VALIDATION OF VARIOUS ANTIMICROBIAL SOLUTIONS ON THE REDUCTIONS OF SURFACE MICROBIAL LOAD OF *E. COLI* O157:H7 ON LEAN BEEF

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Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

2009

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2011

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ACKNOWLEDGMENTS

I would like to take this opportunity to express my most sincere thanks to everyone that was involved with this research project. Especially to my graduate student counterparts it was extremely helpful for all of you to offer help in the completion of this study. Thank you Dr. Morgan for providing me with the opportunity to work under your direction on this degree and for all of the guidance you have given me throughout the entire process, I have became a more worldly person because of your leadership. I owe a large thank you to the other members of my masters committee. Dr. Muriana, thank you for all of the expertise that you provided in the lab and elsewhere to make this project happen. Dr. VanOverbeke and Dr. Hilton, your influences throughout my time as a graduate student have led to a knowledge base of information that I otherwise would not have capitalized on. To Jake Nelson and Kris Novotny both of you have became friends and people who's knowledge I respect great, thank you so much for all of the help you have given me throughout the last two years. Kyle, J.D. and the rest of the crew on the 2nd floor of FAPC I appreciate everything that you have helped me with throughout my time as a graduate student. Kalpana, Dinesh and all the other people working on the 3rd floor of FAPC your assistance in this project could not have been replaced, I appreciate deeply all you have done for me. Wayne Spillner thank you and the team at Ross Industries for your financial and intellectual support of this project. Finally, I would like to thank Jackie as she put up with me throughout this entire process and our work together on both phases of this study were sometimes tiring but extremely beneficial.

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CHAPTER I

INTRODUCTION

Meeting consumer expectations for product quality and consistency (particularly for tenderness) has been identified as a high priority by the U.S. beef industry (NCBA, 1998). As a result many methods have been developed in order to ensure tenderness and palatability in beef products (blade tenderization, injection and reconstruction). Scientists have investigated the palatability aspects of these processes for many years. Until recently there has been little research conducted addressing the potential microbial human health concerns associated with non-intact beef products. The National Advisory Committee on Microbiological Criteria for Foods and the FSIS defines an intact beefsteak as "a cut of whole muscle(s) that has not been injected, mechanically tenderized or reconstructed" (FSIS, 2005). The focus of Phase I of this study is to evaluate the performance of various antimicrobial products on their effectiveness of reduction of *E. coli* O157:H7 on lean beef surfaces prior to the mechanical blade tenderization process. Using a real world applicable approach in order to help the industry make decision on antimicrobial usage that will ultimately lower potential risk for public health concerns.

CHAPTER II

REVIEW OF LITERATURE

History of Meat Tenderization in the Beef Industry

The versatility of beef as a product in today's marketplace is greatly due to advancements in processing and fabrication methods. In relation to whole muscle cuts, tenderness is perhaps the most important factor used by consumers to judge palatability, quality and overall acceptance (Carpenter, 1975).

There have been extensive dollars and research time devoted to the study of improving meat tenderness. The most effective means available to drastically improve tenderness in beef cuts is grinding (Johnston, 1979). Disappointingly though, the issue of acceptable tenderness is not that simple to solve because grinding is not a sustainable method of tenderization for many cuts, such as beef steaks and roasts. As a result, other methods of tenderization have been studied and developed. Prior to 1960, the most accepted practice of improved postmortem tenderization was to "hang" carcasses in refrigeration for extended periods of time to allow for natural enzymatic tenderization, otherwise know as "aging". Numerous research trials have shown postmortem aging as an effective means of tenderization, yet it presents some major problems for industry today. Aging carcasses results in substantial moisture loss, surface spoilage and requires massive amounts of space and energy, making it unfeasible in today's mass production settings.

In the 1970's, researchers focused a great deal of effort on analyzing the effect of various feeding practices on tenderness. Grain-fed cattle produce well-marbled and more tender beef than grass-fed cattle (Kropf et al., 1975). Following the onset of an American grain shortage in 1974, which greatly increased the cost of feeding grain dense finishing diets and increased the number of grass fed cattle being sent to market (Johnston, 1979), researchers began investigating newly developed mechanical methods of tenderization. Throughout the 1970's there were several studies that reported positive improvement of meat tenderness associated with mechanical tenderization (Davis et al., 1975; Glover et al., 1977; Schwartz and Mandigo, 1974). It was estimated, in 1975, that over 90% of hotel, restaurant, and institutional operations used blade tenderization (Miller, 1975). Initially, there was some consumer concern over the process; however, its use and impact on the beef industry today is overwhelming.

***Escherichia coli* O157:H7**

Escherichia coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak of hemorrhagic colitis (HC) (Riley et al., 1983). Throughout the 1990's, *Escherichia coli* O157:H7 evolved from a clinical novelty to a global public-health concern, leading to the illness of 5,000 Japanese school children, death of 20 people in Scotland and the recall of millions of pounds of ground beef in the USA (Mead et al., 1999). There are numerous strains of *Escherichia coli* that exist, which have the potential to cause disease. Karmali et al. (1983) reported an association between infection with *E coli* that produce Shiga toxins and post-diarrheal haemolytic uraemic syndrome (HUS), a clinical condition defined by acute renal injury, thrombocytopenia, and microangiopathic hemolytic anemia. *Escherichia coli* O157:H7 became the first strain known as an enterohemorrhagic *E. coli* (EHEC), which is believed to account for around 90% of all HUS cases (Karmali et al., 1998). During the past 20 years, *E. coli* O157:H7 has emerged as a major disease causing pathogen, capable of causing high morbidity and mortality numbers among humans that become infected (Altekruse et al., 1997).

E. coli O157:H7 is the cause of the majority of severe, life threatening gastrointestinal illnesses related to *E. coli* (Fratamico et al., 2002; Peacock et al., 2001). Severity of symptoms

related to *E. coli* most typically depends on the age and health status of the person infected; obviously young children and people with immune system deficiencies are more susceptible to severe episodes.

E. coli O157:H7 prevelance on hides and within fecal samples has been shown to be present at very high levels; 13% and 23%, respectively (Elder et al., 2000). These figures range significantly with seasonality, with peak fecal shedding being in late summer and early fall (Hancock et al., 1997; Donkersgoed et al., 1999). This same time frame correlates directly with the peak of human outbreaks in North America, July through August (Armstrong et al., 1996). These studies quantify the level of public threat *E. Coli* O157:H7 presents to the consuming public.

Concerns of Blade Tenderization

There are an extensive number of research trials that have been conducted with respect to blade tenderization and its impact on sensory traits. However, only a limited number of studies have focused on the microbiological impact blade tenderization may impose. Boyd et al. (1978) determined that one pass through a tenderizer yielded significantly lower ($P < 0.05$) anaerobic bacterial counts than two, three or four passes during a four week shelf life trial.

Like meat processing equipment, sanitation programs are extremely vital when using blade tenderization machines and unsanitary conditions can result in shorter shelf life, and in the presence of pathogens, a public health hazard (Raccach and Henrickson, 1979). This same study (Raccach and Henrickson, 1979) concluded that using iodine based solution to sanitize both the blades and conveyor belt on a tenderization machine was adequate in controlling contamination of tenderized product.

