

**METHODS FOR CONTROLLING *ESCHERICHIA COLI* O157:H7 AND
SALMONELLA SURROGATES DURING THE PRODUCTION OF NON-
INTACT BEEF PRODUCTS**

A Thesis

by

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ABSTRACT

This study evaluated methods for controlling *Escherichia coli* O157:H7 and *Salmonella* non-pathogenic bacterial surrogates during the production of marinated non-intact beef products. Hot (~30°C) boneless, beef strip loins ($n = 54$, Institutional Meat Purchase Specification 180) were inoculated with one of two levels (approximately 5.8 and 1.9 log₁₀ CFU/cm², hereafter referred to as high- and low-inoculated, respectively) of non-pathogenic, rifampicin-resistant *E. coli* organisms used to simulate harvest floor contamination. The inoculated beef strip loins were chilled at 2°C for 24 h, and then vacuum packaged and aged for 7 to 24 days at 2°C. The beef strip loins were subjected to one of five treatments or control (no treatment). Spray treatments were: 2.5% L-lactic acid, 5.0% L-lactic acid, 1,050 ppm acidified sodium chlorite, 205 ppm peroxyacetic acid, and tap water. Lactic acid treatments were applied at ~53°C, whereas the other sprays were applied at room temperature (~25°C). Treated and control pieces were tumble marinated using a commercial marinade. Sample counts were collected throughout the experiment to track reductions in inoculated microorganisms as impacted by antimicrobial treatment and processing. For the high-inoculated strip loins, the 5.0% L-lactic acid treatment was most effective ($P < 0.05$) across treatments and control at reducing surrogate organisms on meat surfaces before marination, producing a 2.6 log₁₀ CFU/cm² reduction. The water treatment accounted for the least ($P < 0.05$) reductions across treatments and control of surrogate organisms on the meat surface before marination. Peroxyacetic acid produced the greatest reduction of surface surrogate

organisms in the finished, marinated product. The water treatment resulted in greater internalization of surrogate microorganisms when compared to the control.

Furthermore, certain less effective antimicrobial sprays such as water may facilitate internalization of surface bacteria, more so than non-treated subprimals. It is important that producers of non-intact beef products focus on using effective antimicrobial sprays that maximize reductions and minimize internalization of surface bacteria into the finished product.

DEDICATION

I dedicate this work to my family and friends. I am fortunate that I am blessed with such wonderful people in my life. Thank you for your love and support. Without your encouragement, accomplishing my goals would have proven to be much more difficult.

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NOMENCLATURE

ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention (Atlanta, GA)
HACCP	Hazard Analysis Critical Control Points
IMPS	Institutional Meat Purchase Specifications
PBS	Phosphate Buffered Saline
Rif ^R	Rifampicin Resistant (100 mg/l)
Rif-TSA	Rifampicin-Tryptic Soy Agar
STEC	Shiga Toxin-Producing <i>Escherichia coli</i>
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
USDA-FSIS	United States Department of Agriculture - Food Safety and Inspection Service (Washington, DC)

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CHAPTER I

INTRODUCTION

In late 1992 and early 1993, a deadly foodborne outbreak occurred on the nation's west coast involving ground beef contaminated with *Escherichia coli* O157:H7. The outbreak included hundreds of illnesses and the deaths of children. The severity of the outbreak caught the attention of the nation, and raised questions regarding the safety of the U.S. beef supply. In response, in 1994, the United States Department of Agriculture - Food Safety and Inspection Service (USDA-FSIS) declared *E. coli* O157:H7 an adulterant in ground beef under the Federal Meat Inspection Act (63). Since then, new mandatory measures have been set forth by the FSIS in an attempt to reduce the likelihood of producing unsafe foods. These measures include the development and implementation of a Hazard Analysis and Critical Control Point (HACCP) plan, implementation of sanitation standard operating procedures, and microbiological testing for *E. coli* and *Salmonella* to reduce incidence of foodborne illness. As part of the HACCP plan, meat processing facilities must identify hazards that are likely to occur and implement effective methods for controlling or eliminating the potential hazard. There is no "one size fits all" practice when it comes to pathogen interventions; however, many plants use organic acid sprays, food sanitizing chemical sprays, or a combination thereof to reduce microbial contamination during slaughter, before fabrication, and before further processing.

From 1996 to 2000, the U.S. meat and poultry industries spent approximately \$380 million annually and made another \$580 million in long-term investments to comply with HACCP requirements (58). Several guidance documents and processing regulations have been produced to minimize production of adulterated beef. These guidance documents include, “Guidance for Minimizing the Risk of *Escherichia coli* O157:H7 and *Salmonella* in Beef Slaughter Operations” (62), and “Guidance for Beef Grinders and Suppliers of Boneless Beef and Trim Products - Guide for Minimizing Impact Associated with Food Safety Hazards in Raw Ground Meat and Other FSIS Regulated Products” (61). However, despite the best efforts of the USDA-FSIS and meat producers, recalls (65, 67, 68) and foodborne illnesses (17, 18) tied to *E. coli* O157:H7 and other pathogens such as *Salmonella* have not become a thing of the past.

In 1999, the USDA-FSIS clarified its policy regarding raw beef products contaminated with *E. coli* O157:H7 in a notice published in the Federal Register (60). The agency pointed out that the public health risk from *E. coli* O157:H7 contamination and transmission was not limited to ground beef, but included non-intact beef products, such as all comminuted, mechanically tenderized, marinated, enhanced, and reconstructed beef products. Therefore, on January 19, 1999, USDA-FSIS declared *E. coli* O157:H7 an adulterant in non-intact beef products (60). This adulterant-status expansion was made based on evidence that identified the opportunity for possible introduction or translocation of surface pathogens, such as *E. coli* O157:H7, into the deep, internal tissues of non-intact beef, such as tenderized and marinated beef products.

Harmful bacteria, if present, should only be on the surface of intact beef products. Surface temperatures achieved during cooking, even when cooked to a low degree of doneness, are sufficient to kill these surface bacteria and make the product safe to eat. In contrast, if pathogens are in the interior portions of non-intact beef products, the internal temperature achieved during cooking will determine whether or not the product is safe to eat. Therefore, it has been recommended that non-intact beef products should never be consumed at lower degrees of doneness than rare (internal temperature 60°C) (57). At this time, USDA-FSIS has considered labeling requirements for non-intact beef products (64). These labeling requirements might include a non-intact statement and recommended final internal cooking temperature. Given the low infectious dose of *E. coli* O157:H7 associated with foodborne disease outbreaks and the very severe consequences of an *E. coli* O157:H7 infection, the USDA-FSIS believes that, under the Federal Meat Inspection Act of 1906, the safety of beef products contaminated with *E. coli* O157:H7 must depend on whether there is adequate assurance that subsequent handling of the product will result in food that is not contaminated when consumed (59).

Numerous studies have reported the efficacy of antimicrobial treatments applied to hot and cold beef to control enteric pathogens (12, 14, 15, 16, 27, 29, 33, 38). However, the meat industry and the USDA-FSIS are always seeking new information for the latest and currently used antimicrobial treatments and to evaluate their effectiveness for various production processes. Internalization through use of contaminated marinade (44), blade tenderization of contaminated beef (42), and translocation and control of

pathogens on vacuum-packaged beef destined for non-intact production has been studied (40). The purpose of this study was to evaluate five different spray treatments in comparison to a control that could be applied to contaminated beef subprimals destined for marination to control surface-inoculated *E. coli* O157:H7 and *Salmonella* surrogate organisms counts and to minimize subsequent internalization during the marination process.

