 1.9 log CFU/cm^2 for *Salmonella* Typhimurium, indicating that, when combined with prechill treatments, application of lactic acid post-chilling can be effective in reducing pathogens on carcasses and ground beef from those carcasses. Another study conducted by Castillo et al. (2001a), determined that this method was effective in an actual in-plant setting, as it consistently reduced *E. coli* and coliforms to undetectable levels.

Acidified Sodium Chlorite. A study by Gill and Badoni (2004) evaluated the effects of a 0.16% citric acid acidified sodium chlorite solution on the natural flora of the distal surfaces of pieces of chilled brisket obtained from two different slaughtering plants (plant A and plant B), compared to 0.02% peroxyacetic acid and 4% lactic acid. Each piece of meat was treated by spraying with about 50 ml of distilled water or one of the specified solutions at approximately 7° C, and samples were taken about 60 minutes later. The 0.02% peroxyacetic acid and 0.16% acidified sodium chlorite both resulted in reductions of less than 0.5 log units, while the 4% lactic acid produced reductions of greater than 1 log unit for aerobes for meat from plant A. However, 0.02% peroxyacetic acid and 0.16% acidified sodium chlorite both produced reductions of about 1 log unit and 4% lactic acid showed reductions of greater than 2 log units for aerobes when applied to meat samples from plant B. Both the 0.02% peroxyacetic acid and 0.16% acidified sodium chlorite showed almost no reductions, while the 4% lactic acid resulted in reductions of up to 1 log unit for coliforms on meat from plant A. The 0.02% peroxyacetic acid and 0.16% acidified sodium chlorite produced reductions of about 1 log unit, and the 4% lactic acid solutions showed reduction of about 1.5 log units for coliforms on meat from plant B. As these results show, nearly all treatments were less effective at reducing the natural flora present on meat from plant A than on meat from plant B. The researchers suggest that these differences in reductions could be due to differences in the compositions of the flora on carcasses from the two plants, which could be the result of different processing treatments at the plants. These findings indicate that 4% lactic acid may be generally useful as an antimicrobial intervention, and

that 0.02% peroxyacetic acid and 0.16% acidified sodium chlorite are far less effective when applied to chilled beef.

Peroxyacetic Acid. King et al. (2005) conducted a study in which peroxyacetic acid was used in different concentrations as a post-chilling microbial intervention. Inoculated beef sides were treated with a water wash with and without 2% lactic acid treatment before chilling to simulate industry procedures. In scenarios where chilled carcasses were treated with 200 ppm peroxyacetic acid at 43° C for 15 s, no effects on microbial counts for any of the organisms measured were shown. However, samples that were taken prechilling, after the 2% lactic acid treatment was applied, showed that lactic acid reduced microbial counts of *E. coli* Type I, coliforms, *E. coli* O157:H7, and *S.* Typhimurium, which has been shown in previous research. In another experiment, the peroxyacetic acid was applied at varying concentrations (200, 600, and 1000 ppm) and temperatures (45 and 55° C) and compared to a 4% lactic acid treatment applied at 55° C. Temperature and concentrations of 200 and 600 ppm had no effect on reductions. Peroxyacetic acid concentrated at 1000 ppm resulted in reductions of 1.7 and 1.3 log CFU/cm^2 for *E. coli* and *S.* Typhimurium, respectively, however, the 4% lactic acid solution resulted in greater reductions of 2.7 and 3.4 log CFU/cm^2, respectively. Results from this study show that peroxyacetic acid is not an effective pathogen intervention when applied to chilled beef carcasses, even when applied in concentrations that far exceed the approved level.

Eschirichia coli O157:H7 and *Salmonella* Surrogates

Surrogate organisms display similar or identical properties (especially thermal and acid resistance) to one or more pathogens and therefore can be used as biological markers for those pathogens. These surrogates are useful in research to help validate pathogen intervention strategies in a laboratory or production setting without exposing the product, equipment, facilities, researchers, or consumers to contamination from the pathogen. The perfect surrogate, as defined by the U.S. Food and Drug Administration, is the pathogen itself that is transformed, through genetic engineering, into a nonpathogenic form (USDHHS-FDA, 2011). Due to the possibility of the organism reverting back to a pathogen and the possibility of the facility receiving false positives when sampling for the pathogen, this isn't done often. Therefore, suitable surrogate organisms should be nonpathogenic, have well defined characteristics, be easily enumerated, have durability to processing parameters similar to the target organism, and be easily differentiable from other microorganisms that may be present (USDHHS-FDA, 2011).

