ESCHERICHIA COLI O157:H7 AND *SALMONELLA* TYPHIMURIUM RISK

ASSESSMENT DURING THE PRODUCTION OF MARINATED BEEF INSIDE

SKIRTS AND TRI-TIP ROASTS

A Thesis

by

TIFFANY MARIE MURAS

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

MASTER OF SCIENCE

August 2009

Major Subject: Food Science and Technology

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	Kerri B. Harris
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ABSTRACT

Escherichia coli O157:H7 and *Salmonella* Typhimurium Risk Assessment during the

Production of Marinated Beef Inside Skirts and Tri-tip Roasts. (August 2009)

Tiffany Marie Muras, B.A., Southern Methodist University

Co-Chairs of Advisory Committee: Dr. Jeffrey W. Savell
        Dr. Kerri B. Harris

This study was conducted to determine the survival of *Escherichia coli* O157:H7

and *Salmonella* Typhimurium in marinade that was used to vacuum tumble beef inside

skirts and beef tri-tip roasts. The depth of penetration of each microorganism into the

individual meat products, and the survival of these microorganisms in the products as

well as marinade stored over time were evaluated. Two commercial marinades were

used, Reo TAMU Fajita Marinade and Legg's Cajun Style Marinade. Eighteen beef

inside skirts and 18 tri-tips were used during this study. Both inside skirts and tri-tips

were vacuum tumbled for a total of 1 h. Samples of products were tested immediately

following tumbling (day 0), or were vacuum packaged and stored in the cooler

(approximately 2˚C) to be tested 7 and 14 days following tumbling. Samples of the

spent marinade were taken and tested initially following tumbling (day 0), and were also

stored in a cooler and tested 3 and 7 days after the marinade was used. The results of the

study showed that with both marinades *S.* Typhimurium and *E. coli* O157:H7 penetrated

throughout the skirt meat. After having been stored for 7 days following tumbling, the

log value of both *S.* Typhimurium and *E. coli* O157:H7 decreased in the meat. After 14

days of storage following tumbling, the log value of both *S.* Typhimurium and *E. coli* O157:H7 continued to decrease; however, both pathogens were still detectable. The penetration of the pathogens in the tri-tip roast varied depending on the thickness of the roast. The thicker roasts had undetectable levels of both pathogens in the geometric center; however, the thinner tri-tip roasts had detectable levels at the geometric center. The spent marinade tested on day 0, 3, and 7 showed that the microorganisms were able to survive in the marinade at refrigerated temperatures. The results of this study demonstrated that pathogens may penetrate into the interior of beef skirts and tri-tips during vacuum tumbling with contaminated marinade, and that pathogens survive during refrigerated storage of spent marinade. Industry should consider these data when evaluating potential food safety risks associated with the production of vacuum tumbling beef products.

DEDICATION

I dedicate this work to my husband and family. Without their love and support, this journey would have proven to be much more difficult.

ACKNOWLEDGEMENTS

Cajun Marinade and processing parameters, which served as Marinade B in this experiment.

Thank you to Lisa Lucia, for her commitment, hard work, and assistance throughout the entirety of the project.

Finally, thanks to my family for their encouragement and to those closest to me for their patience, love, and words of wisdom.

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CHAPTER I

INTRODUCTION

Following the foodborne outbreak in late 1992 and early 1993 caused by *E. coli* O157:H7 associated with ground beef, the safety of beef products has been highly scrutinized. In 1994, USDA's Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 an adulterant in raw ground beef. In 1999, FSIS clarified that the public health risk by raw beef products contaminated with *E. coli* O157:H7 was not limited to ground beef, but also included non-intact beef products (*30*). These non-intact beef products include beef that has been injected, mechanically tenderized, or reconstructed. This definition of non-intact is consistent with the Food and Drug Administration's (FDA) food code, which defines whole muscle, intact beef, as beef that is not injected, mechanically tenderized, reconstructed, or scored and marinated (*36*).

Since the outbreaks of 1993, beef producers and packers have spent more than $420 million dollars on beef safety research (*20, 24*). These investments have resulted in the publication of a significant amount of research data, best practices, and information to assist the industry in assessing the overall risk of their processes and products. Due to FSIS's expansion of adulteration to include non-intact beef products, a great deal of research surrounding food safety questions have been related to the risk associated with the production of non-intact beef. While the

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This thesis follows the style of the *Journal of Food Protection*.

results of studies (*24*) have shown the prevalence of pathogens such as *E. coli* O157:H7 on the surface of beef subprimals is rare (*10,13*), recalls and illnesses associated with non-intact beef products have been reported (*33, 34*).

In January of 2002, the National Advisory Committee on Microbiological Criteria in Foods (NACMCF) (*18*) reviewed available data, primarily from a study by Kansas State University (KSU) (*27*), and concluded that "non-intact, blade tenderized beef steaks do not present a greater risk to consumers if the meat is oven broiled and cooked to an internal temperature of 140˚F or above". KSU also concluded that "blade tenderized beef steaks would present a greater risk when compared to intact beef steaks if they are cooked to an internal temperature below 140˚F" (*18*).

The KSU study demonstrated that blade tenderization could transfer pathogens from the surface of beef steaks to the interior tissue. However, several gaps and questions still remain related to the production of non-intact products, specifically related to marinating. For example, does the same translocation of bacteria from outside to inside occur during marination of beef roasts that occurs during tenderization of beef steaks, and if so, how far do the pathogens penetrate?

The importance of food safety and the emphasis of production practices to reduce pathogens are crucial to the success of the industry. The more knowledge gained on food safety production practices will enhance consumer confidence and reduce the risk of foodborne illness.

To provide additional information that establishments can use to strengthen their food safety programs, this project evaluated the effect of inoculated marinade used in vacuum tumbling on pathogen penetration into two different commonly marinated beef cuts. The marinade was inoculated with *S.* Typhimurium and *E. coli* O157:H7 before the product was enhanced by vacuum tumbling. The overall result showed that during the process of vacuum tumbling with inoculated marinade, the microorganisms penetrated the same distance as the marinade into the beef product. Also, the microorganisms survived in spent marinade and in beef product while being stored under refrigerated conditions.

CHAPTER II

LITERATURE REVIEW

Originally isolated in 1975 (*12*), *E. coli* O157:H7 is an microorganism that is commonly associated with foodborne illnesses that occur due to consumption of contaminated undercooked ground beef or undercooked beef products (*21*), contaminated fruits and vegetables, or contaminated water. Hundreds of thousands of kilograms of beef product are recalled every year due to *E. coli* O157:H7 contamination and illness (*21*). An estimated 62,000 cases of symptomatic *E. coli* O157:H7 infections occur annually due to foodborne exposure (*31*). Symptoms of the illness associated with *E. coli* O157:H7 include bloody diarrhea and abdominal cramping. When an individual is infected with *E. coli* O157:H7 there is usually no fever present. Infected immunocompromised individuals run the chance of the infection causing hemolytic uremic syndrome (HUS), which occurs in as many as 3,000 cases per year (*31*). HUS is a medical condition where the red blood cells of the body are destroyed, and the kidneys begin to fail. Death may occur in extreme situations where the infected individual develops HUS.

Infectious microorganisms occur naturally in beef cattle intestines. During harvesting techniques, care is taken to reduce cross contamination of feces, milk, and ingesta material from the internal organs and hide of the animal to the carcass that is meant for consumption. Research has shown that *E. coli* O157:H7 was "detected in 17% of 30 lots of carcasses sampled post processing after entering the cooler" (*7*).

