# **EVALUATION OF PEROXYACETIC ACID AS A POTENTIAL PRE-GRINDING TREATMENT FOR CONTROL OF ENTERIC PATHOGENS ON FRESH BEEF TRIM**

A Thesis

by

## JOHN WAYNE ELLEBRACHT

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

## MASTER OF SCIENCE

August 2004

Major Subject: Animal Science

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## **PRE-GRINDING TREATMENT FOR CONTROL OF ENTERIC PATHOGENS**

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Approved as to style and content by:

Jeff W. Savell Gary R. Acuff (Chair of Committee) (Member)

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#### **ABSTRACT**

Evaluation of Peroxyacetic Acid as a Potential Pre-Grinding Treatment for Control of Enteric Pathogens on Fresh Beef Trim. (August 2004) John Wayne Ellebracht, B.S., Texas A&M University Chair of Advisory Committee: Dr. Jeff W. Savell

Peroxyacetic acid was evaluated in four separate trials for ability to reduce populations of *Escherichia coli* O157:H7 and *Salmonella* serotype Typhimurium (ATCC 13311) on fresh beef trim. Trial 1 examined the effectiveness of peroxyacetic acid on individual pieces of fresh beef trim. Trial 2 was performed to evaluate the effectiveness of peroxyacetic acid at low levels of contamination on batches of trim. Trial 3 studied the washing effect of the dip due to water. Lastly, Trial 4 compared the effectiveness of peroxyacetic acid to lactic acid. At various inoculation levels, peroxyacetic acid reduced populations of rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium by approximately 1.0 log CFU/cm<sup>2</sup>. Much of the reductions recorded in Trials 1 and 2 may have been due to the washing effect of the dip. Trial 3 showed that approximately half of the reduction was due to the water dip. In addition, as shown in Trial 1, increases in concentrations (> 200 ppm) did not significantly increase log reductions of rifampicinresistant *E. coli* O157:H7 and *S.* Typhimurium. Following a water dip in Trial 4, peroxyacetic acid caused a reduction of 0.7 log CFU/cm2 in *E. coli* O157:H7 and 1.0 log  $CFU/cm<sup>2</sup>$  in *S*. Typhimurium, whereas lactic acid caused reduction of 1.3 log CFU/cm<sup>2</sup>

in *E. coli* O157: H7 and 2.1 log CFU/cm<sup>2</sup> in *S.* Typhimurium following the water dip. Peroxyacetic acid was not more effective than 2% L-lactic acid in reducing pathogens on fresh beef trim.

# **DEDICATION**

The author dedicates this thesis to Elizabeth Duffy and his parents for their love and support.

#### **ACKNOWLEDGEMENTS**

 The author takes this opportunity to thank everyone for their help and support. First, the author thanks Dr. Jeff W. Savell for his guidance, support, and leadership during my research. The opportunities and training under his supervision will benefit me for many years to come.

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#### **CHAPTER I**

#### **INTRODUCTION**

 Consumers have become increasingly aware of food safety issues because of numerous outbreaks caused by *E. coli* O157:H7 and *Salmonella* associated with ground beef. In response to these outbreaks, the USDA's (United States Department of Agriculture) Food Safety Inspection Service called for mandatory HACCP (Hazard Analysis Critical Control Point) plans in all meat facilities (USDA, 1996). HACCP systems are scientifically based systems that rely on controlling the process to prevent hazards, instead of testing for acceptability of the final product. HACCP based systems do this through the use of critical control points (CCP) that are defined as points at which hazards can be reduced, controlled or eliminated. The major problem with production of fresh beef is that there is no kill step, such as cooking, to reduce or eliminate pathogen hazards.

 Numerous methods have been implemented in order to control biological hazards, such as bacterial contamination, and ultimately produce cleaner beef cuts and trim. Steam pasteurization, hot water, steam vacuum, lactic acid, and peroxyacetic acid are all being used in the industry to help reduce the number of bacteria on beef carcass surfaces. Many studies have reported the effectiveness of both hot water and lactic acid on beef carcasses surfaces. Additionally, Ellebracht, Castillo, Lucia, Miller and Acuff (1999) studied the use of lactic acid on fresh beef trim to reduce pathogens. However,

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The style and format conform to the journal, *Meat Science*.

the beef industry is always searching for new methods of intervention to control microbial populations that will not negatively affect the quality and palatability of the final product.

Peroxyacetic acid has been studied for use as a surface sanitizer; however, little research exists regarding its use on fresh beef trim. Therefore, the purpose of these studies was to evaluate the effectiveness of peroxyacetic acid in reducing the level of enteric bacterial pathogens on fresh beef trim.

#### **CHAPTER II**

#### **LITERATURE REVIEW**

The control of bacteria on fresh beef and beef trimmings remains a high priority in the meat industry. In 1992-1993, an outbreak of *E. coli* O157:H7 was linked to undercooked hamburgers produced by a major fast food restaurant and caused bloody diarrhea and hemolytic uremic syndrome (Bell et al., 1994). Microbial contamination of meat occurs inevitably in the conversion of live animals to fresh meat products. The majority of the contamination originates from the dirt, dust, and fecal matter associated with the hide and occurs as the hide is removed (Ayres, 1955; Elder, Keen, Siragusa, Barkocy-Gallagher, Koohmaraie & Laegreid, 2000). The use of strict sanitation procedures during harvesting and fabrication has been shown to reduce aerobic plate counts and extend microbial shelf life of primals and cuts from beef carcasses (Dixon et al., 1991). Interventions, such as organic acid and hot water rinses, have been effective at reducing the microbial load on hot carcass surfaces prior to chilling (Hardin, Acuff, Lucia, Oman & Savell, 1995; Castillo, Lucia, Goodson, Savell & Acuff, 1998); however, as beef carcasses are processed into primals, subprimals, and trim, the freshly cut surfaces are exposed to bacteria.

Beef processors must control microbial loads on fresh meat during processing in order to control spoilage on meat products and extend shelf-life. At the same time, controlling the microbial load may also decrease the number of pathogens on fresh meat which may reduce the incidence of foodborne illnesses. Many studies have been

conducted to find a way to reduce or eliminate the number of microorganisms on fresh meat.