Marshall et al. (2005) compared survival traits such as temperature sensitivity and acid resistance of five *E. coli* isolate indicators to five clinical strains of *E. coli* O157:H7. All isolates evaluated showed similar temperature sensitivity to the clinical strains. Isolates were exposed to seven treatments, in all of which at least two isolates showed reductions similar to the clinical strains, which suggests that the isolates could be used in combination to represent contamination with *E. coli* O157:H7. Niebuhr et al. (2008) subjected these five *E. coli* isolates and a mixture of five *Salmonella* strains to

microbial interventions comparable to those used in the industry. The *E. coli* isolates showed similar reductions to the *Salmonella* strains, with four of the isolates exhibiting a higher survival rate, indicating that the isolates could be used as *Salmonella* indicators. Cabrera-Diaz et al. (2009) compared the growth, acid and thermal resistance, and attachment properties of the nonpathogenic *E. coli* strains to those of *E. coli* O157:H7 and *Salmonella* strains. The researchers found that thermal and acid resistance of the nonpathogenic *E. coli* strains were not different or slightly higher than that of the *E. coli* O157:H7 and *Salmonella* strains, enabling them to be used as a sufficient surrogate organism for validating hot water and lactic acid interventions on beef carcasses. Another study was conducted by Keeling et al. (2009) to evaluate the effect of processes such as freezing, refrigerating, fermentation, and thermal inactivation on the *E. coli* biotype I isolates compared to *E. coli* O157:H7. The study showed that three of the isolates, BAA-1427, BAA-1429, and BAA-1430, showed no difference in the refrigeration study and had slightly better survival in the frozen, fermentation, and thermal inactivation studies than *E. coli* O157:H7, which would allow for a margin of safety.

CHAPTER III

MATERIALS AND METHODS

Preparation of Inoculum

Three nonpathogenic *E. coli* Biotype I strains (ATCC 1427, 1428, and 1430) were obtained from the American Type Culture Collection (ATCC – www.ATCC.org) for use in this study. These strains then were selected in the Food Microbiology Laboratory at Texas A&M University for their inherent ability to naturally resist rifampicin. These marker organisms were selected for use in a "cocktail" to represent possible contamination with enteric pathogens of fecal origin such as *Salmonella* or *E. coli* O157:H7. Through previous scientific research, these surrogate organisms have demonstrated identical thermal and acid resistance to the human pathogen *E. coli* O157:H7 (Cabrera-Diaz et al., 2009; Keeling et al., 2009; Marshall et al., 2005).

Approximately 48 h before each collection day the rifampicin-resistant cultures of *E. coli* organisms (American Type Culture Collection #: BAA-1427, BAA-1428, BAA-1430) were propagated by transferring 0.1 ml of the stored microorganisms from a tryptic soy broth (TSB) tube to a fresh 10 ml TSB tube and incubated at 35°C for 18 to 24 h. Each culture was then transferred individually by pipetting 0.1 ml into conical centrifuge tubes containing 10 ml TSB before incubating for 18 h at 35°C. Following incubation, cells from each culture were harvested by centrifugation at 1,620 × g for 15 min. The supernatant was discarded and the pellet suspended in 10 ml of phosphate-buffered saline (PBS). Each cell suspension was centrifuged again (1,620 × g for 15

min) and this procedure was repeated once. The final pellets were each suspended in 10 ml of PBS and combined to form a cocktail of rifampicin-resistant, nonpathogenic *E. coli* organisms (ATCC#: BAA-1427, BAA-1428, BAA-1430).

Product Procurement

Beef loin, strip loin, boneless (Institutional Meat Purchase Specifications [IMPS] 180) subprimals (n = 120) were obtained from cattle slaughtered at a commercial beef processor on five occasions (n = 24 each time). Before leaving for the beef processor, an insulated container was filled with hot water. Immediately before entering the plant, the hot water was removed from the container. This helped to keep the container warm, along with the strip loins that were transported in it. The subprimals were removed from both sides of the carcass immediately upon entering the blast chill, labeled according to carcass number and side (left or right), placed in the insulated container, and transported to the Food Microbiology Laboratory, Texas A&M University. Upon arrival, surface temperature and pH readings were taken from the dorsal surface of each strip loin.

Before inoculation, one 10 cm^2 sample was taken from the dorsal surface of 15 randomly chosen strip loins (n = 3 each collection day) to determine if naturally occurring rifampicin-resistant organisms were present. Samples were pummeled individually in the stomacher in 99 ml of 0.1% buffered peptone water, for 1 min, at 260 RPM. Samples were plated using appropriate decimal dilutions on rifampicin tryptic soy agar (rif-TSA) plates with a sterile bent glass rod. The rif-TSA was prepared by adding

0.1 g of rifampicin dissolved in 5 ml of methanol to 1 liter of autoclaved and cooled (55° C) TSA.