Petersohn et al. (1979) analyzed microbial levels in needle tenderized meat. The researchers mechanically tenderized boneless strip loins, vacuum packed them in a barrier film bag and analyzed the strip loins over a ten day storage period. Plate counts were obtained for total aerobic, anaerobic and psychotrophic bacteria both from the surface and the interior of parts

of the meat. None of the total aerobic plate counts for the tenderized or the non-tenderized steaks differed significantly ($P > 0.05$) on any of the sampling days throughout the 10 day storage period (Petersohn et al., 1979). However, it was noted that tenderized samples had consistently higher ($P < 0.05$) aerobic microbial counts than controls (Petersohn et al., 1979). Furthermore, tenderized beef samples had significantly higher ($P < 0.05$) psychotropic counts than controls on day 2 and 5 of storage (Petersohn et al., 1979). With regard to the interior of the meat, plate counts showed no significant ($P > 0.05$) differences in aerobic, anaerobic and phychrotrophic microbes between the tenderized steaks and controls (Petersohn et al., 1979). However, like the surface plate counts, tenderized steaks did consistently display higher numbers when compared to the controls (Petersohn et al., 1979).

Following the passage of legislation that required roast beef to be cooked to an internal temperature of 62.8^{o}C, Johnson et al. (1979) conducted an inoculation study of blade tenderized beef rounds. Within the study, both surface and deep tissue core samples were analyzed for presence and quantity of *Salmonella newport*. In this investigation, *Salmonella* was discovered within the core samples of both the tenderized and non-tenderized rounds; however, levels of the bacteria were higher within the tenderized cores (Johnson et al., 1979). Following this sector of the experiment, rounds were cooked to an internal temperature of 54.4^{o}C, which resulted in *Salmonella* still being detected in some core samples. These findings highlight the importance of proper cooking guidelines to prevent a potential public health hazard associated with blade-tenderized product.

A study conducted at Kansas State University focused on the assessments of translocation of *E. coli* O157:H7 into the deep muscle tissue of beef top sirloin subprimals following surface inoculation with high levels of the pathogen (10^6 cfu/cm^2) and one pass through a needle tenderization unit (Phebus et al., 2000). Following evaluation of the core samples, the needle tenderization process used showed about 3.0 logs of *E. coli* being translocated up to 6 cm from the surface into the deep tissues. Inoculation with low levels of the pathogen yielded similar results, with 1.8 logs of the bacteria being transferred into the deep tissue. When adequate

cooking guidelines were being tested during this same study, researchers found that internal temperatures of 60^{o} C and higher were needed to eliminate *E. coli* O157:H7 by broiling. Luchansky et al. (2008) solidified these prior findings with another similar study concluding the blade tenderization transfers *E. coli* O157:H7 primarily into the top most 1 cm, but also into the deeper tissues of beef subprimals.

Antimicrobial Interventions

Various solutions of organic acids have been studied extensively as a source of antimicrobial treatments for beef carcasses post-harvest. Specifically, lactic acid (1-3%) solutions have been shown to reduce bacterial numbers on carcass tissue by 1-3 logs (Castillo et al., 1998; Gorman et al., 1995, 1997; Hardin et al., 1995; Kochevar et al., 1997; Reagan et al., 1996; Smulders and Greer, 1998; Smulders et al., 1986). Experiments have shown that lactic acid is effective in reducing both *E. coli* O157:H7 and *L. monocytogenes* (Delmore, 1998). Lactic acid is used expansively in meat processing facilities in the United States and is a very effective method for reduction of bacteria, especially when used in combination with hot water spraying. Relative to concerns, lactic acid may enhance the selection of acid resistant organisms that increase product spoilage, have an undesirable effect on product appearance, and cause equipment corrosion (Gill, 1998; Smolders and Greer, 1998).

Exposing carcasses to water above 70^{o}C has been found effective (1-3 log reduction) against pathogenic bacteria, including: *Salmonella, Y. enterocolitica, E. coli* O157:H7 *and L. monocytogenes* (Castillo et al., 1998; Davey and Smith, 1989; Gorman et al., 1995; Kochevar et al., 1997; Smith, 1992). A method of thermal decontamination known as steam pasteurization is being used in the industry today and has been found to reduce bacterial counts by 1-2 logs (Gill, 1998).

Building upon the topic of thermal decontamination, steam vacuuming is another technology that combines steam pasteurization with a more spot oriented approach that effectively eliminates the need for knife trimming. Results have shown that steam vacuuming can

effectively reduce levels of *E coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes* by 1.0-2.7 logs (Castillo et al., 1999; Dorsa et al., 1997). However, both these studies proved that using steam vacuuming in combination with organic acid and hot water rinses was significantly more effective at eliminating bacteria than just the single use of steam vacuuming.

There are a variety of commercially available products on today's market that are being used as antimicrobial interventions at difference stages of processing. Lactic acid bacteria, acidified sodium chlorite and lactic acid have all been shown to demonstrate significant ($P < 0.05$) log reductions of *E. coli* O157:H7 on beef subprimals (Echeverry et al., 2009). Calicioglu et al. (2002) showed that the application of non-ionic surfactant, Tween 20, prior to treatment with lactic acid increased log reductions of simple lactic acid application. Other combination treatments have shown promise, such as: acidic calcium sulfate and lactic acid, citric acid-activated acidified sodium chlorite (Castillo et al., 1999), and cetylpyridinium chloride (Kim and Slavik, 1996).

Researchers in the food industry have put a tremendous amount of resources and effort into investigating a variety of other methodologies for the reduction of surface microorganisms both pre- and post-harvest, including: ionizing radiation, hydrostatic pressure, electric fields, pulsed light, sonication and microwaves (Bawcom et al., 1995; Bolder, 1997; Dunn et al., 1995; Farkas, 1998; Hoover, 1993, 1997; Lillard, 1994). Ionizing radiation is the most effective means of reduction and is approved for use in the United States. However, due to a lack of knowledge about the long term impact it may have on human health and a general lack of consumer approval, it is not being used extensively.

Pre-harvest intervention steps have also been validated as effective means of reducing pathogenic bacteria. A "competitive exclusion" product by the trade name Preempt ^{TM} works by introducing a unique mixture of 29 bacteria that compete with pathogens in the gut effectively reducing the prevalence of Salmonella in young chicks (Hume et al., 1998). Water has been shown to be a primary reservoir of *E. coli* O157:H7 (Besser & Hancock, 2001), and treatment of water with chlorine is a proven means of controlling this hazard (Rice et al., 1999). Anderson,

Callaway et al. (2001) and Anderson, Buckley et al. (2001) have reported the oral administration of sodium chlorate reduced intestinal levels of *E. coli* O157:H7 in pigs.

Disease Outbreaks Linked to Blade Tenderized or Enhanced Meat

In October 1994, the Food Safety and Inspection Service of the U.S. Department of Agriculture (FSIS-USDA) pronounced *E. coli* O157:H7 to be an adulterant in raw ground beef. This decision occurred in response to a multi-state outbreak that was associated with the consumption of contaminated beef patties, resulting in 400 illnesses and four deaths (Barret et al., 1994). Subsequently, FSIS mandated the implementation of a Hazard Analysis and Critical Control Point (HACCP) system in all operating meat and poultry plants in order to identify potentially hazardous practices that account for microbial contamination.