Salmonella Typhimurium is an infectious microorganism that is commonly found in intestinal tracts of birds, mammals, humans, and farm animals (*12*). Because of its ability to become wide spread through multiple vectors including animals and insects, it is found on multiple food commodities. *S*. Typhimurium optimum growth is at neutral pH of 7.0, but it can also grow at a minimum pH of 4.0 and a maximum pH of 9.0 (*12*). This microorganism is rod-shaped, non-spore forming, and is Gram-negative. Symptoms of the illness caused by the microorganism include nausea, abdominal cramps, vomiting, diarrhea, fever, and headache, which can last for 3 days (*6*). *S*. Typhimurium can be transferred from human to animal, animal to human, and human to human. Common household food commodities that are not properly cleaned, prepared, or stored are may be carriers of *S*. Typhimurium to humans.

Due to the public health risk associated with *E. coli* O157:H7, FSIS has declared *E. coli* O157:H7 as an adulterant under the Federal Meat Inspection Act (*32*). *E. coli* O157:H7 adulterated beef includes raw ground beef, raw non-intact beef products, and raw intact cuts of beef that are to be further processed into non-intact beef products (*31*). Due to recalls associated with ground beef and non-intact products, FSIS issued Notice 05-09 to remind establishments that "*E. coli* O157:H7 is a hazard that establishments that receive, grind, or otherwise process raw beef products need to address in their hazard analysis" (*35*).

Originally, processing was intended to preserve meat products before refrigeration was available. Through the evolution of meat processing consumers

found favorable attributes that increased the acceptability of products such as increased flavor, juiciness, and tenderness (*22*). Over time, this process added variety to meat products, and convenience. There are more women working outside of the home compared to previous years, this has lead to an increased demand of marinated meat products purchased (*22*). A study done by Marriott et al. found that vacuum tumbling bone out hams can increase cure penetration rate (*16*). They also found that the bone out hams vacuum tumbled for 4 or 6 hours allowed hams to fully cure by 28 to 36 days compared to those normally cured for 70 days (*16*). The vacuum tumbled bone out hams also required less curing salt due to the vacuum tumbling (*16*). Krause et al. found that vacuum tumbling hams that were previously injected with brine retained more brine compared to hams that were only injected with brine (*14*). This implies there was more retention of brine or marinade due to vacuum tumbling compared to non-vacuum tumbling. Because of the increased use of marinated products, the population at risk is widespread from individuals who are young, elderly, or those who are immunocompromised. Marinated products that are used in school lunch programs and those used in elderly home facilities and hospitals could cause major problems if they are not prepared properly. The other group that would be detrimentally affected is the product manufacturer, and other producers of marinated products. In the event contaminated product enters commerce and individuals become ill, a recall would be required and the possible financial shut down of the company would result.

Due to FSIS policy and illnesses associated with non-intact beef products, researchers have performed numerous studies on blade tenderization and intervention strategies and to determine the prevalence of *E. coli* O157:H7. According to Sporing (*27*) blade tenderization transfers 3-4% of surface contamination to the interior of the muscle. Gill et al. (*8*) also recovered microorganisms in the deep tissue of mechanically tenderized retail cuts. Luchansky et al. (*15*) also found that blade tenderization transferred *E. coli* O157:H7 primarily into the topmost 1 cm, but also in the deeper tissues of beef subprimals. Therefore, it has been well documented that blade tenderization can translocate pathogens from the surface to the interior tissue of beef. Fortunately studies have shown that *E. coli* O157:H7 is not commonly found on beef subprimals (*10, 13*).

Popularity of marinated products has increased throughout the United States due to their quick preparation time and increased eating experience. Marination of beef is often used to increase consumer acceptability and improve the value (*25*). In a study conducted by Behrends et al. (*1*), marinated steaks cooked to at least a medium well degree of doneness were found to have a higher flavor than unmarinated steaks. According to Molina et al. (*17*) marinated, needle-pumped, and vacuum tumbled products were generally more palatable than untreated beef steaks. As described above, research has shown the benefits of marination in order to improve beef quality; however, there is limited information on the effect of vacuum tumbling marination on translocation of pathogens.

All inspected establishments that manufacture meat and poultry products, including those producing non-intact beef, are required to have a Hazard Analysis and Critical Control Point (HACCP) plan in place (*29*). A HACCP plan is defined as "a management system focused on prevention of problems in order to assure the production of food products that are safe to consume" (*25*). There are several intervention methods that are commonly used as part of an establishments HACCP/food safety system to reduce contamination and prevent the spread of both *S*. Typhimurium and *E. coli* O157:H7 (*4, 5, 9*).

Based on FSIS's requirement to address *E. coli* O157:H7 in HACCP plans for non-intact products (*35*), establishments must fully understand the implications of marination on the food safety hazards of concern. Because it is known that beef products are a common carriers of microorganisms, facilities that process beef products in marinade have to be cautious of cross-contamination. Some processing facilities use spent marinades several times before discarding and making a new batch. Some marinades may have antimicrobial attributes due to the pH, salt concentration, or added spices. *E. coli* O157:H7 has a minimum pH value for growth of 4.5, and a maximum pH value of 9. *E. coli* O157:H7 can tolerate salt to certain percentages (*12*). "Four point five percent NaCl in broth causes a threefold increase in doubling time, whereas at 6.5%, a 36-hour lag was noted with a generation time of 31.7 hours" (*12*).

In conclusion, *E. coli* O157:H7 and *S*. Typhimurium are microorganisms that have detrimental effects on the beef industry. If not addressed properly,

contaminated marinade used to process multiple batches of non-intact beef product

could contribute to widespread illnesses, death, and recalls of product.

CHAPTER III

MATERIALS AND METHODS

3.1 Raw Material

Eighteen vacuum packaged, beef inside skirts (Institutional Meat Purchase
Specifications, IMPS, #121C), and 18 vacuum packaged tri-tip roast (IMPS #185D)
were obtained for this project from a commercial processing facility and shipped to
the Rosenthal Meat Science and Technology Center at Texas A&M University
(College Station, TX) (*19*).   Products were kept in a temperature controlled
environmental room (Frigitemp, College Station, TX) at 4.4˚C. Two different
marinade formulations, Reo TAMU Fajita Marinade (Reo Spice & Seasoning, Inc.,
Huntsville, TX), and Legg's Cajun Marinade (A. C. Legg, Inc., Calera, AL), and
associated processing parameters were obtained from commercial processing
facilities in Texas.

3.2 Preparation of Marinade

The dry marinade was mixed according to manufacturer's instructions.
Legg's Cajun Marinade consisted of 215.40 g of dry seasoning added to 986.87 ml
of distilled water. Reo TAMU Fajita Marinade consisted of 272.15 g of dry
seasoning, 29.93 g of sodium tripolyphosphate and 1,391.14 ml of distilled water.
Both marinades were stirred using a hot plate/stirrer (VWR, Suwannee, GA) for 30
min.

3.3 Bacterial Cultures

Previously described (*9*), rifampicin-resistant strains derived from parent strains of *S*. Typhimurium and *E. coli* O157:H7 obtained from the Food Microbiology Laboratory, Texas A&M University, were used to inoculate the marinades used in this study. The selected rifampicin-resistant cultures were maintained on tryptic soy agar (TSA, Becton Dickinson, Sparks, MD) slants at 25˚C. Cultures were transferred once a month throughout the project.