CHAPTER II

LITERATURE REVIEW

Escherichia coli

Escherichia coli is a rod-shaped Gram-negative bacterium belonging to the family *Enterobacteriaceae*. *Escherichia coli* is a facultative anaerobe, can ferment lactose, and is motile by peritrichous flagella. The optimal growth temperature for *E. coli* is 37°C; however, *E. coli* can still grow from 7°C up to 50°C. This bacterium is considered to be enteric or part of the naturally occurring microflora inside the intestines of most warm-blooded animals (37). *Escherichia coli* serogroups are distinguished by three antigens, the “O” or somatic antigen, the “K” or capsular antigen, and the “H” or flagellar antigen (37). There are over 200 O-serotypes and approximately 30 H serotype variations of *E. coli* (37). As scientific methods improve, more serotypes are discovered. Some serogroups of *E. coli* are pathogenic to humans whereas others are nonpathogenic. *Escherichia coli* was first discovered in 1885 by Theodor Escherich, though based on DNA sequencing, *E. coli* has been determined to be closely related to, and possibly diverged from, the *Salmonella* lineage approximately 100 million years ago (36).

***Escherichia coli* Virulence Groups**

Serotypes of *E. coli* are grouped into six different virulence groups. These groups are recognized as enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and diffusely adherent (DAEC) *Escherichia coli* (28). Of the six virulence groups, EHEC, EIEC,

EPEC, and ETEC have been implicated in illnesses connected to meat products (48, 72). However, only certain EHEC have been identified as adulterants in non-intact beef products. Children, the elderly, and the immunocompromised are more susceptible to illness caused by food or waterborne pathogens (20).

***Escherichia coli* O157:H7 Pathogenicity**

The exact infectious dose for *E. coli* O157:H7 is not known; however, it has been estimated to be as low as ten organisms (72). Once ingested, attachment, colonization and effacement of the intestine is dependent on the encoding of the *eaeA* gene that encodes for specialized proteins responsible for attachment and effacement (37). *E. coli* strains identified as Shiga toxin-producing can produce two toxins that are very similar to Shiga toxin produced by *Shigella dysenteriae*. These toxins were commonly referred to as Shiga-like toxins 1 and 2; however, today they are called Shiga toxins 1 and 2 or abbreviated as Stx₁ and Stx₂. Shiga toxins 1 and 2 consist of a single enzymatically active A subunit and five B subunits. Once the Shiga toxin enters the cell, the A subunit binds to and releases an adenine residue from the 28S ribosomal RNA of the 60S ribosomal subunit, which inhibits protein synthesis (37). Shiga toxins attach to host cells surrounded with the toxin receptor, globotriaosylceramide (Gb₃). Therefore, cells containing large amounts of Gb₃ become targets for and are more susceptible to Shiga toxins. Human renal tubule tissue and central nervous tissue contain abundant amounts of Gb₃, which explains why patients suffering from an EHEC illness may develop hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (37, 47). Shiga toxins also cause hemorrhagic colitis resulting in bloody diarrhea, severe abdominal

cramps, nausea, vomiting, and fever (72). Enterohemorrhagic *E. coli* illnesses are very severe and potentially fatal if not treated in a timely manner. Based on data from 2000 to 2008, the CDC estimated that there were around 175,905 foodborne illnesses caused by Shiga toxin-producing *E. coli* in the United States each year (56). Roughly 36% of the annual estimated STEC foodborne illnesses were caused by *E. coli* O157:H7 (18, 56). Therefore, data show that non-O157:H7 STEC may cause almost twice as many STEC-mediated foodborne illnesses as *E. coli* O157:H7 (56). Other CDC estimates rank *E. coli* O157:H7 fifth for number of hospitalizations (2,138) resulting from foodborne illness (19, 56). These same data estimated that 20 deaths per year are caused by *E. coli* O157:H7 foodborne illness, whereas no deaths have been attributed to non-O157:H7 STEC (56). Although no deaths are reported by the CDC for non-O157:H7, this does not mean that they are less virulent. Non-O157:H7 STEC are still capable of causing the same detrimental effects in their hosts and therefore have the same potential to cause illness and even death.

Presence of Non-O157:H7 STEC in Beef Processing

Brooks et al. (9) conducted a survey of non-O157:H7 Shiga toxin-producing *E. coli* infections in the United States from 1983 to 2002. The six most prevalent non-O157:H7 serotypes responsible for foodborne illness were O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%), and O145 (5%) (9). As a result, in 2011, the FSIS declared these six non-O157:H7 STEC adulterants in non-intact beef products (66). However, a study conducted by Bosilevac and Koochmarai (8) has shown that these six non-O157:H7 STECs are not the most prevalent Shiga toxin-producing *E. coli* serotypes

in the U.S. meat supply. Bosilevac and Koohmaraie (8) evaluated 4,133 ground beef samples from different U.S. ground beef suppliers. Presence of Shiga toxin was found in 1,006 (24.3%) of the samples tested, although other studies have observed a lower prevalence of Shiga toxin in beef products (5, 50, 52). A total of 99 STEC serotypes were isolated from 7.3% of the 4,133 samples from this study (8). Of the 99 isolated STEC, only four of the FSIS six non-O157:H7 STEC adulterants (O26, O103, O121, and O145) were present in ground beef samples and at low prevalence compared to other non-O157 STEC (8). The most prevalent STEC isolates found were O113:H21 (9.5%), followed by O8:H19 and O117:H7 (4.4% and 4.7%, respectively) (8). Research (8, 9, 37) stated that an increased risk of HUS in the host is associated with STEC that produced stx₂, rather than just stx₁. However, Bosilevac and Koohmaraie (8) stated that only 3.0% of the STEC isolates recovered in their study could be classified as having the potential to cause severe disease based on their molecular risk assessment profile.

Barlow et al. (5) conducted a study in which 285 ground beef samples were collected over a 52 week period from 31 different outlets. Each ground beef sample was assayed for presence and identification of STEC. Eighteen different O serotypes of Shiga toxin-producing *E. coli* were identified in 16% of the ground beef samples. However, 20 STEC isolates from the Barlow et al. (5) study were untypable. Furthermore, no STEC O157, O26, O111, O103, O121, O45, O145 isolates were identified in the Barlow et al. (5) study.

Salmonella

Salmonella species are motile Gram-negative rods. There are around 2,500 serovars of *Salmonella* (75). *Salmonella* is grouped into just two species, *S. enterica* and *S. bongori*, and their subspecies (37). *Salmonella* reservoirs are primarily the intestines of warm-blooded animals and humans. Optimal pH for growth of *Salmonella* spp. is around neutrality or between 6.6 to 8.2, while a pH below 4.0 or above 9.0 will result in the death of *Salmonella* organisms (37). Most *Salmonella* grow optimally at a temperature of 37°C, whereas no growth has been reported below 5.3°C or above around 45°C (37). Cattle presented for slaughter are known reservoirs for *Salmonella*. One U.S. study (23) detected 280 different *Salmonella* isolates in the feces of feedlot beef cattle. Another study (4) found *Salmonella* species present in 4.4%, 71.0%, and 12.7%, of feces, hides, and pre-eviscerated beef carcass samples, respectively. Furthermore, seasonal and locational differences associated with *Salmonella* prevalence have been observed (4, 54). Nonetheless, the potential for *Salmonella* contamination during slaughter is present. If harvest floor interventions are unsuccessful at eliminating the pathogen, it is likely that they could internalize during non-intact processing of subsequent beef cuts.

***Salmonella* Pathogenicity**

Symptoms of foodborne illness caused by *Salmonella*, known as salmonellosis, are usually seen 12-14 hours after ingestion of the bacteria and can last for two to three days. Salmonellosis is characterized by nausea, vomiting, abdominal pains, headache, chills, and diarrhea, as well as prostration, muscular weakness, faintness, moderate fever,

restlessness, and drowsiness (37). The normal infectious dose of *Salmonella* is estimated around 10^6 - 10^8 CFU; however, it is thought that the infectious dose can be as low as a few cells depending on the host's immune system, the virulence of the organism, and the food that is ingested (55). Illness is caused when ingested organisms survive the acidic environment of the stomach, and subsequently attach to and colonize the intestines. After colonization of the distal ileum, ruffling of the membrane occurs and eventually leads to endocytosis of the bacterial cells (11). After endocytosis, an enterotoxin is released into the lamina propria of the intestine. The enterotoxin stimulates the production of cAMP in host cells, which leads to electrolyte imbalance, fluid accumulation, and membrane inflammation. CDC estimates that *Salmonella* spp. are responsible for over 1 million incident foodborne illnesses in the U.S. each year (19). Estimates also indicate that annual incident hospitalization and deaths due to foodborne illness caused by *Salmonella* spp. are around 20,000 and 378, respectively (19).

