IN-PLANT VALIDATION OF TWO ANTIMICROBIAL AGENTS APPLIED DURING THE PRODUCTION OF TENDERIZED AND/OR ENHANCED BEEF PRODUCTS

A Thesis

by

KAYLA MICHELLE NELSON

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Chair of Committee,	Kerri B. Harris
Committee Members,	Jeffrey W. Savell
	T. Matthew Taylor
Head of Department,	H. Russell Cross

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ABSTRACT

Numerous outbreaks of foodborne illness have been attributed to non-intact beef (e.g., tenderized, marinated, and enhanced) products contaminated with *Escherichia coli* O157:H7. Organic acids are commonly utilized in the beef industry as antimicrobial interventions, which must be validated to eliminate or reduce *E. coli* O157:H7 to an undetectable level. Rifampicin-resistant Biotype I *E. coli* O157:H7 surrogate microorganisms (ATCC BAA-1427, BAA-1428, and BAA-1430) were applied as a cocktail (7.8 $\log_{10}$ CFU/ml) to three beef products (boneless strip loins, top sirloin butts, and bottom sirloin flaps) prior to treatment with an antimicrobial intervention (2.5% Beefxide or 2.9% lactic acid). Products were then subjected to a single or multiple pass tenderization and/or marination process. Beefxide and lactic acid treatments resulted in statistically significant log reductions of the microorganisms ($P < 0.05$) on the surfaces for all three products. Surrogate microorganisms were recovered from interior samples of all three products after mechanical tenderization. Additionally, surrogate concentrations recovered from flap surface and internal samples taken post-tumbling and marination were statistically similar ($P < 0.05$). These data indicate that tenderization and marination processes can transfer microorganisms into the interior of whole-muscle cuts, and suggest Beefxide and lactic acid may be similar in their efficacy as an antimicrobial applied as an intervention in the production of non-intact beef products.

DEDICATION

To my parents, husband, and fellow graduate students. Without their continued support and motivation this would not have been possible.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Between 1992-1993, a multi-state foodborne outbreak on the west coast of the United States involving ground beef contaminated with *Escherichia coli* O157:H7 resulted in over 500 incident cases, 151 hospitalizations, 45 cases of hemolytic uremic syndrome (HUS), and 3 deaths *(5)*. As a result, 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 (21 U.S.C. §§ 601 et seq). The USDA-FSIS also required new food safety measures be taken including the development and application of a Hazard Analysis and Critical Control Point (HACCP) plan, implementation of Sanitation Standard Operating Procedures (SSOP), and microbiological testing. As part of the HACCP plan, meat-processing facilities must identify hazards that are reasonably likely to occur and implement critical control points designed to prevent, eliminate, or reduce to an acceptable level the pathogen of concern. Various antimicrobial interventions, such as hot water, lactic acid, acetic acid, and other organic acid sprays are applied during harvest to reduce contamination on the carcass *(15)*. With the declaration of *E. coli* O157:H7 as an adulterant in non-intact beef products, many further processors are also applying antimicrobial interventions *(13)*. All processors are required to validate their systems to ensure that the pathogens of concern are adequately controlled.

This project was designed to validate the in-plant application of two different antimicrobial interventions, lactic acid and Beefxide (Birko Corporation, Henderson, Colorado), applied to multiple processing schemes (e.g., single pass or multiple pass tenderization, and marination/vacuum tumbling). Through the use of the non-pathogenic surrogate organisms these data will help establishments validate their specific in-plant pathogen reduction processes *(27)*.

***Escherichia coli* O157:H7**

Shiga-toxigenic *E. coli* (STEC) including *E. coli* O157:H7 cause an estimated 63,153 incident cases of foodborne illness annually in the United States, as well as 2,138 hospitalizations and 20 deaths per year *(31)*. *E. coli* O157:H7 was first identified as a foodborne pathogen in 1982 *(30)*. This pathogen was the cause of the deadly multistate foodborne outbreak 1992-1993 that would forever change the face of our nation's food safety regulations and policies.