3.4 Inoculum Preparation

Two days prior to each experiment, the rifampicin-resistant cultures of *E. coli* O157:H7 and *S*. Typhimurium and were propagated by transferring 1 loop of the stored microorganism to a new TSA slant and incubated at 37˚C for 24 h. Each culture was transferred individually using a sterile loop, to 10 ml of tryptic soy broth (TSB, Becton Dickinson) and was incubated for 18 h at 37˚C. Prior to dispensing 1 ml of each pathogen into two 99 ml milk dilution bottle of sterile buffered peptone water (BPW, Becton Dickinson), each 18 h culture was vortexed in order to provide a uniform distribution of the microorganism.

In order to inoculate the marinade, 60 ml (120 ml total) was taken from each of the 99 ml (*E. coli* O157:H7 and *S*. Typhimurium) inoculated BPW, and was added to 960 ml of marinade, resulting a total volume of 1200 ml of marinade and inoculum. The inoculated marinade was added to the BIRO vacuum tumbler Model

VTS-42 (Biro MFG Co., Marblehead, OH). Three tri-tips or three inside skirts were added to the tumbler on each sampling day.

3.5 Product Inoculation

The product was tumbled for 15 min, and then was allowed to rest for 5 min, and the tumbler drum was manually rotated. This tumble/rest procedure was formulated in order to accomplish maximum marinade pick up within the product. This tumble/rest process was completed 3 additional times for a total of 1 h tumbling/rest time. The 3 pieces of product were then removed and placed on sterile foil. One of these pieces was sampled immediately and represented 0 day. The 2 remaining pieces of product were individually vacuum packaged (KOCH Vacuum Packaging Machine, Kansas City, MO) and held at 4˚C to be sampled at 7 days and 14 days.

3.6 Sampling and Microbiological Examinations

Before inoculation, negative control surface samples of tri-tip roast and inside skirt were collected to measure possible natural presence of marker microorganism by excising a 10 cm^2 x 3 mm in depth sample using a sterile stainless steel borer, scalpel and forceps. The sample was placed in a sterile stomacher bag to which 99 ml of BPW were added and pummeled for 1 min at 230 rpm using a Stomacher® 400 (Seaward, England). Counts were determined by plating 1 ml (.25 ml on each of four plates) of the sample homogenate, 0.1 ml of the homogenate and

the appropriate 10-fold dilutions of the same on pre-poured and dried lactose-sulfite phenol red rifampicin (LSPR) agar plates (*4*). Non-inoculated marinade was plated on LSPR, by dispensing 1 ml (0.25 ml on each of four plates) to determine the natural presence of marker microorganisms. LSPR is a selective and differential medium designed for the simultaneous enumeration of both marker pathogens. LSPR plates were incubated for 24 h at 35˚C before counting and reporting the number of rifampian-resistant *E. coli* O157:H7 and *S*. Typhimurium/cm^2.

3.7 Inoculated Samples

The inoculum cocktail containing the marker organisms *S.* Typhimurium and *E. coli* O157:H7 were plated on LSPR each sampling day in order to determine the initial bacterial population of the individual microorganisms. Two samples of the spent marinade were taken immediately following tumbling of the inoculated marinade and product. One sample was plated immediately to determine the log value of the microorganism after being introduced to the marinade and having been tumbled under vacuum pressure with meat product for 1 h. The other sample was stored at 4˚C and plated on day 3 and day 7.

3.8 Product Sampling

The product was removed from the tumbling drum and placed on foil, using a flamed scalpel blade, and forceps, two 5 cm x 5 cm square plugs were removed from the center of each tri-tip and inside skirt. The square plugs were placed

individually on foil and the depth measurement of each plug was recorded as shown in Fig. 1.

Using forceps dipped in 95% ethanol and then flamed, the plug was removed from the foil and was dipped into 95% ethanol, covering the bottom and all four sides, avoiding the top from being submerged as shown in Fig. 2. The four plug sides and bottom were further flamed (BonJour Professional Cooking Torch, Taiwan) in order to ensure the reduction of microorganisms on the outer surface. This was to reduce cross contamination from exterior to interior when sampling as shown in Fig. 3. The plug was placed on a new piece of foil. Again, using a flamed scalpel blade and forceps, a cross-sectional cut was made by an approximate 3 mm in depth upper layer. This cross sectional sample was placed in a sterile Petri dish as shown in Fig. 4.

The plug was again torched on all 4 sides and placed on new foil. The second cross-sectional sample was taken using flamed scalpel blade and forceps with an approximate depth of 3 mm. This second cross-sectional sample was placed in a sterile Petri dish. This process was repeated on all tri-tip samples until the geometric center of the plug was reached. There were 2 samples taken from the inside skirt plug, exterior and interior. The cross sectional samples in the Petri dishes were sampled by excising a 10-cm^2 sample using a flamed stainless-steel borer, scalpel, and forceps, as shown in Figs. 5 and 6, and placed into sterile stomacher bags to which 99 ml BPW were added.
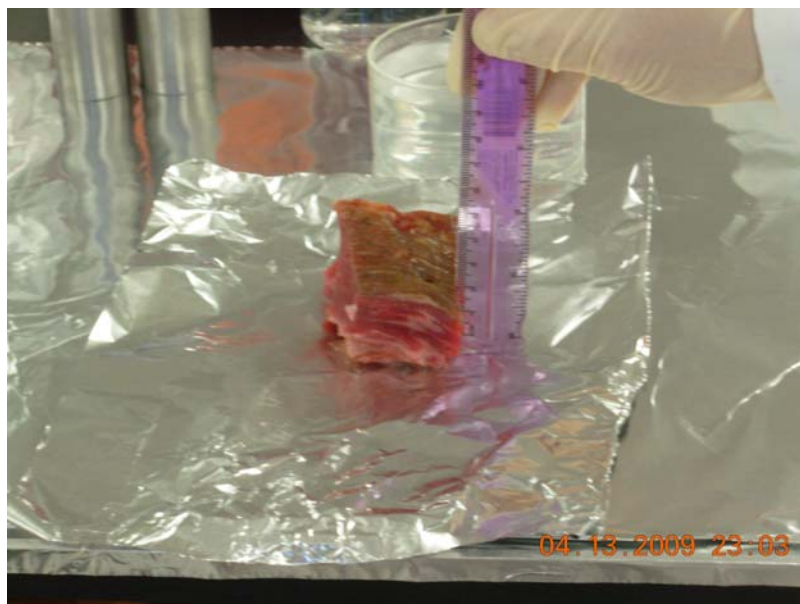
FIGURE 1. *Depth measurement of meat product plug sample.*

FIGURE 2. *Meat plug dipped in 95% ethanol on all 4 sides and bottom.*

FIGURE 3. *Meat plug flamed on all four sides and bottom.*

FIGURE 4. *Cross sectional sample taken from top of plug.*

FIGURE 5. *Core taken from cross sectional sample in Petri dish.*

FIGURE 6. *10-cm² sample taken with core from cross sectional sample.*

Colonies were counted, recorded and reported as log CFU/cm^2 following

Culture Methods for Enumeration of Microorganisms (*28*).

3.9 Weights of Product, pH of Marinade, Salt Concentration of Marinade

Tri-tips and inside skirts were weighed on a Mettler digital calibrated scale

(VWR, Suwanee, Georgia) both before tumbling and after tumbling to determine

percent pick up. The pH was determined on all batches of non-inoculated marinade

using a Symphony pH meter (VWR, Suwanee, Georgia). Salt concentration was

determined using an Orion Model concentration meter (Orion Research, Inc.,

Beverly, MA).