Non-Intact Beef

Non-intact beef products include beef that has been injected/enhanced with solutions, mechanically tenderized by needling, cubing, or pounding devices, or reconstructed into formed entrées (e.g., beef that has been scored to incorporate a marinade, beef that has a solution of proteolytic enzymes applied to or injected into the cut of meat, or a formed and shaped product such as beef gyros). In addition, non-intact beef products include comminuted products that are chopped, ground, flaked, or minced (e.g., fresh veal sausage and fabricated beef steak) (59). "Whole-muscle, intact beef" means whole muscle beef that is not injected, mechanically tenderized, reconstructed, or

scored and marinated, from which beef steaks may be cut (73). Intact beef cuts of muscle include such cuts as steaks, roasts, briskets, and stew beef. In these intact cuts, the interior remains protected from pathogens that may exist on the exterior, so it is highly unlikely that pathogens would be found below the surface (60). A study done by Heller et al. (35) found that the prevalence of *E. coli* O157:H7 on 1,014 beef subprimals sampled from six U.S. beef processors was 0.2% (2 positives out of 1,014 samples); positive samples were contaminated with <0.375 CFU/cm², as determined by most probable number method. All of the cuts sampled in the study were intended for blade tenderization or moisture enhancement. Although the presence of *E. coli* O157:H7 on beef subprimals is rare, the threat to consumers is still existent. The process by which non-intact beef products are made may allow internalization of surface pathogens through processes including needling, injecting, marinating, and grinding. Evidence of bacterial internalization in non-intact beef products has been reported in numerous studies (32, 35, 42, 53), and has even been reported by Heller et al. (35) to be greater in moisture enhanced products than blade tenderized products.

Interventions

Research has been conducted for many intervention methods applied to meat to reduce pathogens. Most of this research has focused on applications intended for use on the slaughter floor or on chilled subprimals and trimmings. These same interventions may reduce surface pathogens and pathogen internalization during non-intact beef production; however, their efficacy needs to be evaluated. Possible interventions include

trimming of meat surfaces or use of antimicrobial sprays, dips, and even the addition of an antimicrobial to the enhancement solution.

Lactic Acid Solutions

Lactic acid gained Generally Recognized As Safe (GRAS) status from the U.S. Food and Drug Administration (FDA) in 1978 (70). Lactic acid may be applied to beef subprimals at a concentration of 2.0% to 5.0% and shall not exceed a temperature of 55°C, according to FSIS Directive 7120.1 (69). The use of lactic acid as an antimicrobial on beef has been studied extensively. Lactic acid treatments have been shown to be more effective at reducing bacterial contamination on adipose tissues compared to lean tissue (22, 33). After application of organic acid, the adipose tissue surface pH remains low enough for an extended period of time, thereby creating a prolonged environment that is unfavorable for many microorganisms (25). Similar pH durations and effects on microorganism survival were reported in the Hardin et al. (33) and Dickson (25) studies. However, Cutter and Siragusa (22) reported that the surface pH of acid treated lean tissue did not differ from untreated pieces 24 h after acid application. Dickson (25) theorized that the pH differences between the lean and adipose tissues were likely due to acid dilution caused by differences in lean and adipose tissue water content, 75% and 20%, respectively, or even different buffering mechanisms.

Hardin et al. (33) found that water washing followed by 2.0% organic acid solution (55°C) significantly reduced *E. coli* O157:H7 and *Salmonella* Typhimurium on

beef. Furthermore, reduction of *E. coli* O157:H7 was greatest for those receiving the lactic acid spray compared to those that received the acetic acid spray (33). Harris et al. (34) found no significant differences among organic acid types or concentration (lactic or acetic; 2.0% or 4.0%) and acidified sodium chlorite sprays in their ability to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium on beef trim. However, ground beef samples from the acidified sodium chlorite treated trimmings generally had a greater amount of *E. coli* O157:H7 present. Yoder et al. (76) stated that organic acids including lactic acid are generally more effective as concentration increases from 1.0 to 5.0%. Lactic acid and acetic acid sprays did not differ statistically in their ability to reduce *Salmonella* Typhimurium in the Hardin et al. (33) study. However, a study conducted by Anderson et al. (1), showed that 3.0% lactic acid was more effective than acetic acid at reducing *Salmonella* Typhimurium on inoculated beef muscle cores. In fact, the 3.0% lactic acid treatment was the most effective acid treatment for reducing *Salmonella* Typhimurium at all application temperatures (20°C, 45°C, and 70°C) (1). Furthermore, reduction of *Salmonella* Typhimurium increased as lactic acid application temperature increased. A 2.0 log reduction of *Salmonella* Typhimurium was achieved using the 3.0% lactic acid (70°C) dip, while reductions between 1.0 and 1.5 log were still achieved with the 20°C and 45°C lactic acid applications (1). Castillo et al. (16) discovered that application of a 2.0% lactic acid solution (55°C) following a water wash to inoculated pre-chilled beef rounds reduced *E. coli* O157:H7 and *Salmonella* Typhimurium by 5.2 log cycles each. Application of a 4.0% lactic acid solution (55°C) to chilled, inoculated beef rounds reduced *E. coli* O157:H7 and *Salmonella* Typhimurium an additional 2.0 and 1.6 log

cycles, respectively. Cutter and Siragusa (22) reported a 2.6 log reduction in *E. coli* O157:H7 on beef carcass tissues following a 5.0% lactic acid (24°C) spray treatment. Finally, Heller et al. (35) observed a 1.1 log reduction of *E. coli* O157:H7 on inoculated outside rounds destined for moisture enhancement or blade tenderization after application of a 5.0% lactic acid (55°C). Because lactic acid has been shown to effectively reduce *E. coli* and *Salmonella* across many different applications and parameters, it has become a very common intervention implemented by numerous beef processors.

Acidified Sodium Chlorite Solutions

Acidified sodium chlorite solutions used as antimicrobial sprays are regulated under Title 21 of the Code of Federal Regulations, Part 173.325 (71). In accordance to regulations, an acidified solution having a pH of 2.3 to 2.9 consisting of 500 to 1,200 ppm sodium chlorite may be applied to red meat carcasses and parts by either spray or dip (69, 71). The sodium chlorite solution must be acidified using a GRAS acid.

Castillo et al. (14) tested the efficacy of two acidified sodium chlorite solutions on their ability to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces following a water wash. Both treatments were 1,200 ppm sodium chlorite solutions; however, the acidulant used for one was phosphoric acid, whereas the other solution was acidified by the addition of citric acid. The mean pH for the phosphoric-acid activated sodium chlorite (PASC) solution and the citric-acid activated sodium chlorite (CASC) before application was 2.62. The citric-acid activated sodium chlorite solution was more effective at reducing both pathogens in the study than the phosphoric-

acid activated sodium chlorite solution, or water washing alone. Reduction of *E. coli* O157:H7 by treatment was 2.3, 3.8, and 4.5 log cycles for water washing, water washing followed by PASC, and water washing followed by CASC, respectively. Reduction of *Salmonella* Typhimurium by treatment was 2.3, 3.9, and 4.6 log cycles for water washing, water washing followed by PASC, and water washing followed by CASC, respectively. Spreading of *E. coli* O157:H7 and *Salmonella* Typhimurium to uninoculated areas was observed in samples from each treatment and control. Castillo et al. (14) believed that most of the spreading was caused by the high pressure water washing that each piece received before treatment. Although spreading of the inoculated pathogens occurred regardless of treatment or control, counts for both pathogens were typically very low and often below limits of detection for samples that had received either acidified sodium chlorite treatment (14).

