THE SURVIVABILITY, GROWTH AND HEAT SUSCEPTIBILITY OF *E. COLI* O157:H7 IN ENHANCED BEEF BRINE SOLUTIONS CONTAINING POTASSIUM LACTATE AND LACTIC ACID PRODUCING BACTERIA

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ASHLEY R. ROSENBERG, B.S.

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Approved

J. Chance Brooks
Co-chairperson of the Committee

Mindy M. Brashears
Co-chairperson of the Committee

Markus F. Miller

Guy H. Loneragan

Fred Hartmeister
Dean of the Graduate School

May, 2009

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ABSTRACT

Meat enhancement is used to increase consumer satisfaction through improved palatability and uniformity. Brine solutions that are re-covered or reused during the processing of enhanced meat cuts have a high risk of cross contamination. The objectives of the present study were to determine the effect of potassium lactate and lactic acid bacteria on the survivability and heat susceptibility of *E. coli* O157:H7 in brine solutions used to enhance beef products and to determine the effect of these interventions on consumer sensory scores and shelf life characteristics. To characterize safety, beef strip loins were enhanced with brine solutions (0.3% sodium chloride and 0.35% phosphate at 10% pump level) inoculated with high or low levels of *E. coli* O157:H7 and one of the following interventions: 0, 1.5, 2.5% potassium lactate or lactic acid bacteria (LAB 10^7 CFU/ml). Treated subprimals were fabricated into steaks and randomly allotted to one of the following internal endpoint temperatures: 0 (not cooked), 50, 55, 60, 65, 70 and 75°C. Once endpoint temperature was reached, the interior of each steak was sampled and *E. coli* O157:H7 was enumerated (high level) or detected (low level). To characterize palatability and shelf life, beef strip loin subprimals were enhanced with brine solutions containing previously mentioned interventions plus a non-enhanced control. Consumer panelists evaluated palatability at 14 d postmortem and lean color on days 1, 3, and 7 of display (after a 14 d postmortem dark storage period). Display steaks were packaged in high-oxygen (80% O_2 / 20%CO_2) or low-oxygen (0.4%CO/30%CO_2/69.6%N_2)

modified atmosphere packages. Data analysis showed no significant interactions between intervention treatment and steak temperature endpoints, indicating *E. coli* O157:H7 from treated brine solutions were not more susceptible to heat during cooking. Results also indicate the transference of pathogens into meat products was low for all interventions, regardless of inoculation level. Internal steak temperature (especially 70 and 75 °C) remains the most effective way to reduce pathogen levels in steaks enhanced with inoculated brine solutions. Steaks packaged in high-oxygen MAP and enhanced with a brine solution containing 1.5 or 2.5% potassium lactate maintained more desirable lean color scores throughout display and were more likely to be purchased by consumers than steaks enhanced with other treatments. Finally, the presence of potassium lactate (1.5 and 2.5%) and lactic acid producing bacteria had no detrimental impact on consumer palatability, while enhanced steaks were more desirable than non-enhanced controls.

CHAPTER I

INTRODUCTION

Foodborne disease is a concern to all segments of the meat industry. Microorganisms such as *Escherichia coli* O157:H7 receive the greatest attention because of their virulence and pathogenicity in humans, and because they are considered an adulterant by USDA-FSIS *(16)*. *Escherichia coli* O157:H7 is resilient and able to survive at low pH, high salt concentrations and refrigeration temperatures *(32).* Because of these characteristics, *E. coli* O157:H7 has the potential to survive in brine solutions used to marinate whole muscle cuts.

In 2004, 16% of whole muscle cuts of beef in US fresh meat retail cases were enhanced *(2).* Today, the process of adding non-meat ingredients to fresh meat to improve the eating quality, lean color, and ultimate case-life of the retail product is defined as 'enhancement'. Enhancement improves meat tenderness and juiciness, which is desired by consumers. However, when consumers cook enhanced cuts, internal temperatures may not be sufficient to kill pathogenic microorganisms if they have been transferred to the interior of the product during the enhancement process. This could increase the likelihood of a foodborne illness from contaminated meat products.

First designated as a foodborne pathogen in 1982, *(29, 32, 34, 42)* verotoxigenic *E. coli* O157:H7 has become of pathogen of concern, namely for its ability to cause hemolytic uremic syndrome (HUS) and hemorrhagic colitis in individuals with underdeveloped or compromised immune systems. The

organism has the ability to produce shiga-toxins, which are potentially responsible for human pathogenesis *(32, 34, 41).* Five distinct verotypes of *E. coli* which cause diarrheal disease are recognized. Of these, the O157:H7 serotype is classed as enterohemorrhagic (EHEC) *E. coli*. This potentially fatal pathogen is commonly isolated from the gastrointestinal tracts of cattle *(10, 37).*

E. coli O157:H7 can be present on beef carcasses due to hide, feces or ingesta contamination during harvest *(10).* When carcasses are fabricated the contamination can be spread to other meat subprimals. The internal surfaces of whole muscle cuts are generally sterile. However, when products are enhanced, there is a risk of bacterial contamination from either the surface of the meat or contaminated brine solutions. This contamination is introduced into the interior of the meat products via needle penetration.

Meat is mechanically enhanced using a multi-needle injector or a hand-held stitch pump. Both methods are designed to reuse brine solution by collecting the brine run-off during injection. The brine solution can become contaminated with pathogenic bacteria present on the surface of raw meat products and systematically distribute it to all enhanced product injected with the recovered brine. Therefore, there is a need to develop novel methods to reduce pathogens which may contaminate brine solutions.

Food grade additives can be included in the brine solution to reduce *E. coli* O157:H7 levels. Lactic acid bacteria (LAB) are generally recognized as safe

(GRAS) and an approved ingredient in meat products. Lactic acid bacteria are known for their antagonistic properties against pathogenic microorganisms *(25, 36).* Such modes of action include the generation of organic acids, bacteriocins and hypothiocyanate *(25, 30).* In previous research, the addition of LAB to beef products reduced *E. coli* O157:H7 populations in meat products without detrimental effects on consumer palatability scores *(36).*

Salt and alkaline phosphates are common ingredients in enhancement solutions. Salt is generally used at levels of 0.25 to 0.50% in the final product while phosphate levels typically comprise 0.25 to 0.45% of the final product weight. However, USDA regulations stipulate that phosphate levels cannot exceed 0.50% *(17).* Lactates, including potassium lactate and sodium lactate, are recommended at 2.5 to 3.0%. Potassium lactate has been found to improve the color of fresh beef under modified atmosphere packaging (MAP) *(24).* Flavoring agents, such as rosemary oleoresins, may also be added to the solution to extend shelf life.

The intrinsic properties of brine solutions can be antagonistic towards the survival of bacteria. The solution is typically held at refrigeration temperatures and contains sodium chloride and other antimicrobial ingredients. The environment in the solution can potentially kill or injure *E. coli* O157:H7 cells. Injured cells are of great concern because they retain their virulent and pathogenic properties despite their damaged state *(27).* It is necessary to have a recovery method to identify these cells when investigating antimicrobial

interventions. The thin agar layer (TAL) has been found to be a viable method for recovering injured cells and differentiating between microorganisms. Stressed *E. coli* O157:H7 cells have been successfully recovered and repaired for enumeration by utilizing a combination of MacConkey sorbital agar (MSA) and TAL medium *(42).* TAL, which is conventionally used to recover heat, cold and acid-injured foodborne pathogens, can be used to recover sodium chloride-injured *E. coli* O157:H7 *(23).*

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Consumer acceptability is an important factor in determining which ingredients will be added to a brine solution. Enhanced meat cuts have been successfully marketed at the retail level. Enhanced meat products have increased flavor, tenderness and juiciness over non-enhanced meat of similar quality *(8).* Injection of fresh cuts with phosphate/lactate solutions provides the beef industry a means to improve product quality and consistency *(41).* It has been shown that enhancing strip loin steaks with a solution containing sodium chloride, phosphate, and sodium lactate improved the sheer force, sensory tenderness and juiciness over non-enhanced controls and loins pumped with distilled water only *(41).*

The process of subjecting meat to solutions containing spices, flavorings and other ingredients to tenderize and impart flavors to the product via absorption or osmosis is referred to as marination. In order to successfully

market these items to consumers for retail, other terms such as “deep basting” or “enhancement” have been developed. The ingredients used are similar to a traditional brine solution without the presence of sodium nitrite and erythorbate.