3.10 Replication of Experiment

The inoculation process of the marinade and the tumbling process occurred

each time prior to tumbling. This process was run in triplicate for each meat by

marination combination (tri-tip and Marinade A, tri-tip and Marinade B, inside skirt

and Marinade A, inside skirt and Marinade B). The number of cross-sectional

samples taken from the square 5 cm x 5 cm plug of the tri-tip varied from 4-7

samples depending on the thickness of the tri-tip. When evaluating the inside skirt,

2 samples were taken from each 5 cm x 5 cm plug, exterior and interior.

3.11 Statistical Analysis

Data were analyzed using PROC GLM of SAS (SAS Institute, Inc., Cary, NC). Least squares means were generated for main effects and separated using PDIFF option when appropriate with an alpha-level ($P < 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Inside Skirt and Tri-tip Roast Analysis

The initial concentration of *S.* Typhimurium inoculum in the Marinade A

before tumble ranged from of 4.7 to 5.5 log CFU/ml, and the inoculum level after

tumble was a minimum of 4.6 to a maximum of 5.1 log, on the three tumbles of

inside skirt meat (3 tumbles of 3 inside skirts each). The initial concentration of *E.*

coli O157:H7 inoculum in the Marinade A before tumble ranged from of 4.7 to 5.5

log CFU/ml, and the inoculum level after tumble ranged from of 4.4 to 4.7 log

CFU/ml, on the 3 tumbles of inside skirt meat (3 tumbles of 3 inside skirts each). It

is important to note that the initial log value used to inoculate the marinades used in

this experiment is higher than what would be expected in marinade found in a plant.

However, this is an initial inoculum level commonly used in laboratory experiments

in order to determine levels of log reductions. Using lower levels of pathogens or

those levels more comparable to what may be found in processing make it difficult

to evaluate reductions in a laboratory setting. The decrease in levels of

microorganisms in the marinade after tumble could be attributed to salt

concentration and pH. There were no antimicrobial ingredients identified in the

marinade.

After tumbling inside skirt meat in the inoculated marinade, the penetration

of the microorganisms was evident throughout the inside skirt meat as shown by

Table 1. For the purpose of discussion, it was determined that at least a 1 log difference would indicate a microbiological difference, while statistically less than a 1 log change may have differed ($P < 0.05$). The 1 log was chosen for microbiological difference because it has been commonly referenced when evaluating antimicrobial interventions. The counts of both microorganisms on the exterior inside skirt meat treated with Marinade A was the same as the level on the interior inside skirt meat, both statistically and microbiologically. It was evident that once the product was stored for 7 days, in refrigerated temperature, after being tumbled in inoculated Marinade A, there was a similar log count, both statistically and microbiologically, of *S.* Typhimurium compared to that of day 0 for samples. The statistical significant difference ($P < 0.05$) of a decrease in log count in *S.* Typhimurium occurred when the product was stored for 14 day. Although this was statistically different, it was not microbiologically different. A study done by Jacob et al. (*11*) on kippered beef during extended refrigeration storage shows concurrent data of a decrease in log value of *E. coli* O157:H7 and *S.* Typhimurium on processed beef product. One parameter to be considered was the Jacob et al. (*11*) paper used processed beef with a lower a_w compared to that of the current project being reported.

The inside skirt meat had a significant decrease in *E. coli* O157:H7 log value on 7 day compared to the log value found on inside skirt meat at 0 day. The statistical significant difference ($P < 0.05$) of a decrease in log count in *E. coli* O157:H7 occurred on the product after being stored for 7 day, and again after the

TABLE 1. *Least squares means for interior and exterior layers effect on counts (log$_{10}$ CFU/cm^{2}) of* S. *Typhimurium and* E. coli *O157:H7 for Marinade A treated inside skirts.*

	Layer	
	Interior	Exterior
S. Typhimurium	4.1[a]	4.2[a]
	(0.1)[1]	*(0.2)*[1]
E. coli O157:H7	3.5[a]	3.6[a]
	(0.2)[1]	*(0.2)*[1]

[a] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).
[1]SEM = is the standard error of the least squares means.

product was stored for 14 day as shown in Table 2. There was a greater than 1 log difference when comparing day 0 to day 14. The decrease in log value could be attributed to several factors in the inoculated skirt including: salt concentration, pH, and refrigerated vacuum packaging. Studies done by Calicioglu et al. (*2, 3*) observing the fate of *E. coli* O157:H7 and *S*. Typhimurium in marinated and non-marinated jerky strips post drying for 28 days of storage reflect similar results of decreasing log value on both surfaces of marinated and non-marinated product.

The initial concentration of *S*. Typhimurium in the Marinade B before tumble ranged from 4.7 to 6.5 log CFU/ml, and the inoculum level after tumble ranged from 4.8 to 5.2 log CFU/ml, on the 3 tumbles of inside skirt meat (3 tumbles of 3 inside skirts each). The initial concentration of *E. coli* O157:H7 inoculum in Marinade B before tumble ranged from 4.4 to 6.5 log CFU/ml, and the inoculum level after tumble ranged from 4.8 to 5.6 log CFU/ml, on the 3 tumbles of skirt meat (3 tumbles of 3 inside skirts each). The decrease in log value in the marinade could be attributed to salt concentration and pH.

After tumbling skirt meat in inoculated marinade, the penetration of the microorganisms was evident throughout the skirt meat as shown in Table 3. The microbiological counts of both microorganisms was similar in exterior and interior samples of inside skirt meat treated with Marinade B. There was a significant difference ($P < 0.05$) in log values of both microorganisms when comparing samples

TABLE 2. *Least squares means for treatment day effect on counts (log_{10} CFU/cm^2) of* S. *Typhimurium and* E. coli *O157:H7 for Marinade A treated inside skirts.*

	Day		
	0	7	14
S. Typhimurium	4.4^a	4.2^a	3.8^b
	(0.1)[1]	*(0.1)*[1]	*(0.1)*[1]
E. coli O157:H7	4.2^a	3.5^b	3.0^c
	(0.1)[1]	*(0.1)*[1]	*(0.1)*[1]

[a-c] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).

[1]SEM = is the standard error of the least squares means.

TABLE 3. *Least squares means for interior and exterior layers effect on counts (log$_{10}$ CFU/cm^2) of* S. *Typhimurium and* E. coli *O157:H7 for Marinade B treated inside skirts.*

	Layer	
	Interior	Exterior
S. Typhimurium	3.9[a]	3.9[a]
	(0.1)[1]	(0.1)[1]
E. coli O157:H7	3.5[a]	3.4[a]
	(0.2)[1]	(0.2)[1]

[a] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).
[1]SEM = is the standard error of the least squares means.

from 0 day to samples taken on 7 day. Although there was not a greater than 1 log difference when comparing day 0 to day 7 of *S*. Typhimurium, there was a greater than 1 log difference in *E. coli* O157:H7 on those days. When comparing the results of 7 days to that of the samples on 14 days there was a statistical significant difference ($P < 0.05$) in log value of both microorganisms as shown in Table 4; however, there was not a microbiological difference. Comparing day 0 to day 14 there was a greater than 1 log difference in presence of *S*. Typhimurium, as seen in Table 4. The decrease in log value could be attributed to several factors in the inoculated inside skirt including: salt concentration, pH, and refrigerated vacuum packaging.