There are numerous methods currently being used to decontaminate fresh beef. One method, proven to be effective, is the use of organic acid rinses, such as lactic acid and acetic acid (Hardin et al., 1995; Castillo, Lucia, Mercado & Acuff, 2001a; Dorsa, Catherine & Siragusa, 1998). Extensive studies have been conducted to search for the most effective organic acid wash. Hamby, Savell, Acuff, Vanderzant, and Cross (1987) conducted a study using intermittent spraying of beef carcasses during chilling with water, 1% acetic acid or 1% lactic acid and a single spray treatment of 1% acetic acid or 1% lactic acid. Intermittent sprays were 30 s in duration per hour for a period of 12 h, while single sprays were 30 s in duration applied once to beef carcasses as they entered the chilling cooler. They found that mean aerobic plate counts (APCs) were significantly reduced by intermittent sprays of 1% acetic or 1% lactic acid. In another study, Castillo, Lucia, Roberson, Stevenson, Mercado, and Acuff (2001b) reported significant reductions of *E. coli* O157:H7 and *S.* Typhimurium when lactic acid was applied to beef carcasses after chilling. They reported that these reductions might be large enough to recommend lactic acid sprays as a post-chill intervention to be used prior to fabrication.

The use of organic acids has been used to decontaminate the surface of primal and subprimal cuts as well. Goddard, Mikel, Conner, and Jones (1996) studied the effect of spraying 10 ml of a mixture containing 2% lactic acid and 2% acetic acid on beef strip loins subsequently vacuum packaged in Cryovac BG bags and stored at -1°C for 112

days. They discovered that the use of organic acids significantly reduced the population of anaerobic and lactic acid-producing bacteria by 0.9 and 0.8 log CFU/g, respectively. Also, these data suggested that organic acids improved shelf life of beef strip loins stored at -1°C that were sprayed before packaging. In another study, Acuff, Vanderzant, Savell, Jones, Griffin, and Ehlers (1987) decontaminated beef strip loins with organic acid sprays containing 1.0% lactic acid, 1.0 % acetic acid or a mixture of 1.0% lactic acid, 2.0% acetic acid, 0.25% citric acid and 0.1% ascorbic acid. They found that steaks produced from acid treated loins did not differ in APCs from steaks produced from control loins that received no treatment. A study also was conducted by Brackett, Hoa, and Doyle (1994) that tested the inhibitory effect of acetic, citric, and lactic acid applied at temperatures of 20°C and 55°C on slices of fresh, raw beef sirloin tips (obtained from local butchers) inoculated with *E. coli* O157:H7. Of the three acids, lactic acid was shown to be the most effective; however, the reductions, which were statistically significant, were minimal  $(< 0.3 \log_{10} CFU/g)$ . They also observed minimal effect of acids on surface pH. In fact, at a temperature of 20°C, 1.5% concentrations of acetic, citric, and lactic acid lowered the surface pH of the meat from 5.41 to 5.18, 5.12, and 5.16, respectively. Fu, Sebranek, and Murano (1994) compared APCs of loins from pork carcasses that were sprayed with acetic, citric or lactic acid. They found that loins from carcasses treated with acetic and lactic acid had lower ( $P < 0.05$ ) APCs when compared to untreated control loins for up to 14 days of storage and all acid treated loins were significantly lower in APCs ( $P < 0.05$ ) at day 42. However, all treatments showed

minimal reductions of *E. coli* or fecal and total coliforms. This could be attributed to carcasses in this study not retaining organic acids due to processing into loins.

Organic acids also have been used to decontaminate the surfaces of retail cuts. Kotula and Thelappurate (1994) reported that ribeye steaks treated with lactic acid had significantly lower *E. coli* counts after treatment with lactic acid. Lactic acid caused a significant reduction of 0.4 log CFU/g for both total colony forming units and *E. coli*  counts when used at a temperature of 2°C and a concentration of 1.2% for 120 s. In addition, Kotula and Thelappurate (1994) showed that concentration and time of treatment were directly proportional to inhibition of microorganisms. Anderson (1990) studied the use of lactic acid on meat cores dipped in 1%, 2% or 3% solutions for 15 s at 25, 40, 55, or 70°C to test the effect of concentration and temperature on reducing the microbial population. Anderson (1990) showed that concentration of lactic acid was less significant at 70°C, but as temperature was lowered to 25°C, concentration of lactic acid became a more significant variable. Therefore, at low temperatures (25°C), one might expect a greater increase in microbial reduction from an increase in the concentration of lactic acid from 1% to 3%.

Applying organic acids to the surface of retail cuts can cause undesirable discoloration and sensory attributes. Many studies have been conducted to test the effects of organic acids on the appearance and palability of cuts. Bell, Marshall, and Anderson (1986) conducted a study of this type in which one-centimeter cubes of semimembranosus and adductor muscles were decontaminated by dipping for 1, 10, and 100 s in solutions of 0.6%, 1.2%, 1.8% or 2.4% acetic acid or a mixture of 0.6% acetic

acid and 0.046%, 0.092%, 0.184% or 0.230% formic acid. Only samples dipped for one minute in 0.6% acetic acid did not significantly differ from the untreated control in mean sensory color scores, and as the concentration of acetic acid increased, the color scores differed more. Another study conducted by Garcia Zepeda, Kastner, Kenney, Campbell, and Schwenke (1994) compared aroma profiles of beef chuck rolls sprayed with chlorine (200 ppm) and lactic acid (3%) for 2 min at a temperature of 20°C. Beef chuck rolls decontaminated with chlorine tended  $(P = 0.08)$  to have higher acceptability scores than those treated with lactic acid; however, chlorine decontamination did not cause significant reductions in microbial populations.

Organic acid treatments also have been used to decontaminate beef trimmings. Ellebracht et al. (1999) applied treatments of hot water and lactic acid to decontaminate fresh beef trim from both young and mature beef cattle. Levels of 4.3 and 4.3 log CFU/g of *E. coli* O157:H7 and 3.8 and 3.9 log CFU/g of *S.* Typhimurium were reported on trimmings produced from young cattle and old cattle, respectively. Therefore, trimming type did not show significant effects ( $P < 0.05$ ) on levels of both pathogens found on the trimmings. Also, they reported that treatment of hot water alone significantly reduced the level of *E. coli* O157:H7 by 0.5 log CFU/g and *S.* Typhimurium by 0.7 log CFU/g. Hot water followed by lactic acid produced an additional reduction of 1.1 and 1.8 log CFU/g for both *E. coli* O157:H7 and *S.* Typhimurium, respectively. In a similar study, Conner, Kotrola, Mikel, and Tamblyn (1997) reported that spraying beef trimmings with 1 ml combinations of both acetic and lactic acid at 55°C slightly reduced the inoculated level of *E. coli* O157:H7 present in fresh ground beef by 0.1 and 0.2 log CFU/g for 2%

and 4% mixtures, respectively; however, counts still remained high after the application of a combination of both acids. They suspected that it would be more difficult to achieve sufficient reductions of pathogens due to extensive handling and grinding associated with ground beef operations. In another study by Anderson, Marshall, Stringer, and Naumann (1979), plate beef was decontaminated with cold water (15.6°C), hot water (76<sup>o</sup>C), steam (95<sup>o</sup>C), sodium hypochlorite, or 3% acetic acid sprays. Water washes were applied at 14 kg/cm<sup>2</sup> pressure, 12.81/min volume, and 10 cm/s speed of meat travel beneath the spray. Sanitizers were applied at  $14 \text{ kg/cm}^2$  pressure, 6.81/min volume, and 2 cm/s speed of meat travel beneath the spray. It was shown that the treatment with 3% acetic acid was most effective and increased the microbial shelf life by 18 to 21 days under refrigeration at 3.3 °C.