E. coli, like many other human pathogens, (*Salmonella*, *Shigella*, *Yersinia*), is a member of the *Enterobacteriaceae* family *(32)*. *E. coli* is classified as a coliform, which is a general term used to describe Gram-negative asporogenous rods that ferment lactose, forming acid and gas within 48 h at 35°C *(4)*. Isolates are serologically distinguished based on surface antigens. O (somatic) antigens are used to identify the serogoup of a particular strain, while H (flagellar) antigens are used to identify the serotype of the strain. From there, diarrheagenic *E. coli* isolates are categorized into specific groups known as pathotypes. *E. coli* O157:H7 falls within the enterohemorrhagic *E. coli*

(EHEC), which among the *E. coli* strains, cause the most foodborne illness in the United States *(24)*.

The organism is mesophilic, growing at temperatures from 7 to 50°C, depending on the strain, with an optimum growth temperature of 37°C, although growth at temperatures outside these ranges has been reported *(9)*. These growth temperatures are one characteristic that make *E. coli* a common microorganism in the gastrointestinal tracts of many species *(9, 32)*. However, the presence of these organisms in the GI tract of cattle and ability to be shed via feces make cattle the primary source of this pathogen in the meat industry. Illness due to infection with *E. coli* O157:H7 begins with ingestion of contaminated food or water. For humans, the infectious dose of *E. coli* O157:H7 has been estimated to be as low as 10 organisms *(1)*. Acid resistance mechanisms allow *E. coli* O157:H7 to survive the low pH of the stomach and pass through the small intestine to the colon, where it attaches to host cells and damages microvilli *(24)*. This process of attachment and effacement creates a lesion on the host cell, and is thought to be the mechanism by which *E. coli* O157:H7 induces non-bloody diarrhea *(26)*. Complications attributed to *E. coli* O157:H7 infection include hemorrhagic colitis (HC), characterized by severe abdominal cramps, watery diarrhea followed by bloody diarrhea, and, in some cases, fever, and hemolytic uremic syndrome (HUS), characterized by acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia (*(8, 37)*. Approximately 2 to 15% of *E. coli* O157:H7 infections progress to HUS. Bloody diarrhea and HUS resulting from *E. coli* O157:H7 infection are attributed to production of Shiga toxins, which induce cell death via inhibition of protein synthesis *(13)*. Shiga

toxins create lesions on blood vessels in the colon, resulting in bloody diarrhea, and can damage renal cells, leading to HUS.

Arthur et al. *(2)* reported the recovery of *E. coli* O157 from the hides of more than 75% of cattle sampled at two commercial beef processing plants. Barkocy-Gallagher et al. *(3)* used similar methods to identify isolates of the *E. coli* O157 serogoup. These isolates were further analyzed to determine serotype. The investigators reported that, of the samples from which *E. coli* O157 isolates were obtained, 91% contained isolates that were further characterized as *E. coli* O157:H7. Overall, *E. coli* O157:H7 was recovered from the hides of 60.6% of cattle sampled and from 1.2% of postintervention carcasses sampled prior to entering the cooler. Kennedy et al. *(18)* failed to detect *E. coli* O157:H7 in any of 1,199 subprimal beef cuts intended for mechanical tenderization or enhancement collected from five further-processing establishments. Heller et al. *(14)* recovered *E. coli* O157:H7 from 2 (0.2%) of 1,014 beef subprimal cuts intended for blade tenderization or moisture enhancement collected from six U.S. processing establishments over a five week period. The investigators reported both positive samples had an *E. coli* O157:H7 concentration of <0.375 CFU/cm^2 based on Most Probable Number (MPN) analysis.

Non-intact beef

Non-intact beef products are defined by the USDA-FSIS as products that have been "injected with solutions, moisture enhanced, mechanically tenderized by needling, cubing, Frenching, or pounding devices, and beef that has been reconstructed into