A study conducted by Lim and Mustapha (41) compared three chemical treatments and their efficacy towards reducing *E. coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus* on inoculated beef cubes. The chemical treatments included sprays of 0.5% cetylpyridinium chloride (CPC), 0.12% acidified sodium chlorite (ASC), 0.1% potassium sorbate (PS), or an equal mixture of any two solutions. The acidified sodium chlorite solution was responsible for a 4.62 log reduction in *E. coli* O157:H7 throughout storage. Log reductions, greatest to least, of *E. coli* O157:H7 throughout storage can be ranked by treatment in the following order: ASC (4.62), CPC and ASC (4.0), ASC and PS (3.67), CPC (2.78), CPC and PS (2.09), and PS (0.64). ASC reduced *Listeria monocytogenes* and *Staphylococcus aureus* by 1.81 and 5.09 log cycles, respectively.

CPC was more effective than ASC at reducing *Listeria monocytogenes*, although ASC's ability to reduce *Staphylococcus aureus* did not differ from all other chemical treatments.

Harris et al. (34) reported that ASC reduced *E. coli* O157:H7 and *Salmonella* Typhimurium on beef trimmings approximately 1.5 logs, and did not differ from trimmings treated with lactic or acetic acid at concentrations of 2.0 or 4.0%. However, *E. coli* O157:H7 and *Salmonella* Typhimurium counts were higher in fresh ground beef made from ASC treated trimmings compared to organic acid treated trimmings. Despite greater pathogen counts immediately after grinding for ASC, counts taken from ground beef after 1, 5, and 30 days of refrigerated or frozen storage did not differ by treatment. Furthermore, Harris et al. (34) found that all treatments in the study including ASC reduced *E. coli* O157:H7 and *Salmonella* Typhimurium to undetectable levels when applied to the low-level (1.0 log CFU/g) inoculated trimmings. Based on these studies, acidified sodium chlorite has been shown to be an effective antimicrobial against multiple pathogens on beef.

Peroxyacetic Acid Solutions

Certain peroxyacetic acid solutions permitted for use as antimicrobials on red meat carcasses and parts are listed in FSIS's Directive 7120.1 (69). The peroxyacetic acid solution used in this study contained peroxyacetic acid, hydrogen peroxide, acetic acid, 1-hydroxyethylidene-1, 1-diphosphonic acid (HEDP), and is permitted at levels not to exceed 220 ppm peroxyacetic acid, 85ppm hydrogen peroxide, 11 ppm HEDP (69).

This peroxyacetic acid solution was approved in 2009 with the release of FDA's Food Contact Substance Notification (FCN) No. 887 (74).

Yoder et al. (76) conducted a study that tested the efficacies of eight chemical sprays to reduce pathogens on meat surfaces. The eight chemicals used in the study were citric acid, lactic acid, acetic acid, peroxyacetic acid, acidified sodium chlorite, chlorine dioxide, sodium hypochlorite, and ozone-enriched water. Peroxyacetic acid was applied at two concentrations, 200 and 1,000 ppm. The 1,000 ppm peroxyacetic acid treatment was responsible for 3.78 and 4.76 log CFU/cm² reductions of *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively. However, according to current regulations, a 1,000 ppm peroxyacetic acid solution exceeds the maximum concentration FSIS allows for application to meat carcasses, parts, or trim (69). However, Yoder et al. (76) found that a 200 ppm peroxyacetic acid solution, which meets FSIS regulations, reduced *E. coli* O157:H7 and *Salmonella* Typhimurium 0.44 and 1.03 log CFU/cm² respectively. Although reductions were accomplished, these reductions did not differ from those achieved using tap water alone. All other treatments were more effective than the 200 ppm peroxyacetic acid solution at reducing *E. coli* O157:H7 and *Salmonella* Typhimurium.

Penney et al. (49) studied the efficacy of peroxyacetic acid and water washing to control *E. coli* O157:H7 on beef and bob veal carcasses. Treatments evaluated in the study were: 180 ppm peroxyacetic acid, water wash, and water wash followed by 180 ppm peroxyacetic acid wash. The treatments were tested on hot-boned bob veal and beef flaps inoculated with *E. coli* O157:H7 at levels of approximately 6.0 and 3.0 log

CFU/cm². Water washing followed by peroxyacetic acid spray resulted in reductions of 2.73 and 3.21 log CFU/cm² of *E. coli* O157:H7 on veal and beef, respectively. The peroxyacetic acid treatment alone was most effective at reducing *E. coli* O157:H7 on veal and beef with reductions of 3.56 and 3.59, respectively. Water wash alone was responsible for approximately 1.5 log reduction of *E. coli* O157:H7. Application of the peroxyacetic acid to hot rather than chilled beef may have enhanced the antimicrobial effectiveness of peroxyacetic acid as reported in a study by King et al. (38).

King et al. (38) evaluated the effects of peroxyacetic acid and its ability to reduce *E. coli* O157:H7 and *Salmonella* Typhimurium on beef carcass cuts. Multiple variables including peroxyacetic acid treatment before or after chilling, following a water wash, and at different concentrations and application temperatures were evaluated.

Application of 200 ppm peroxyacetic acid to chilled beef resulted in no reduction of *E. coli* O157:H7 or *Salmonella* Typhimurium. Spreading of pathogens was noticed and was attributed to water washing before chilling. The next treatment involved the same fecal material removal by water washing, plus a 2.0% lactic acid spray before chilling, and then the same post chill 200 ppm peroxyacetic acid spray. Water washing and the 2.0% lactic acid spray accounted for a 2.0 and 2.9 log CFU/cm² reduction of *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively. However, the post-chill 200 ppm peroxyacetic acid spray was still ineffective at reducing the inoculated pathogens. Next, the previous protocol was repeated; however, the post-chill peroxyacetic acid was applied at concentrations of 200, 600, and 1,000 ppm, and at temperatures of 45°C and 55°C. Still, all peroxyacetic acids solutions at concentrations of 200 and 600 ppm were

not successful for reducing *E. coli* O157:H7 or *Salmonella* Typhimurium. The 1,000 ppm peroxyacetic acid solution applied at 55°C produced significant reductions of *E. coli* O157:H7 and *Salmonella* Typhimurium, whereas the 45°C 1000 ppm solution did not produce significant reductions. The reduction caused by the 55°C 1,000 ppm peroxyacetic acid spray did not differ from that caused by a 4.0% lactic acid application. Finally, King et al. (38) applied a 200 ppm peroxyacetic acid solution to hot carcasses following a water wash. Pathogen reductions of 0.7 log CFU/cm² were achieved using this method. However, this was insignificant considering that water washing alone produced reductions of 1.9 and 2.0 log CFU/cm² for *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively.

Water Washes

There are several reasons why use of water interventions has gained interest among beef processors. For the most part, water is widely available, relatively inexpensive, presents little danger to workers, and is far less corrosive than some antimicrobial interventions. Water washes have been shown to reduce microorganism populations on the surface of beef carcasses and subprimals. Application parameters for water treatments include temperature, pressure, nozzle type and number, distance from nozzle to meat surface, angle, and time of exposure. Of these parameters, temperature and pressure are the most widely studied. Reductions caused by water treatments vary by study; however, they are attributed primarily to the temperature of the water and its ability to thermally inactivate the microorganism (6, 7). Castillo et al. (13) found that *Salmonella* Typhimurium and *E. coli* O157:H7 on beef carcasses were reduced

significantly when washed with warm (35°C) and hot water (95°C) versus warm water only. The addition of the hot water wash following the 35°C warm water wash in the Castillo et al. (13) study accounted for an additional 0.7 to 2.2 log CFU/cm² reduction in the pathogens tested. In another study conducted by Castillo et al. (16), automated water washing (5L, 9s, 250 - 400 psi, 35°C) of hot beef rounds accounted for log reductions of 2.4 and 2.6 log CFU/cm² for *E. coli* O157:H7 and *Salmonella* Typhimurium, respectively. Cold or warm water sprays ($\leq 40^{\circ}\text{C}$) were generally less effective at reducing bacterial populations in comparison to hot water sprays ($\geq 70^{\circ}\text{C}$) (13, 31, 39, 51). Additionally, cold and warm water sprays are more likely to spread contamination to uncontaminated regions of carcasses or subprimals since little or no thermal inactivation occurs (7, 33, 40). Although effective at reducing bacteria, hot water washes, when applied for extended periods of time, are known to discolor meat surfaces (3, 24). Davey and Smith (24) noted that carcasses treated with 83°C water for 20s, while exhibiting a bleached appearance, were acceptable in color following a 48h chill. Another important washing parameter is the pressure of the water wash application. Multiple studies (21, 30) suggest that higher pressures are more effective at reducing surface bacteria on beef carcasses. However, some evidence suggests that high pressures may facilitate the penetration of bacteria and fecal material beneath the carcass surface (2). The maximum recommended pressure found by Pordesimo et al. (51) for use on red meat cuts and carcasses was 2,070 kPa or 300 psi. Pressure not only contributes to the force necessary to dislodge bacteria, but is also very important in hot

water washes since it, in part, determines spray volume and droplet size, which affect the actual temperature of the spray contacting the meat surface (3).