Bacterial attachment to meat surfaces can increase resistance of bacteria to methods of decontamination of fresh meat surfaces. Selgras, Marin, Pin, and Casas (1993) reported that bacterial attachment to meat surfaces is affected by numerous factors, such as type of meat surface, pH, temperature, surface charge, and chemical residues. In addition, Lillard (1988) stated that bacteria became entrapped in crevices on tissue surfaces and might act as a barrier to antimicrobial effectiveness. In order to search for a solution to this complex problem, Stivarius, Pohlman, McElyea, and Waldroup (2002) studied the effect of different application methods of hot water and lactic acid (5%) on microbial inhibition. In this study, they applied hot water (82°C) or lactic acid (5%) to beef trim by tumbling either aerobically or anaerobically (559 mm/Hg vacuum) for 3 min at 16 rpm. They suspected that the vacuum might allow more

effective penetration of treatments; however, they reported that vacuum application of lactic acid or hot water did not significantly enhance  $(P > 0.05)$  effectiveness of either antimicrobial treatment in reducing numbers of *E. coli, S.* Typhimurium, coliforms, or APCs. In another attempt at a solution to this complexity, Kang, Koohmaraie, Dorsa, and Siragusa (2001) used multi-step processes consisting of various combinations of water, hot water, hot air or lactic acid to decontaminate beef trim. Treatment combinations consisted of the following: 1) water wash at 65 psi for five passes, 2) water and 2% lactic acid applied at room temperature for three passes at 30 psi, 3) water and hot water applied at 65<sup>o</sup>C for one pass at 30 psi followed by hot air at 510<sup>o</sup>C for four passes and lactic acid, 4) water and hot water applied at 82°C for one pass followed by hot air at 510°C for five passes and lactic acid, and 5) water and hot water applied at 82°C for three passes followed by hot air at 510°C for six passes and lactic acid. They reported greater microbial reductions from adipose surfaces than from lean surfaces. In addition, their data indicated that certain treatment combinations significantly reduced total coliform counts on fat-covered lean beef trim by approximately 4.0 logs  $CFU/cm<sup>2</sup>$ from an initial level of 6.5 log  $CFU/cm^2$ . In a similar study, Castelo, Kang, Siragusa, Koohmaraie, and Berry (2001) researched several combinations of treatments on the inhibition of microorganisms on pork trim. Their results were similar to the previously mentioned study on beef trim. For all treatments, it was found that microbial contamination was significantly lower on fat-covered pork trim than on the lean pork trim tissue. In addition, all microbial populations were lowered immediately after treatment with water plus lactic acid, combination 1 (water plus hot water [65.6°C, 15s]

and hot air [510°C, 60s] plus lactic acid), combination 2 (water plus hot water [82.2°C, 15s] and hot air [510°C, 75s] plus lactic acid), and combination 3 (water plus hot water  $[82.2^{\circ}C, 45s]$  and hot air  $[510^{\circ}C, 90s]$  plus lactic acid). Castelo et al. (2001) also reported that with any of the above mentioned combination treatments, color and emulsion stability was significantly  $(P < 0.05)$  affected.

Another organic acid that has been used to control microbial growth is peroxyacetic acid. Ransom, Belk, Sofos, Stopforth, Scanga, and Smith (2003) studied the effect of peroxyacetic acid, as well as other organic acid treatments, on fresh beef trim. In this study, 0.02% peroxyacetic acid applied for 30 s at 55°C caused a 1.4-log CFU/cm<sup>2</sup> and a 1.0-log CFU/g reduction in *E. coli* O157:H7 when applied by dipping to beef carcass tissue or lean tissue pieces, respectively. However, this study also showed that 2% lactic acid and 0.02% acidified sodium chlorite were the most effective organic acids approved for commercial use.

 Peroxyacetic acid also has been used as an antimicrobial agent on food contact surfaces (Farrell, Ronner & Wong, 1998) and on fruit (Wisniewsky, Glatz, Gleason & Reitmeier, 2000). Peroxyacetic acid (0.2 %) was shown to cause a 2.6-log reduction (from 3.3 log CFU/cm<sup>2</sup> to 0.7 log CFU/cm<sup>2</sup>) in *E. coli* O157:H7 when used as a sanitizer on a meat grinder auger surface for 2 min at ambient temperature (Farrell et al., 1998). In addition, peroxyacetic acid was shown to reduce the percentage of positive samples taken from stainless steel chips  $(1 \text{ cm}^2)$  that were glued to the auger housing portion of a meat grinder. Stopforth, Samelis, Sofos, Kendall, and Smith (2003) studied the effects of two sanitizers, peroxyacetic acid (150 ppm) and quaternary ammonium compound

(200 ppm), on the growth of *E. coli* O157:H7. Both sanitizers were applied to stainless steel coupons by submerging in 50 ml of each solution for 15, 30 or 60 s. They reported that *E. coli* O157:H7 cells were more sensitive to peroxyacetic acid sanitizer than a quaternary ammonium compound sanitizer, regardless of time of storage at 15°C. In another study, Bagge-Ravn, Gardshodn, Gram, and Vogel (2003) compared the effects of fog sanitization with 10% peroxyacetic acid to foam sanitization with 1,000 to 1,250 ppm sodium hypochlorite on the environment (APCs) and *L. monocytogenes.* Fog sanitization was applied until a dense fog cloud formed for 30 min and foam sanitization was allowed a contact time of 20 min. Fog sanitization with peroxyacetic acid caused a greater percentage of samples to contain < 10 CFU per sampling site; however, the prevalence of *L. monocytogenes* remained the same for both treatments. These findings support that peroxyacetic acid is an effective sanitizer against *E. coli* O157:H7 when used on fruit and food contact surfaces, and therefore may possibly be effective when used on fresh beef trim.