Strip Loin Inoculation, Treatment, and Sampling

All strip loins were inoculated with approximately 10^7 CFU/cm^2 of the nonpathogenic, rifampicin-resistant *E. coli* cocktail. One ml of inoculum was applied to the dorsal surface of each strip loin and spread using a sterile disposable plastic spreader. The strip loins were allowed 30 min for microbial attachment before treatments were applied.

After the 30 min attachment period, another surface temperature and pH reading were taken on the dorsal surface of the strip loins. One strip loin from each carcass was randomly selected to be placed in a refrigerated cooler (1°C) for 24 h, while the other strip loin from that carcass was selected for treatment before chilling, which was designed to represent "hot" carcass surfaces. Post-inoculation, pre-treatment samples were then taken on strip loins that were selected to be treated hot (30°C) to determine the level of microbial attachment for each strip loin. Using a sterile stainless-steel borer, scalpel and forceps, two 10-cm^2 (2 mm in depth) samples were excised from random locations on the dorsal surface of each strip loin, composited (20-cm^2 total area) into a sterile stomacher bag with 99 ml of 0.1% buffered peptone water, and pummeled in the stomacher for 1 min at 260 RPM. Samples were then plated on rif-TSA plates.

For chemical treatment, each strip loin was suspended from the posterior end using a flame-sterilized, stainless-steel, meat hook, and the dorsal surface was sprayed

for 12 s at a rate of ~2.5 ml/s (total of 30 ml) with one of the interventions listed in Table 1.

Immediately after treatment, surface temperature and pH measurements were taken again on the dorsal surface of each strip loin. Post treatment samples were then taken from each loin to determine level of reduction. Using a sterile stainless-steel borer, scalpel and forceps, two 10-cm^2 (2 mm in depth) samples were excised from random locations (that didn't overlap previous sample locations) on the dorsal surface of each strip loin, composited (20-cm^2 total area) into a sterile stomacher bag with 99 ml of 0.1% buffered peptone water, and pummeled in the stomacher for 1 min at 260 RPM. Samples were then plated on rif-TSA plates.

Strip loins that were chilled in the refrigerated cooler (1°C) were removed 24 h later and surface temperature and pH readings were taken on the dorsal surface of each strip loin. Post-inoculation, pre-treatment samples were then taken to determine the level of microbial attachment for each chilled strip loin. Using a sterile stainless-steel borer, scalpel and forceps, two 10-cm^2 (2 mm in depth) samples were excised from random locations on the dorsal surface of each strip loin, composited (20-cm^2 total area) into a sterile stomacher bag with 99 ml of 0.1% buffered peptone water, and pummeled in the stomacher for 1 min at 260 RPM. Samples were then plated on rif-TSA plates.

The chilled strip loins (n = 60) were then suspended from the posterior end using a flame-sterilized, stainless-steel meat hook and the dorsal surface of each strip loin was sprayed for 12 s at a rate of ~2.5 ml/s (total of 30 ml) with one of the interventions listed in the table above. Both strip loins from a carcass were treated with the same chemical

intervention, one of the strips was treated hot (~30°C) while the other was treated chilled (~7°C). Immediately after treatment, surface temperature and pH measurements were taken again on the dorsal surface of each strip loin. Post treatment samples were then taken from each loin to determine level of reduction. Using a sterile stainless-steel borer, scalpel and forceps, two 10-cm^2 (2 mm in depth) samples were excised from random locations (that didn't overlap previous sample locations) on the dorsal surface of each strip loin, composited (20-cm^2 total area) into a sterile stomacher bag with 99 ml of 0.1% buffered peptone water, and pummeled in the stomacher for 1 min at 260 RPM. Samples were then plated on rif-TSA plates.

Plates were incubated for 24 h at 35°C. Colonies were counted, recorded, and reported as log CFU per square centimeter. In total, 240 strip loin surface composites (120 from hot strip loins, 120 from chilled strip loins) were analyzed. A total of 420 surface pH and 420 surface temperature measurements were taken (120 upon arrival, 120 after attachment, 60 post-treatment on subprimals treated hot, 60 pre-treatment on subprimals chilled for 24 h, 60 post-treatment on subprimals chilled for 24 h).