CHAPTER III

MATERIALS AND METHODS

Bacterial Cultures

The inoculum used in this study consisted of a “cocktail” of three nonpathogenic *E. coli* Biotype I isolates deposited with the American Type Culture Collection under accession numbers BAA-1427, BAA-1428, and BAA-1430. These three isolates were selected to express rifampicin resistance (100 mg/liter) for use in this study by incubating and growing the organisms in the presence of the antibiotic and then selecting for isolates demonstrating stable resistance. Previous research (10, 43, 46) has shown that these isolates exhibit similar lactic acid and thermal resistance to *Escherichia coli* O157:H7 and five serotypes of *Salmonella*. Therefore, these isolates were used to model the contamination of the enteric pathogens *E. coli* O157:H7 and *Salmonella* during slaughter processes (pre-rigor and pre-chilled) on beef strip loins, so that survival and translocation of the surrogates could be studied throughout chilling, cold storage, application of a pre-further processing intervention, and marination.

Product Procurement

Hot, pre-rigor boneless beef strip loins (n = 54) were selected from a commercial cull cow beef processing plant in Texas. The boneless beef strip loins were removed from beef carcasses to comply with Institutional Meat Purchase Specification #180 as described by USDA (45). Strip loins were cut from the carcass immediately after slaughter and before chilling. During slaughter, a 500-1200 ppm acidified sodium

chlorite spray (pH 2.3-2.9) was applied to each carcass as required by the plant's beef slaughter HACCP plan. After removal, the strip loins were transported immediately after collection in an insulated container to the Rosenthal Meat Science and Technology Center at Texas A&M University (College Station, TX). Procurement of the strip loins was spread out over three collection days. Every strip loin from each of the three collection days (n = 21) was assigned to a corresponding treatment repetition and one of six antimicrobial spray treatments or control.

Upon arrival, initial microbiological samples were taken randomly from the surfaces of three strip loins on each collection day to ensure that no naturally occurring rifampicin-resistant (Rif^R) organisms were detected on the surface of the strip loins before inoculation. A surface temperature was taken using a Taylor 9878E thermometer (Taylor Precision Products, Oak Brook, IL) on each strip loin at the time of arrival or just before inoculation. Each strip loin then was halved by a cut perpendicular to the long axis of the strip loin, and mid way between the anterior and posterior ends. A numbered plastic tag was attached to the corner of each strip loin piece for identification purposes throughout the study.

Inoculum Preparation

At 48 h before each collection day, the Rif^R cultures of *E. coli* organisms (ATCC BAA-1427, BAA-1428, BAA-1430) were propagated by transferring a loop of the stored microorganism from a tryptic soy agar (TSA, Becton, Dickinson and Co., Sparks, MD) slant to a fresh 10 ml tryptic soy broth (TSB, Becton, Dickinson and Co.) tube and incubated aerobically at 37°C for 18 to 24 h. Each culture then was transferred

individually by pipetting 0.1 ml into Falcon™ (Thermo-Fisher Scientific, Waltham, MA) conical centrifuge tubes containing 10 ml TSB before incubating for 18 h at 37°C. After incubation at 37°C for 18 h, cells from each culture were harvested by centrifugation at $1,620 \times g$ 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 ($1,620 \times g$ for 15 min) and the procedure was repeated once. The final pellets were suspended in 10 ml of PBS each. Following the final suspension in 10 ml of PBS, cell suspensions from each culture were combined to form a high inoculum ($8.0 \log_{10}$ CFU/ml) cocktail of Rif^R *E. coli* organisms. The low inoculum cocktail ($4.0 \log_{10}$ CFU/ml) was created by pipetting 1.0 ml of the high inoculum cocktail into 99 ml of sterile 0.1% peptone water. Following vortexing, 1.0 ml was taken from the 100 ml suspension and was pipetted into 99 ml of sterile 0.1% peptone water to create the low inoculum.

Inoculation of Hot Strip Loin Pieces

Strip loin pieces were paired so that they received the same antimicrobial treatment or control application. However, one piece was inoculated using a low concentration inoculum ($\sim 4.0 \log_{10}$ CFU/cm²), whereas the other piece received a high concentration inoculum ($\sim 8.0 \log_{10}$ CFU/cm²). The inoculum was a cocktail of three non-pathogenic, Rif^R *E. coli* organisms and applied to the (29.8°C) strip loins. One milliliter of inoculum was applied to each side (dorsal and ventral) of each hot (29.8°C) strip loin piece and spread using a sterile plastic spreader. The strip loins were allowed 30 minutes for microbial attachment before they were transported to a plastic rack inside

a refrigerated (2°C) room. After chilling for 24 h, each inoculated strip loin piece was vacuum packaged in a Cryovac® B6620 bag (Cryovac Food Packaging and Food Solutions, Sealed Air Corp., Duncan, SC) and returned to the refrigerated room until the appropriate treatment day. Strip loin pieces were aged inside of the refrigerated room for 7 to 24 days before being pulled for treatment application and marinating.

Treatment Preparation

Lactic acid was prepared by diluting 28.4 ml and 56.8 ml of 88% L-lactic acid concentrate (Purac America, Inc., Lincolnshire, IL), respectively, into tap water to achieve concentrations of 2.5 and 5.0% lactic acid mixtures. Acid titrations were conducted according to the manufacturer's directions using the supplied test kit to confirm proper lactic acid concentrations. Titrations were performed by pipetting 1 ml of lactic acid test solution into a clean test tube. Next, one drop of Phenolphthalein indicator solution was added. Finally, individual drops of 0.25 N Sodium Hydroxide were added and then shaken to stir the solution until the test solution turned from clear to pink in color. The number of N Sodium Hydroxide drops added was recorded, and divided by ten to calculate the percent lactic acid concentration. Acidified sodium chlorite solution was made by adding 14.2 ml of sodium chlorite to 1.0 L of tap water to achieve a final concentration of 1,050 ppm. Powdered citric acid was dissolved in the sodium chlorite solution until a pH of 2.7 to 2.8 was achieved. Peroxyacetic acid was mixed by adding 26.0 ml of peroxyacetic acid to 1.0 L of tap water to achieve a concentration of 205 ppm. Lactic acid solutions were heated by circulating hot water around the solution container until the solution temperature reached the desired

temperature (close to but not exceeding 55°C) for application in this study. All chemical treatments except the lactic acid treatments were applied at room temperature.