In May 2005, FSIS-USDA published notice that organizations producing mechanically tenderized beef were required to make a reassessment of their HACCP protocols due to recent outbreaks of *E. coli* O157:H7 associated with blade tenderized products. Listed below is a review of the outbreaks, which helped prompt the FSIS-USDA actions previously mentioned.

Outbreak 1

The first reported outbreak occurred in Michigan, in August 2002 (FSIS, 2005). Following analysis with a process called pulsed field gel electrophoresis (PFGE), a technique commonly used to determine the relatedness of bacteria, the Michigan Department of Community Health identified two matching human *E. coli* O157:H7 strains. Follow up studies revealed the consumption of rare-medium degree of doneness prepared steaks as the possible cause for the outbreak. This episode did not result in any recall of product, however; the steak supplier was forced to reassess and change sanitation protocols related to their tenderization machines and implement an *E. coli* O157:H7 testing program.

Outbreak 2

The second outbreak involved mechanically tenderized products produced by a company in Chicago, Illinois, between the dates of March 17 and March 22, 2003. These products were sold by door-to-door vendors and after epidemiological studies were linked to *E. coli* O157:H7 infections in the states of Minnesota, Michigan, Kansas, Iowa, and North Dakota (FSIS, 2005). These incidents were linked to steaks that had been injected with a marinade solution, which likely transferred the bacteria into the interior of the steaks. The establishment voluntarily recalled 739,000 pounds of beef and immediately implemented changes to their sanitation procedures. Specifically, they began dismantling and disinfecting their injection equipment on a daily basis as opposed to once a week (FSIS, 2005; Laine et al., 2005).

Outbreak 3

In August 2004, the third outbreak involving mechanically tenderized beef occurred in the Denver, Colorado area. The Colorado Department of Public Health and Environment conducted a microbial analysis using PFGE and confirmed four cases of human infection with *E. coli* O157:H7. Like before, an epidemiological study was carried out and the outbreak was linked to consumption of marinated beef steaks (FSIS, 2005); resulting in the recall of 406,000 pounds of beef produced on June 23, 2004 by an establishment located in Bolingbrook, Illinois.

Conclusion

Due to the widespread production of these non-intact products which are associated with the outbreaks previously mentioned, the expansion of the *E. coli* O157:H7 adulteration act is extremely important and concerning to the beef industry. It is evident that the processes of blade tenderization, needle tenderization and moisture enhancement have the potential for translocation of microbial flora into deep muscle tissue of beef. There are a variety of organic acids and other antimicrobial interventions that exist today; however, there is a genuine lack of research related to the use of these technologies on non-intact beef products.

CHAPTER III

METHODOLOGY

ABSTRACT

A study was conducted to examine the effectiveness of several antimicrobial products of differing chemistries in order to determine the most effective solutions that can be applied to varying industry situations. Antimicrobials (n=14) were tested for effectiveness on lean beef surfaces (5.08 cm diameter, 0.4 cm thick) within a Ross TC 700MC tenderizer (Ross Industries, Midland, VA) equipped with a Dosatron (Clearwater, FL) custom-built spray cabinet. Lean beef wafers (n=80) for each antimicrobial, which were fabricated from boneless top butt sirloins (IMPS #184), were subjected to spray treatment within this piece of equipment. Prior to treatment, samples were inoculated with 0.1 mL of 2 X 10^8 CFU/ml of *E. coli* O157:H7 cocktail (ATCC 43890, ATCC 43894, ATCC 43895, ATCC 35150). After processing samples were plated in order to achieve surface reduction effectiveness of each antimicrobial at 1 h, 1 d, 7 d, and 14 d post treatment. BeefXide was the most effective ($P < 0.05$) antimicrobial at 1 hr post processing. AvGard-Xp, AFTEC 3000, and Cytoguard Plus were the most effective ($P < 0.05$) antimicrobials at surface reduction after 1 day of vacuum-sealed, refrigerated storage (2^o C). After 7 days of storage (2^o C) under the same conditions AvGard-XP was the most effective ($P < 0.05$) at reduction of *E. coli* O157:H7, AvGard-XP remained the most effective ($P < 0.05$) antimicrobial tested after 14 days of storage.

Introduction

Researchers have studied extensively the issue of improving product tenderness in the beef industry. Grinding is the most effective means of improving beef tenderness (Johnston, 1979), however; this method is obviously not applicable to beef steaks and roasts. Meeting consumer expectations for product quality and consistency (particularly for tenderness) has been identified as a high priority by the U.S. beef industry (NCBA, 1998). Methods of tenderization have been implemented (mechanical tenderization and injection) in order to meet industry demands surrounding beef tenderness. Palatability aspects of these processes have been investigated substantially. This study focuses on the reductions of surface microbial load by the application of various antimicrobial solutions of top sirloin butt muscle prior to the mechanical blade tenderization process.

Materials and Methods

Bacterial Strains. A four strain cocktail of *E. coli* O157:H7 (ATCC 43890, ATCC 43894, ATCC 43895, ATCC 35150) were used in this experiment. These strains were outbreak isolates associated with beef. It should be noted that these strains were made constitutively resistant to gentamycin (10 µg/ml) (Sigma-Aldrich, St. Louis, MO) and rifamycin (10 µg/ml) (MP Biomedicals LLC., Solon, OH) by passage on media containing these antibiotics. The strains were made resistant to two antibiotics in order to selectively recover from non-sterile meat by plating on media containing the antibiotics at these levels. Stock strains were grown separately in Difco TM Tryptic Soy Broth (Becton, Dickinson & Company; Sparks, MD) at 37^{o} C for 24 hours, then mixed to obtain a cocktail in a 50 ml centrifuge tube.

Processing of Lean Beef Wafers. Top butt beef subprimals (IMPS #184) were acquired from a local wholesale distributor. After purchasing, subprimals were processed using a coring device **(Figure 1)**, which was used to generate lean wafers 5.08 cm in diameter (20.25 cm^{2}). Lean core wafers were then removed from the whole cut and tempered for one hour at -26.1^{o} C. After

tempering, the cores were processed on a Bizerba (Bizerba GmbH & Co. KG. Balingen, Germany) slicer to a thickness of .635 cm to create lean wafers **(Figure 2)**.