#### **CHAPTER III**

#### **MATERIALS AND METHODS**

#### **Bacterial cultures**

Rifampicin-resistant strains derived from *S.* Typhimurium ATCC 13311, and *E. coli* O157:H7 (from ground beef implicated in an outbreak in Washington in 1993; supplied courtesy of P.I. Tarr, Children's Hospital and Medical Center, Seattle, WA) were used to inoculate fresh beef trim pieces. Strains were stored on Protect™ Bacterial Preservers (Key Scientific Products, Round Rock, TX) at -80°C and were revived by placing into 9 ml sterile tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) and incubating at 37°C for 24 h. Rifampicin-resistance was confirmed by streaking cultures onto lactose-sulfite-phenol-red rifampicin (LSPR) plates (Castillo et al., 1998) and incubating for 24 h at 37°C. Rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium characteristic colonies (*E. coli* O157:H7 colonies appeared yellow, while *S.*  Typhimurium colonies had a black center surrounded by a pink halo) were inoculated into TSB and incubated for 24 h at 37°C. Cultures were maintained weekly by transferring to fresh TSB and incubating at 37°C for 24 h. To prepare the inoculum for the trials, 1 ml of the TSB culture was transferred to 50 ml of fresh TSB and incubated for 18 to 24 h. Previous work indicated that 18-to 24-h cultures would grow to a level of  $10^9$  CFU/ml.

## **Trial 1**

## *Purpose*

Previous check-off funded research performed with peroxyacetic acid by Savell, Harris, Castillo, Acuff, and King (2003) indicated peroxyacetic acid sprayed on carcass surfaces was minimally effective at reducing the levels of *E. coli* O157:H7, *S.* Typhimurium, *E. coli* or coliforms. Research was designed, therefore, to test peroxyacetic acid in a scenario that would likely promote bacterial reduction. Trial 1 evaluated the effect of single pieces of inoculated fresh beef trim dipped in peroxyacetic acid to allow for optimum surface contact. In addition, various concentrations were evaluated.

## *Inoculum preparation*

Inocula were prepared by adding 5 ml each of 18- to 24-h cultures of rifampicinresistant *E. coli* O157:H7 and *S.* Typhimurium to 3 1-L Erlenmeyer flasks containing 490 ml of 0.1% sterile peptone water (Difco). The total 500-ml mixtures were transferred to each of 3 sterile stomacher bags, and inoculum levels were confirmed to be 8.1 log CFU/ml for *E. coli* O157:H7 and 7.8 log CFU/ml for *S.* Typhimurium.

## *Application of inoculum and peroxyacetic acid*

Beef trimmings were obtained from the Texas A&M University Rosenthal Meat Science and Technology Center and separated into pieces  $(n = 12)$  approximately 100  $\text{cm}^2$  and 3 cm thick. Nine pieces of trim were placed individually in a sterile stomacher

bag containing 500 ml of inoculum and gently agitated for 30 s. The remaining 3 pieces served as non-inoculated controls, which received no treatment. Peroxyacetic acid (Inspexx 200, Ecolab Inc., St. Paul, MN) was prepared according to the manufacturer's directions at a constant temperature (43°C) to concentrations of 200, 500, and 1000 ppm. Inoculated pieces of trim were treated by submerging in different concentrations of peroxyacetic acid for 15 s. Dipping was chosen as a treatment procedure to allow more complete contact with the surface of the trimmings than spraying, providing optimal opportunity for peroxyacetic acid to effectively reduce pathogenic bacteria.

## *Sampling*

Excise samples were collected from pieces of trim before and after dipping in peroxyacetic acid. Excise samples were obtained by removing a 10-cm<sup>2</sup> x 2-mm surface area from pieces of trim using a sterile scalpel and forceps. Each sample was placed in a sterile stomacher bag containing 99 ml of sterile 0.1% peptone water (Difco) and pummeled in a Tekmar 400 Lab Blender Stomacher (Tekmar, Cincinnati, OH) for 1 min. Counts were determined by surface plating appropriate decimal dilutions on LSPR agar plates. The plates were incubated for 24 h at 37°C and rifampicin-resistant *E. coli*  O157:H7 and *S.* Typhimurium colonies were counted.

## **Trial 2**

## *Purpose*

The purpose of Trial 2 was to test the ability of peroxyacetic acid to reduce low

contamination levels of bacteria. Additionally, the fresh beef trim was ground after peroxyacetic acid treatment to determine efficacy of peroxyacetic acid in controlling pathogens in the final ground product.

#### *Inoculum preparation*

Inocula were prepared to achieve a lower concentration of cells, in relation to previous, on the fresh beef trim. *E. coli* O157:H7 culture (18-24 h) was diluted by transferring 1 ml into 9 ml of sterile 0.1% peptone water. Then, 0.8 ml of the previous dilution was transferred into a sterile Nalgene™ polypropylene sterilizing tray (VWR International, Suwanee, GA) containing 8 L of sterile 0.1% peptone water. *S.*  Typhimurium culture (18-24 h) was diluted by transferring 1 ml into 9 ml sterile 0.1% peptone water, and then transferring 1 ml of this dilution into 9 ml sterile 0.1% peptone water. Then, 0.8 ml of this diluted culture was transferred into a sterile Nalgene™ polypropylene sterilizing tray containing 8 L of sterile 0.1% peptone water. The inoculum was measured at a level of 3.5 log CFU/ml for *E. coli* O157:H7 and 3.3 log CFU/ml for *S.* Typhimurium, which resulted in a mean initial inoculum level on trim surfaces of 2.0 and 1.3 log  $CFU/cm^2$  for *E. coli* O157:H7 and *S.* Typhimurium, respectively.

## *Application of inoculum and peroxyacetic acid*

Beef trimmings were obtained from the Texas A&M University Rosenthal Meat Science and Technology Center and separated into batches  $(n = 4)$  weighing 2 kg each.

Each batch was placed in a sterile basket made from 19-gauge galvanized wire. The sterile basket then was placed in a Nalgene™ polypropylene sterilizing tray containing the inoculum and the basket was gently shaken back and forth for 30 s. Following inoculation, the basket was removed from the inoculum and placed in a sterile Nalgene™ polypropylene sterilizing tray for an additional 30 s dwell time to allow for attachment of both pathogens. The basket then was placed for 15 s in another sterile Nalgene™ polypropylene sterilizing tray containing 8 L of peroxyacetic acid (Inspexx 200) that was prepared according to manufacturer's directions (43°C) to 200 ppm. Following treatment, the batches were ground first through a 1.27-cm grinder plate and then subsequently through a 0.23-cm grinder plate using a sterile meat grinder (Hobart, Model 4612, Troy, Ohio, U.S.A.).