For the tri-tips, the initial log CFU/ml concentration of *S*. Typhimurium inoculum in the Marinade A treated before tumble ranged from 5.0 to 6.0 log CFU/ml, and the inoculum level after tumble ranged from 4.6 to 4.9 log CFU/ml, on the 3 tumbles (3 tumbles of 3 tri-tip each). The initial concentration of *E. coli* O157:H7 inoculum in the Marinade A before tumble ranged from 4.9 to 5.9 log CFU/ml, and the inoculum level after tumble ranged from 4.7 to 5.1 log CFU/ml, on the 3 tumbles of tri-tip (3 tumbles of 3 tri-tip).

There was a significant difference ($P < 0.05$) in log values of both organisms as the depth increases and samples are taken towards the geometric center as shown in Table 5. Tri-tips treated with Marinade A indicated similarities in log values of *S*. Typhimurium between 0-3 and 3-6 mm depths. There was a statistical significant difference ($P < 0.05$) between log values of *S*. Typhimurium at depths of 0-3 and 6-9

TABLE 4. *Least squares means for treatment day effect on counts (log_{10} CFU/cm^2) of* S. *Typhimurium and* E. coli *O157:H7 for Marinade B treated inside skirts.*

	Day		
	0	7	14
S. Typhimurium	4.3[a]	3.9[b]	3.6[c]
	(0.1)[1]	(0.1)[1]	(0.1)[1]
E. coli O157:H7	4.4[a]	3.2[b]	2.9[c]
	(0.1)[1]	(0.1)[1]	(0.1)[1]

[a-c] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).

[1]SEM = is the standard error of the least squares means.

TABLE 5. *Least squares means for depth (mm) effect on counts (log_{10} CFU/cm^2) of* S. *Typhimurium and* E. coli *O157:H7 for Marinade A treated tri-tips.*

	Depth (mm)						
	0-3	3-6	6-9	9-12	12-15	15-18	18-21
S. Typhimurium	4.1[a]	3.5[ab]	3.2[bc]	2.7[cd]	2.4[d]	2.3[d]	2.5[cd]
	(0.2)[1]	(0.2)[1]	(0.2)[1]	(0.2)[1]	(0.2)[1]	(0.3)[1]	(0.4)[1]
E. coli O157:H7	3.9[a]	3.4[ab]	3.1[bc]	2.7[bc]	2.5[c]	2.4[c]	2.3[bc]
	(0.2)[1]	(0.2)[1]	(0.2)[1]	(0.2)[1]	(0.2)[1]	(0.3)[1]	(0.5)[1]

[a-d] Least squares means within a row lacking a common letter significantly differ (*P* < 0.05).

[1]SEM = is the standard error of the least squares means.

mm, however there are similarities between depths of 3-6 and 6-9 mm. There are

similarities in log values of *S.* Typhimurium between depths of 6-9 and 9-12 mm;

however, there are statistical significant differences ($P < 0.05$) between depths of 3-6

and 19-12 mm. As shown in Table 5, there are similarities for *S.* Typhimurium

between depths of 9-12, 12-15, 15-18, and 18-21 mm; however, there was a

statistical significant difference ($P < 0.05$) between depths of 6-9, 12-15, and 15-18

mm. There are similarities for both microorganisms between depths of 6-9 and 18-21

mm. This similarity may be attributed to the 7th sample being taken through the

geometric center of the meat core where log values would be expected to begin

increasing in value because penetration of the marinade would occur from both

sides. Tri-tips treated with Marinade A show there to be similarities in log values of

E. coli O157:H7 between depths of 0-3 and 3-6 mm. There are statistical significant

differences ($P < 0.05$) in log value of *E. coli* O157:H7 between depths of 0-3 and 6-9

mm, however depths of 3-6 mm are similar to log values at depths of 6-9 mm.

Similarities are found amongst depths of 6-9 and 9-12 mm; however, there are

statistical significant differences ($P < 0.05$) between depths of 3-6 and 9-12 mm.

Similarities were found between depths of 9-12, 12-15, 15-18, and 18-21 mm. There

were also similarities found between depths of 6-9 mm and depths of 18-21 mm as

shown in Table 5. As stated above, the similarity may be attributed to the 7th sample

being taken through the geometric center of the meat core. There was a greater than

1 log difference between depths of 0-3 mm and 9-12 mm of both microorganisms.

Patel et al. (*21*) found that "it is highly unlikely for pathogens to migrate below the

surface of intact untreated meat; however, blade tenderization may allow the introduction of pathogens below the surface rendering the beef steak as nonintact cuts of beef". Patel el al. also found that there was cross contamination into the interior muscle, and that cooking to lower degrees of doneness resulted in survival of *E. coli* O157:H7 in the interior (*21*). This experiment coincides with the survival of microorganisms in interior muscles due to penetration of inoculated marinade. Analysis of variance revealed statistical significant differences ($P < 0.05$) of log values for both microorganisms between 0 day and 7 day treated with Marinade A. However, there were similarities between 0 day and 14 day for *S.* Typhimurium, as well as similarities between 7 day and 14 day as shown in Table 6. There were no microbiological differences between any of the days for either of the microorganisms.

For Marinade B, the initial concentration of *S.* Typhimurium inoculum before tumble ranged from 4.5 to 4.8 log CFU/ml, and the inoculum level after tumble ranged from 4.4 to 4.9 log CFU/ml, on the 3 tumbles of tri-tip (3 tumbles of 3 tri-tip each). The initial concentration of *E. coli* O157:H7 inoculum in the Marinade B before tumble ranged from 4.6 to 4.8 log CFU/ml, and the inoculum level after tumble ranged from 4.9 to 5.1 log CFU/ml, on the 3 tumbles of tri-tip (3 tumbles of 3 tri-tip each).

There was a statistical significant difference ($P < 0.05$) in log values of both organisms as the depth increased and samples were taken towards the geometric center as shown in Table 7. Tri-tips treated with Marinade B are similar in log

TABLE 6. *Least squares means for treatment day effect on counts (log_{10} CFU/cm^2)*
of S. *Typhimurium and* E. coli *O157:H7 for Marinade A treated tri-tips*.

	Day		
	0	7	14
S. Typhimurium	2.7[b]	3.8[a]	2.8[ab]
	(0.2)[1]	(0.2)[1]	(0.4)[1]
E. coli O157:H7	2.8[b]	3.6[a]	2.8[ab]
	(0.2)[1]	(0.2)[1]	(0.3)[1]

[a,b] Least squares means within a row lacking a common letter significantly differ (P < 0.05).

[1]SEM = is the standard error of the least squares means.

counts of *S.* Typhimurium between depths of 0-3 and 3-6 mm, and similarly between depth of 0-3 and 6-9 mm; however, there was a statistical significant difference ($P < 0.05$) in depth at 3-6 and 6-9 mm. There was a statistical significant difference ($P < 0.05$) between 6-9 and 9-12 mm in log value of *S.* Typhimurium. Depth of 9-12, 12-15, 15-18, and 18-21 mm all have similar log values. There was a greater than 1 log difference from depth 0-3 mm to 12-15 mm. Tri-tips treated with Marinade B had similarities in microbiological counts of *E. coli* O157:H7 at depths of 0-3, 3-6, and 6-9 mm. There were also similarities between depths of 9-12, 12-15, 15-18, and 18-21 mm. However, depths at 0-3, 3-6, and 6-9 mm were statistically significantly different ($P < 0.05$) from depths of 9-12, 12-15, 15-18, and 18-21 mm as seen in Table 7. There was a greater than 1 log difference for *E. coli* O157:H7 when comparing depth 0-3 mm to 15-18 mm.