Antimicrobial Treatment Preparation

Lactic acid solutions of 2.5% were prepared by diluting 29 ml of 88% L-lactic acid concentrate (Purac America, Inc., Lincolnshire, IL) into 1000 ml of either distilled or municipal water. Lactic acid solutions of 5% were prepared by diluting 56 ml of 88% L-lactic acid concentrate (Purac America, Inc., Lincolnshire, IL) into 1000 ml of either distilled or municipal water. Lactic acid solutions were also prepared in order to achieve

a common pH (~2.2) using the 2 different water sources (tap and distilled). For the tap water lactic acid solution (pH of ~2.2), ~25.2 ml of 88% L-lactic acid concentrate (Purac America, Inc., Lincolnshire, IL) were diluted into 1000 ml of tap water. For the distilled water lactic acid solution (pH of ~2.2), ~11.2 ml of 88% L-lactic acid concentrate (Purac America, Inc., Lincolnshire, IL) were diluted into 1000 ml of distilled water.

Acidified sodium chlorite solutions of 1200 ppm were prepared by diluting 16.2 ml of sodium chlorite into 1000 ml of either tap or distilled water. Acidified sodium chlorite solutions of 500 ppm were prepared by diluting 6.75 ml of sodium chlorite into 1000 ml of either tap or distilled water. Powdered citric acid was dissolved into the acidified sodium chlorite solutions so that the pH of the solutions was ~2.3.

Peroxyacetic acid solutions of 210 ppm were prepared by diluting 1.2 ml of peroxyacetic acid into 1000 ml of either distilled or tap water. Peroxyacetic acid solutions of 150 ppm were prepared by diluting 0.9 ml of peroxyacetic acid into 1000 ml of either distilled or tap water.

All acid solutions were put in garden pump sprayers to be sprayed. The sprayers were pumped the same number of times for each application and the pressure was relieved after each application to ensure a common flow rate. Lactic acid solutions that were heated were placed into the sprayers, and the sprayers were placed into a hot water bath. Temperature of the solutions was measured at the spray nozzle immediately prior to spraying in order to accurately determine solution temperature.

Statistical Analysis

Microbiological count data were transformed into logarithms before obtaining means and performing statistical analyses. The level of reduction was determined by taking the difference of microbiological count of the post-treatment from the pretreatment. In the case of counts below the minimum detection level, a number between 0 and the minimum detection limit was used in order to facilitate the data analysis. All data were analyzed using JMP software (JMP Pro, Version10.0, SAS Institute Inc., Cary, NC). The fit model function was used for analysis of variance, using the pretreatment microbiological count as a covariate in the model. Interactions were determined from the full model, and when significant differences were found, means were separated to evaluate the least squares means comparisons using a student's t-test. These means then were analyzed in order to determine the impact of antimicrobial intervention and processing practices on the numbers of the pathogen surrogate organisms.

CHAPTER IV

RESULTS AND DISCUSSION

Before inoculation 15 samples were taken from randomly chosen strip loins and plated to determine if there were any preexisting rifampicin-resistant organisms present on the strip loins. As expected, there were no detectable counts of these organisms present.

The initial inoculum level of the strip loins had to be high enough to ensure that sufficient reduction could be detected. Strip loins were inoculated with 7.89 log CFU/ml of the surrogate organisms (Table 2). After the strip loins were inoculated and allowed time for attachment, pre-treatment samples from strip loins that were to be treated hot were collected, while those that were to be treated after chilling were placed in a refrigerated cooler for 24 h before pre-treatment samples were taken. Average attachment level varied ($P < 0.05$) between hot and chilled strip loins (Table 3). This difference indicates that chilling reduced the number of surrogate bacteria by 1.49 logs.

The pH of the strip loin surfaces differed ($P < 0.05$) between hot and chilled products prior to application of a treatment (Table 4). It was surprising that there was this much difference in pH between the hot and the chilled strip loins. It is also noted that both pH values were higher than would be expected for most fresh beef products.

Before application of the acid treatments to the strip loins, a pH measurement was taken on each acid. Water source had a significant effect on the pH of all acids (Table 5). In this interaction the greatest differences in pH values were between the

peroxyacetic treatments mixed with the tap and distilled water sources. This difference was at least 1.5 pH units for both treatments. All acids mixed with tap water had a significantly ($P < 0.05$) higher pH than those mixed with distilled water, although the value differences for all other treatments were not as great as those observed in the peroxyacetic treatments. Most often in controlled studies in labs in university and government settings distilled water is used to prepare various acids for intervention trials. However, in typical industry practices the water used to mix these acids is tap water. Based on these findings, it appears that source of water may play a key role in determining the pH of the specific acids that are being applied for these antimicrobial interventions. In this study, tap water was a single source, but industry water may vary greatly based on municipal and/or well sources and may not have been accounted for in determining efficacy of in-plant antimicrobial interventions.