Consumer perception of fresh beef cuts in retail display is an important factor in purchasing decisions. Appearance determines how consumers perceive quality and serves as a predictor of what their eating experience should be like. Consumers prefer to purchase bright red beef and consider purple or brown meat colors a deviation of quality *(7).* Color is used as a consumer indicator of freshness; bright red color being the most desired *(7).*

Limited information is available to determine the effect of brine ingredients on *E. coli* O157:H7 injury and susceptibility to subsequent thermal processing. Government and industry leaders have begun to question the benefits of enhancement over non-enhanced products because of three foodborne illness outbreaks attributed to marinated meat products. Consequently, research must be conducted to identify the safety of enhanced and tenderized beef cuts, and address possible interventions to control these potential hazards.

The objectives of the present study were to determine the effect of potassium lactate and lactic acid bacteria on the survivability and heat susceptibility of *E. coli* O157:H7 in brine solutions used to marinate beef for retail, and to determine the effect of these enhancement solutions on consumer

perception of the sensory and shelf life characteristics of enhanced beef strip steaks.

CHAPTER II

LITERATURE REVIEW

Consumers have become increasingly concerned about the safety of their food supply - especially beef. This concern has stemmed from media attention over *E. coli* O157:H7 contaminated beef recalls, outbreaks, human sicknesses and deaths. Although the industry and government has been extremely proactive in addressing this contamination issue, the system is not perfect. Beef producers, packers and retailers continue to work diligently to ensure the safety and quality of the nation's beef supply.

Escherichia coli O157:H7

E. coli O157:H7 is considered an adulterant in ground beef products as determined by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) *(16).* The organism obtained this status due to its pathogenicity and virulence when ingested by humans. The bacteria are also readily transmitted through contaminated water and direct contact with infected people or animals *(32).* Primarily responsible for affecting immunocompromised individuals, *E. coli* O157:H7 infection is most often expressed in children, the elderly, AIDS patients and pregnant women. This demographic has an increased chance of developing a potentially deadly clinical illness due to immune system suppression. Generic *E. coli* is a fecal coliform that is a normal inhabitant of the intestinal tracts of all animals, including humans.

E. coli functions to provide a background flora in the intestines to competitively inhibit harmful bacteria and to synthesize small amounts of vitamins. However, a specific strain of *E. coli* with serovar O157:H7 is responsible for causing human illness by releasing toxins that damage the intestinal wall *(40).*

Human infection with *E. coli* O157:H7 is associated with asymptomatic shedding, non-bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS), and death. *E. coli* O157:H7 may be shed in the stool for several weeks following the resolution of symptoms *(32).* The average interval between exposure to the organism and illness is 3 days, with incubation periods as short as 1 day and as long as 8 days have been reported *(32).*

Hemorrhagic colitis is characterized by severe cramping, abdominal pains, and watery and or bloody diarrhea with little or no fever *(42).* HUS is defined by acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia. *E. coli* O157:H7 is thought to account for over 90% of all HUS cases, however only 5% of *E. coli* O157:H7 infections result in HUS development in the patient *(32).*

E. coli O157:H7 is a type of shiga-toxin or verotoxin producing bacteria which can also be classified as enterohemorrhagic *E. coli* (EHEC). Enteropathogenic, enterotoxogenic, enteroaggregative and enteroinvasive *E. coli* are the other four classes of the pathogen which cause diarrheal disease *(32)*. The O157:H7 nomenclature references the 157th somatic (O) antigen and the 7th

flagellar (H) antigen *(32).* This strain was first identified *(29, 32, 34, 42)* in 1982 when it was associated with hemorrhagic colitis outbreaks in Oregon and Michigan. The illnesses were later linked to eating hamburgers at the same fast food outlets. Laboratory tests conducted by Riley et al. (1983) confirmed *E. coli* O157:H7 did not produce either a heat-labile or heat-stable enterotoxin - meaning the pathogen would be susceptible to the cooking process *(34),* indicating that the hamburger patties from the outbreaks in the fast food outlets did not reach sufficient internal temperature to kill the pathogen.

Like all enteropathogenic bacteria; *E. coli* O157:H7 has the ability to adhere to human intestinal epithelia cells and produce lesions. The adhesion to the mucosal cells of the large bowel potentially disrupts the brush border, causing diarrhea in the patient. However the mechanisms by which *E. coli* O157:H7 cause human pathogenesis such as HUS and hemorrhagic colitis are not fully understood. Public health officials and doctors can be proactive about *E. coli* O157:H7 infections by closely monitoring patients with bloody diarrhea or HUS, and by educating others on about hazards of eating undercooked ground beef and unpasteurized milk and juices *(32).*

Since the organism was initially identified, *E. coli* O157:H7 is estimated to be responsible for a total 73,480 illnesses, hospitalizations and deaths per year as reported by Mead et al. (1999) but accounts for only 2.9% of all foodborne illnesses with a 0.83% case fatality rate. In comparison, to the estimated 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths resulting from all

foodborne diseases in the United States each year *(31).* These estimates include illnesses in which the patient did not seek medical attention in addition to documented hospital visits.

The discovery of *E. coli* O157:H7 is relatively recent considering its pathogenicity and highly publicized outbreaks. Prior to 1982, the Centers for Disease Control and Prevention laboratory had detected only one strain of *E. coli* O157:H7 among over 3,000 *E. coli* organisms stereotyped since 1973. The strain was isolated from a 50-year old California woman in 1975 during an acute, self-limited illness with severe abdominal cramps followed by bloody diarrhea. This was the first documented case of *E. coli* O157:H7 human illness previous to a substantial outbreak in Oregon and Michigan *(34).* This rare *E. coli* serotype quickly gained the attention of the scientific and medical communities.

In October of 1994, FSIS declared *E. coli* O157:H7 to be an adulterant in raw ground beef and implemented a sampling program for the pathogen in all federally inspected beef establishments and retail stores *(16).* Later in 1999, FSIS released a statement further clarifying that *E. coli* O157:H7 was a public health risk in not only raw ground beef products, but also intact raw beef products including trimmings further processed into non-intact products *(39).* It was determined by FSIS that if these contaminated cuts were to be used in human consumption, they must be further processed into ready-to-eat products with a certified cooking process or be deemed adulterated *(39).*

E. coli is a known inhabitant of the gastrointestinal tract of healthy cattle and is shed in the feces of animals that are carriers. It has been shown that cattle are a reservoir of *E. coli* O157:H7 and that contamination of carcasses during the slaughter process is the primary route to beef and beef product contamination; thereby transmitting the organism to man *(10).* Beef cattle fed in confinement are most often associated with the pathogen. Smith and others have isolated *E. coli* O157:H7 from the rectal contents of 23% of cattle in feedlot pens. They also reported 100% of feedlot pens tested had at least one animal positive for the organism *(37).* This data indicate *E. coli* O157:H7 is common in fed cattle confinement operations and therefore has the potential to effectively enter the slaughter facility.

Research conducted by Smith and others (2001) concluded *E. coli* O157:H7 prevalence correlated only with muddy pen conditions ($P = 0.01$) and did not correlate to animal weight, cleanliness of water tanks, pH of feed or number of days on feed *(37).* Muddy pen conditions could more easily facilitate the transfer of the organism from animal to animal, in addition to providing a more ideal environment for the bacteria to live. *E. coli* O157:H7 in cattle and sheep manure may survive for months under wet environmental conditions, whereas recovery of the organism is less likely from dried layers of manure *(11).* In this way, fecal matter from the feedlot can be attached to the animal's hide - cross contaminating the beef carcass with *E. coli* O157:H7 during the harvesting process.