formed entrees" *(13)*. Mechanical tenderization and moisture enhancement are used by the meat industry to increase the tenderness of products derived from muscles of lower tenderness *(33)*. Such products may also undergo marinating or restructuring processes. A 2007 USDA-FSIS survey of federally inspected beef establishments found that 850 (37%) of establishments surveyed conducted mechanical tenderization operations, while 472 (20%) of establishments surveyed conducted enhanced product operations, either by marinating or injecting products *(1)*. Several studies have reported the translocation of exterior microorganisms to the interior of otherwise intact product during mechanical tenderization or enhancement *(16, 20, 25, 33)*. Sporing *(34)* examined the translocation of *E. coli* O157:H7 from the surface to the interior of beef subprimals following single-pass blade tenderization. Subprimal lean surfaces were sprayed with a liquid inoculum of rifampicin-resistant *E. coli* O157:H7, resulting in a surface concentration of either 10^3 CFU/cm^2 or 10^6 CFU/cm^2. Subprimals were passed once through a blade tenderizer and stored at 0°C for 3 hours. Core samples were taken using a sterile coring device and sliced into cross-sectional strips, then homogenized and serially diluted onto TSA with rifampicin. Results showed that approximately 3-4% of the surface inoculum was translocated to the geometric center of the core sample, regardless of the concentration of surface inoculum. Luchansky et al. *(20)* reported similar results in a study comparing *E. coli* O157:H7 penetration into subprimals inoculated on either the lean or fat side and passed through a blade tenderizer either once or twice. Further, the study found no significant difference in the penetration of subprimals with surface-inoculated *E. coli* O157:H7 based on whether the inoculum was applied to the fat or lean side or the

number of passes through the blade tenderizer. Gill and McGinnis *(12)* examined the translocation of aerobic microorganisms from the surface to the interior of top butts during mechanical tenderization, observing the amount of bacteria recovered from internal samples was significantly associated bacterial concentrations on the surface of the top butts. Johns et al. *(16)* used a strain of *E. coli* made resistant to 200 ppm nalidixic acid to investigate the cross-contamination of noninoculated subprimals by processing equipment used to blade-tenderize an inoculated subprimal. An inoculum solution with a concentration of 8.21 to 10.06 log CFU/ml was sprayed onto the first subprimal to be passed. The inoculated subprimal was passed once through a blade tenderizer; five noninoculated subprimals were subsequently passed through the same blade tenderizer. *E. coli* was recovered from each of the five noninoculated subprimals passed after the inoculated subprimal, suggesting surface contamination on a subprimal can be translocated not only to the interior of the subprimal, but to subprimals processed subsequently after the contaminated subprimal as well.

Translocation has been demonstrated to occur through processes other than mechanical tenderization as well. Muras et al. investigated *E. coli* O157:H7 and *Salmonella* Typhimurium translocation to the interior of beef inside skirt steaks and tri-tip roasts during vacuum tumbling with a 5 log CFU/mL marinade inoculum. Statistically similar concentrations of both microorganisms were recovered from interior and exterior samples of inside skirt steaks. Levels of both microorganisms decreased in tri-tip roast samples as sample depth approached the geometric center; however, samples at a depth of 18-21 mm from the surface of tri-tips vacuum tumbled with marinade A

and B yielded mean *E. coli* O157:H7 concentrations of 2.3 and 1.4 log CFU/cm^2, respectively, and mean *Salmonella* Typhimurium concentrations of 2.5 and 1.6 log CFU/cm^2, respectively. The study also examined the ability of the two pathogens to survive in spent marinade samples stored at 4°C. No significant decreases in concentration were reported for either microorganism after 7 days *(25)*.

The demonstrated translocation of pathogenic microorganisms to internal portions of mechanically tenderized and/or enhanced beef products has raised the question of whether consumer and commercial cooking practices for intact beef products are adequate for non-intact beef products. Luchansky et al. *(21, 22)* reported the recovery of *E. coli* O157:H7 survivors from inoculated brine-injected steaks cooked on an open-flame gas grill to internal temperatures ranging from 37.8°C to 71.1°C, as well as from inoculated blade-tenderized beef steaks cooked on an open-flame gas grill to internal temperatures ranging from 48.9°C to 71.1°C. Porto-Fett et al. *(29)* investigated the effect of typical commercial cooking, searing, and holding practices on the inactivation of *E. coli* O157:H7 in prime rib, reporting 3.2 to 5.2 log CFU/g reductions at various time-temperature combinations, but noting the recovery of *E. coli* O157:H7 survivors at all combinations of cooking and holding temperatures and times.