Strip Loin Treatment

On each processing day, the appropriate strip loin pieces (n = 6) were pulled from the refrigerated room for each treatment that was to be applied. The low-inoculated strip loins for each treatment were always processed first, followed by the high-inoculated strip loins. The strip loin pieces were aseptically removed from their vacuum package and sampled to determine the numbers of Rif^R microorganisms that survived the aging process in the vacuum package. Next, the strip loin pieces received one of the following five chemical treatments or control:

1. 2.5% L-Lactic Acid: mean temperature 53.3°C, mean pH 2.60
2. 5.0% L-Lactic Acid: mean temperature 52.8°C, mean pH 2.44
3. Acidified Sodium Chlorite: 1,050 ppm acidified sodium chlorite, mean temperature 18.4°C, mean pH 2.78
4. Peroxyacetic Acid: 205 ppm peroxyacetic acid, mean temperature 19.8°C, mean pH 5.22
5. Water: mean temperature: 26.1°C, mean pH 8.61

Spray treatments were applied using a Chad (Chad, Inc., Olathe, KS) spray cabinet with 6 nozzles (3 on top and 3 on bottom) running at a belt speed of 5.08 cm/sec, spraying at a pressure of 1.4 atm, delivering 0.42 L of liquid per sec per nozzle. After treatment, the strip loin pieces were allowed 5 min to drip before the post-treatment samples were taken for microbiological analysis. The strip loin pieces then were

weighed so that the marinade could be mixed according to the manufacturer's instructions.

Marination of Strip Loins

The marinade seasoning used for all of the treatments and control was REO TAMU Fajita Marinade (REO Spice & Seasoning, Inc., Huntsville, TX). The dry marinade seasoning was mixed according to the manufacturer's instructions. The marinade consisted of 18.14 g of seasoning, mixed with 88.9 g tap water with 2.00 g sodium tripolyphosphate dissolved, per 454 g of meat. The marinating process was performed in a Leland Southwest VT500 Vacuum Tumbler (Leland Southwest, Fort Worth, TX) at a speed of 5.5 rpm and a force of 0.07 g. A vacuum of 0.6 atm was pulled before tumbling. The product was tumbled for three 15 min periods. Each 15 min tumbling period was followed by a 5 min resting period. The entire tumbling and resting periods were conducted under vacuum for a total of one hour.

Sampling and Microbiological Examinations

After the marinating process was finished, the strip loin pieces were transported to the Texas A&M University Food Microbiology Lab. Surface samples were taken from each piece to determine the level of Rif^R microorganisms that had survived the marinating process. Internal samples were taken by cutting two plugs from the center of each strip loin piece. These pieces were submerged into 95% ethanol and then charred using a Bunsen burner and a butane torch to sterilize the outside surface. Next, a sterile scalpel and forceps were used to aseptically expose the geometric center of the plug. A

sterile 10 cm² stainless steel borer and scalpel then was used to excise a 10 cm² × 2 mm sample from the inside of the plug.

All samples were a composite of two 10 cm² × 2 mm excisions. Each sample was transferred to a sterile stomacher bag containing 99 mL sterile 0.1% peptone water. The samples were pumped for 1 min at 260 rpm using a Stomacher-400 (Tekmar Company, Cincinnati, OH). For each sample, counts were determined by plating appropriate decimal dilutions on pre-poured and dried rifampicin-tryptic soy agar (rif-TSA) plates with a sterile bent glass rod. Rif-TSA was prepared by adding a solution of 0.1 g of rifampicin (Sigma-Aldrich, St. Louis, MO) dissolved in 5.0 mL methanol to 1 L of autoclaved and tempered (55°C) TSA. Plates were incubated 24 h at 37°C. Colonies were counted, recorded, and reported as log₁₀ CFU/cm² according to published methods (26). For surface samples from low inoculated pieces post treatment and post marination, 10.0 ml of stomached sample was transferred to 90.0 ml of Rif-Nutrient Broth. After the addition of the 10.0 ml of stomached sample, the Rif-Nutrient Broth samples were incubated at 37°C for 24 h. After incubation the Rif-Nutrient Broth samples were checked by eyesight for presence of turbidity. Samples exhibiting turbidity were considered positive for presence of an organism or organisms capable of surviving in the presence of rifampicin (100 mg/l) and were streaked for isolation on MacConkey agar. The streaked MacConkey Petri dishes were incubated at 37°C for 24 h. After incubation the streaked MacConkey plates were examined for presence of colonies exhibiting characteristic *E. coli* phenotypic appearance (pink to red colonies surrounded by reddish bile precipitate). Rif-Nutrient Broth was prepared by adding 0.1

g of rifampicin dissolved in 5.0 ml methanol to 1.0 L of autoclaved and cooled nutrient broth (Nutrient Broth, Becton, Dickinson and Co.). Rif-Nutrient Broth was dispensed into sterile bottles using an autoclaved graduated cylinder before capping with a sterile cap.

Sanitation Procedures

All equipment including the vacuum tumbler and spray cabinet were rinsed with hot tap water between low and high-inoculum runs of the same treatment. If two treatments were applied in one day, a complete cleaning and sanitation was performed after the first treatment and before the second treatment. The process consisted of a warm tap water rinse, soap and scrub, hot tap water rinse, and application of 200 ppm BiQuat chemical sanitizer (Birko Corporation, Henderson, CO). Equipment surfaces were rinsed before every run to minimize effects of residual sanitizer compounds.

Statistical Analysis

Microbiological count data were transformed into logarithms before calculating reductions and conducting statistical analyses. In the case of counts below the detection limit of the counting method, a number between 0 and the lowest detection limit was used in order to facilitate the data analysis. Data were analyzed using PROC GLM of SAS (SAS Institute Inc., Cary, NC) to perform analysis of variance. The data set was sorted by inoculum level. Means were separated using the pdiff function of SAS.

CHAPTER IV

RESULTS AND DISCUSSION

Analysis of High Inoculated Strip Loin Pieces

The initial concentration of Rif^R surrogate organisms on high-inoculated strip loin pieces after attachment ranged from 5.7 to 5.9 log₁₀ CFU/cm². Based on the Heller et al. (35) findings, this concentration was much higher than the amount of contamination that might be expected on meat products in an inspected processing establishment. However, for the sake of determining reductions in a laboratory setting, a high-level inoculum concentration of the *E. coli* cocktail was used in addition to a more industry-realistic, low inoculum concentration. After the strip loins were chilled for 24 h post inoculation, surface organisms were reduced up to 0.4 log₁₀ CFU/cm². Vacuum packaging and cold storage for 7 to 24 days resulted in reductions of surrogates up to 1.8 log₁₀ CFU/cm² in reference to the initial contamination level.

Initial and after treatment surface samples were taken from each strip loin piece to determine the mean reduction in surrogate organisms. Data showed that there were no statistical differences in reductions when stratified by repetition. However, reductions between treatments were significant ($P < 0.05$) (Table 1). After treatment with one of the five antimicrobial sprays, surrogate organisms surface numbers ranged from 3.2 to 5.0 log₁₀ CFU/cm². The 5.0% lactic acid spray was the most effective treatment at reducing surrogate organisms on the meat surfaces. Similar results regarding the efficacy of 5.0% lactic acid were reported by Yoder et al. (76). The least effective

treatment for reducing the surrogate organisms on the meat surface was the water treatment. The water treatment used in this study was applied close to room temperature (26.1°C), which is likely why it was relatively ineffective. Bolder (6) and Bolton et al. (7) identified water temperature as the primary application variable determining its efficacy against microorganisms.

TABLE 1. Least squares means of surface surrogate organism reduction (\log_{10} CFU/cm²) stratified by treatment \times sampling time for high- and low-inoculated strip loin pieces

Treatment	Sampling Time			
	Post 24 Hour Chill	Post Aging	Post Treatment	Post Marination
High inoculum^h				
Control	0.3 A ^g	1.2 B	1.2 B ^f	2.1 AB
2.5% Lactic Acid ^a	0.0 B	0.8 B	1.5 B	2.0 BC
5.0% Lactic Acid ^b	0.3 AB	1.8 A	2.6 A	2.3 AB
Acidified Sodium Chlorite ^c	0.3 A	0.9 B	1.4 B	2.1 AB
Peroxyacetic Acid ^d	0.4 A	1.2 B	1.3 B	2.4 A
Water ^e	0.2 AB	0.7 B	0.7 C	1.7 C
Low inoculumⁱ				
Control	0.4 AB	0.9 ABC	0.9 BC ^f	1.4 A
2.5% Lactic Acid ^a	0.2 AB	0.8 BC	1.0 AB	0.9 B
5.0% Lactic Acid ^b	0.5 A	1.1 AB	1.4 A	1.3 A
Acidified Sodium Chlorite ^c	0.2 AB	1.0 ABC	1.3 A	1.2 AB
Peroxyacetic Acid ^d	0.3 AB	1.2 A	1.3 A	1.5 A
Water ^e	0.1 B	0.6 C	0.5 C	1.1 AB

^a 2.5% L-Lactic acid, mean temperature: 53.3°C, mean pH: 2.60.