In a study by Chapman et al. (1993), *E. coli* O157 was isolated from 30% of carcasses from cattle where the organism had been previously isolated from rectal contents; while *E. coli* O157 was also isolated from 8% of carcasses were the organism had not been previously isolated from rectal contents *(10)*. The prevalence of *E. coli* O157 in the intestinal tract or on the hide of live cattle magnifies the risk of contamination during further the processing.

The economic impact of *E. coli* O157:H7 contamination to the beef industry is significant. Product rework, antimicrobial interventions, bacterial testing, and recalls greatly affect profitability and the success of a company. More recently, *E. coli* O157:H7 recalls have had a considerable impact on U.S. consumers. It has been estimated that *E. coli* O157:H7 has cost the beef industry $2.8 billion in the past ten years *(28).* Of this expense, $400 million is spent on food safety intervention strategies. However, the most significant cost the industry is associated to loss of consumer demand from negative publicity and beef recalls from *E. coli* O157:H7 contaminated product *(28).* It is estimated that $1.6 billion is has been lost due in reduced consumer demand. Major recalls have a lingering effect on public purchasing decisions, as consumer trust is particularly challenging to regain. Such large dollar sums attributed to *E. coli* O157:H7 contamination only reiterate its economic significance in the industry and the continued need to resourcefully but effectively control this pathogen.

Retailers, packers and producers have all suffered economic hardships associated to one substantial *E. coli* O157:H7 outbreak. In January, 1993 a

physician reported an increase in children with HUS to the Washington Department of Health. The case further progressed to 602 patients with bloody diarrhea or HUS documented to the state health department *(9).* Laboratory test confirmed *E. coli* O157:H7 infections in all patients. Affected individuals had either eaten ground beef patties at Jack-in-the-Box restaurants (88%) or in close contact with people confirmed to be infected (11%) *(9).* At the conclusion of the outbreak, four deaths were associated with *E. coli* O157:H7 infection complications. The median age of the patients was 7.5 years *(9).*

While the majority of *E. coli* O157:H7 associated outbreaks in beef are associated with undercooked ground hamburger patties, there are also reports of product recalls related to whole muscle cuts. In 2007 HFX Inc., a firm in Pennsylvania, voluntarily recalled over 250,000 pounds of beef steaks associated with *E. coli* O157:H7 illnesses *(14).* In 2003, a Chicago, Illinois establishment voluntarily recalled 739,000 pounds of beef steaks. Stampede Meats, Inc. was responsible for distributing vacuum packaged steaks potentially contaminated with *E. coli* O157:H7 sold to consumers through door-to-door sales. The product was distributed to restaurants and retail stores nationwide, in addition to Canada *(15).* Typically, *E. coli* O157:H7 contamination in steaks does not pose a risk to the consumer. FSIS recommends that consumers cook enhanced steaks products to an internal temperature of 160°F as determined by a food thermometer *(14, 15).*

Traditionally, ground beef is the culprit for *E. coli* O157:H7 illness outbreaks. *E. coli* O157:H7 contaminated beef trim is distributed throughout the product when it is passed through a meat grinder and produces ground beef. This allows the pathogen to be present on the inside of ground beef patties. If these patties are not cooked to sufficient internal temperatures to kill the organism, the consumer has an increased risk of contracting a foodborne illness. Beef steaks are considered a low risk to consumers for *E. coli* O157:H7 infection due to the constitution of whole muscle cuts. The inside of muscle is considered sterile and free of bacteria. The surface of the steak is the only area with the potential to be contaminated with pathogenic organisms. However mechanically enhanced beef steaks have been associated with *E. coli* O157:H7 recalls *(14, 15).*

Mechanical Enhancement

Tenderness is the most important palatability factor to beef consumers *(35).* Retailers add value to lower quality cuts of beef by mechanically enhancing their products to increase consumer satisfaction. Mechanical enhancement consists of series of needles that are driven through a whole muscle subprimal while simultaneously injecting a brine solution. A brine is an aqueous solution containing various compounds used in meat curing or enhancement. Mechanical enhancement contributes to steak juiciness and enhances flavor.

A traditional brine solution consists of water, sodium chloride (salt) and phosphate. Water serves as a medium to transport and distribute the ingredients of the brine while adding moisture and improving juiciness of the product. Phosphates contribute to the improvement of water holding capacity in the meat and may not exceed 0.5% of the final product weight *(17)*. By increasing water holding capacity, phosphates act to improve the cooking yields of the product which increases the tenderness and juiciness. Meat color is positively affected by phosphates which offer protection against browning during storage *(1)*. Salt functions to solubilize proteins and develop flavor in the meat. It is used in low amounts (0.25% to 0.50%) and is restricted to 2.5% of the final product weight *(17).* However even at these low concentrations, salt maintains antimicrobial properties for meat safety.

Other ingredients may be added to a brine solution. Potassium lactate is a viscous ingredient that can be added up to 2.0% of final product weight *(17).* Potassium lactate is a lactic acid salt, derived from lactic acid producing bacteria. This ingredient is beneficial for its antimicrobial and flavor enhancing properties which mimics sodium chloride. Potassium lactate acts to inhibit bacteria by interfering with the metabolism of the organism and increasing its lag phase. Therefore, this nonmeat additive does not directly kill bacteria, but rather serves as a bacteriostat *(13).*

Lactic acid bacteria (LAB) are a culture of live organisms that can be added to a brine solution and can serve as a biological antimicrobial against

pathogens. Lactic acid bacteria are responsible for producing a microbial metabolite or lactic acid, which lowers the environmental pH and aids in increasing the lag phase of pathogen growth. Other inhibitory actions include hydrogen peroxide production *(36).*

In addition to manufacturing lactic acid, LAB cultures also inhibit harmful bacteria through competitive inhibition. Competitive inhibition involves the use of non-pathogenic bacteria to compete with pathogens for nutrients and specific niches in environments *(4).* The addition of LAB to the brine solution could cause the bacteria to compete with the *E. coli* O157:H7 for nutrients and adhesion to the surface of the meat at refrigeration temperatures. Growth of LAB in meat products is undesirable as they may cause spoilage and other detrimental sensory characteristics *(4).*

Lactic acid bacteria can be used to improve the safety and quality of meat products in an alternative approach to traditional chemical treatments, which may be seen as unwholesome by some consumers. Additionally they have been used in food products for years, functioning as a preservative in fermented meat products. Specific strains must be carefully selected to ensure the desired antimicrobial properties will be generated, but there is no growth at refrigeration temperatures *(5).* Ideal LAB strains can inhibit pathogens and spoilage organisms at refrigeration temperatures while not growing themselves. It is important that the bacteria do not alter the components of the food. An exception to this would be under temperature abuse conditions *(5).* In this scenario, LAB

can also serve as an indicator organism in refrigerated foods, causing spoilage and altering the consumer to mishandled product *(5).*

Amezquita and Brashears (2002) reported bacteriostatic or bacteriocidal activity can be observed, depending on the LAB strain present when acting against *Listeria monocytogenes (5).* A strain identified as *Pediococcus acidilactici* was a potential bacteriocin producer, while *Lactobacillus casei* and *Lactobacillus paracasei* were associated with antilisterial activity resulting from organic acid production *(5).* Lactic acid bacteria can have applications in ground beef as well. When 10^7 CFU/g of the bacteria were added to *E. coli* O157:H7 inoculated ground beef, there was significant 2.0 log reduction of the pathogen ($P < 0.05$) after 3 days of storage in a study conducted by Smith et al. (2005) *(36).* These results indicate that LAB can serve as a beneficial pathogen intervention for ground beef producers who are limited in their antimicrobial additives.

Hypothiocyanate is an antagonistic molecule produced by LAB which has the potential to control pathogens *(25).* Gram negative bacteria demonstrate the greatest potential to be controlled by hypothiocyanate *(25, 30)* as they are particularly sensitive. Levels as low as 5mM have been reported to inhibit *E. coli (25, 30)*, however in a study conducted by Jones et al. (2008) *(25)* LAB strains did not generate concentrations sufficient for detection.