Inoculation and Spray Treatment of Lean Beef Wafers. A TC 700MC tenderizer (Ross Industries, Midland, VA) equipped with a Dosatron (Clearwater, FL) multiple nozzle custom-built spray system with 3 nozzles spraying from above the conveyor belt and 2 nozzles below the belt was used for application of the antimicrobial and water sprays to the lean beef wafers. The needle heads were removed from the Ross tenderizer since no actual tenderization was taking place. Lean wafers were inoculated with 0.1 mL of 2 X 10^8 cfu/ml of the *E. coli* O157:H7 cocktail while resting in stainless steel trays. After inoculation, the cocktail was spread over the surface of the wafers using a double-gloved finger and allowed to sit at 4^o C for 30 minutes allowing bacterial attachment. Samples were then subjected to each antimicrobial product (n=14), in which the exposure time was 18 seconds. **Figure 3.** depicts the sampling procedure, three control groups were used: 1) deionized water spray, 2) inoculated wafers absent spray treatment or 3) un -inoculated wafers; lean wafers subjected to these control treatments (n=24) were further processed at 1 h after spray, an individual set of controls was processed for each antimicrobial. In order to ensure that potential residual effects of prior antimicrobials was accounted for and previous microbial presence was eliminated, the spray reservoir was rinsed thoroughly 3 times with hot water (70^OC) and the spray machine was continually operated for 2 minutes allowing hot water to spray through nozzles. Four treatment groups existed based on the time between spraying and the plating procedure (1 h, 1 d, 7 d and 14 d). Lean wafers for 1 h (n=16), 24 h (n=16), 7 d (n=16) and 14 d (n=8) were placed on the conveyor belt (inoculated side up) of the Ross Tenderizer and subjected to an 18 second dwell time. The spray system nozzles expelled each particular antimicrobial and control treatment at a rate of 5.68 L per minute with 2.46 kg pounds per cm^2 pressure. Wafers were then collected from the opposite end of the Ross machine and placed in stainless steel trays. The same process was conducted for the water and the uninoculated controls (subjected to spray treatment by each antimicrobial tested). After retrieving samples from the Ross Tenderizer wafers randomly selected with 2 wafers being place

into sterile Stomacher filter bags (Nasco Whirl-Pak ®). The 1d, 7 d, and 14 d treatment groups were then vacuum-sealed and stored at 2^{o} C until the time of further processing.

Microbiological Sampling of Lean Beef Wafers. Each sample bag received 40.54 milliliters of Difco™ D/E Neutralizing Broth (Becton, Dickinson & Company; Sparks, MD) and was stomached on the high setting for 30 sec on each side of the filter bag with a Stomach 400 (Seward Laboratory Systems Inc., Behemia, New York). Samples were then direct plated, at the appropriate dilutions, onto Bacto™ Tryptic Soy Broth(TSA) (Becton, Dickinson & Company; Sparks, MD) containing Gentomicin Sulfate Salt (Sigma-Aldrich; St. Louis, MO) and Rifamycin SV Sodium Salt (MP Biomedicals, LLC; Solon, OH). The plates were then incubated at 40^{O}C for 48 hours, at which time the plates were counted manually for *E.coli* O157:H7 colonies. This same protocol was followed for treatment groups processed following 1 d, 7 d and 14 d of refrigerated storage.

Antimicrobial Solutions. There were fourteen antimicrobials evaluated in this study: disodium metasilicate (AvGard-XP; Danisco A/S, Copenhagen, Denmark), cetylpyridinium chloride (Cecure; Safe Foods Corp, Little Rock, Arkansas), copper sulfate pentahydrate (Preserv; Envirogreen Global Solutions, Miami, Florida), Na chlorite/citric acid/Na hydroxide (Stabilized Na Chlorite; Alliance Analytical Laboritories Inc., Coopersville, MI) Peracetic acid (Perasan; Enviro Tech Chemical Services Inc, Modesto, CA), lauric arginate & peroxyacetic acid (CytoGuard Plus (CytoGuard; A&B Ingredients, Fairfield, NJ) acidified sodium chlorite (XG-940; Dan Mar Co., Arlington, Texas), sodium chlorite & citric acid (acidified sodium chlorite; Crimson Chemicals, Fort Worth, Texas), lactic & citric acids (BeefXide; Birko Corporation, Henderson, Colorado), hydroxypropanoic acid (Lactic Acid FCC 88%; Archer Daniels Midland Company, Decatur, Illinois), hydrochloric & citric acids (Syntrx 3300; Synergy Technologies Inc., Shreveport, Louisiana), buffered sulfuric acid (AFTEC 3000; Advanced Food Technologies, LLC, Shreveport, LA), hydrochloric and citric acids (Citrilow; Safe Foods Corp, Little Rock, Arkansas), and hydrobromic acid (HB2; Enviro Tech Chemical Services, Modesto, CA). The application strength (strength of actual product dilution is listed, not active ingredient concentrations; the active

ingredient concentrations are protected for proprietary purposes by respective manufacturers), and pH for each particular antimicrobial is presented in **Table 1**.

Statistical analysis. For each set of treatments duplicated plates were obtained at each dilution level for each set of two lean samples at each time tested and averages were calculated. The cellular surface counts of *E. coli* O157:H7 on lean beef were transformed into log CFU per square cm form. Standard deviation of the log CFU/cm^2 values associated with each antimicrobial were calculated using the statistical function option offered with Microsoft Excel 2003 software (Redmond, WA). Log reduction values were considered dependent variables. Data were analyzed using version 12 of the Sigma Plot statistical package (Systat Software Inc., San Jose, CA). A one-way analysis of variance was performed and pairwise multiple comparison procedures (Holm-Sidack method) were used for mean separation of log reduction values.

Results and Discussion

Results

***E. coli* O157:H7 surface counts following 1 hour post treatment without refrigerated storage.** Microbial surface counts of lean beef wafers 1 h post treatment with various antimicrobials revealed that BeefXide was the most effective ($P < 0.05$) at reducing surface microbial load (~ 1.46 log CFU/cm^2) of *E. coli* O157:H7 (Figure. 4) on lean wafers. AFTEC 3000, Cytoguard Plus, Citrilow, and AvGard-XP were not as effective ($P < 0.05$) as BeefXide at surface reduction after 1 h; however, these solutions proved more effective ($P < 0.05$) than the remaining tested antimicrobials: Lactic Acid, XG-940, Stabilized NA-Chlorite, Perasan MP2, Acidified NA-Chlorite, Syntrx 3300, HB2, Cecure, Preserv, Water, and the inoculated controls (Inoc. CTL's) used to obtain surface attachment levels of *E. coli* O157:H7 (Figure 4).

***E. coli* O157:H7 surface counts following 1 day post treatment refrigerated storage.** Microbial surface counts of lean beef wafers after 1 day of refrigerated storage and treatment with various antimicrobials revealed that AvGard-XP, AFTEC 3000, and Cytoguard Plus were the

most effective ($P < 0.05$) at reducing surface microbial load (~2.08–1.92 log CFU/cm^2) of *E. coli* O157:H7 (Figure 5). Citrilow and HB2 were not as effective ($P < 0.05$) as AvGard-XP, AFTEC 300, and Cytoguard Plus; however, HB2 and Citrilow were more ($P < 0.05$) effective at reducing surface microbial loads than Lactic Acid, Cecure, XG-940, Acidified Sodium Chlorite, Stabilized Sodium Chlorite, Perasan-MP2, BeefXide, Syntrx 3300, Preserv and Water. As expected, Inoculated controls (Inoc. CTL's) had the highest ($P < 0.05$) surface microbial load of *E. coli* O157:H7 and were used to determine the surface attachment levels of *E. coli* O157:H7 on the top sirloin butt lean wafer samples.