Strip loin surface pH measurements were taken following application of the acid treatment on each strip loin. Water source had a significant effect on post-treatment strip loin surface pH for strip loins treated with the peroxyacetic 210 and 1200 ASC treatments (Table 6). Strip loins treated with the 210 peroxyacetic x tap water combination had a higher ($P < 0.05$) surface pH than strip loins treated with the 210 peroxyacetic x distilled water combination. Strip loins treated with the 1200 ASC x tap water combination had a lower ($P < 0.05$) surface pH than strip loins treated with the 1200 ASC x distilled water combination. All other strip loin surface pH values did not differ for the remaining acid treatment x water source combinations. The surface of the strip loins may have buffered the pH of the acids after application so that the differences

in acid pH found and reported in Table 5 did not remain significant after application to the surface of the strip loins.

Surface temperatures were taken prior to treatment application for all strip loins (Table 7). As expected, surface temperatures of the hot strip loins were significantly higher ($P < 0.05$) than those that were chilled. Surface temperatures were also taken immediately after treatment application (Table 7). The differences between hot and chilled strip loin surface temperatures remained significant ($P < 0.05$) after treatment application. However, the surface temperature of the chilled product rose approximately 8°C after application of the treatments.

Because lactic acid treatments were applied at two different temperatures (25°C and 53°C), these data were analyzed separately. There was a three-way interaction on post-treatment strip loin surface temperature between hot vs. chilled strip loins x acid treatment x temperature of application (Table 8). There were no significant differences in post-treatment meat surface temperature for the various combinations of acid treatment or acid temperature when applied to the hot strip loins. There were significant differences in post-treatment meat surface temperatures for the various combinations of acid treatment or acid temperature when applied to the chilled strip loins, with the lowest post-treatment surface temperature observed for chilled strip loins treated with 2.5% lactic acid applied at 53°C.

Means for all strip loin surface pH and temperature readings taken throughout the process are shown in Table 9.

There were no differences ($P > 0.05$) in reduction within the acid treatments based on water source (tap or distilled). There were also no differences ($P > 0.05$) between reductions for the hot or chilled products based on water source. However, reductions observed on chilled products treated with acids mixed using tap water were greater ($P < 0.05$) than those observed on hot products treated with acids mixed using either water source (Table 10).

There were differences ($P < 0.05$) in reductions between the different antimicrobial treatments applied to hot and chilled strip loins (Table 11). Within the strip loins treated hot, the greatest log reductions were observed on strip loins treated with 5% lactic acid. The 2.5% lactic acid treatment provided greater reduction ($P < 0.05$) than both the 150 peroxyacetic and the 500 ASC treatments. Within the strip loins treated after being chilled, all treatments resulted in at least a one log reduction, with fewer significant differences in reductions between treatments. Reductions for the 1200 ASC and 150 peroxyacetic treatments were significantly greater ($P < 0.05$) on chilled strip loins compared to strip loins treated hot. All acid treatments provided greater reductions on chilled strip loins than hot strip loins. This could be due to the fact that the surrogate organisms were weakened during chilling and therefore more susceptible to the interventions used. In general, the 5% lactic acid treatment resulted in the greatest reductions, similar to the results shown by Gill and Badoni (2004).

There were no differences ($P > 0.05$) in reduction within the individual acid treatments mixed with the two water sources (data not shown), indicating that the significant differences in pH shown in Table 5 didn't result in differences in reduction.

Data for only the 2.5% and 5.0% lactic acid solutions applied at 25° C and 53° C were analyzed. There was a three-way interaction for log reductions for hot vs. chilled strip loins x acid treatment x acid temperature (Table 12). No differences ($P > 0.05$) in reduction were seen within chilled strip loins, or within strip loins treated with 53°C lactic acid. However, the 5.0% lactic acid solution applied at 25° C to the hot strip loins showed greater reduction ($P < 0.05$) than all other lactic acid treatment combinations when applied to both hot and chilled strip loins. All other combinations when directly compared between the 25°C and 53°C showed no differences ($P > 0.05$) in reduction, which conflicts with the results of previous research that shows heated lactic acid can produce greater reductions (Anderson and Marshall, 1989; Greer and Dilts, 1992). Although factors such as temperature and concentration of a particular treatment are important, it is clear that effectiveness of the treatment can differ based on the temperature of the product to which it is being applied.