Analysis of variance revealed significant differences ($P < 0.05$) of *S.* Typhimurium in tri-tips treated with Marinade B from 0 day to 7 day; however, there were similarities between 0 day and 14 day, and between 7 day and 14 day as shown in Table 8. Analysis of variance revealed significant difference ($P < 0.05$) in counts of *E. coli* O157:H7 between 0 day and counts from 7 day and 14 day; however, there were similarities between 7 day and 14 day as shown in Table 8. The decrease in log value could be attributed to several factors in the inoculated inside skirt including: salt concentration, pH, and refrigerated vacuum packaging. The increase in log value of the microorganisms from 7 day to 14 day could be attributed to the microorganisms stabilizing in the environment.

TABLE 7. *Least squares means for depth (mm) effect on counts (log_{10} CFU/cm^2) of* S. *Typhimurium and* E. coli *O157:H7 for Marinade B treated tri-tips.*

	Depth (mm)						
	0-3	3-6	6-9	9-12	12-15	15-18	18-21
S. Typhimurium	3.3[ab]	3.5[a]	2.9[b]	2.4[c]	2.1[c]	1.9[c]	1.6[c]
	(0.2)[1]	*(0.2)*[1]	*(0.2)*[1]	*(0.2)*[1]	*(0.2)*[1]	*(0.3)*[1]	*(0.4)*[1]
E. coli O157:H7	2.9[a]	3.2[a]	2.7[a]	2.2[b]	2.0[b]	1.9[b]	1.4[b]
	(0.2)[1]	*(0.2)*[1]	*(0.2)*[1]	*(0.2)*[1]	*(0.2)*[1]	*(0.3)*[1]	*(0.4)*[1]

[a, b] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).

[1]SEM = is the standard error of the least squares means.

TABLE 8. *Least squares means for treatment day effect on counts (log_{10} CFU/cm^2) of* S. *Typhimurium and* E. coli *O157:H7 for Marinade B treated tri-tips.*

	Day		
	0	7	14
S. Typhimurium	3.0[a]	2.5[b]	2.7[ab]
	(0.2)[1]	(0.2)[1]	(0.2)[1]
E. coli O157:H7	3.0[a]	2.2[b]	2.2[b]
	(0.1)[1]	(0.1)[1]	(0.2)[1]

[a,b] Least squares means within a row lacking a common letter significantly differ ($P < 0.05$).
[1]SEM = is the standard error of the least squares means.

4.2 Marinade Analysis

Samples of marinade were plated as controls, and the samples were run for pH and salt concentration. Analysis of variance revealed no differences ($P \geq 0.05$) in log CFU/ml for *E. coli* O157:H7 (day 0: 4.9; day 7: 4.9; day 14: 4.5) and *S.* Typhimurium (day 0: 4.8; day 7: 4.5; day 7: 4.4) for Marinade A that treated tri-tip meat on Day 0, 3, or 7. There were no differences ($P \geq 0.05$) in log CFU/ml for *E. coli* O157:H7 (day 0: 4.4; day 7: 3.7; day 14: 4.2) and *S.* Typhimurium (day 0: 4.0; day 7: 4.5; day 7: 4.4) for Marinade B that treated tri-tip meat on Day 0, 3, or 7. There were no differences ($P \geq 0.05$) in log CFU/ml for *E. coli* O157:H7 (day 0: 4.6; day 7: 3.8; day 14: 4.6) and *S.* Typhimurium (day 0: 4.9; day 7: 4.7; day 7: 4.4) for Marinade A that treated inside skirt meat on Day 0, 3, or 7. There were no differences ($P \geq 0.05$) in log CFU/ml for *E. coli* O157:H7 (day 0: 5.2; day 7: 4.4; day 14: 5.2) and *S.* Typhimurium (day 0: 5.0; day 7: 4.7; day 7: 4.7) for Marinade B that treated inside skirt meat on Day 0, 3, or 7.

There were no significant differences in initial weights or final weights of either the inside skirt product or tri-tip product as shown in Table 9. Marinade A had a pH of 6.58 and did not have a significant difference between the 3 salt concentrations used in 3 tumbling replications (rep 1: 3340.0 ppm; rep 2: 3483.5 ppm; rep 3: 3260.0 ppm). Marinade B had a pH of 6.96 and did not have a significant difference between the 3 salt concentrations used in 3 tumbling replications (rep 1: 2624.5 ppm; rep 2: 2640.0 ppm; rep 3: 2695.0 ppm). The microorganisms were likely not able to increase in log value due to being stored in

refrigeration temperatures and in vacuum package environment with high

concentrations of NaCl.

TABLE 9. *Initial weight (g), final weight(g), retention %, and thickness (cm) of product for inside skirt and tri-tip products processed with Marinade A and Marinade B.*

| | Inside Skirts | | | | | | Tri-tip | | | | | |
| | Marinade A | | | Marinade B | | | Marinade A | | | Marinade B | | |
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Initial Weight (g)	437.00[a]	595.00[a]	413.00[a]	673.00[a]	568.00[a]	585.00[a]	1162.00[a]	983.00[a]	839.00[a]	1196.00[a]	1030.00[a]	1080.67[a]
Final Weight (g)	636.33[a]	893.33[a]	636.33[a]	952.67[a]	827.00[a]	866.33[a]	1369.67[a]	1168.67[a]	1012.00[a]	1389.00[a]	1236.00[a]	1323.67[a]
Retention %	32.17[a]	33.67[a]	34.85[a]	32.72[a]	31.29[ab]	29.33[b]	15.87[a]	15.61[a]	17.43[a]	14.25[a]	16.94[a]	17.99[a]
Thickness (cm)	1.36[a]	1.50[a]	1.33[a]	1.42[a]	1.38[a]	1.33[a]	4.17[a]	3.83[a]	3.17[a]	4.17[a]	3.67[a]	2.67[b]

[a, b]LS Means for Means for Inside Skirt and Tri-tip Initial Weight (g), Final Weight (g), Retention %, and Thickness (cm), treated with Marinade A and B.
[1]LS Means within a row with different letters (a-b) differ ($P < 0.05$).

CHAPTER V

CONCLUSIONS

E. coli O157:H7 and *S.* Typhimurium are a major food safety concern to the beef industry. Although the *E. coli* O157:H7 prevalence is typically low on beef subprimals, processors must still be concerned about contaminating marinade. If processors reuse spent marinade, they need to be aware of the potential risk of contaminating products during subsequent marination. The survival of pathogens in spent marinade, and the transfer of pathogens into the interior during vacuum tumbling may both contribute to potential food safety concerns. Processors of non-intact beef products must consider all potential hazards and properly address them in their food safety program.

REFERENCES

1.   Behrends, J. M., K. J. Goodson, M. Koohmaraie, S. D. Shackelford, T. L. Wheeler, W. W. Morgan, J. O. Reagan, B. L. Gwartney, J. W. Wise, and J. W. Savell. 2005. Beef consumer satisfaction: USDA quality grade and marination effects on consumer evaluations of top round steaks. *J. Anim. Sci*. 83:662-670.

2.   Calicioglu, M., J. N. Sofos, and P. A. Kendall. 2003. Fate of acid-adapted and non-adapted *Escherichia coli* O157:H7 inoculated post-drying on beef jerky treated with marinades before drying. *Food Microbiol*. 20:169-177.

3.   Calicioglu, M., J. N. Sofos, J. Samelis, P. A. Kendall, and G. C. Smith. 2003. Effects of acid adaptation and modified marinades on survival of post-drying *Salmonella* contamination on beef jerky during storage. *J. Food Prot*. 66:396-402.