## *Sampling*

Excise samples (three  $10$ -cm<sup>2</sup> x 2-mm surface areas) were obtained from inoculated beef trim before and after treatment in peroxyacetic acid and ground samples (10 g) were taken after subsequent grinding. Excise samples were pummeled and plated as described in Trial 1. Ground samples were obtained by placing a 10-g representative sample in a sterile stomacher bag, adding 90 ml of sterile 0.1% peptone water, and pummeling in a Tekmar 400 Lab Blender Stomacher for 1 min. Counts were determined by surface plating appropriate serial dilutions on LSPR agar plates according to Castillo et al. (1998). The plates were incubated for 24 h at 37°C and rifampicin-resistant *E. coli*  O157:H7 and *S.* Typhimurium colonies (per previous description) were counted.

## **Trial 3**

## *Purpose*

The purpose of Trial 3 was to evaluate how much pathogen reduction might occur due to a washing effect of the water in the peroxyacetic acid solution and to compare that to the effect of peroxyacetic acid on the pathogen level on the fresh beef trim.

#### *Inoculum preparation*

Inoculum was prepared by adding 0.8 ml of both 18- to 24-h cultures of rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium to a sterile Nalgene™ polypropylene sterilizing tray containing 8 L of sterile 0.1% peptone water, as previously described. The concentration of the inoculum was 5.2 log CFU/ml for *E. coli* O157:H7 and 5.2 log CFU/ml for *S.* Typhimurium.

## *Application of inoculum and peroxyacetic acid*

Beef trimmings were obtained from the Texas A&M University Rosenthal Meat Science and Technology Center and separated into batches  $(n = 8)$  weighing 2 kg each. Each batch was placed in a sterile basket made from 19-gauge galvanized wire mesh. The sterile basket then was placed in a Nalgene™ polypropylene sterilizing tray containing the inoculum and the basket was gently shaken back and forth for 30 s. Following inoculation, the basket was removed from the inoculum and placed in a sterile Nalgene<sup>™</sup> polypropylene sterilizing tray for an additional 30 s dwell time to allow for

attachment of both pathogens. At this point, half of the batches were dipped for 15 s in a Nalgene™ polypropylene sterilizing tray containing 8 L sterile distilled water at 43°C and then removed and allowed a dwell time of 30 s before sampling to allow sufficient time for excess water to drain. All batches then were dipped for 15 s in another sterile Nalgene<sup>™</sup> polypropylene sterilizing tray containing 8 L of 200 ppm peroxyacetic acid (Inspexx 200) at 43°C. This was followed by a dwell time of 30 s to allow sufficient time for excess peroxyacetic acid to drain.

#### *Temperature and pH collection*

All temperatures were recorded with a Traceable® Total-Range Thermometer (VWR International) and all pH values were determined with a Thermo Orion portable pH meter equipped with a Gel Epoxy Flat Surface Combination pH Electrode (Thermo Orion, Beverly, MA). Meat surface temperature values were obtained by inserting the temperature recording probe 1 mm below the surface of the fresh beef trim. Meat surface pH was obtained by placing the pH probe directly on the surface of the fresh beef trim and recording the pH. Meat surface temperatures and pH were collected prior to inoculation and after dipping in peroxyacetic acid for all batches. In addition, the temperature and pH was recorded for the peroxyacetic acid solution before and after dipping. Both temperature and pH for the peroxyacetic acid solution were obtained by placing either the temperature recording probe and the pH probe directly into the peroxyacetic acid solution. The temperature of the distilled water also was recorded for the batches that were dipped in water.

### *Sampling*

Excise samples (three  $10$ -cm<sup>2</sup> x 2-mm surface areas) were obtained before and after dipping in peroxyacetic acid for the half of the batches that did not receive a water dip. The batches that did receive a water dip were sampled before and after dipping in water and then after subsequent dipping in peroxyacetic acid. The samples were processed and plates were counted following the same procedure described in Trial 1.

## **Trial 4**

### *Purpose*

The purpose of Trial 4 was to compare peroxyacetic acid to lactic acid, which has been previously reported to be effective at reducing the microbial load on meat surfaces. Additionally, this trial ensured that parameters of treatments were applied correctly by using lactic acid treatment as a positive control.

## *Inoculum preparation*

Inoculum was prepared by adding 0.8 ml of both 18- to 24-h cultures of rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium to a sterile Nalgene™ polypropylene sterilizing tray containing 8 L of sterile 0.1% peptone water, as previously described. The concentration of the inoculum was 5.3 log CFU/ml for *E. coli* O157:H7 and 5.3 log CFU/ml for *S.* Typhimurium.

#### *Application of inoculum and peroxyacetic acid*

Beef trimmings were obtained from the Texas A&M University Rosenthal Meat Science and Technology Center and separated into batches  $(n = 8)$  weighing 2 kg each. Each batch was placed in a sterile basket made from 19-gauge galvanized wire mesh. The sterile basket then was placed in a Nalgene™ polypropylene sterilizing tray containing the inoculum and the basket was gently shaken back and forth for 30 s. A dwell time of 30 s was allowed before sampling to allow for attachment of both pathogens. Each batch was placed in another Nalgene™ polypropylene sterilizing tray containing 8 L of distilled water at room temperature and dipped for 15 s. Another dwell time of 30 s was allowed before sampling to allow sufficient time for excess water to drain. Following the water dip, half of the batches were dipped for 15 s in another Nalgene™ polypropylene sterilizing tray containing 8 L of 200 ppm peroxyacetic acid (Inspexx 200) at 43°C. The remaining four batches were dipped for 15 s in another Nalgene<sup>™</sup> polypropylene sterilizing tray containing 8 L of 2% L-lactic acid (Purac Inc., Arlington Heights, IL) at 55°C. A dwell time of 30 s was allowed before final sampling of both treatments to allow sufficient time for excess peroxyacetic acid to drain.

#### *Temperature and pH collection*

All temperatures were recorded with a Traceable® Total-Range Thermometer and all pH values were determined with a Thermo Orion portable pH meter equipped with a Gel Epoxy Flat Surface Combination pH Electrode. Meat temperatures and surface pH were recorded before inoculation and after dipping in peroxyacetic acid or lactic acid. In addition, the temperature and pH were recorded for the peroxyacetic acid and lactic acid solutions prior to and after dipping. The temperature of the distilled water also was recorded for all batches.