The data collected from strip loins treated with the four lactic acid solutions that were mixed to a pH of ~2.2 were analyzed separately and no differences ($P > 0.05$) in reduction were shown between these treatments (data not shown). Because all treatments had approximately the same pH, although having different concentrations, no differences in reduction were shown, this suggests that pH may be an important factor to monitor to ensure efficacy of a food safety program. As expected, post-treatment surface pH of strip loins treated with these four solutions was lower ($P < 0.05$) compared to all other acids (Table 13).

CHAPTER V

CONCLUSIONS

Overall, data from this project clearly demonstrate that not all intervention parameters are critical to the efficacy of the intervention, and not all intervention parameters can be assumed to be effective when applied to different surfaces (hot vs. chilled). Of the interventions used, 5% Lactic acid generally resulted in the greatest reduction. Although significant differences in pH were observed between acids mixed with tap and distilled water, they didn't result in significant differences in reduction. Although having different concentrations, lactic acid solutions mixed to a pH of ~2.2 showed no significant differences in reduction, indicating that pH could have been the main cause of reduction rather than concentration. Therefore, these data support the importance of conducting in-plant validation studies utilizing the specific intervention parameters being applied.

REFERENCES

Anderson, M. E., and R. T. Marshall. 1989. Reducing microbial populations on beef tissues: concentration and temperature of lactic acid. J. Food Safety 10: 181-190.

Cabrera-Diaz, E., T. M. Moseley, L. M. Lucia, J. S. Dickson, A. Castillo, and G. R. Acuff. 2009. Fluorescent protein marked *Escherichia coli* Biotype I strains as surrogates for enteric pathogens in validation of beef carcass interventions. J. Food Prot. 74: 295-303.

Castillo, A., M. D. Hardin, G. R. Acuff, and J. S. Dickson. 2002. Reduction of microbial contaminants on carcasses. In: V. K. Juneja and J. N. Sofos, editors, Control of foodborne microorganisms. Marcel Dekker, Inc., New York. p. 351-381.

Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1998a. Comparison of water wash, trimming, and combined hot water and lactic acid treatments for reducing bacteria of fecal origin on beef carcasses. J. Food Prot. 61: 823-828.

Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1998b. Use of hot water for beef carcass decontamination. J. Food Prot. 61: 19-25.

Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1999a. Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. J. Food Prot. 62: 146-151.

Castillo, A., L. M. Lucia, G. K. Kemp, and G. R. Acuff. 1999b. Reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces using acidified sodium chlorite. J. Food Prot. 62: 580-584.

Castillo, A., L. M. Lucia, I. Mercado, and G. R. Acuff. 2001a. In-plant evaluation of a lactic acid treatment for reduction of bacteria on chilled beef carcasses. J. Food Prot. 64: 738-740.

Castillo, A., L. M. Lucia, D. B. Roberson, T. H. Stevenson, I. Mercado, and G. R. Acuff. 2001b. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. J. Food Prot. 64: 58-62.

Centers for Disease Control. 2011. CDC Estimates of Foodborne Illness in the United States. Accessed Feb. 4, 2013. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html.

Centers for Disease Control. 2013. Trends in Foodborne Illness in the United States. Accessed Feb. 6, 2013. http://www.cdc.gov/foodborneburden/trends-in-foodborne-illness.html.

Dorsa, W. J., C. N. Cutter, G. R. Siragusa, and M. Koohmaraie. 1996. Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. J. Food Prot. 59: 127-135.

Gill, C. O., and M. Badoni. 2004. Effects of peroxyacetic acid, acidified sodium chlorite or lactic acid solutions on the microflora of chilled beef carcasses. International Journal of Food Microbiology 91: 43-50.

Gill, C. O., and J. Bryant. 1997. Decontamination of carcasses by vacuum-hot water cleaning and steam pasteurizing during routine operations at a beef packing plant. Meat Sci. 47: 267-276.

Greer, G. G., and B. D. Dilts. 1992. Factors affecting the susceptibility of meatborne pathogens and spoilage bacteria to organic acids. Food Research International 25: 355-364.

Hardin, M. D., G. R. Acuff, L. M. Lucia, J. S. Oman, and J. W. Savell. 1995. Comparison of methods for decontamination from beef carcass surfaces. J. Food Prot. 58: 368-374.