Bacterial Contamination

Surface contamination is of particular importance to whole muscle cuts intended for mechanical enhancement. The concern is the enhancement process will translocate pathogens from the surface of the product to the interior. This could cause potential shielding of the internal pathogens by the subsequent muscle tissue; escaping the lethal effects of cooking. A study conducted by Stopforth and others evaluated the incidence of *E. coli* O157:H7 contamination of fresh beef whole muscle cuts *(38).* Product testing positive for *E. coli* O157:H7 out of 1,022 samples included top sirloin butt (0.9%), and ball tip (2.1%). There was no indication the pathogen was associated with corresponding total aerobic plate counts, total coliform count, or *E. coli* counts ($P \geq 0.05$) *(35).* However, *E. coli* O157:H7 was found exclusively on cuts deriving from the sirloin area of the beef carcass. A similar research study conducted by Kennedy et al. (2006) explored the prevalence of *E. coli* O157:H7 contamination on fresh beef subprimals intended for mechanical enhancement in different seasons (summer and winter). *E. coli* O157:H7 was not detected on any of the 1,199 samples collected with an overall incidence of less than 0.083% *(29).*

Because of the intrinsic properties of meat, there are typically large numbers of bacteria present on the surface of cuts that may or may not be pathogenic *(21, 22, 38).* Higher bacteria counts on the surface of subprimal cuts increase the opportunity for organisms to be translocated deep into the muscle tissues *(20).* A study conducted by Gill et al. (2008) found increased

contamination of deep tissues when surface contamination was higher when meat was tenderized with a *Lumar* machine *(20).* Another factor to consider in addressing contamination in mechanically enhanced or tenderized product is ease and accessibility to cleaning the parts of the equipment. In the same study by Gill et al. (2008), boiled meat cuts which passed through a cleaned Ross tenderizer machine yielded up to 2.0 log CFU/cm^2 aerobes on the surface of the meat. However, few aerobes were recovered from the deep tissues of the product *(20).*

As previously mentioned, the brine solution can be a source of bacterial contamination. Solutions that are recovered or reused during the processing of multiple cuts have the highest risk of cross contamination. It has been found, by Geer and others (2004) that peak bacteria counts (log CFU/ml) are recovered from brine solutions used to marinate pork loins after 2.5 h (of samples taken at 0, 0.75, 1.75 and 2.5 h) of continuous processing *(22).* After 2.5 h, total plate counts were present at 4.5 CFU/ml, lactic acid bacteria at 2.99, *pseudomonads* at 3.95, and *enteric* at 3.01 CFU/ml. *L. monocytogenes* was also found to increase over time in the brine solution, reaching a maximum of 2.34 log CFU/100 ml at 2.5 h. Therefore, brine solutions have the ability to harbor large populations of bacteria, and can also serve as a significant source of pathogen contamination on meat products *(22).*

Brine solutions will continue to remain a challenge in preventing cross contamination of pathogens. However, the safety of a meat product improves

substantially when consumers adequately cook to an internal temperature sufficient to kill internalized pathogens. The use of heat to inactivate pathogens is a critical control point and the definitive way of assuring the microbiological safety of foods *(26).* Particular attention needs to be given to avoid over-estimating the heat resistance of a pathogen, because this may have a negative impact on the quality of the product. However, under-estimating the heat resistance of a pathogen increases the likelihood of micro-organism survival and contraction of a food borne illness *(26).*

Work conducted by Gill et al. (2008) *(19)* evaluated the survivability of *E. coli* and *L. innocua* in a brine solution (2 or 5% NaCl and sodium tripolyphosphate) that was injected into 3 cm thick beef steaks. Steaks were cooked to different endpoint temperatures and revealed a final temperature between 60 and 65 °C was sufficient to inactivate all bacteria in the product. In order to ensure microbiological safety of meat, FSIS recommends that all parts of a product be heated to 63, 65 or 68 °C for times of 180, 60 or 15 seconds, respectively or a temperature greater than 70 °C with no time requirement *(18).* These time and temperature associations are based on bacterial decimal reduction times (D-values) for *Salmonella* spp. The D-value for an organism is defined as the time (in minutes) required to reduce a microbial population by 1 log or 90% of its initial value under specified conditions. However, precise maintenance at these specified temperatures for this short amount of time is extremely difficult *(19).*

Properties of the beef steak, cooking equipment, and procedures are factors to consider. Temperatures probes, when inserted into the geometric center of a steak, revealed core steak temperatures continue to rise after the meat has been removed from its heating source *(19).* In some steaks, the core temperatures rose to several degrees above the cooking temperature, while others rose little above the desired temperature. Therefore, with the D-values listed for pathogen reduction, it cannot be assumed that a steak cooked to a temperature of 60°C or more will maintain this temperature long enough to for an effective kill.

Brine ingredients can have the ability to interact with each other to effectively inactivate *E. coli* O157:H7, making the organism more susceptible to the thermal process *(26)*. A study by Juneja et al. (1999) examined the effectiveness of heating temperature, sodium chloride and sodium pyrophosphate on the heat resistance of *E. coli* O157:H7. The thermal resistance of the pathogen was lowered by combining the intrinsic factors of these ingredients. Interestingly, salt (6%) was found to have a protective effect on *E. coli* O157:H7 by increasing the D-value of the organism in beef gravy at a pH of 4. However when salt was combined with a phosphate (sodium pyrophosphate 0.3%) in the gravy, the sensitivity of *E. coli* O157:H7 increased resulting in a decrease in the D-value by 58.3% from the control *(26)*.Therefore, phosphates may improve meat quality and safety when used as described above.

Thin Layer Method

The applied intervention treatments of salt, phosphate, heat, potassium lactate, and lactic acid all serve to inhibit or kill pathogens present on a meat product. Despite the effects of these treatments, some bacteria can survive in an injured state *(27).* These injured bacteria maintain their virulent properties and are able to cause foodborne illness*.* Due to the harsh qualities of the selective components in *E. coli* O157:H7 media, sublethal injured cells are not able to recover and enumerate. For this reason, a method to recover heat-injured bacteria cells was developed and called the *thin agar layer* (TAL) method. The process involves applying a thin layer of a nutrient rich agar, typically tryptic soy agar (TSA), to the top of a selective pathogen media such as MacConkey agar for *E. coli* O157:H7 enumeration. Heat injured cells are able to repair and recover via the nourishment from the TSA, while the underlying MacConkey agar maintains its selective properties to only allow *E. coli* O157:H7 cells to grow *(43).* TSA as a lone medium is not effective because it allows injured cells to grow, and lacks the selective properties to differentiate targeted bacteria from background flora *(43, 44).*

In a study conducted by Hajimeer and others (2001), it was found that NaCl-injured *E. coli* O157:H7 cells had the highest recovery rate on TSA medium, followed by TAL medium and finally MacConkey sorbital agar (MSA). As NaCl concentration increased (0%, 5%, 7.5%) cell injury increased ($P \leq 0.05$)

regardless of media used *(23).* Overall, the TAL method was either more effective or equivalent to MSA in recovering injured cells. Healthy *E. coli* O157:H7 are able to withstand the harsh selective ingredients of MSA such as bile salts, neutral red, and crystal violet. These same components are responsible for the lethal destruction of injured cells. With the innovative TAL recovery method, pathogens injured from interventions such as heat, acid *(43)* or salt can be selected for without negative impacts on the recovery of the organism *(43,44).*

Consumer Perceptions

Consumer demand ultimately influences beef production and price. The purchases made by consumers indicate what further processed beef products are desired. Beef tenderness is the predominant palatability trait influencing consumer satisfaction *(35)* however juiciness and flavor are important as well *(30)*. The U.S. beef industry consistently produces steaks of varying tenderness within the same USDA quality grade *(33).* This provides for an inconsistent and potential unpleasant eating experience for the consumer. From a study in 2001, the industry produced 15 – 20% tough steaks sold at retail *(33).*