In 1999, USDA-FSIS issued a notice clarifying its policy regarding beef products contaminated with *E. coli* O157:H7 *(13)*. In the notice, USDA-FSIS stated that non-intact beef products contaminated with *E. coli* O157:H7 are considered adulterated unless further processed into ready-to-eat (RTE) products. In 2002, USDA-FSIS required manufacturers of raw beef products to reassess their HACCP plans to determine

if contamination with *E. coli* O157:H7 was reasonably likely to occur in their process *(35)*.

In 2003, a multi-state outbreak of *E. coli* O157:H7 infections was attributed to steaks sold by door-to-door vendors *(18)*, representing the first reported outbreak associated with steak products. The steaks were blade-tenderized, marinade-injected, and vacuum packed. Twelve (12) incident cases were identified, including three hospitalizations and one incident of HUS. The steaks involved in this outbreak originated from a single processing establishment in Illinois, prompting a recall of 739,000 pounds of frozen beef products *(36)*. The establishment was reported to have been breaking down, cleaning and sanitizing its injection needles once per week at the time of the outbreak. The establishment revised its SSOPs to require injection and tenderization-related processing equipment to be broken down, cleaned, and sanitized on a daily basis.

In May of 2005, FSIS published a notice that required establishments that produced non-intact beef products to reassess their HACCP plan to determine whether the plan adequately addressed *E. coli* O157:H7 as a biological hazard *(10, 11, 36)*. In addition to the 2003 outbreak discussed previously, the notice cited outbreaks involving mechanically tenderized beef products in 2000 and 2004 in Colorado and Michigan, respectively, as events that might necessitate the reassessment of an establishment's HACCP plan. FSIS suggested that, as part of its reassessment, establishments producing mechanically tenderized beef products require incoming product to be treated by suppliers to eliminate or reduce *E. coli* O157:H7 to an undetectable level.

In 2013, USDA-FSIS proposed a rule requiring raw and partially cooked mechanically tenderized beef products to be bear the descriptive designation of "mechanically tenderized" on their label *(37)*. The proposed rule cited a petition submitted in 2010 by the Conference for Food Protection stating that consumers may not realize the product they are consuming is a non-intact product, potentially posing a health risk to the consumer, as non-intact meat perceived as intact may not be cooked to a degree of doneness necessary to kill internalized pathogens.

Control of *E. coli* O157:H7 using organic acids

The use of organic acid sprays as processing interventions is common in the beef industry *(15)*. Organic acids are typically applied to the entire carcass surface, though further processing establishments such as those that produce mechanically tenderized product utilize them as well. Organic acids exhibit greatest antimicrobial activity in their undissociated form, as this form facilitates greater penetration of the lipid bilayer of the cell membrane *(8)*. The near neutral internal pH of bacterial cells causes the acid to dissociate, acidifying the cell's cytoplasm. The bacterial cell must then use adenosine triphosphate (ATP) to pump protons out of the cell across the cytoplasmic membrane, depleting the cell of its energy source. Lactic acid is the most commonly used organic acid, though many others have been and continue to be researched as well *(3, 15)*. Acetic acid (21 CFR 184.1005), citric acid (21 CFR 184.1033) and lactic acid (21 CFR 184.1061) are identified as "direct food substances affirmed as generally recognized as safe (GRAS)" by the U.S. Food and Drug Administration (FDA). FSIS Directive 7120.1

lists substances approved by FSIS for use in the production of meat, poultry, and egg products *(21)*. Solutions of acetic acid and citric acid are approved by the FSIS for use as an antimicrobial as part of a carcass wash to be applied prior to chilling at a concentration up to 2.5%. Solutions of up to 4% acetic acid are approved as an antimicrobial spray solution applied to dried and fermented sausages. Solutions of up to 5% citric acid are approved for use as an antimicrobial spray solution applied to beef subprimals and beef trimmings prior to grinding; solutions of up to 10% citric acid are approved for use as an antimicrobial solution applied to bologna prior to slicing. Solutions of up to 5% lactic acid are approved for use as an antimicrobial applied both prior to and following chilling of livestock carcasses; 2 to 5% solutions of lactic acid at temperatures not to exceed 55°C are approved for application to beef and pork subprimals and trimmings.