Keeling, C., S. E. Niebuhr, G. R. Acuff, and J. S. Dickson. 2009. Evaluation of *Escherichia coli* Biotype I as a surrogate for *Escherichia coli* O157:H7 for cooking, fermentation, freezing, and refrigerated storage in meat processes. J. Food Prot. 72: 728-732.

King, D. A., L. M. Lucia, A. Castillo, G. R. Acuff, K. B. Harris, and J. W. Savell. 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces. Meat Sci. 69: 401-407.

Marquez-Gonzalez, M., K. B. Harris, and A. Castillo. 2010. Interventions for hazard control in foods during harvesting. In: V. K. Juneja and J. N. Sofos, editors, Pathogens and Toxins in Foods: Challenges and Interventions. ASM Press, Washington, DC. p. 379-395.

Marshall, K. M., S. E. Niebuhr, G. R. Acuff, L. M. Lucia, and J. S. Dickson. 2005. Identification of *Escherichia coli* O157:H7 meat processing indicators for fresh meat through comparison of the effects of selected antimicrobial interventions. J. Food Prot. 68: 2580-2586.

Niebuhr, S. E., A. Laury, G. R. Acuff, and J. S. Dickson. 2008. Evaluation of nonpathogenic surrogate bacteria as process validation indicators for *salmonella enterica* for selected antimicrobial treatments, cold storage, and fermentation in meat. J. Food Prot. 71: 714-718.

Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Accessed Feb. 6, 2013. http://dx.doi.org/10.3201/eid1903.111866.

USDHHS-FDA. 2011. Kinetics of microbial inactivation for alternative food processing technologies -- Overarching principles: kinetics and pathogens of concern for all technologies. Accessed Jan. 15, 2013. http://www.fda.gov/Food/ScienceResearch/ResearchAreas/SafePracticesforFoodProcesses/ucm100198.htm.

APPENDIX A

Table 1. Treatment parameters for all antimicrobial interventions.

Acid	Temp (°C)	conc. (ppm)	conc. (%)	pH	Water Source
Lactic	25		2.5	2.14	tap
Lactic	25		2.5	2	distilled
Lactic	25		5	1.91	tap
Lactic	25		5	1.83	distilled
Peroxyacetic	25	210		5.07	tap
Peroxyacetic	25	210		3.44	distilled
Peroxyacetic	25	150		5.64	tap
Peroxyacetic	25	150		3.78	distilled
Lactic	25		2	2.21	tap
Lactic	25		1	2.21	distilled
Lactic	53		2	2.18	tap
Lactic	53		1.3	2.23	distilled
ASC	25	500		2.39	tap
ASC	25	500		2.29	distilled
ASC	25	1200		2.44	tap
ASC	25	1200		2.36	distilled
Lactic	53		2.5	2.18	tap
Lactic	53		2.5	2.03	distilled
Lactic	53		5	1.93	tap
Lactic	53		5	1.85	distilled

Table 2. Mean and SEM for log CFU of inoculums.

	Log CFU	SEM
Inoculum	7.89	0.124

[a-b] Means lacking a common letter differ ($P < 0.05$).

Table 3. Least squares means and SEM for log CFU/cm^2 for hot vs. chilled strip loins after inoculation, before treatment.

	Log CFU	SEM
Hot	5.62[a]	0.158
Chilled	4.13[b]	0.158

[a-b] Means lacking a common letter differ ($P < 0.05$).

Table 4. Least squares means (SEM) for strip loin surface pH immediately prior to treatment on hot and chilled strip loins.

	Hot	Chilled
Surface pH	6.08[b]	6.56[a]
	(0.108)	(0.102)

[a-b] Means lacking a common letter differ ($P < 0.05$).

Table 5. Least squares means (SEM) for acid pH values for acid treatment x water source.

	Water Source	
Acid Treatment	Tap	Distilled
150 Peroxyacetic	5.64[a]	3.78[c]
	(0.005)	(0.005)
210 Peroxyacetic	5.07[b]	3.44[d]
	(0.005)	(0.005)
500 ASC	2.39[f]	2.29[h]
	(0.005)	(0.005)
1200 ASC	2.44[e]	2.36[g]
	(0.005)	(0.005)
2.5% Lactic[1]	2.16[i]	2.02[j]
	(0.003)	(0.003)
5.0% Lactic[1]	1.92[k]	1.84[l]
	(0.003)	(0.003)

[a-l] Means lacking a common letter differ ($P < 0.05$).

[1] Represents both 25°C and 53°C lactic acid treatments.

Table 6. Least squares means (SEM) for meat pH after treatment for acid treatment x water source.