To improve consistency and uniformity in palatability traits, steak enhancement has been utilized to increase consumer satisfaction. Retail survey results have indicated that consumers can differentiate tenderness levels in

steaks and are willing to pay more for enhanced product that improves the quality of the beef *(8).* Consumers scored enhanced steaks for tenderness nearly one full score higher ($P \leq 0.05$) than control steaks. In addition, enhanced steaks were scored higher ($P \leq 0.05$) for juiciness, beef flavor and overall quality *(8).* When consumers were asked if they would pay more for "guaranteed tender" steaks by a retailer, 75% would pay more while 25% would not ($P \leq 0.05$). The results indicated retailers or packers could profit by marketing guaranteed tender products. Trained sensory panels were also conducted for $CaCl_2$ enhanced strip loins. Similar results were observed, with trained panel scores for sustained juiciness and sustained mouthfeel averaging one score higher than control steaks *(8).*

Meat color is used by consumers as in indicator or meat quality and safety, and is the primary driver of purchase decisions. The appearance of the product strongly influences the expected eating experience to the shopper. For these reasons, it is important packaged meat have an attractive look to increase its likelihood of being purchased. The most desired color of beef amongst consumers is bright red, rather than purple or brown *(7).* Consumers were found to use color as an indicator of meat freshness and would not purchase meat products when brown discoloration was evident. Brine ingredients can also affect to meat color. Enhanced meats can display different color shades; dependent on the components of the brine. Pork loins pumped to 110% of the original weight with a brine solution containing 1.83% potassium lactate were

found to be darker in color (have a lower L* value) and loins pumped with a 0.35% phosphate were lighter in color (have a higher L* value) than control pork loins *(24).*

Modified atmosphere packaging (MAP) is a method widely used at the retail level to extend the shelf life of meat products. It is defined as packaging that encloses a food in an atmosphere that is different from air. Specific gases can be used in combinations to create an environment conducive to improving meat color, safety and quality. High oxygen (80% O_2, 20% CO_2) and low oxygen (0.4% CO, 30% CO_2, 69.6% N_2) atmospheres are two gas mixtures commonly utilized in the industry. Additionally, MAP improves the presentation of the product to the consumer and increases shipping distances.

A study conducted by Brooks et al. (2008) evaluated the spoilage and safety characteristics of ground beef in low and high oxygen packages. Researchers found the use of MAP does not mask meat spoilage, nor did it increase the risk of pathogen concentration *(6). E. coli* O157:H7 inoculated ground beef was found to have no significant differences in pathogen load until day 14 of retail display. At d 14, *E. coli* O157:H7 counts for MAP packages ranged from 4.51 to 4.73 log CFU/g while counts in the control package on day 14 were 5.34 log CFU/g. The same trend continued through day 21 with MAP packages having lower *E. coli* O157:H7 counts than control packages *(6).* These reductions in pathogen loads could be due to the CO_2 and CO content in high and low oxygen packages, respectively.

The objectives of the present study were to determine the effect of potassium lactate and lactic acid bacteria on the survivability and heat susceptibility of *E. coli* O157:H7 in brine solutions used to marinate beef for retail, and to determine the effect of these enhancement solutions on consumer perception of the sensory and shelf life characteristics of enhanced beef strip steaks.

References

1. Aberle, E.D., J.C. Forrest, D.E. Gerrard, E.W. Mills (4th ed.). 2001. *Principles of Meat Science*. Kendall/Hunt Publishing Co., Dubuque, Iowa.

2. American Meat Institute. 2007. Meat matters. Consumer's guide to enhanced meats. Available at: www.meatami.com. Accessed 25 September 2008.

3. American Meat Science Association. 1991. Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat. National Live Stock and Meat Board, Chicago, Illinois.

4. Amezquita, A., and M.M. Brashears. 2002. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. *J. Food Prot.* 65:316-325.

5. Brashears, M.M., and W.A. Durre. 1999. Antagonistic action of *Lactobacillus lactis* toward *Salmonella* spp. and *Escherichia coli* O157:H7 during growth and refrigerated storage. *J. Food Prot.* 62:1336-1340.

6. Brooks, J.C., M. Alvarado, T.P. Stephens, J.D. Kellermeier, A.W. Tittor, M.F. Miller and M.M. Brashears. 2008. Spoilage and safety characteristics of ground beef packaged in traditional and modified atmosphere packages. *J. Food Prot.* 71:293-301.

7. Carpenter, C.E., D.P. Cornforth, and D. Whittier. 2001. Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Sci.* 57:359-363.

8. Carr, M.A., K.L. Crockett, C.B. Ramsey, and M.F. Miller. 2004. Consumer acceptance of calcium chloride-marinated top loin steaks. *J. Anim. Sci.* 82:1471-1474.

9. Centers for Disease Control. 1993. Update: Multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers - western United States, 1992-1993. *MMWR*. 42:258-263.

10. Chapman, P.A., C.A. Siddons, D.J. Wright, P. Norman, J.Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.* 111:439-447.

11. Dargatz, D.A., S.J. Wells, L. Thomas, D.D. Hancock, and L.P. Garber. 1997. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. *J. Food Prot*. 60:466-470.

12. Fegan, N., P. Vanderlinde, G. Higgs, and P. Desmarchelier. 2004. The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. *J. App. Microbiol.* 97:362-370.

13. Fik, M., A. Leszczynska-Fik. 2007. Microbiological and sensory changes in minced beef treated with potassium lactate and sodium diacetate during refrigerated storage. *Int. J. Food Properties*. 10:589-598.

14. Food Safety and Inspection Service, USDA. 2007. Pennsylvania firm recalls beef products for possible *E. coli* O157:H7. *Recall Release.* FSIS-RC-019-2007.

15. Food Safety and Inspection Service, USDA. 2003. Illinois firm recalls beef products for possible *E. coli* O157:H7. *Recall Release*. FSIS-RC-028-2003.

16. Food Safety and Inspection Service, USDA. 1994. *E. coli* contamination of beef products. *FSIS Fed. Regist. Notice*. 9 CFR part 417. Docket no. 00-022N. pg. 62325-62334.

17. Food Safety and Inspection Service, USDA. 2002. Safe and suitable ingredients used in the production of meat and poultry products. *FSIS Derivative 7120.1.* Available at: http://www.fsis.usda.gov/Regulations_&_Policies/Ingredients_Guidance/index.asp. Accessed on: 5 November 2008.

18. Food Safety and Inspection Service, USDA. 1999. Performance standards for the production of certain meat and poultry products. *Federal Register.* 64:732-749.

19. Gill, C.O., L.F. Moza, and S. Barbut. 2008. Survival of bacteria in less than thorough cooked, brine-injected steaks. *Food Control*. Accepted Manuscript. Doi: 10.1016/j.foodcont.2008.07.019.

20. Gill, C.O., B. Uttaro, M. Badoni, and S. Zawadski. 2008. Distributions of brine and bacteria in beef primal cuts injected with brine without, or before or after mechanical tenderizing. *Meat Sci.* 79:181-187.

21. Gill, C.O., and J.C. McGinnis. 2005. Factors affecting the microbiological condition of the deep tissues of mechanically tenderized beef. *J. Food Prot.* 68:796-800.

22. Greer, G.G., F. Nattress, B. Dilts and L. Baker. 2004. Bacterial contamination of recirculating brine used in the commercial production of moisture-enhanced pork. *J. Food Prot.* 67:185-188.

23. Hajmeer, M.N., D.Y.C. Fung, J.L. Marsden, and G.A. Millilken. 2001. Effects of preparation method, age, and plating technique of thin agar layer media on recovery of *Escherichia coli* O157:H7 injured by sodium chloride. *J. Microbiol. Methods.* 47:249-253.

24. Jensen, J.M., K.L. Robbins, K.J. Ryan, C. Homco-Ryan, F.K. McKeith, and M.S. Brewer. 2003. Effects of lactic and acetic acid salts on quality characteristics of enhanced pork during retail display. *Meat Sci.* 63:501-508.

25. Jones, R.J., H.M. Hussein, M. Zagorec, G. Brightwell, and J.R. Tagg. 2008. Isolation of lactic acid bacteria with inhibitory activity against pathogens and spoilage organisms associated with fresh meat. *Food Microbiol.* 25:228-234.

26. Juneja, V.K., B.S. Marmer, and B.S. Eblen. 1999. Predictive model for the combined effect of temperature, pH, sodium chloride, and sodium pyrophosphate on the heat resistance of *Escherichia coli* O157:H7. *J. Food Safety.* 19:147-160.