E. coli* O157:H7 *surface counts following 7 days post treatment refrigerated storage. After 7 days of refrigerated storage microbial surface counts on lean beef wafers displayed that AvGard-XP was the most effective ($P < 0.05$) antimicrobial tested (Figure 6) for the reduction of *E. coli* O157:H7 (~3.61 Log CFU/cm^2). HB2, AFTEC 3000, Cytoguard Plus & Stabilized Sodium Chlorite (~ 2.30–1.89 Log CFU/cm^2) were not as effective as ($P < 0.05$) as the previously mentioned AvGard-XP. However, these antimicrobials remained more effective at the surface reduction of *E. coli* O157:H7 on lean beef wafers than Citrilow, Lactic Acid, Perasan MP2, Acidified Sodium Chlorite, Cecure, BeefXide, Syntrx 3300 and Preserv. Inoculated controls were again used to determine surface attachment levels of *E. coli* O157:H7.

E. coli* O157:H7 *surface counts following 14 days post treatment refrigerated storage. Following the completion of 14 days of refrigerated storage, surface counts of lean been wafers revealed that AvGard-XP remained the most effective ($P < 0.05$) at the reduction (~4.18 Log CFU/cm^2) of *E. coli* O157:H7 surface microbial load (Figure 7). HB2 was the next most effective ($P < 0.05$) antimicrobial used for surface reduction (~3.28 Log CFU/cm^2) of *E. coli* O157:H7 after 14 days post processing. Cytoguard Plus, Stabilized Sodium Chlorite and AFTEC 3000 were not as effective as AvGard-XP and HB2, however; these antimicrobial achieved higher ($P < 0.05$) log reductions (~2.71–2.36 Log CFU/cm^2) than Acidified Sodium Chlorite, Citrilow, Lactic Acid, Cecure, Perasan MP2, XG-940, Syntrx 3300, Preserv and BeefXide. Inoculated

controls were once again used to establish surface attachment levels of *E. coli* O157:H7 on lean beef wafers.

Discussion:

The Food Safety and Inspection Service of the United Stated Department of Agriculture (FSIS-USDA) declared *E. coli* O157:H7 an adulterant in raw ground beef in 1994, following a multi-state outbreak related to the consumption of ground beef patties (Barret et al. 1994). More recently, concerns and subsequent research efforts have shifted from ground beef to non-intact whole muscle beef products that have been injected, mechanically tenderized, or reconstructed. Outbreaks related to these non-intact products have motivated the FSIS-USDA to evaluate the safety of meat products subjected to these processes (FSIS, 2005; Laine et al., 2005).

There has been extensive research conducted that evaluates the effectiveness of injected, mechanically tenderized or reconstructed meat products on sensory attributes. Until recently, the potential microbial effects of such practices have not received much attention. It is accepted that the internal portion of whole muscle is sterile, unless subjected to grinding or some other form of reconstruction (Gill et al., 1978; Gill and Penney 1979). Injection and blade tenderization have now been identified as potential methods by which microbial cells can be translocated into the interior of whole muscle cuts of beef (Luchansky et al., 2008). This issue has became more prevalent recently due to several outbreaks linked to illnesses derived from *E. coli* O157:H7 that was transferred into the interior of meat via these methods. Echeverry et al. (2009) reported that internalization of *E. coli* O157:H7 from surface to internal muscle occurred at ~2.0 – 4.0 Log after mechanical tenderization. Theoretically, the smaller the surface microbial load of *E. coli* O157:H7 on lean beef, the less likely such a pathogen is to be transferred into the interior of products subjected to mechanical blade tenderization. The findings of this study indicate that there are different antimicrobial solutions that offer advantages over others dependent upon the conditions they will be utilized. BeefXide performed the best ($P < 0.05$) directly after treatment (~1.46 Log CFU/cm^2). However, upon vacuum storage under refrigerated conditions the performance of this product became much less effective. Indicating that there

were potential injured cells, which apparently recovered throughout the storage periods tested. Others, most notable HB2 increased in effectiveness throughout the storage process. Possessing this antimicrobial's lowest numerical reduction of *E. coli* O157:H7 (~0.34 Log CFU/cm^2), 1 hour after treatment and it highest numerical reduction (~3.28 Log CFU/cm^2) after 14 days of storage. AvGard-XP possessed a similar trend with increased efficacy over each allotted sample time. Additionally, there is potential that lactic acid producing background microflora that is commonly associated with fresh meat products could have contributed to a perceived increase in efficacy of some antimicrobials over time. Furthermore, the absence of oxygen in ground beef products has been proven to cause a decline in *E. coli* O157:H7 microbial loads, while the presence of this gas increased its viability (Brooks et al., 2008).

These variations indicate that there are industry situations in which one antimicrobial vs. another may be more suitable and vice versa. For instance, this study involved mineral acids, organic acids, and several other combinations of these. Obviously there were difference within the performance of these classes of chemistries, and some that performed at a high level are not applicable in "organic" food products. This provides an example of how industry personnel must use the data provided in this study to make decisions specific to the operations in which they work. Price structure, potential environmental implications, worker hazard issues, cost effectiveness, and other issues of this nature were not evaluated in this study. These criteria are obviously very important when making decisions regarding the use of antimicrobials in practical industry settings. Phase II of this study will focus on the transfer of surface microorganisms into the interior of whole muscle cuts after treatment with various antimicrobials. More research relative to potential antimicrobials, application rates, inoculums level, bacterial strains, and microbial attachments strengths as must be analyzed in order to successfully build a pool of information that helps our industry make relevant and informed decisions for antimicrobial use.

TABLE 1. *Antimicrobial products used for potential reduction of E. coli* O157:H7 *on lean beef wafer, active ingredients of these products, applied dilution strength and pH upon application.*

Trade Name	Active Ingredients	pH	Application Strength
AvGard-XP	Disodium Metasilicate	13.1	6% SMS (w/w)
HB2	Hydrobromic Acid	7.5	300 ppm Br
Cecure	Cetylpyridinium Chloride	7.0	0.4%
Preserv	Copper Sulfate Pentahydrate	6.8	3000 ppm
Stabilized Na Chlorite	Na Chlorite/Citric Acid/Na Hydroxide	6.5	*<1%, <1%, <1% (w/v)
XG-940	Acidified Sodium Chlorite	6.5	200 ppm
Perasan MP2	Peroxyacetic Acid	3.2	220 ppm
CytoGuard Plus	Lauric Arginate & Peroxyacetic Acid	3.0	50 ppm LAE, 220 ppm PAA
Acidified Sodium Chlorite	Sodium Chlorite acidified with Citric Acid	2.7	1100 ppm
BeefXide	Lactic & Citric Acids	2.1	*2.4%
Lactic Acid	Hydroxypropanoic Acid	1.9	5% LA (w/v)
Syntrx 3300	HCl & Citric Acids	1.2	*3%
AFTEC 3000	Buffered Sulfuric Acid	1.0	175 ppm
Citrilow	HCL & Citric Acid	0.8	*18%

*NOTE For proprietary reasons the actual concentrations have not been disclosed; the 'application strength' listed is the dilution level of the concentrate provided by the manufacturer.