4.   Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1997. Use of hot water for beef carcass decontamination. *J. Food Prot*. 61:19-25.

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

6.   D'Aoust, J. Y. 1997. *Salmonella* species, p. 129-158. *In* M.P. Doyle, L.R. Beauchat, and T.J. Montville (ed.), Food microbiology: fundamentals and frontiers. American Society for Microbiology, Washington, DC.

7. Elder, R., J. E. Keen, G. Siragusa, J. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. U. S. A. 97(7), 2000-3003.

8. Gill, C. O., and J. C. McGinnis. 2004. Microbiological conditions of mechanically tenderized beef cuts prepared at four retail stores. *J. Food Microbiol.* 95:95-102.

9. Hardin, M. D., G. R. Acuff, L. M. Lucia, J. S. Oman, and J. W. Savell. 1994. Comparison of methods for decontamination from beef carcass surfaces. *J. Food Prot.* 58:368-374.

10. Heller, C. E., J. A. Scanga, J. N. Sofos, K. E. Belk, W. Warren-Serna, G. R. Bellinger, R. T. Bacon, M. L. Rossman, and G. C. Smith. 2007. Decontamination of beef subprimal cuts intended for blade tenderization or moisture enhancement. *J. Food Prot.* 70:1174-1180.

11. Jacob, R. A., C. S. Porto-Fett, J. E. Call, and J. B. Luchansky. 2009. Fate of surface-inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium on kippered beef during extended storage at refrigeration and abusive temperatures. *J. Food Prot.* 72:403-407.

12. Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern food microbiology. Springer Science+Business Media, LLC, New York, NY.

13. Kennedy, J. E., S. K. Williams, T. Brown, and P. Minerich. 2006. Prevalence of *Escherichia coli* O157:H7 and indicator organisms on the surface of intact subprimal beef cuts prior to further processing. *J. Food Prot.* 69:1514-1517.

14. Krause, R. J., H. W. Ockerman, B. Krol, P. C. Moerman, and R. F. Plimpton Jr. 1978. Influence of tumbling, tumbling time, trim and sodium tripolyphosphate on quality and yield of cured hams. *J. of Food Sci.* 43: 853-855.

15. Luchansky, J. B., R. K. Phebus, H. Thippareddi, and J. E. Call. 2008. Translocation of surface-inoculated *Escherichia coli* O157:H7 into beef subprimals following blade tenderization. *J. Food Prot.* 71:2190-2197.

16. Marriott, N. G., P. P. Grahman, J. W. Boling, and W. F. Collins. 1984. Vacuum tumbling of dry-cured hams. *J. of Anim Sci.* 58:1376-1381.

17. Molina, M. E., D. D. Johnson, R. L. West, B. L. Gwartney. 2005. Enhancing palatability traits in beef chuck muscles. *Meat Sci*. 71:52-61.

18. National Advisory Committee on Microbiological Criteria for Foods. 2002. *Escherichia coli* O157:H7 in blade-tenderized, non-intact beef. USDA FSIS. Washington, DC.

19. North American Meat Processors Association (NAMP). 2007. The meat buyer's guide. John Wiley and Sons, Inc., Hoboken, NJ.

20. National Cattlemen's Beef Association (NCBA). 2004. A basic look at *E. coli* O157:H7. Facts for beef producers. Centennial, CO. Available at: www.beefresearch.org/CMDocs/BeefResearch/E. Coli a Basic Look.pdf. Accessed: 01 June 2009.

21. Patel, J. R., A. C. Williams-Campbell, M. N. Liu, and M. B. Solomon. 2005. Effects of hydrodynamic pressure treatment and cooking on inactivation of *Escherichia coli* O157:H7 in blade-tenderized beef steaks. *J. Muscle Foods.* 16:342-353.

22. Pearson, A. M., and T. A. Gillett. 1999. Processed meats, Third Edition. Aspen Publication. Gaithersburg, MD.

23. Rojas, M. C., S. E. Martin, R. A. Wicklund, D. D. Paulson, F. A. Desantos, and M. S. Brewer. 2007. Effect of high-intensity pulsed electric fields on survival of *Escherichia coli* K-12 suspended in meat injection solutions. *J. Food Safety*, 27:411-425.

24. Rossman, M. 2006. Non-intact beef products unlikely to pose pathogen concern. Issues Update 2006. National Cattleman's Beef Association, Centennial, CO.

25. Scanga, J. A., R. J. Delmore Jr., R. P. Ames, K. E. Belk, J. D. Tatum, and G. C. Smith. 2000. Palatability of beef steaks marinated with solutions of calcium chloride, phosphate, and (or) beef-flavoring. *Meat Sci.* 55:397-401.

26. Scott, V. N., K. E. Stevenson (ed.). 2006. HACCP: A systematic approach to food safety, fourth edition. The Food Products Association, Washington, DC.

27. Sporing, S. B. 1999. *Escherichia coli* O157:H7 risk assessment for production and cooking of blade tenderized beef steaks. U. S. Department of Agriculture, Washington, DC. Available at:

http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/01-013N/Blade3.pdf. Accessed: 01 June 2009.

28. Swanson, K. M. J., R. L. Petran, and J. H. Hanlin. 2001.   Culture methods for enumeration of microorganisms, p. 53-62. *In* F. P. Downes and K. Ito (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, DC.

29. US Department of Agriculture, Food Safety and Inspection Service. USDA-FSIS. 1996. Pathogen reduction: hazard analysis and critical control point (HACCP) systems. Final Rule. United States Department of Agriculture, Food Safety Inspection Service, Washington, DC. 9 CFR 417.1-417.8.

30. USDA-FSIS. 1999. FSIS policy on non-intact raw beef products contaminated with *E. coli* O157:H7. Available from: http://www.fsis.usda.gov/OA/background/O157policy.htm. Washington, DC. Accessed: 01 June 2009.

31. USDA-FSIS. 2002. Comparative risk assessment for intact (non-tenderized) and non-intact (tenderized) beef: executive summary. Risk Assessment Division, Office of Public Health Service. Washington, DC.

32. USDA-FSIS. 2004. Federal meat inspection act. Available at: http://www.fsis.usda.gov/Regulations_&_Policies/FMIA/index.asp#Poultry%20 Products%20Inspection%20Act. Washington, DC. Accessed 8, June, 2009.

33. USDA FSIS. 2005. HACCP plan reassessment for mechanically tenderized beef products. 70:30331-30334. Washington, DC.

34. USDA FSIS. 2007. Michigan firm recalls beef products due to possible *E. coli* O157:H7 contamination. Available from: http://www.fsis.usda.gov/News_&_Events/Recall_001_2008_Release/index.asp Accessed: 01 June 2009.

35. USDA-FSIS. 2009. Measures to address *E. coli* O157:H7 at establishments that receive, grind, or otherwise process raw beef products, 05-09. Washington, DC.

36. United States Department of Health and Human Services, Food and Drug Administration (USDHHS-FDA). 2005. Food Code. Public Health Service Food and Drug Administration. College Park, MD.

APPENDIX A

Media Preparation

Preparation of Tryptic Soy Broth (TSB)

1. Suspend 40 g tryptic soy broth (Becton Dickinson, Spark MD) in 1 liter distilled

   water.