27. Kang, D., and D.Y.C. Fung. 2000. Application of thin agar layer method for recovery of injured *Salmonella typhimurium. International J. of Food Microbiol.* 54:127-132.

28. Kay, S. 2003. *E. coli* has cost the industry $2.8 billion. *Cattle Buyers Weekly.* Available at: http://www.cattlebuyersweekly.com/users/newsletters.php/2003 /0310.txt. Accessed 16 September 2008.

29. Kennedy, Jr., 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.

30. Marshall, V., and B. Reiter. 1980. Comparison of the antibacterial activity of hypothiocyanate anion towards *Streptococcus lactis* and *Escherichia coli. J. Gen. Microbiol*. 120:513-516.

31. Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Diseases*. 5:607-625.

32. Mead, P.S., and P.M. Griffin. 1998. *Escherichia coli* O157:H7. *The Lancet.* 352:1207-1212.

33. Miller, M.F., M.A. Carr, C.B. Ramsey, K.L. Crockett, and L.C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. *J. Anim. Sci.* 79:3062-3068.

34. Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681-685.

35. Savell, J.W., R.E. Branson, H.R. Cross, D.M. Stiffler, J.W. Wise, D.B. Griffin, and G.C. Smith. 1987. National consumer retail beef study: Palatability evaluations of been loin steaks that differed in marbling. *J. Anim. Sci.* 52:517-519.

36. Smith, L., J.E. Mann, K. Karris, M.F. Miller, and M.M. Brashears. 2005. Reduction of *Escherichia coli* O157:H7 and *Salmonella* in ground beef using lactic acid bacteria and the impact on sensory properties. *J. Food Prot*. 68:1587-1592.

37. Smith, D., M. Blackford, S. Younts, R. Moxley, J. Gray, L. Hungerford, T. Milton, and T. Klopfenstein. 2001. Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pen. *J. Food Prot.* 64:1899-1903.

38. Stopforth, J.D., M. Lopes, J.E. Shultz, R.R. Miksch and M. Samadpour. 2006. Microbiological status of fresh beef cuts. *J. Food Prot.* 69:1456-1459.

39. United States Department of Agriculture. 1999. Beef products contaminated with *Escherichia coli* O157:H7. *Fed. Regist*. 64:2803-2805. Availible at: http://fr.cos.com/cgi-bin/getRec?id=19990119a9. Accessed 13 November 2008.

40. U.S. Food and Drug Administration. 2001. *Escherichia coli* O157:H7. *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook.* Available at: www.cfsan.fda.gov/~mow/intro.html. Accessed on 18 September 2008.

41. Vote, J.D., W.J. Platter, J.D. Tatum, G.R. Schmidt, K.E. Belk, G.C. Smith, and N.C. Speer. 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. *J. Anim. Sci.* 78:952-957.

42. Wells, J.G., B.R. Davis, K. Wachsmuth, L.W. Riley, R.S. Remis, R. Sokolow, and G.K. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clinical Microbiol*. 18:512-520.

43. Wu, V.C.H., X. Qiu, and Y.P. Hsieh. 2008. Evaluation of *Escherichia coli* O157:H7 in apply juice with *Cornus* fruit (*Cornus officinalis Sieb.et Zucc*.) extracted by conventional media and thin agar layer method. *Food Microbiol*. 25:190-195.

44. Wu, V.C.H. and D.Y.C. Fung. 2001. Evaluation of thing agar layer method for recovery of heat-injured foodborne pathogens. *J. Food Sci.* 66:580-583.

CHAPTER III

THE SURVIVABILITY, GROWTH AND HEAT SUSCEPTIBILITY OF *E. COLI* O157:H7 IN ENHANCED BEEF BRINE SOLUTIONS CONTAINING POTASSIUM LACTATE AND LACTIC ACID PRODUCING BACTERIA

Abstract

Meat enhancement is used to increase consumer satisfaction through improved palatability and uniformity. Brine solutions that are re-covered or reused during the processing of enhanced meat cuts have a high risk of cross contamination. The objectives of the present study were to determine the effect of potassium lactate and lactic acid bacteria on the survivability and heat susceptibility of *E. coli* O157:H7 in brine solutions used to enhance beef products and to determine the effect of these interventions on consumer sensory scores and shelf life characteristics. To characterize safety, beef strip loins were enhanced with brine solutions (0.3% sodium chloride and 0.35% phosphate at 10% pump level) inoculated with high or low levels of *E. coli* O157:H7 and one of the following interventions: 0, 1.5, 2.5% potassium lactate or lactic acid bacteria (LAB 10^7 CFU/ml). Treated subprimals were fabricated into steaks and randomly allotted to one of the following internal endpoint temperatures: 0 (not cooked), 50, 55, 60, 65, 70 and 75°C. Once endpoint temperature was reached, the interior of each steak was sampled and *E. coli* O157:H7 was enumerated (high level) or detected (low level). To characterize palatability and shelf life, beef strip loin subprimals were enhanced with brine solutions containing previously mentioned

interventions plus a non-enhanced control. Consumer panelists evaluated palatability at 14 d postmortem and lean color on days 1, 3, and 7 of display (after a 14 d postmortem dark storage period). Display steaks were packaged in high-oxygen (80% O_2 / 20%CO_2) or low-oxygen (0.4%CO/30%CO_2/69.6%N_2) modified atmosphere packages. Data analysis showed no significant interactions between intervention treatment and steak temperature endpoints, indicating *E. coli* O157:H7 from treated brine solutions were not more susceptible to heat during cooking. Results also indicate the transference of pathogens into meat products was low for all interventions, regardless of inoculation level. Internal steak temperature (especially 70 and 75 °C) remains the most effective way to reduce pathogen levels in steaks enhanced with inoculated brine solutions. Steaks packaged in high-oxygen MAP and enhanced with a brine solution containing 1.5 or 2.5% potassium lactate maintained more desirable lean color scores throughout display and were more likely to be purchased by consumers than steaks enhanced with other treatments. Finally, the presence of potassium lactate (1.5 and 2.5%) and lactic acid producing bacteria had no detrimental impact on consumer palatability, while enhanced steaks were more desirable than non-enhanced controls.

Introduction

Consumers have become increasingly concerned about the safety of their food supply – especially due to *E. coli* O157:H7 contamination in beef. First designated as a foodborne pathogen in 1982, *(29, 32, 34, 42)* verotoxigenic *E. coli* O157:H7 has become of pathogen of concern, namely for its ability to cause hemolytic uremic syndrome (HUS) and or hemorrhagic colitis in individuals with suppressed or underdeveloped immune systems. The organism has the ability to produce shiga-toxins, which are responsible for human pathogenesis *(32).*

E. coli O157:H7 is a known inhabitant of the gastrointestinal tract of healthy cattle and is shed in the feces of animals that are carriers *(12).* The pathogen *E. coli* O157:H7 has the potential to be cross-contaminated onto the beef carcass during harvest. *E. coli* O157:H7 is resilient and able to survive at low pH, high salt concentrations and refrigeration temperatures *(32).* Because of these characteristics, *E. coli* O157:H7 has the potential to survive in brine solutions used to marinate whole muscle cuts.

While the majority of *E. coli* O157:H7 associated outbreaks in beef are with undercooked ground hamburger patties, there are also reports of product recalls related to whole muscle cuts. Beef steaks are considered a low risk to consumers for *E. coli* O157:H7 infection due to the fact the inside of muscle is generally considered sterile. The surface of the steak is the area with the potential to be contaminated with pathogenic organisms and is of particular

importance to whole muscle cuts intended for mechanical enhancement. Mechanical enhancement has the ability to translocate pathogens from the surface of the product to its interior. Many steaks are not cooked to an internal temperature of 160° F, allowing pathogens to escape the lethal effects of cooking and increase the risk of foodborne illness. Mechanically enhanced beef steaks have been associated with *E. coli* O157:H7 recalls *(14, 15).*

Mechanical enhancement contributes to steak juiciness and enhances flavor. A traditional brine solution consists of water, sodium chloride (salt) and phosphate. Other ingredients may be added to a brine solution. Potassium lactate is a viscous ingredient that can be added up to 2.0% of final product weight *(17)* Potassium lactate is a lactic acid salt, an ingredient beneficial for its antimicrobial and flavor enhancing properties which mimics sodium chloride.