Figure 1. *Coring device used for fabrication of lean beef wafer from top butt sirloins obtained for sampling procedure.*

Figure 2. *Top sirloin butt (IMPS #184) lean beef wafers used for surface inoculation samples of E. coli* O157:H7 *and application of various antimicrobial solutions.*

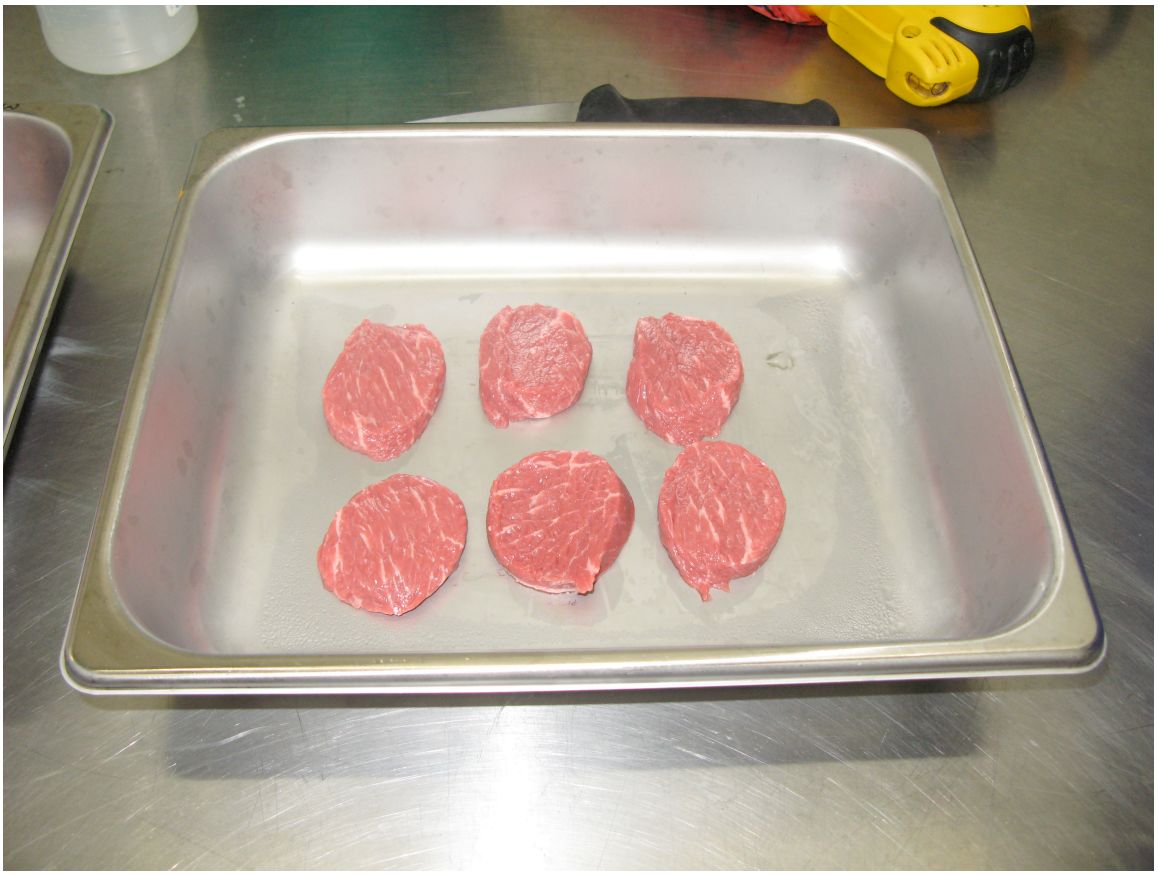


FIGURE 3. *Sampling protocol used for the collection of lean beef wafers for further analysis of surface E. Coli* O157:H7 *microbial load and subsequent antimicrobial intervention effectiveness on reduction.*