## *Sampling*

Excise samples (three  $10$ -cm<sup>2</sup> x 2-mm surface areas) were obtained before and after dipping in water for all batches. In addition, excise samples were collected after treatment in either peroxyacetic acid or lactic acid. The samples were processed and plates were counted as described for Trials 1 and 3.

## **Statistical analysis**

Data from all trials were analyzed using SAS (SAS Institute, Cary, NC). Simple statistics for temperature and pH data were generated using the PROC MEANS procedure. Microbial reductions were tested for significance  $(P < 0.05)$  by analysis of variance using PROC GLM.

#### **CHAPTER IV**

#### **RESULTS AND DISCUSSION**

#### **Trial 1**

The least-squares means of  $log_{10}$  counts for rifampicin-resistant *E. coli* O157:H7 and *S*. Typhimurium on inoculated fresh beef trimmings before and after application of peroxyacetic acid via dipping are presented in Table 1. The initial inoculum level on the trim was a mean of 5.2 log CFU/cm<sup>2</sup> and 4.8 log CFU/cm<sup>2</sup> for *E. coli* O157:H7 and *S.* Typhimurium, respectively. These initial inoculum levels were sufficient to study the effectiveness of peroxyacetic acid when applied to fresh beef trim. Peroxyacetic acid dipping resulted in a reduction of 0.6 and 1.0 log CFU/cm2 reduction in *E. coli* O157:H7 and *S*. Typhimurium, respectively. In a similar study, Ransom et al. (2003) reported a reduction of 1.4 log  $CFU/cm<sup>2</sup>$  when beef carcass tissue surfaces were dipped in peroxyacetic acid. Savell et al. (2003) reported slightly lower reductions on fresh beef carcass surfaces that might possibly be attributed to the application method (dipping versus spraying).

The least-squares means for  $log_{10}$  counts and  $log_{10}$  reductions of *E. coli* O157:H7 and *S*. Typhimurium before and after application of peroxyacetic acid at each concentration via dipping are presented in Table 2. The 200-ppm peroxyacetic acid dip caused a significant reduction (*P* < 0.05) in numbers of inoculated *E. coli* O157:H7 and *S*. Typhimurium. Additionally, *S*. Typhimurium was reduced (*P* < 0.05) by the 500-ppm Table 1

Least-squares means ( $n = 9$ ) of counts (CFU/cm<sup>2</sup>) for rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium on inoculated fresh beef trim surfaces before and after application of peroxyacetic acid dip pooled across all concentrations



LS means within same column lacking common letters  $(a,b)$  differ  $(P < 0.05)$ .

Table 2

Least-squares means (n = 3) of  $log_{10}$  counts (CFU/cm<sup>2</sup>) and  $log_{10}$  reductions (CFU/cm<sup>2</sup>) of rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium on fresh beef trim surfaces treated with 200, 500 or 1000 ppm peroxyacetic acid

$\mu$ and $\mu$		E. coli O157:H7	S. Typhimurium		
Sample	Log <sub>10</sub> Log <sub>10</sub>		Log <sub>10</sub>	Log <sub>10</sub>	
	counts	reductions	counts	reductions	
$200$ ppm					
Pre-peroxyacetic acid	5.4a		5.1a		
Post-peroxyacetic acid	4.7 <sub>b</sub>	0.7	4.1 <sub>b</sub>	1.0	
<b>SEM</b>	0.12	0.22	0.13	0.29	
P > F	0.01		0.005		
500 ppm					
Pre-peroxyacetic acid	5.1		4.6a		
Post-peroxyacetic acid	4.4	0.7	3.6 <sub>b</sub>	1.0	
<b>SEM</b>	0.19	0.22	0.26	0.29	
P > F	0.06		0.05		
1000 ppm					
Pre-peroxyacetic acid	5.1		4.6a		
Post-peroxyacetic acid	4.6	0.5	3.8 <sub>b</sub>	0.8	
<b>SEM</b>	0.12	0.22	0.75	0.29	
P > F	0.06		0.001		

LS means within same column for each concentration lacking common letters  $(a,b)$ differ  $(P < 0.05)$ .

and 1000-ppm treatments. Peroxyacetic acid has been approved by the FDA for use as a food additive on meat carcasses, parts, trim, and organs at a maximum concentration of 220 ppm (CFR, 2003). In fact, no additional bacterial reduction was observed at peroxyacetic acid concentrations greater than 200 ppm. Savell et al. (2003) reported no additional reduction for concentrations of peroxyacetic acid up to 600 ppm when sprayed on chilled beef carcass surfaces for 15 s at application temperatures of 45 or 55°C.

Dipping trimmings in peroxyacetic acid reduced the number of inoculated *E. coli*  O157:H7 and *S.* Typhimurium on fresh beef trim, which is in agreement with the stated hypothesis. However, these reductions could be partially due to the washing action of the dip treatment rather than the lethality of peroxyacetic acid, which is later studied in trial 3. In addition, consistently lower reductions were found for *E. coli* O157:H7 as compared to *S.* Typhimurium.

## **Trial 2**

The least-squares means of  $log_{10}$  counts for rifampicin-resistant *E. coli* O157:H7 and *S*. Typhimurium on inoculated fresh beef trimmings before and after application of peroxyacetic acid and following subsequent grinding are presented in Table 3. The means of the initial inoculum level on the trim were 2.0 log  $CFU/cm<sup>2</sup>$  and 1.3 log CFU/cm<sup>2</sup> for *E. coli* O157:H7 and *S.* Typhimurium, respectively. These low inoculation levels were used to better reflect the use of peroxyacetic acid at low levels of contamination. Peroxyacetic acid dipping resulted in a reduction of 1.2 and 0.8 log

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Table 3

Least-squares means ( $n = 4$ ) of  $log_{10}$  counts<sup>a</sup> for rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium on fresh beef trim inoculated at low levels before and after application of peroxyacetic acid dip and following final grind



LS means within same column lacking common letters  $(x,y)$  differ  $(P < 0.05)$ .

Minimum detection level of counting method =  $0.5 \log_{10} CFU/cm^2$ .