Castillo et al. *(7)* reported 4.2 to 5.0 $\log_{10}$ CFU/cm^2 reductions of *E. coli* O157:H7 on the surface of inoculated hot beef carcasses by applying a 2% lactic acid solution at 55°C in a model carcass spray cabinet following a cleaning treatment of either a high-pressure water wash at 35°C or trimming. Laury et al. *(19)* investigated the efficacy of an antimicrobial composed of a blend of lactic acid (45-60%), citric acid (20-35%), and potassium hydroxide (>1%), known as Beefxide (Birko Corporation, Henderson, Colorado) in reducing *E. coli* O157:H7 and *Salmonella* on beef tips. Beef tips were inoculated by immersion in a solution inoculated with a cocktail of either four *E. coli* O157:H7 strains or three *Salmonella* strains at a concentration of 10^4 CFU/ml. The inoculated tips were held 37°C for 30 minutes to facilitate attachment. Inoculated

controls were sprayed with sterile water in a sanitizing spray cabinet, while treatment samples were sprayed with a 2.5% Beefxide solution in a sanitizing spray cabinet at an application rate of 1 ft/2.5 s at 40 lb/in^2. The external surface of the control and treatment groups of beef tips was swabbed over an area of 100 cm^2. The swab was placed in a sterile bag with 10 ml peptone buffer and the sample serially diluted. Treatment and control samples from beef tips inoculated with the *E. coli* O157:H7 cocktail were plated on MacConkey agar with a thin layer of TSA; treatment and control samples from beef tips inoculated with the *Salmonella* cocktail were plated on xylose lysine deoxycholate agar with a thin layer of TSA. Plates were incubated at 37°C for 24 h to determine *E. coli* and aerobic plate counts (APC). The Beefxide treatment reduced *E. coli* O157:H7 and *Salmonella* populations by 1.4 and 1.1 log CFU/100 cm^2, respectively.

CHAPTER II

MATERIALS AND METHODS

Texas A&M University worked with a commercial further-processing establishment in Texas to complete this project. The establishment is federally inspected, so the project was designed to comply with all USDA regulatory requirements related to process validation and use of surrogate microorganisms. To conduct this investigation, data collection procedures were conducted on two different dates, scheduled to occur when no other production was in process to allow for cleaning and sanitizing of the facility following data collection procedures. The processing environment was sampled following the cleaning and sanitizing procedures to ensure that no residual surrogate organisms used for the purposes of this research investigation existed.

Product selection

Three beef products, boneless strip loin (Institutional Meat Purchase Specifications [IMPS] 180), top sirloin butt-cap off (IMPS 184B), and bottom sirloin flap (IMPS 185A), were selected by the establishment based on availability of product, number of passes through the tenderizer, and the marination process *(28)*. For this experiment, the plant's normal production practices were followed for each cut. The bottom sirloin flaps were passed through the tenderizer one time, split, and then marinated using a vacuum tumbler. The strip loins were passed through the tenderizer two times, and the top sirloin butts were passed through the tenderizer three times. The

facility uses Chad (Chad, Inc., Olathe, KS) and Ross (Ross Industries, Midland, VA) systems for application of antimicrobial interventions. It is noted that this project was not designed to make comparisons between the two application systems; therefore, all three products were treated using both the Chad and the Ross systems. The Ross system was used on the first day of data collection, and the Chad system was used on the second day. However, the production and experimental processes remained the same on both days. Products were processed according to established parameters utilized by the commercial processing establishment on a daily basis. Plant personnel performed preparation of each antimicrobial compound in accordance to the manufacturers' recommendations to ensure that it was the same as their daily preparation. Data collected at the establishment was used to calculate the average operating parameters for the antimicrobials (Beefxide: pH 2.18, temperature 24.4°C, concentration 2.5%; lactic acid: pH 1.97, temperature 27°C, concentration 2.9%). The machine lines were flushed after the use of Beefxide prior to using lactic acid. To ensure proper coverage with the antimicrobial, all products were placed in a single layer with no overlap. For the products that were passed through the tenderizer two and three times, the products did not receive additional antimicrobial treatment but only additional tenderization. A total of 12 cuts per subprimal were used; 6 were treated with Beefxide and 6 with lactic acid.