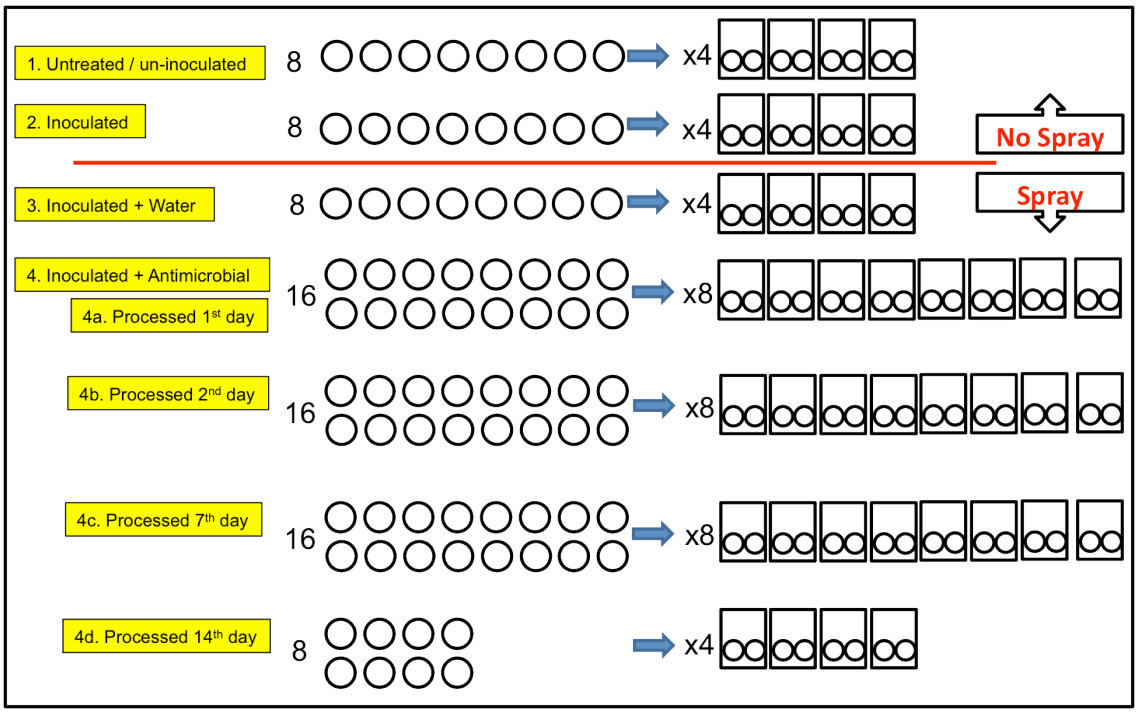
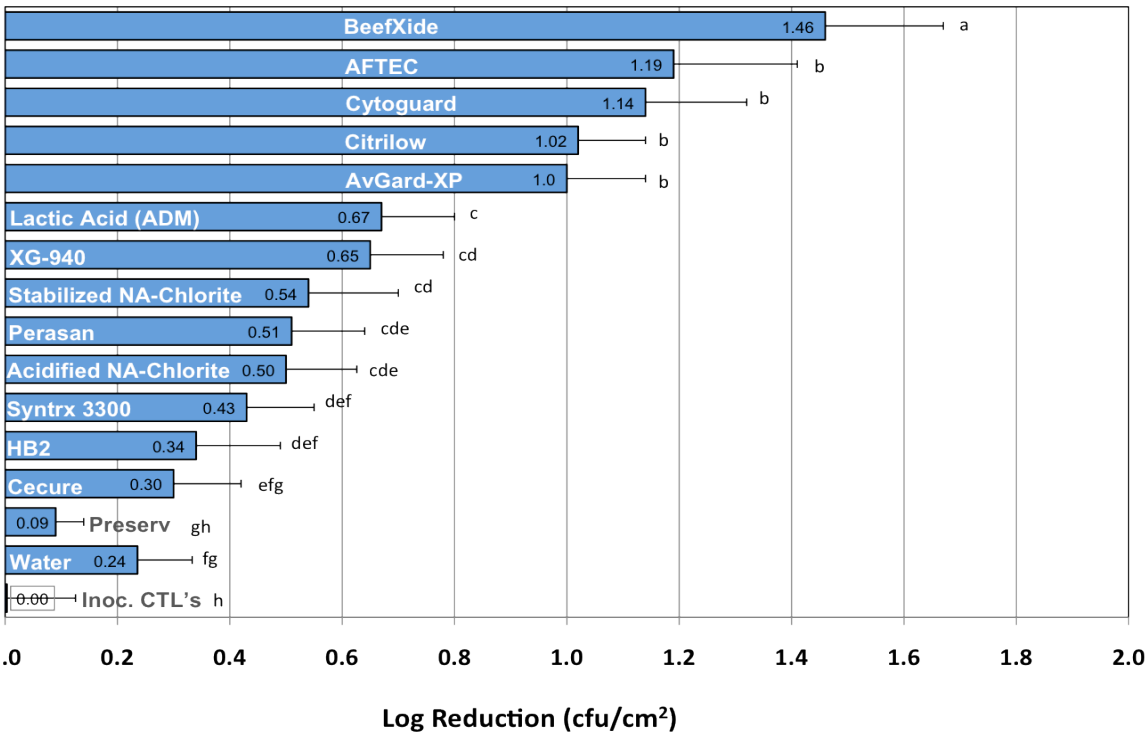
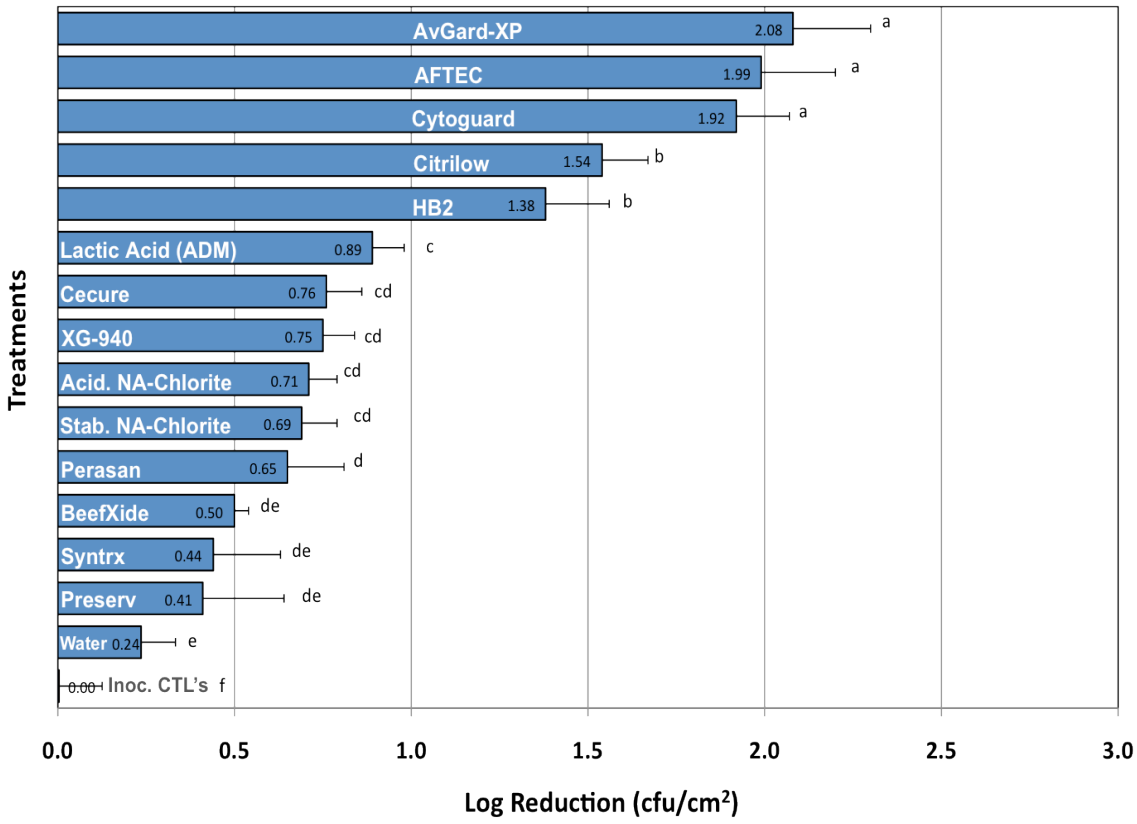


Figure 4. *Log CFU/cm^2 reduction of E. coli* O157:H7 *on lean beef samples after spray treatment with various antimicrobial interventions 1 hour post treatment.*



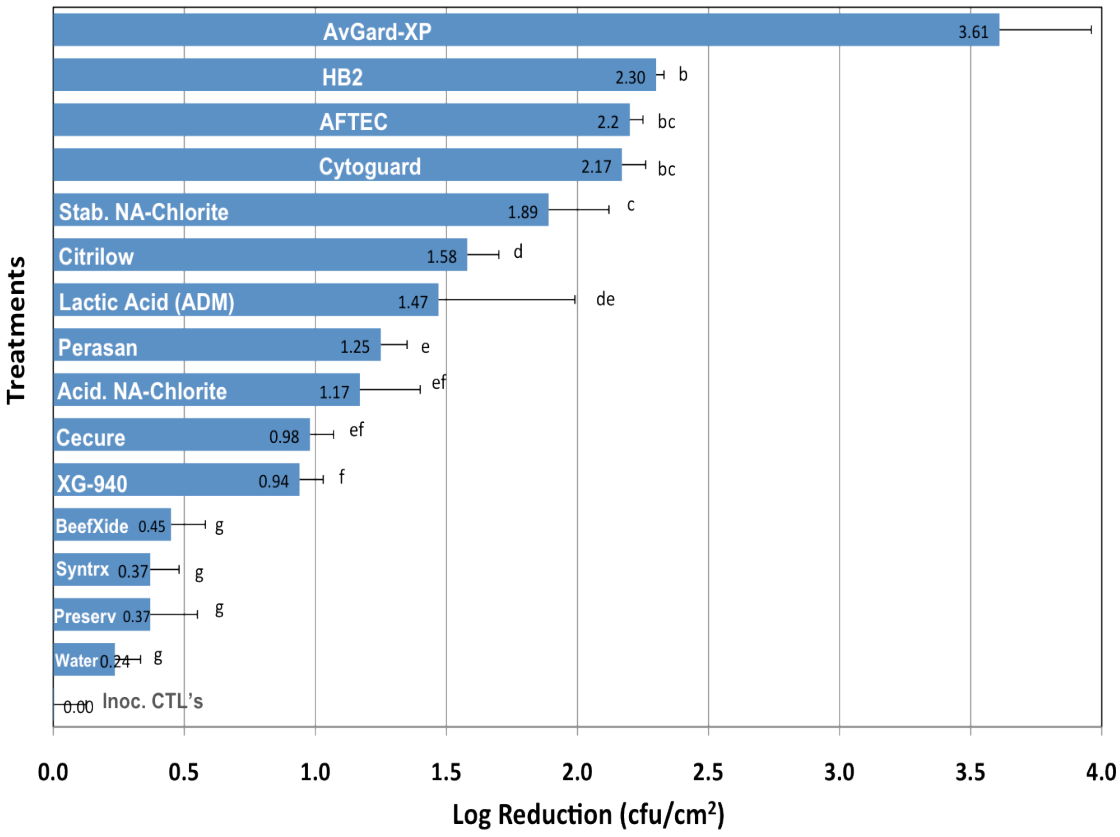
^{a,b,c,d,e,f,g,h} Means lacking a common superscript differ ($P < 0.05$)