^b 5.0% L-Lactic acid, mean temperature: 52.8°C, mean pH: 2.44.

^c 1,050 ppm acidified sodium chlorite, mean temperature: 18.4°C, mean pH: 2.78.

^d 205 ppm peroxyacetic acid, mean temperature: 19.8°C, mean pH: 5.22.

^e Tap water, mean temperature: 26.1°C, mean pH: 8.61.

^f The after treatment reduction for the control was based on the reduction calculated after aging since no treatment was applied to control pieces after aging.

^g Numbers within columns within inoculation levels with different letters differ ($P < 0.05$).

^h Initial attachment: approximately 5.8 \log_{10} CFU/cm².

ⁱ Initial attachment: approximately 1.9 \log_{10} CFU/cm².

The findings of Gorman et al. (31), Kotula et al. (39), and Pordesimo et al. (51) support this with evidence showing hot water sprays ($>40^{\circ}\text{C}$) produce greater bacterial reductions than warm water treatments. All other spray treatments and control did not differ by reduction of surrogate organism on the meat surface. Following treatment application to high-inoculated strip pieces, reductions of surrogate organisms were 2.6, 1.5, 1.4, 1.3, 1.2, and 0.7 \log_{10} CFU/cm², respectively, for the 5.0% lactic acid, 2.5% lactic acid, acidified sodium chlorite, peroxyacetic acid, control, and water spray treatments, respectively. Similar findings, as far as order of antimicrobial effectiveness by treatments, were reported by Yoder et al. (76). Treatments in the Yoder et al. (76) study were ranked most effective to least effective in the following order: organic acid sprays, peroxyacetic acid, chlorinated compounds, and aqueous ozone. However, greater reductions due to antimicrobial spraying, up to 5.32 \log CFU/cm², were achieved by Yoder et al. (76) than in this study.

After treatment, each strip piece was marinated with REO TAMU Fajita Marinade. Following marination, surface surrogate organisms reductions were greater for the peroxyacetic acid treated strip loin pieces when compared to the water treated strip loin pieces. The same relationship was noticed in samples taken before marination of the strip loin pieces, and in the Yoder et al. (76) and Penney et al. (49) studies, which noted greater reductions attributed to peroxyacetic acid treated beef compared to water treated beef. Reductions achieved by peroxyacetic acid treatment on high-inoculated pieces in this study were greater than the water treated pieces. However, this outcome did not agree with the findings of King et al. (38). King et al. (38) achieved greater

reductions of *E. coli* O157:H7 and *Salmonella* Typhimurium using a water wash compared to peroxyacetic acid treatment. However, King et al. (38) used a water wash that was applied at a considerably greater pressure and higher temperature than the water wash used in this study. King et al. (38) also achieved greater reductions of *E. coli* O157:H7 and *Salmonella* Typhimurium when peroxyacetic acid was applied to hot beef, whereas peroxyacetic acid was applied to cold beef in this study.

Internalization of surrogate organisms was greater for the water treated strip loin pieces in comparison to control pieces (Table 2). Internal samples post marination contained 1.1 to 2.1 log₁₀ CFU/cm² of surrogate organisms. Internalization of surrogate organisms for all treatments receiving a spray were similar ($P \geq 0.05$). Surface reduction of surrogate organisms up to the point before marination ranged from 0.7 to 2.6 log CFU/cm². Therefore, the meat surface contained approximately 3.2 to 5.1 log CFU/cm² surrogate organisms when placed into the vacuum tumbler. Mean surrogate organism internalization from highest to lowest was water wash, acidified sodium chlorite and peroxyacetic acid, 2.5 and 5.0% lactic acid, and the control.

Analysis of Low Inoculated Strip Loin Pieces

The initial concentration of Rif^R *E. coli* on low inoculated strip loin pieces after attachment ranged from 1.7 to 2.1 log₁₀ CFU/cm². This inoculation level proved to be challenging to work with when attempting to evaluate microbial reductions. However, this level of inoculation is more industry-realistic and likely to be seen on contaminated meat in comparison to the high inoculum conditions described above.

TABLE 2. Least squares means of internal (\log_{10} CFU/cm²) surrogate organisms for high- and low-inoculated strip loins post marination stratified by antimicrobial treatment or control

Treatments	Inoculation Concentration	
	High ^a	Low ^b
Control	1.1 _B ^h	0.5 _A
2.5% Lactic Acid ^c	1.3 _{AB}	0.5 _A
5.0% Lactic Acid ^d	1.3 _{AB}	0.5 _A
Acidified Sodium Chlorite ^e	1.5 _{AB}	0.5 _A
Peroxyacetic Acid ^f	1.5 _{AB}	0.5 _A
Water ^g	2.1 _A	0.5 _A

^a Initial attachment: approximately 5.8 \log_{10} CFU/cm².

^b Initial attachment: approximately 1.9 \log_{10} CFU/cm².

^c 2.5% L-Lactic acid, mean temperature: 53.3°C, mean pH: 2.60.

^d 5.0% L-Lactic acid, mean temperature: 52.8°C, mean pH: 2.44.

^e 1,050 ppm acidified sodium chlorite, mean temperature: 18.4°C, mean pH: 2.78.

^f 205 ppm peroxyacetic acid, mean temperature: 19.8°C, mean pH: 5.22.

^g Tap water, mean temperature: 26.1°C, mean pH: 8.61.

^h Numbers within a column with different letters differ ($P < 0.05$).

After inoculation and a 24 h chill period, reduction of initial surface surrogate organisms ranged from 0.1 to 0.5 log₁₀ CFU/cm². Aging for 7 to 24 days in a vacuum package resulted in a reduction of initial surrogate contamination equivalent to 0.6 to 1.1 log₁₀ CFU/cm².

Initial and after treatment surface samples were taken from each strip piece to calculate a log₁₀ CFU/cm² reduction from inoculation to treatment. Reduction of surface surrogate organisms through treatment for low inoculated strip pieces are shown in Table 1. Reduction of surrogate organisms was achieved with all treatments and control. The 5.0% lactic acid, acidified sodium chlorite, and peroxyacetic acid treatments were more effective at reducing the surrogate organism when compared to the water treatment. Reduction of surrogate organisms was greater for peroxyacetic acid and acidified sodium chlorite treated product in comparison to water treated and control pieces. 5.0% and 2.5% lactic acid treatments did not differ in their ability to reduce the surrogate organisms. This finding agrees with Harris et al. (34) in that organic acid concentration did not significantly influence reduction of microorganisms. However, reductions as a result of the 2.5% lactic acid treatment did not differ from the pieces that did not receive a treatment. The lack of statistical difference in microbiological reduction between 2.5% lactic acid and control may be explained by the difficulty associated with reduction determination using low (<2.0 log CFU/cm²) levels of surrogate organisms. Microbiological numerical reductions of surrogates by treatment from most to least are as follows: 5.0% lactic acid, acidified sodium chlorite and peroxyacetic acid, 2.5% lactic acid, control, and water treatment.

Reduction of surrogates through marination was greater for those receiving peroxyacetic acid, no treatment, or the 5.0% lactic acid spray in comparison to the 2.5% lactic acid spray. Again, statistical differences in microbiological reduction between treatments and control may have been influenced by the difficulty associated with reduction determination using low ($<2.0 \log \text{CFU}/\text{cm}^2$) levels of surrogate organisms. However, the 5.0% lactic acid and peroxyacetic acid were numerically the most effective treatments at reducing the surrogate organisms, though differences in numerical reductions in loins resulting from these treatments were not statistically different. Acidified sodium chlorite and water treatments did not differ from other treatments or the control in their ability to reduce numbers of the surrogate organisms. However, regardless of treatment or control, presence of internalized surrogates in the finished product was below the level of detection ($0.5 \log_{10} \text{CFU}/\text{cm}^2$). Only one colony was counted during enumeration of the internal low inoculated marinated samples. This colony was isolated from an internal sample taken from a 2.5% lactic acid treated and marinated strip piece.