Preparation of inoculum

Three nonpathogenic *E. coli* Biotype I strains (BAA-1427, BAA-1428 and BAA-1430) were obtained from the American Type Culture Collection (WHERE is it

located?) for use in this study. These strains were then selected in the Food Microbiology Laboratory at Texas A&M University for their inherent ability to naturally resist rifampicin (Rif^R) using the methods published by Kaspar and Tamplin *(17)*. Previous scientific research has validated that these organisms demonstrate similar thermal and lactic acid resistance properties to the human pathogen *E. coli* O157:H7 *(6, 23).* These marker organisms were designed for use in a "cocktail" to represent possible contamination with enteric pathogens of fecal origin such as *E. coli* O157:H7. Cocktail preparation began 48 h before each day of data collection; Rif^R cultures of *E. coli* (ATCC BAA-1427, BAA-1428, BAA-1430) were proliferated by transferring a loop of the microorganism from a tryptic soy agar (TSA, Becton, Dickinson and Co., Sparks, MD) slant to a sterile 10 ml tryptic soy broth (TSB, Becton, Dickinson and Co., Sparks, MD) tube and incubating aerobically at 37°C for 18 to 24 h. Following incubation, each culture was transferred individually by pipetting 0.1 ml into 15 ml Falcon™ (Thermo-Fisher Scientific, Waltham, MA) conical centrifuge tubes containing 10 ml TSB before incubating for 18 h at 37°C. Upon completion of incubation, cells from each culture were collected by centrifugation at 1,620 × *g* for 15 min. The supernatant was decanted and the pellet re-suspended in 10 ml of phosphate buffered saline (PBS). The centrifugation/re-suspension process was repeated twice more and the final pellets were re-suspended in 10 ml of PBS each. After the final re-suspension into 10 ml of PBS, cell suspensions from each culture were combined to form a cocktail of Rif^R *E. coli* Biotype I organisms.

Meat preparation and sampling

Background microbiological samples were taken randomly from six strip loins, top butts, and flaps to show that no naturally Rif^R microorganisms were present prior to inoculation with our cocktail. A total of 240 samples were obtained for microbiological analysis. The top and bottom surfaces of each cut were inoculated with the cocktail; 2 ml (per side) was used for the strip loins and flaps and 1 ml (per side) was used for the top butt. Volumes of inoculum used were based on the surface area of the product. The $\log_{10}$ CFU/ml of initial inoculum cocktail was high (7.8 log CFU/ml) to ensure that a sufficient number of microorganisms could be recovered from the product, before and after the antimicrobial intervention, in order to measure any reduction. After inoculation of the cuts, 30 minutes was allowed for attachment of microorganisms to meat surfaces. Following attachment, microbiological samples were collected from both sides of the product before and after the intervention/tenderization process. Flap surface samples were also taken at two additional processing steps, post-splitting and again after a 20 minute marination/vacuum tumbling. Surface samples were collected at the establishment by excising two pieces of product 10 cm^2 x 2 mm deep via a sterile stainless-steel borer, scalpel and forceps, and compositing them for a total of 20 cm^2 sample area. Each sample was placed in a sterile Whirl-Pak bag (Nasco, Atlanta, GA) placed inside an insulated container, and transported to the Food Microbiology Laboratory at Texas A&M University. Along with the surface samples, the subprimals used were transported by insulated container to the laboratory to allow for the aseptic extraction of internal samples. At the laboratory the subprimals were set on a foil surface