Figure 5. *Log CFU/cm^2 reduction of E. coli* O157:H7 *of lean beef samples treated with various antimicrobial interventions 1 day post spray treatment and after vacuum storage at refrigerated temperature for 1 day.*



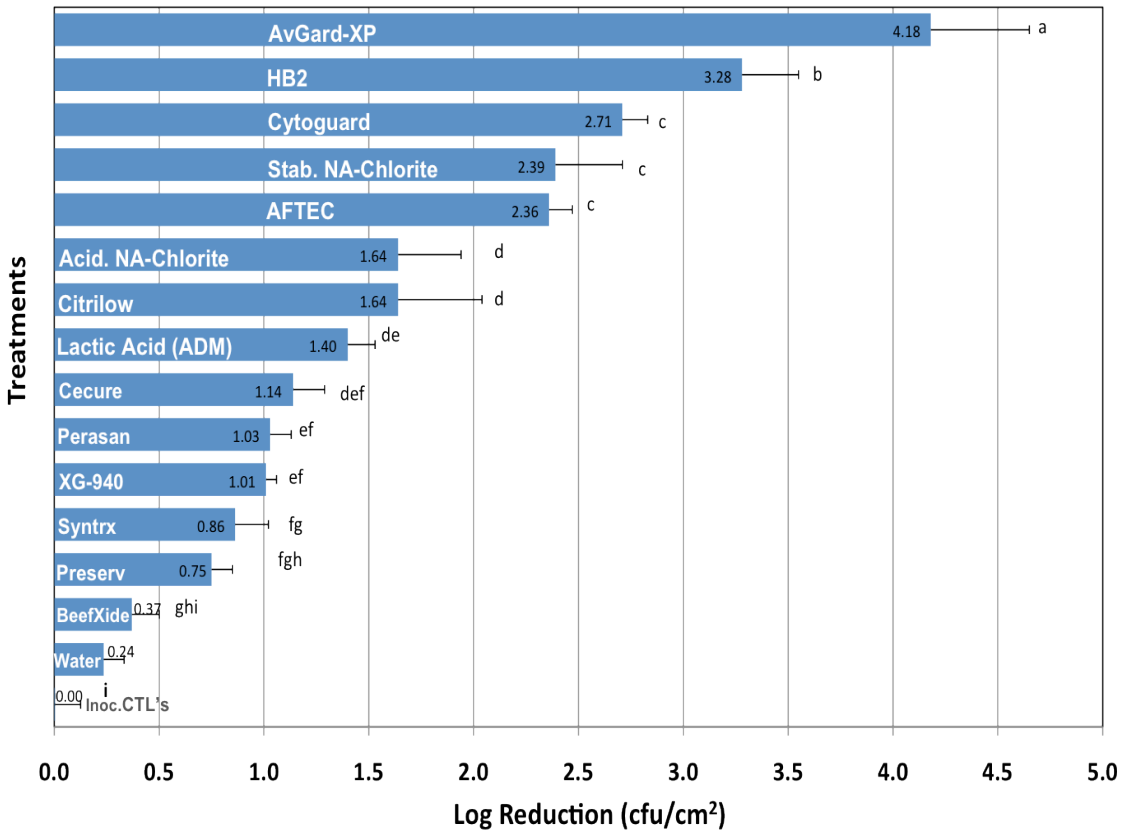
[a,b,c,d,e,f] Means lacking a common superscript differ ($P < 0.05$).

Figure 6. *Log CFU/cm^2 reduction of E. coli* O157:H7 *of lean beef samples treated with various antimicrobial interventions 7 days post spray treatment and after vacuum storage at refrigerated temperature for 7 days.*



[a,b,c,d,e,f,g,h] Means lacking a common superscript differ (P < 0.05).

Figure 7. *Log CFU/cm^2 reduction of E. coli* O157:H7 *of lean beef samples treated with various antimicrobial interventions 14 days post spray treatment and after vacuum storage at refrigerated temperature for 14 days.*



[a,b,c,d,e,f,g,h,I] Means lacking a common superscript differ (P < 0.05).

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VITA

C. Brent Wellings

Candidate for the Degree of

Master of Science

Thesis: VALIDATION OF VARIOUS ANTIMICROBIAL SOLUTIONS ON THE REDUCTION OF SURFACE MICROBIAL LOAD OF *E. COLI* O157:H7 ON LEAN BEEF

Major Field: Animal Science – Meat Science

Biographical:

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Completed the requirements for the Master of Science in Animal Science – Meat Science at Oklahoma State University, Stillwater, Oklahoma in May, 2011.

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Professional Memberships: National Auctioneers Association

Name: C. Brent Wellings Date of Degree: May, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: VALIDATION OF VARIOUS ANTIMICROBIAL SOLUTIONS ON THE REDUCTION OF SURFACE MICROBIAL LOAD OF *E. COLI* O157:H7 ON LEAN BEEF

Pages in Study: 33 Candidate for the Degree of Master of Science

Major Field: Animal Science – Meat Science

Scope and Method of Study: Antimicrobials (n=14) were tested for effectiveness on lean beef surfaces (5.08 cm diameter, 0.4 cm thick) within a Ross TC 700MC tenderizer (Ross Industries, Midland, VA) equipped with a Dosatron (Clearwater, FL) custom-built spray cabinet. Lean beef wafers (n=80) for each antimicrobial, which were fabricated from boneless top butt sirloins (IMPS #184), were subjected to spray treatment within this piece of equipment. Prior to treatment, samples were inoculated with 0.1 mL of 2 X 10^8 CFU/ml of *E. coli* O157:H7 cocktail (ATCC 43890, ATCC 43894, ATCC 43895, ATCC 35150). After processing samples were plated in order to achieve surface reduction effectiveness of each antimicrobial at 1 hr, 1 day, 7 days, and 14 days post treatment.

Findings and Conclusions: BeefXide was the most effective ($P < 0.05$) antimicrobial at 1 hr post processing. AvGard-Xp, AFTEC 3000, and Cytoguard Plus were the most effective ($P < 0.05$) antimicrobials at surface reduction after 1 day of vacuum-sealed, refrigerated storage (2^o C). After 7 days of storage (2^o C) under the same conditions AvGard-XP was the most effective ($P < 0.05$) at reduction of *E. coli* O157:H7, AvGard-XP remained the most effective ($P < 0.05$) antimicrobial tested after 14 days of storage.

Name: Type Name Here Date of Degree: May, 2001

Institution: Oklahoma State University Location: Stillwater, Oklahoma

ADVISER'S APPROVAL: Dr. Brad Morgan