Selective Enrichment for *E. coli* in Surface and Internal Post-Marination Samples from Low Inoculated Strips

Selective enrichment and isolation of *E. coli* are presented in Table 3 and 4. This portion of the study was designed to look further than the enumeration or detection capabilities of the decimal dilutions on rif-TSA plates. Selective enrichment results showed that organisms capable of growing in the presence of rifampicin were present in surface and internal samples taken from all treated and control strip pieces after

marination even in instances where no *E. coli*-typical colonies were enumerated. After the selective enrichment broth was evaluated for turbidity, a sample was streaked on MacConkey agar to determine whether streaked organisms produced a phenotypic appearance typical of *E. coli* and not organisms foreign to the inocula used. Apparent positives for *E. coli* organisms on MacConkey agar were observed in surface and internal samples regardless of the control or treatment received. Six of nine surface after marination samples from 2.5% lactic acid, acidified sodium chlorite, and water treated strip loins tested positive using the MacConkey agar assay. Samples that received the peroxyacetic acid spray had five out of nine samples test positive for *E. coli* present on the surface after marination. The 5.0% lactic acid treated strip loins had the fewest (4/9), but still nearly half of the samples test positive for presences of *E. coli* on the surface of the product after marination. However, MacConkey positive results for internal *E. coli* were more frequent (4/9) for both lactic acid treatments. Acidified sodium chlorite, peroxyacetic acid, and water treated samples had the fewest MacConkey positive results for *E. coli* isolates from internal samples after marination. Again, regardless of treatment or control, none were successful at completely eliminating all recoverable *E. coli* colonies from the surface and internal areas of the marinated strip loins. It is expected that most if not all recovered *E. coli* were from the inocula used in the study, however some uncertainty is left since isolated colonies from the enrichments samples were not genotyped

TABLE 3. Frequency of positive results for selective enrichment of *Escherichia coli* by treatment for post-treatment surface samples

<i>Treatment^c</i>	<i>Selective Enrichment^a</i>	<i>MacConkey Agar^b</i>
2.5% Lactic Acid ^d	(7/9)	(6/9)
5.0% Lactic Acid ^e	(6/9)	(4/9)
Acidified Sodium Chlorite ^f	(9/9)	(6/9)
Peroxyacetic Acid ^g	(5/9)	(5/9)
Water ^h	(7/9)	(6/9)

^a Stomached sample homogenate (10.0 ml) was transferred to 90.0 ml Nutrient Broth containing 0.1 g/L Rifampicin and incubated at 37°C for 24 h. Samples exhibiting turbidity after incubation were streaked for isolation on MacConkey agar. Streaked MacConkey Petri dishes were incubated at 37°C for 24 h.

^b Signifies number of samples bearing at least one colony exhibiting typical appearance of *Escherichia coli*.

^c Treatments were applied using an antimicrobial spray cabinet (conveyor speed 5.08 cm/sec) spraying at a pressure of 1.4 atm, while delivering 0.42 L of liquid per sec per nozzle, containing six nozzles in the cabinet (3 above and 3 below the conveying belt).

^d 2.5% L-Lactic acid: mean temperature: 53.3°C, mean pH: 2.60.

^e 5.0% L-Lactic acid: mean temperature: 52.8°C, mean pH: 2.44.

^f Acidified Sodium Chlorite: 1,050 ppm acidified sodium chlorite, mean temperature: 18.4°C, mean pH: 2.78.

^g Peroxyacetic Acid: 205 ppm peroxyacetic acid, mean temperature: 19.8°C, mean pH 5.22.

^h Tap water: mean temperature: 26.1°C, mean pH 8.61.

TABLE 4. Frequency of positive results for selective enrichment of *Escherichia coli* by treatment for internal post-marination samples

Treatment ^c	Nutrient Broth + Rifampicin ^a	MacConkey Agar ^b
2.5% Lactic Acid ^d	(7/9)	(4/9)
5.0% Lactic Acid ^e	(6/9)	(4/9)
Acidified Sodium Chlorite ^f	(9/9)	(1/9)
Peroxyacetic Acid ^g	(8/9)	(1/9)
Water ^h	(6/9)	(1/9)

^a Stomached sample homogenate (10.0 ml) was transferred to 90.0 ml Nutrient Broth containing 0.1 g/L Rifampicin and incubated at 37°C for 24 h. Samples exhibiting turbidity after incubation were streaked for isolation on MacConkey agar. Streaked MacConkey Petri dishes were incubated at 37°C for 24 h.

^b Signifies number of samples bearing at least one colony exhibiting typical appearance of *Escherichia coli*.

^c Treatments were applied using an antimicrobial spray cabinet (conveyor speed 5.08 cm/sec) spraying at a pressure of 1.4 atm, while delivering 0.42 L of liquid per sec per nozzle, containing six nozzles in the cabinet (3 above and 3 below the conveying belt).

^d 2.5% L-Lactic acid: mean temperature: 53.3°C, mean pH: 2.60.

^e 5.0% L-Lactic acid: mean temperature: 52.8°C, mean pH: 2.44.

^f Acidified Sodium Chlorite: 1,050 ppm acidified sodium chlorite, mean temperature: 18.4°C, mean pH: 2.78.

^g Peroxyacetic Acid: 205 ppm peroxyacetic acid, mean temperature: 19.8°C, mean pH 5.22.

^h Tap water: mean temperature: 26.1°C, mean pH 8.61.

CHAPTER V

CONCLUSIONS

Escherichia coli O157:H7, the non-O157:H7 STEC, and *Salmonella* pose serious threats to consumers of non-intact beef products. While the prevalence of these pathogens on meat is relatively low, if present it is possible for them to become internalized into finished non-intact product. Insufficient cooking of contaminated non-intact beef products will likely lead to foodborne illness. Spray treatments including lactic acid, acidified sodium chlorite, and peroxyacetic acid have shown that they are capable of significantly reducing surface bacteria on beef cuts before marination. If pieces are heavily contaminated before marination, these treatments alone cannot eliminate all contamination and may allow for internalization of pathogens into the final product. Furthermore, some treatments like room temperature water washing may not aid in the decontamination of beef cuts before marination, but may actually promote internalization of surface bacteria more so than applying no treatment at all before marination. If cuts only have a slight amount ($\leq 1.9 \log_{10}$ CFU/cm²) of *E. coli* O157:H7 and *Salmonella* contamination on them, these treatments are capable of reducing pathogens to a point that internalized samples are near or below levels of detection. However, no treatment resulted in all internal samples free of *E. coli* after selective enrichment. Since the *E. coli* colonies from the enrichment samples were not biochemically or serologically confirmed in the study, there is some uncertainty regarding the internalization of surrogate or other *E. coli* organisms into the final

product. Nonetheless, internalization was observed in both the high- and low-inoculated strip loin pieces. A suggestion for further research would be to evaluate the effects of using different application pressures and/or combinations of successive chemical sprays before marination to reduce surface contamination and translocation. Furthermore, the surrogates used in this study have not been validated for the six non-O157:H7 STEC recently identified as adulterants in non-intact beef by USDA-FSIS.

It is in the best interests of beef producers and the FSIS to ensure the production of safe foods for consumers. However, in order to do so, processors of non-intact beef products need to understand and implement different methods for decontaminating the surface of meat destined for non-intact beef production. Lactic acid, acidified sodium chlorite, and peroxyacetic acid solutions have shown potential in reducing pathogens. Applying such treatment may minimize numbers of surface pathogens capable of translocating or internalizing during the production process, and thus create safer beef products.

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