and the internal samples were obtained by removing two plugs from the center of each cut. The square plugs were submerged in 95% ethanol and then flamed to sterilize the exterior surface in order to reduce the transfer of microorganisms to the interior surface. A flamed scalpel and forceps were used to make cross-sectional cuts until the geometric center of the plugs were reached. After each cut, the meat plug, scalpel and forceps were dipped in 95% ethanol, flamed and placed on a new piece of foil. A 10 cm^2 × 2 mm sample was taken from the interior of each plug using sterile stainless steel borer and scalpel. The samples were then placed inside a Whirl-Pak bag. Sterile 99 ml of 0.1% peptone water was added to the Whirl-Pak bag of each sample, including the ones taken at the establishment. The samples were then pummeled for 1 minute at 260 rpm using a Stomacher-400 (Tekmar Company, Cincinnati, OH). For each sample, counts of the surrogate microorganisms were determined by plating the appropriate serial dilutions on pre-poured and dried rifampicin-tryptic soy agar (Rif-TSA, Difco, Becton, Dickinson and Co., Sparks, MD) plates with a sterile bent glass rod. Prior to sample collection, Rif-TSA was prepared by adding a solution of 0.1 g of rifampicin (Sigma-Aldrich, St. Louis, MO) dissolved in 5.0 ml of methanol to 1 liter of autoclaved and tempered (55°C) TSA. The plates were incubated for 24 h at 37°C. Colonies then were counted, recorded, transformed and reported as $\log_{10}$ CFU/cm^2.

Statistical analysis

Microbiological count data were transformed into logarithms before obtaining means and performing 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. All data were analyzed using JMP Software (JMP Pro, v10.0, SAS Institute Inc., Cary, NC). The Fit Model function was used for analysis of variance (ANOVA), determining interactions from the full model, and least squares means comparisons were performed using a Student's t-test. These means then were analyzed in order to determine the impact of antimicrobial intervention and processing practices on the numbers of the pathogen surrogate organisms. Additionally, statistical analysis procedures allowed researchers to scientifically quantify the establishment's use of antimicrobial and processing for the control of *E. coli* O157:H7 presence in non-intact beef products.

CHAPTER III

RESULTS AND DISCUSSION

Initial inoculum level was an important consideration for this project, and to ensure that the level would be sufficient to measure a reduction, an average inoculum level of 7.8 $\log_{10}$ CFU/ ml was used. Microbiological data for surrogate level on the surface of the cut prior to antimicrobial treatment, after antimicrobial treatment/tenderizer, and after marination were analyzed. Data for surrogate level on the interior of the cut after tenderizing and after marination (if applied) were analyzed as well.

Reductions of the surrogate microorganisms from the pre-intervention to post-intervention sampling interval for Beefxide and lactic acid were statistically similar for all three product cuts (Tables 1, 2 and 3). Both antimicrobial treatments resulted in a 1.1 $\log_{10}$ CFU/cm^2 reduction from sampling interval pre-intervention to post-intervention (Table 4). These findings are similar to the 1.4 log reduction (Beefxide) of pathogenic *E. coli* that was reported in previous research conducted by Laury et al. *(19)*.

As previously stated, internal samples were taken to evaluate the internalization of the surrogate organisms. Research conducted previously has confirmed that surface bacteria penetrate meat products that are subjected to mechanical/blade tenderization and/or marination and vacuum tumbling *(16, 20, 25)*. The effect of sampling interval and treatment on internalization values (Table 4) indicate that there was a statistically significant difference ($P < 0.05$) for internalization samples between Beefxide and lactic

acid. Table 5 presents the effect on internalization based on sampling interval and subprimal. The internal values differed ($P < 0.05$) for each subprimal. For the flap, this statistical difference can be explained by the thinness of the cut in relation to the strip loin and top butt. However, further research may need to be conducted in order to better describe the difference between the top butt and strip loin as the original hypothesis that microorganism internalization would increase as the number of tenderization passes increased was refuted by gathered data.

CHAPTER IV

CONCLUSIONS

The production of non-intact beef products is a complex system that varies among specific products, as well as among processors. Therefore, further processors must have the ability to support decisions made in food safety/HACCP programs to demonstrate effective control of *E. coli* O157:H7 in and on these products. The results of this study can provide the partnered establishment as well as those with similar processes with validation for these interventions based on designed trials conducted in a processing establishment setting. Data collected during this project suggest both Beefxide and lactic acid applications are effective in the surface decontamination of beef subprimals .