2. Place magnetic stirrer in flask and bring to a boil and continue to boil one min.

3. Dispense 10 ml using Wheaton Unispense (VWR) into glass test tubes and cap.

4. Autoclave 15 min on liquid cycle.

5. Store cooled TSB at 4˚C.

Preparation of Buffered Peptone Water (BPW)

1. Suspend 20 g of BPW powder (Difco) in 1 liter of distilled water.

2. Place the magnetic stirrer in the flask and bring to a boil for one min.

3. Dispense 10 ml using a Wheaton unispense into glass test tubes and 99 ml into milk dilution bottles.

Preparation of LSPR Agar

Rehydrate tryptic soy agar (TSA) according to the manufacturers' instructions and add the following ingredients:

	g per liter:
Yeast extract	3.0
Beef extract	3.0
Lactose	5.0
Sodium sulfite	2.5
Ferrous sulfate	0.3
Phenol red	0.025
Cycloheximide	0.1
Rifampicin*	0.1 (added after sterilization)

Notes:
1. Phenol red has to be dissolved prior to adding to the medium. Up to 0.1 g phenol red can be dissolved in 3 ml of 0.1 N NaOH and then the volume adjusted with distilled water. Solution is very stable and can be stored long periods of time.
2. The pH of the medium is 7.2 +/- 0.2. Usually it is not necessary to adjust the pH. Adjust if necessary.
3. Sterilize by autoclave at 121˚C for 15 min.
4. After sterilization, cool at 50˚C and add filter-sterilized rifampincin solution with a sterile pipette. Mix and pour into sterile Petri dishes.

Important to note:

- To prepare the rifampicin solution, dissolve (slowly) 0.1 g rifampicin in 5 ml methanol.
- Add to 1 liter of the sterile medium.
- Plates can be stored in cooler for up to 1 wk.
- Plates can not be counted until 22-24 h has passed.

APPENDIX B

Preparation of Marinade

REO TAMU Fajita Marinade

For 25 lbs of meat use the following weights of Sodium Tripolyphosphate (STPP), water, and seasoning.

Beef 25 lb
STPP .11 lb
Water 4.9 lb
Seasoning 1 lb

To determine the amount of water, STPP, and seasoning needed for exact pounds of meat, multiply the pounds of meat by the weight of water, STPP, or seasoning to determine the amount of water, STPP, and seasoning.

0.196 x wt. water lb
0.0044 x wt. STPP (sodium tripolyphosphate) lb
0.04 x wt. seasoning lb

Add ingredients to water and mix thoroughly using a stir bar and hot plate. Test pH and salt content of marinade before inoculating and adding to tumbler.

Legg's Cajun Style Marinade

Each sample package is made for 2.27 kg of meat product.
To calculate the 12% H_2O pick up, multiply 2.27 kg times 12%.
Each package of seasoning will require 0.27 kg H_2O.

Add ingredients to water and mix thoroughly using a stir bar and hot plate. Test pH and salt content of marinade before inoculating and adding to tumbler.

APPENDIX C

Contaminated Equipment SOP

Vacuum Tumbler

1. Put on protective clothing (i.e. gowns, rubber gloves, and boots) before cleaning the vacuum tumbler.
2. Add a minimum of 8% bleach to the drum of the vacuum tumbler.
3. Put vacuum tumbler lid on the drum and pull 20 psi to seal the drum.
4. Vacuum tumble contaminated marinade, and bleach in vacuum tumbler for a minimum of 15 min.
5. The residual marinade and bleach can then be dumped down the drain in the BL2 lab sink.
6. Rinse the tumbler drum and lid in the sink in the autoclave room.
7. Fill the bottom of the vacuum tumbler drum with 3 in. of water.
8. Place the drum in the autoclave machine, place the lid of the vacuum tumbler drum in an autoclave container and put in the same autoclave machine.
9. The tumbler drum and lid will then be autoclaved on a liquid 30 cycle.
10. The tumbler drum and lid will then be washed with soap and water, and rinsed three times with distilled water in the autoclave room once the autoclave cycle is complete.
11. The tumbler drum and lid will then sit on a lab bench to air dry.

Cutting board other equipment

1. Put on protective clothing (i.e. gowns, rubber gloves, and boots) before handling contaminated equipment.
2. Contaminated equipment should be put into an autoclavable container.
3. The bottom of the container should be filled with 2 in. of water.
4. This equipment should be autoclaved on a liquid 30 cycle.
5. The equipment will then be washed with soap and water, and rinsed three times with distilled water in the autoclave room once the autoclave cycle is complete.
6. The equipment will then sit on a lab bench or clean cart to air dry.

APPENDIX D

Sodium Concentration Procedure

EQUIPMENT:

Orion Model 720A pH/ISE meter	Squeeze Bottle for Rinse Solution
Sodium Electrode	Kim Wipes
Scale	Disposable pipettes
Stir Plate	1,000 ml volumetric flask
Stir Bars	150 ml Glass Beakers
Food Processor or Homogenizer	

REAGENTS:
Sodium Known Standard (1,000 ppm Sodium, Orion 841108)
Sodium Ionic Strength Adjustor (Orion 841111)
Reference Electrode Filling Solution
Distilled, Deionized Water
Sodium Electrode Rinse Solution: Add 10 ml of ISA to volumetric flask. Bring to volume using distilled water.

Preparation of Standards:

Beaker ID	Standard (ml)	Water (ml)	Standard (ppm)
0	0	100	0
250	25	75	250
500	50	50	500
1000	100	0	1000

PROCEDURE:
Checking Electrode Operation (Slope):
1. If electrode has been stored dry, condition the electrode. Refer to electrode operating manual for this procedure.
2. Connect electrode to the meter.
3. Place 100 ml distilled water in to a 150 ml beaker. Add 10 ml ISA. Stir thoroughly. Set function switch of the meter to read in mV.
4. Rinse electrode with sodium electrode rinse solution and place in the solution prepared in step 3.
5. Select a sodium standard (1000 ppm Na). Pipet 1 ml of this standard solution into the beaker. Stir thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.
6. Pipet 10 ml of the same standard into the same beaker. Stir thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

7.   The difference between the first and the second potential readings is the slope of the electrode. The difference should be in the range of 54-60 mV, assuming the solution temperature is between 20 an 25°C.

Direct Calibration:
1.   Prepare electrodes according to operating manual instructions.
2.   Connect electrode to meter.
3.   Electrode input will appear on the prompt line CH-1 or CH-2. Set channel to match input of electrode by pressing 2nd then Channel (5) to change the channel is needed.
4.   Press Mode (1) until concentration mode indicator CON appears.
5.   Press Calibrate (2). Calibrate will be displayed.
6.   ENTER NO. STDS will appear on the display, enter 4 and press Yes.
7.   Starting with the least concentrated standard, add 10 ml Sodium ISA solution and stir thoroughly.
8.   Rinse the electrode with sodium electrode rinsing solution and place into beaker.
9.   READY ENTER VALUE will appear (it takes a few minutes). Enter the concentration value standard 0 and press Yes.
10.   Repeat steps 7, 8 & 9 for the 250, 500 and 1,000 ppm standards.
11.   The electrode slope will be calculated and displayed.
12.   The meter will then advance to the MEASURE mode.

Measurement of Samples:
1.   Place 10 g of sample in the blender and add 90 ml distilled water.
2.   Blend for 30 s on high.
3.   Transfer 100 ml of diluted sample to a 150 ml beaker.
4.   Add 10 ml Sodium ISA and stir thoroughly.
5.   Rinse electrode in Sodium Electrode Rinse Solution and place into sample. Continue to stir the sample.
6.   Record concentration directly from the meter display when READY appears.
7.   When finished, rinse electrode thoroughly and store according to operating manual instructions.

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