CFU/cm2 reduction in *E. coli* O157:H7 and *S*. Typhimurium, respectively. These reductions were similar to Trial 1; however, Savell et al. (2003) reported slightly lower reductions of 0.7 log CFU/cm<sup>2</sup> for both *E. coli* O157:H7 and *S.* Typhimurium when 200 ppm peroxyacetic acid was sprayed on hot beef carcass surfaces for 15 s. Also, Ransom et al. (2003) reported similar reductions of 1.4 log  $CFU/cm<sup>2</sup>$  when beef carcass tissue surfaces were dipped in 0.02% peroxyacetic acid for 30 s. The means of the  $log_{10}$ counts were 1.1 log CFU/g and 0.6 log CFU/g for *E. coli* O157:H7 and *S.* Typhimurium, respectively, following grinding. Additionally, Table 4 shows that two batches of inoculated beef trim were reduced to undetectable limits  $(< 0.5)$  in log<sub>10</sub> counts of *S*. Typhimurium after treatment in peroxyacetic acid; however, the pathogen reappeared after subsequent grinding in one of the two samples. As reported in another study (Conner et al., 1997), processing steps, such as grinding, seem to compromise effectiveness of organic acids. Grinding and processing steps also act to expose internally sterile surfaces of meat to bacteria and thus, produce a higher degree of bacterial contamination.

## **Trial 3**

Table 5 shows the least-squares means of  $log_{10}$  counts for rifampicin-resistant  $E$ . *coli* O157:H7 and *S*. Typhimurium on inoculated fresh beef trim before and after application of peroxyacetic acid dip only and before and after application of a water dip followed by dipping in peroxyacetic acid. The initial inoculum level on the batches of trim dipped in peroxyacetic acid only was an average of 3.2 log  $CFU/cm<sup>2</sup>$  and 2.9 log

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The log<sub>10</sub> counts<sup>a</sup> for rifampicin-resistant *S*. Typhimurium on fresh beef trim inoculated before and after application of peroxyacetic acid dip and following final grind



Table 5

Least-squares means ( $n = 4$ ) of  $log_{10}$  counts for rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium on fresh beef trim before and after application of peroxyacetic acid dip only and before and after application of a water dip followed by dipping in peroxyacetic acid



LS means within same column for each treatment lacking common letters (a-c) differ  $(P < 0.05)$ .

CFU/cm<sup>2</sup> for *E. coli* O157:H7 and *S.* Typhimurium, respectively. The initial inoculum level on the batches of trim dipped in water followed by peroxyacetic acid was an average of 3.0 log CFU/cm<sup>2</sup> and 3.2 log CFU/cm<sup>2</sup> for *E. coli* O157:H7 and *S.* Typhimurium, respectively. Peroxyacetic acid, used without an initial water dip, caused a 1.5-log CFU/cm<sup>2</sup> and 1.1-log CFU/cm<sup>2</sup> reduction in *E. coli* O157:H7 and *S.* Typhimurium, respectively. These reductions could have been partially due to a washing effect of the water in the solution. Water was applied to the inoculated fresh beef trim to account for the washing effect of the application method. When water was applied followed by peroxyacetic acid, a reduction of 0.8 log  $CFU/cm<sup>2</sup>$  and 0.9 log CFU/cm<sup>2</sup> in *E. coli* O157:H7 and *S.* Typhimurium, respectively, was observed. Reductions caused by water were seen in other studies as well. Ellebracht et al. (1999) reported a 0.5 log CFU/g in *E. coli* O157:H7 and a 0.7 log CFU/g reduction *S.*  Typhimurium when beef trim was dipped in hot water (95°C) for 3 s. A reduction of 2.9 log cycles for *E. coli* O157:H7 was reported when warm water (35°C) was applied as a prechill intervention to beef carcass surfaces using a hand pump sprayer (Castillo, 2001b). Approximately half of the total reduction, 0.4 log CFU/cm<sup>2</sup> for *E. coli* O157:H7 and  $0.5 \log CFU/cm^2$  for *S*. Typhimurium, could be attributed to the water dip.

 The initial and final means for pH and temperature values of the meat, peroxyacetic acid solution, and water dip are shown in Table 6. The mean initial surface pH of the fresh beef trim dipped in peroxyacetic acid only and water followed by peroxyacetic acid was 5.4 and dropped to 4.9. The mean initial pH of the peroxyacetic acid solution was 3.5 for peroxyacetic acid treatment and 3.3 for water and peroxyacetic

#### Table 6

Initial and final means ( $n = 8$ ) of pH or temperature data of meat, peroxyacetic acid dip solution or water dip for treatments of peroxyacetic acid dip only and water and peroxyacetic acid dip

	Initial		Final				
	Value	<b>SD</b>	Value	<b>SD</b>			
Peroxyacetic acid dip							
Meat $pH^a$	5.4	0.10	4.9	0.36			
Meat temperature <sup>b</sup> ( $^{\circ}$ C)	2.7	0.30	13.6	0.70			
Peroxyacetic acid pH <sup>c</sup>	3.5	0.16	3.6	0.17			
Peroxyacetic acid temperature <sup>d</sup>	42.5	0.35	39.3	0.52			
$({}^{\circ}C)$							
Water and peroxyacetic acid							
Meat $pH^a$	5.4	0.16	4.9	0.24			
Meat temperature <sup>b</sup> ( $^{\circ}$ C)	2.5	0.35	17.9	1.20			
Peroxyacetic acid pH <sup>c</sup>	3.3	0.06	3.5	0.00			
Peroxyacetic acid temperature <sup>d</sup>	42.1	0.52	38.0	0.55			
$(^{\circ}C)$							
Water temperature <sup>d</sup> ( $^{\circ}$ C)	42.0	0.72					
<sup>a</sup> Values were obtained by placing pH probe directly on surface of fresh beef trim.							

<sup>b</sup>Values were obtained by inserting temperature recording probe 1 mm below the surface of the fresh beef trim.

c Values were obtained by placing pH probe directly into the peroxyacetic acid solution. d Values were obtained by placing temperature recording probe directly into either the peroxyacetic acid solution or water.

acid treatment, and the pH of peroxyacetic acid solution for both treatments was slightly higher after dipping. The mean surface temperature of the meat dipped in peroxyacetic acid only was raised from 2.7°C to 13.6°C, while the mean surface temperature of the meat dipped in water followed by peroxyacetic acid was 2.5°C and was raised to 17.9°C. The increase in meat surface temperature was greater for the second treatment mentioned, which would be expected since the beef trim received an addition dip in a warm (43°C) solution.