	Water Source	
Acid Treatment	Tap	Distilled
150 Peroxyacetic	6.36[a]	5.78[a]
	(0.230)	(0.230)
210 Peroxyacetic	6.11[a]	5.02[b]
	(0.232)	(0.231)
500 ASC	3.60[cd]	3.80[c]
	(0.240)	(0.242)
1200 ASC	3.49[cde]	4.67[b]
	(0.239)	(0.230)
2.5% Lactic[1]	3.57[cd]	3.14[de]
	(0.170)	(0.163)
5.0% Lactic[1]	3.17[de]	2.96[e]
	(0.163)	(0.169)

[a-e] Means lacking a common letter differ ($P < 0.05$).

[1] Represents both 25°C and 53°C lactic acid treatments.

Table 7. Least squares means (SEM) for strip loin surface temperature (°C) for hot or chilled strip loins.

	Hot	Chilled
Pretreatment temperature	23.73[a]	7.38[b]
	(0.215)	(0.203)
Post-treatment temperature	23.12[a]	15.56[b]
	(0.240)	(0.227)

[a-b] Means within a row lacking a common letter differ ($P < 0.05$).

Table 8. Least squares means (SEM) for strip loin surface temperature (°C) post-treatment for hot vs. chilled strip loins x acid treatment x acid temperature.

Hot vs. Chilled strip loin	Acid Temperature	
2.5% vs. 5.0% Lactic	25°C	53°C
Chilled		
2.5%	18.95[b]	14.71[d]
	(0.431)	(0.440)
5.0%	18.56[b]	16.51[c]
	(0.437)	(0.446)
Hot		
2.5%	23.51[a]	24.67[a]
	(0.450)	(0.429)
5.0%	24.08[a]	24.34[a]
	(0.472)	(0.429)

[a-d] Means lacking a common letter differ ($P < 0.05$).

Table 9. Means for strip loin surface pH and temperature at various steps.

	pH	Temperature(°C)
All strip loins		
Upon arrival to lab	6.16	30.18
Post-inoculation, pre-treatment	6.21	24.02
Strip loins treated hot		
Post-treatment	3.80	23.82
Strip loins treated cold		
Post-chilling, pre-treatment	6.32	7.78
Post-treatment	3.97	16.10

Table 10. Least squares means (SEM) for log CFU/cm^2 reductions for hot vs. chilled strip loins x water source.

	Reduction	
Water Source	Hot	Chilled
Tap	0.68^{c}	2.02^{a}
	(0.262)	(0.247)
Distilled	1.20^{bc}	1.56^{ab}
	(0.257)	(0.255)

$^{a-c}$ Means lacking a common letter differ ($P < 0.05$).

Table 11. Least squares means (SEM) for log CFU/cm^2 reductions for hot vs. chilled strip loins x acid treatment.

	Reduction	
Acid Treatment	Hot	Chilled
500 ASC	0.05^{ef}	1.04^{cde}
	(0.511)	(0.451)
1200 ASC	0.53^{def}	2.30^{bc}
	(0.466)	(0.450)
150 Peroxyacetic	-0.39^{f}	1.34^{cde}
	(0.463)	(0.470)
210 Peroxyacetic	0.60^{def}	1.68^{bcd}
	(0.459)	(0.484)
2.5% Lactic	1.42^{cd}	1.81^{bc}
	(0.319)	(0.330)
5.0% Lactic	3.45^{a}	2.57^{ab}
	(0.321)	(0.339)

[a-f] Means lacking a common letter differ ($P < 0.05$).
[1] Represents both 25°C and 53°C lactic acid treatments.

Table 12. Least squares means (SEM) for log CFU/cm^2 reductions for hot vs. chilled strip loins x acid treatment x acid temperature.

Hot vs. Chilled strip loin	Acid Temperature	
2.5% vs. 5.0% Lactic	25°C	53°C
Chilled		
2.5%	1.59^{bc} (0.499)	1.50^{bc} (0.509)
5.0%	2.31^{b} (0.506)	2.29^{b} (0.516)
Hot		
2.5%	0.64^{c} (0.521)	1.81^{bc} (0.50)
5.0%	4.33^{a} (0.546)	2.22^{b} (0.496)

$^{a-c}$ Means lacking a common letter differ ($P < 0.05$).

Table 13. Least squares means (SEM) for strip loin surface pH post-treatment for lactic acids mixed to 2.2 pH vs. all other acids.

	2.2 pH Lactic	All other treatments
Strip loin surface pH	3.29[b]	4.03[a]
	(0.232)	(0.116)

[a-b] Means lacking a common letter differ ($P < 0.05$).