Lactic acid bacteria (LAB) are a culture of live organisms that can be added to a brine solution. It can be used to improve the safety and quality of meat products in an alternative approach to traditional chemical treatments, which may be seen as unwholesome by some consumers. Lactic acid bacteria have been used in food products for years, functioning in preservation of fermented meat products. Lactic acid bacteria are responsible for producing a microbial metabolite or lactic acid, which lowers the environmental pH and aids in increasing the lag phase of pathogen growth. Other inhibitory actions include hydrogen peroxide production *(35).*

The brine solution can be a source of bacterial contamination. Solutions that are re-covered or reused during the processing of multiple cuts have the highest risk of cross contamination. It has been found by Geer and others (2004), that peak bacterium counts (log CFU/ml) are recovered from brine solutions used to marinate pork loins after 2.5 h (of samples taken at 0, 0.75, 1.75 and 2.5 h) of recirculation *(22).* Therefore, interventions added to brine solutions to inhibit microbial loads would be beneficial in reducing product cross-contamination.

Despite the antimicrobial properties of the brine, some bacteria are able to survive, but in an injured state. The injured bacteria maintain their virulent properties and are able to cause foodborne illness. Due to the harsh qualities of the selective components in *E. coli* O157:H7 media, sublethal injured cells are not able to recover and enumerate. For this reason, a method to recover heat-injured bacteria cells was developed and called the *thin agar layer* (TAL) method. In a study conducted by Hajimeer and others (2001) it was found that NaCl-injured *E. coli* O157:H7 cells had the highest recovery rate on TSA medium, followed by TAL medium and finally MacConkey sorbital agar (MSA) *(23).*

The U.S. beef industry consistently produces steaks of varying tenderness within the same USDA quality grade *(33).* This provides for an inconsistent and potential unpleasant eating experience for the consumer. To improve consistency and uniformity in palatability traits, steak enhancement has been utilized to increase consumer satisfaction. Retail survey results have indicated

that consumers can differentiate tenderness levels in steaks and are willing to pay more for enhanced product that improves the quality of the beef *(8).* Consumers scored enhanced steaks for tenderness nearly one full score higher ($P \leq 0.05$) than control steaks. In addition, enhanced steaks were scored higher ($P \leq 0.05$) for juiciness, beef flavor and overall quality *(8).*

The objectives of the present study were to determine the effect of potassium lactate and lactic acid bacteria on the survivability and heat susceptibility of *E. coli* O157:H7 in brine solutions used to marinate beef for retail, and to determine the effect of these enhancement solutions on consumer perception of the sensory and shelf life characteristics of enhanced beef strip steaks.

Methods and Materials

Quality. USDA Select, boneless beef strip loins IMPS 180, USDA; (n = 10) were obtained from a commercial processor and transported to the Texas Tech University (Lubbock, Texas) Gordon W. Davis Meat Science Laboratory – a USDA-FSIS inspected facility. Strip loins were divided randomly and equally into treatment groups (5 treatment groups, 2 strip loins per treatment) and were stored under vacuum in high-barrier bags for 14 days at 4ºC prior to processing. Five chilled brine solutions where formulated to provide the following ingredients and ingredient levels in the beef product after a 10% (avg. 11.8%) pump level: (i)

0% potassium lactate (PURASAL HiPure P, PURAC America Inc., Lincolnshire, Ill.), 0.3% sodium chloride (A.C. Legg Packing Co., Inc., Birmingham, Ala.) and 0.35% phosphate (Brifisol 85, BK Gilulini Corp., Simi Valley, Calif.), (ii) 1.5% potassium lactate, 0.3% sodium chloride and 0.35% phosphate, (iii) 2.5% potassium lactate, 0.3% sodium chloride and 0.35% phosphate, (vi) lactic acid bacteria cultures at 1 x 10^7 cfu/ml (NPC Meat Cultures, Nutrition Physiology Corp., Guymon, Okla.), 0.3% sodium chloride and 0.35% phosphate, (v) control (non-enhanced strip loin).

Strip loins were enhanced using a low-pressure (model PI 16/32, Gunther, Hausansechrift, Dieburg, Germany) multi-needle pickle injector with conveyor belt. The machine was cleaned by scrubbing with soap and water, and rinsed between each brine solution change. Uniform, 2.54 cm-thick steaks were cut from the anterior aspect of each strip loin and allocated to consumer shelf-life or taste panel analysis. Consumer panelists (n = 98) were recruited from the community. Steaks were prepared on a conveyorized belt grill (model TBG-60 MagiGrill, MagiKitch'n Inc., Quakertown, Pa.) to an internal temperature of 70ºC (avg. 70.9°C ± 1.64), portioned into uniform samples (approximately 1.25 cm^3) and served warm to panelists housed in individual booths under red lighting. Participants were provided unsalted crackers, apple juice and rinse water as pallet cleansers between samples. Consumer sensory characteristics were quantified using an 8-point verbally-anchored scale for tenderness, juiciness, flavor and overall liking (1 = extremely tough, dry, unbeef-like, and dislike; 8 =

extremely tender, juicy, beef-like and like) *(3).* Demographic information was also voluntarily collected (Table 15).

Consumer panelists (n = 32 to 34 per day) also evaluated steaks for changes in meat color over time (1, 3, and 7 days). Steaks were packaged in high-oxygen (80% O_2 / 20% CO_2) or low-oxygen (0.4% CO / 30% CO_2 / 69.6% N_2) modified atmosphere packages (MAP). Steaks were placed (1 per tray) in ridged black plastic trays (oxygen transmission rate [OTR] = 0.1 cc oxygen per tray per 24 h at 22.7°C and 0% relative humidity; moisture vapor transmission [MVT] = 2.0 g water vapor per 64,516 cm^2 per 24 h at 37.8°C and 100% relative humidity; [Cryovac, Inc., Duncan, S.C.]) containing black absorbent pads (model UZSP-40, UltraZap, Paper Pak Ind., La Verne, Calif.). Packages were flushed with the targeted atmosphere and sealed with a high-barrier film (LID 1050, OTR < 20 cc of oxygen per cm^2 per 24 h at 40°F [4.4°C], and 100% relative humidity; Cryovac) using a tray sealing machine (model CV/VG-S, Semi-Automatic 320 by 500, G. Mondini, Brescia, Italy). Gas mixtures were achieved using a gas mixer (model 9700260, Checkmate 9900, PBI Dansensor, Glen Rock, N.J.) or certified, pre-mixed cylinders of compressed gases (ultra-high purity) that were purchased locally (Airgas Inc., Lubbock, Texas). The modified atmospheres were validated by testing packages, which were not part of the trial, at the beginning, middle, and end of each packaging treatment using a head-space analyzer (model 333 Pac Check, Mocon, Minneapolis, Minn.). Packaging for the trial proceeded if

values were within ± 0.5% of the targeted O_2, N and CO_2 levels and 0.4% CO was measured in the tri-gas treatment with less than 0.5% residual O_2 present.