#### **Trial 4**

Concentrations of rifampicin-resistant *E. coli* O157:H7 and *S*. Typhimurium on inoculated fresh beef trim before and after application of a water dip followed by subsequent dipping in either peroxyacetic acid or lactic acid are shown in Table 7. The initial inoculum level on batches of trim dipped in water followed by peroxyacetic acid was 3.8 log CFU/cm<sup>2</sup> and 3.4 log CFU/cm<sup>2</sup> for *E. coli* O157:H7 and *S.* Typhimurium, respectively. The initial inoculum level on batches of trim dipped in water followed by lactic acid was 3.5 log  $CFU/cm^2$  and 3.7 log  $CFU/cm^2$  for *E. coli* O157:H7 and *S.* Typhimurium, respectively. Peroxyacetic acid caused similar reductions to Trial 1, 2, and 3 of 0.7 log CFU/cm<sup>2</sup> for *E. coli* O157:H7 and 1.0 log CFU/cm<sup>2</sup> for *S*. Typhimurium following the water dip, while lactic acid caused reduction of 1.3 log  $CFU/cm^2$  for  $E$ . *coli* O157:H7 and 2.1 log CFU/cm<sup>2</sup> for *S*. Typhimurium following the water dip. Therefore, peroxyacetic acid (200 ppm at 43°C) was not more effective than L-lactic

Table 7

Least-squares means  $(n = 4)$  of  $log_{10}$  counts for rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium on fresh beef trim before and after application of a water dip followed by subsequent dipping in either 200 ppm peroxyacetic or 2% lactic acid treatments



LS means within same column for each treatment lacking common letters (a,b) differ  $(P < 0.05)$ .

acid (2% at 55°C) when applied by similar methods for the same amount of time. Savell et al. (2003) reported that concentrations of peroxyacetic acid at 1000 ppm (5 times the recommended usage) reduced *E. coli* O157:H7 by 1.7 log CFU/cm<sup>2</sup> and *S*. Typhimurium 1.3 log CFU/cm<sup>2</sup> when sprayed on chilled beef surfaces; however, 4% lactic acid reduced these organisms by 2.7 and 3.4  $\log$  CFU/cm<sup>2</sup>, respectively.

The initial and final means of pH or temperature data of meat, organic acid dip solution, and water dip for both treatments are shown in Table 8. The mean initial meat surface pH was 5.8 and 5.9 for the peroxyacetic acid and lactic acid treatments, respectively. The mean meat surface pH dropped from 5.8 to 5.2 after dipping in peroxyacetic acid; however, the mean meat surface pH dropped from 5.9 to 4.1 after dipping in lactic acid. The drop in meat surface pH after peroxyacetic acid treatment was similar to Trial 3 (Table 6). Additionally, the drop in meat surface pH after lactic acid treatment was greater than the peroxyacetic acid treatment. Furthermore, the reduction of *E. coli* O157:H7 and *S.* Typhimurium (1.3 and 2.1 log CFU/cm<sup>2</sup>, respectively) caused by lactic acid treatment was greater than the reduction caused by peroxyacetic acid treament (0.7 log CFU/cm<sup>2</sup> for *E. coli* O157:H7 and 1.0 log CFU/cm<sup>2</sup> *S.* Typhimurium). Kang et al. (2001) reported a decrease in meat surface pH from 5.9 to 3.7 after lean beef trim tissue was sprayed with water and 2% lactic acid at 30 psi for three passes. Peroxyacetic acid solution pH rose from 3.6 to 3.8 after dipping, while pH of lactic acid solution rose from 2.8 to 3.0. Meat temperatures for both treatments increased after dipping.

#### Table 8

Initial and final means  $(n = 4)$  of pH or temperature data of meat, organic acid dip solution, and water dip for both peroxyacetic and lactic acid treatments

	Initial		Final				
	Value	<b>SD</b>	Value	<b>SD</b>			
Water & peroxyacetic acid dip							
Meat $pH^a$	5.8	0.04	5.2	0.10			
Meat temperature <sup>b</sup> ( $^{\circ}$ C)	3.9	0.24	16.4	2.26			
Peroxyacetic acid pH <sup>c</sup>	3.6	0.06	3.8	0.06			
Peroxyacetic acid temperature <sup>d</sup> $(°C)$	42.7	0.59	39.5	0.3			
Water temperature <sup>d</sup> ( $^{\circ}$ C)	23.5	0.21					
Water & lactic acid dip							
Meat $pH^a$	5.9	0.11	4.1	0.06			
Meat temperature <sup>b</sup> ( $^{\circ}$ C)	4.3	0.38	22.9	2.70			
Lactic acid $pHc$	2.8	0.09	3.0	0.07			
Lactic acid temperature <sup>d</sup> ( $^{\circ}$ C)	55.8	1.21	51.1	0.63			
Water temperature <sup>d</sup> ( $^{\circ}$ C)	23.4	0.15					
<sup>a</sup> Values were obtained by placing pH probe directly on surface of fresh beef trim.							

<sup>b</sup>Values were obtained by inserting temperature recording probe 1 mm below the surface of the fresh beef trim.

<sup>c</sup>Values were obtained by placing pH probe directly into the peroxyacetic acid solution. d Values were obtained by placing temperature recording probe directly into either the peroxyacetic acid solution, lactic acid solution or water.

#### **CHAPTER V**

#### **CONCLUSIONS**

The beef industry continues to search for new and effective methods of bacterial decontamination of fresh beef carcass surfaces and beef trim. A commercially available solution consisting mainly of peroxyacetic acid was evaluated for ability to reduce pathogens on fresh beef trim. Due to previous research by Savell et al. (2003) showing limited results for the use of peroxyacetic acid on beef carcasses, four trials were conducted to evaluate peroxyacetic acid on fresh beef trim under ideal conditions using the solution in a "best case" scenario. At various inoculation levels, peroxyacetic acid reduced populations of rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium by approximately 1.0 log  $CFU/cm^2$ . Most of the reductions in Trials 1 and 2 may have been due to the washing effect of the dip, as Trial 3 showed that approximately half of the reduction was due to a washing effect. In addition, Trial 1 showed that log reductions of rifampicin-resistant *E. coli* O157:H7 and *S.* Typhimurium did not differ across solution concentrations.

The effectiveness of peroxyacetic acid was further compared to lactic acid, which has been previously shown to be effective. Following a water dip, peroxyacetic acid caused a reduction of 0.7 log CFU/cm<sup>2</sup> in *E. coli* O157:H7 and 1.0 log CFU/cm<sup>2</sup> in *S*. Typhimurium, whereas lactic acid caused reduction of 1.3 log CFU/cm<sup>2</sup> in *E. coli* O157:H7 and 2.1 log  $CFU/cm<sup>2</sup>$  in *S*. Typhimurium following the water dip. Peroxyacetic acid was not more effective than 2% L-lactic acid in reducing pathogens under the experimental conditions applied in this study.

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