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APPENDIX A

TABLES

TABLE 1. *Least squares means of biotype I* Escherichia coli *at various processing steps on beef bottom sirloin flaps treated with lactic acid or Beefxide.*

Sampling Interval[1]	Beefxide (log_{10} CFU/cm^2)[2]	Lactic acid (log_{10} CFU/cm^2)[2]
Pre-intervention[3]	5.8[a]	5.8[a]
Post-intervention[4]	4.9[b]	4.9[b]
Post-splitting[5]	3.6[d]	2.8[e]
Post-tumbling[6]	4.5[bc]	4.3[c]
Internal[7]	4.0[cd]	4.0[cd]

[1] Beef flaps passed through tenderizer once.
[2] Means lacking a common letter differ ($P < 0.05$).
[3] Surface sample taken after the 30 min attachment period.
[4] Surface sample taken after application of antimicrobial and one pass through the tenderizer.
[5] Internal surface sample taken in-plant after splitting of the flap.
[6] Surface sample taken after marination and 20 min vacuum tumbling step.
[7] Internal sample taken aseptically in the microbiology laboratory.

TABLE 2. *Least squares means of Biotype I* Escherichia coli *surrogates at various processing steps for beef strip loins treated with lactic acid or Beefxide.*

Sampling Interval[1]	Beefxide (log CFU/cm^2)[2]	Lactic acid (log CFU/cm^2)[2]
Pre-intervention[2]	5.7[a]	5.5[a]
Post-intervention[3]	4.3[b]	4.2[b]
Internal[4]	2.8[c]	1.4[d]

[1] Beef strip loins passed through tenderizer twice.
[2] Means lacking a common letter differ ($P < 0.05$).
[3] Surface sample taken after the 30-minute attachment period.
[4] Surface sample taken after application of antimicrobial and two passes through the tenderizer.
[5] Internal sample taken aseptically in the microbiology laboratory.

TABLE 3. *Least squares means of Biotype I* Escherichia coli *surrogates at various processing steps for beef top sirloin butts treated with lactic acid or Beefxide.*

Sampling Interval[1]	Beefxide (log CFU/cm^2)[2]	Lactic acid (log CFU/cm^2)[2]
Pre-intervention[3]	5.5[a]	5.5[a]
Post-intervention[4]	4.3[b]	4.2[b]
Internal[5]	1.5[c]	1.2[c]

[1] Beef top butts passed through tenderizer three times.
[2] Means lacking a common letter differ ($P < 0.05$)
[3] Surface sample taken after the 30-minute attachment period.
[4] Surface sample taken after application of antimicrobial and three passes through the tenderizer.
[5] Internal sample taken aseptically in the microbiology laboratory.

TABLE 4. *Least squares means for sampling interval x treatment effect on log$_{10}$ CFU/cm^2 of internalization of Biotype I* Escherichia coli *surrogates.*

	Sampling Interval		
Treatment	Pre-intervention[1]	Post-intervention[2]	Internal[3]
Beefxide	5.6[a]	4.5[b]	2.9[c]
Lactic acid	5.6[a]	4.5[b]	2.1[d]

[a-d] Means lacking a common letter differ ($P < 0.05$).
[1] Surface sample taken after the 30-minute attachment period.
[2] Surface sample taken after application of antimicrobial and passes through the tenderizer.
[3] Flap internal samples taken post-splitting in-plant prior to marination and tumbling. Strip, Top Butt internal samples taken aseptically in the microbiology laboratory.

TABLE 5. *Least squares means for sampling interval x subprimal effect on counts log CFU/cm^2 of internalization of Biotype I* Escherichia coli *surrogates.*

	Sampling Interval		
Subprimal	Pre-intervention[1]	Post-intervention[2]	Internal[3]
Flap	5.8[a]	4.9[c]	3.2[e]
Strip Loin	5.6[ab]	4.3[d]	2.1[f]
Top Butt	5.5[b]	4.3[d]	1.4[g]

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

[1] Surface sample taken after the 30-minute attachment period.

[2] Surface sample taken after application of antimicrobial and passes through the tenderizer.

[3] Flap internal samples taken post-splitting in-plant, prior to marination, tumbling. Strip, Top Butt internal samples taken aseptically in the microbiology laboratory.