Packages were stored under retail display conditions using coffin-style meat cases (Model M1, Hussmann Corp., Bridgeton, Mo.) at 0°C (actual 0.05°C ± 0.72) under fluorescent lighting (approximately 2000 lx [avg. 2094.0] using high-output bulbs with a color temperature rating of 3500ºK and a color rendering index of 73). Shelf-life characteristics were quantified using a verbally-anchored numeric scale to determine if steaks had good color (1 = Very Strongly Agree; 6 = Very Strongly Disagree) and how likely panelists would be to purchase (1 = Definitely Would Purchase; 6 = Definitely Would Not Purchase) the package based on color *(3).*

Total microbial loads were determined for the meat in each package type (high oxygen and low oxygen) over time (1, 3, and 7 days). Packages were opened and a 50 cm^2 (Biotrace International Plc, Bridgend, UK) swab was aseptically collected from the lean surface of each steak and added to 10 ml of buffered peptone water (BPW; Oxoid Ltd., Basingstoke, Hampshire, England) which was mechanically shaken at 250 rpm for 2 min. Samples were subjected to 1:10 serial dilutions into 9 ml buffered peptone water (BPW) (Oxoid Ltd.). Total aerobic plate counts (APC) were determined by plating onto petrifilm aerobic count plates (3M Corporation, St. Paul, Minn.), *Lactobacillus* were determined by plating onto deMan, Rogosa and Sharpe agar (MRS; Oxoid Ltd.) and total coliforms and generic *E. coli* were determined by plating onto petrifilm

E. coli /coliform count plates (ECC and TCC) (3M Corporation). All plates were incubated at 37ºC for 48 h. Total aerobic psychrophilic bacteria were determined by plating onto petrifilm aerobic count plates (3M Corporation) and incubating at 7ºC for 10 days. All typical colonies were Identified and enumerated according to the manufacturer's instructions.

Safety. USDA Select, boneless beef strip loins IMPS 180, USDA; (n = 10) were obtained from a commercial processor and transported to the BSL II Pathogen Processing Laboratory at Texas Tech University. Strip loins where divided randomly and equally into treatment groups (10 treatment groups, 3 strip loins per treatment). Chilled brine solutions where formulated to provide the following ingredients and ingredient levels in the beef product after a 10% (act. 10.6 ± 0.70) pump level: (i) 0% potassium lactate, 0.3% sodium chloride and 0.35% phosphate, (ii) 1.5% potassium lactate, 0.3% sodium chloride and 0.35% phosphate, (iii) 2.5% potassium lactate, 0.3% sodium chloride and 0.35% phosphate, (iv) lactic acid bacteria cultures at 1 x 10^7 CFU/ml, 0.3% sodium chloride and 0.35% phosphate, (v) non-inoculated control; 0.3% sodium chloride and 0.35% phosphate, (vi) tap water; high inoculation.

Brine solutions 1 thru 4 were prepared in duplicate and inoculated with a four-strain cocktail of *E. coli* O157:H7 (strains A4 966, A5 528, A1 920 and 966 of bovine origin) at a 10^4 level (high), as well as a 10^1 level (low). The high

inoculum was achieved by preparing a concentrated (3.0 x 10^9 CFU/ml) culture of pathogen cocktail as described by Brashears et al. (1999). The cocktail was acquired from a frozen stock culture collection at the Food Microbiology Laboratory, Texas Tech University, prior to inoculating the brine solution. Temperature and pH were determined for all brines. Total *Lactobacillus* was identified from the appropriate brine solutions by making 1:100 serial dilutions into 9.9 ml BPW (Oxoid Ltd.) and plating onto MRS agar (Oxoid Ltd.). Plates were incubated at 37°C for 48 h.

The strip loins were injected with the *E. coli* O157:H7 inoculated brine solution using a hand-held, five needle stitch pump (model 3041, Koch Equipment, Kansas City, Mo.). The machine was cleaned and sanitized with Ala-Quat and Quadra-Quat rotation (Birko Corporation, Henderson, CO) between each brine solution change. Injected strip loins were allowed to marinate for approximately 12 h in individual overwrapped lugs at 10ºC before a 50 cm^2 template (Biotrace International Plc) surface swab was aseptically taken from the posterior end of the strip loin. The swab was diluted in 10 ml of gram-negative broth (GN; Difco, Becton Dickinson Microbiology Systems, Sparks Md.), and incubated at 37°C for 6-18 h for low inoculum samples, and 10 ml BPW (Oxoid Ltd.) for high inoculum samples. The strip loin was then hand cut into uniform 2.54 cm thick steaks using a template.

Steaks were allocated to different endpoint cooking temperatures for each treatment as follow; not cooked, cooked to internal temperatures of 50, 55, 60,

65, 70, and 75°C. Steaks were cooked on a George Forman Grill (model GRP 99, Salton Inc., Miramar, Fla.). Internal temperature was monitored by inserting thermocouple wire (model TT-J-36-SLE, Omega Engineering, Inc., Stamford, Conn.) to the geometric center of each steak. Data was collected using a Personal Daq (model OMB-DAQ-56, Omega Engineering, Inc.) data acquisition program. At desired endpoint temperature steaks went directly to an open ended plastic bag, submerged in ice water (0°C), to chill and decline from peak temperature. An interior sample (2 cm by 5 cm) was aseptically excised from the center through the entire thickness of the steak. This core sample was chopped using a sterilized food processor (model HC306, Black and Decker, Miramar, Fla.) and 10 g of meat was removed and homogenized (model Seward Stomacher 400C, Metrohm USA Inc., Riverview, Fla.) with dilutent; 90 ml GN broth (Difco, Becton Dickinson) for low inoculum samples, and 90 ml BPW (Oxoid Ltd.) for high inoculum samples, at 230 rpm for 2 min. Gram negative broth samples were incubated at 37°C for 6-18 h.

Samples were subjected to serial dilutions and were plated using an automated spiral plater (Autoplate 4000, Spiral Biotech Inc., Norwood, Mass.) onto MacConkey agar (EMD Chemicals, Inc., Gibbstown, N.J.) and MacConkey agar (EMD Chemicals, Inc.) overlayed with tryptic soy agar (TSA; EMD Chemicals, Inc.) using the thin layer method *(24)* and incubated at 37ºC for 24 h. Plates were counted using an automated colony counter (QCount, Spiral Biotech Inc.) for *E. coli* O157:H7. Samples which yielded no *E. coli* O157:H7 colonies via

spiral plating were enriched in GN broth (Difco, Becton Dickison) and processed in a Dynal BeadRetriever (model 701, Thermolabsystems, Helsinki, Finland) for immuno-magnetic separation (IMS) for pathogen detection. The IMS samples were then spread plated on MacConkey agar and incubated at 37ºC for 24 h for enumeration. Each experiment was replicated in its entirety three times.

Results from data analysis of high (log 10^4 CFU/g) and low (log 10^1 CFU/g) *E. coli* O157:H7 inoculated brine solutions are presented in Figures 1-6. Data quantifying *E. coli* O157:H7 counts were analyzed using the MIXED model procedures of SAS (vs. 9.1) with a model that included intervention treatment, steak temperature endpoint and the interaction of the two variables. Least squares means were separated using the PDIFF option of SAS and considered significant at a $P < 0.05$. Data analysis of the percent *E. coli* O157:H7 positive samples were analyzed using the GLIMMIX procedures of SAS (vs. 9.1) with a model that included intervention treatment, steak temperature endpoint and the interaction of the two variables. Main effects were pre-determined to be significant at $P < 0.05$.

Both statistical models (MIXED and GLIMMIX) indicated there were no significant interactions between intervention treatment and steak temperature endpoints. Therefore, there is no evidence to suggest the interventions used in

this study cause *E. coli* O157:H7 to be more susceptible to heat (during cooking) than *E. coli* O157:H7 not treated with an intervention.

Results and Discussion

Quality. Consumer sensory scores are presented in Table 1. Data analysis showed non-injected control steaks were ranked lower ($P < 0.05$) in tenderness, juiciness, flavor and overall liking when compared to other treatments. Consumers found no differences in tenderness, juiciness, flavor and overall liking between beef strip loins injected with solutions containing 0, 1.5, 2.5% potassium lactate or lactic acid bacteria. Data indicate the food safety interventions used in this study would not have detrimental effects on consumer rankings of palatability traits.

Consumer rankings of lean beef color and purchase intent for steaks packaged in high-oxygen modified atmosphere packaging (80% O_2 / 20%CO_2 MAP) are presented in Tables 2 – 3. No statistical difference existed between treatments for lean color scores on day 1 of simulated retail display. On day 3 of display, steaks injected with brine solutions containing 1.5, and 2.5% potassium lactate had more desirable lean color than control steaks, which were similar to steaks treated with lactic acid bacteria and 0% potassium lactate. By day 7 of display, steaks injected with solutions containing 1.5 and 2.5% potassium lactate had significantly better lean color scores than control, lactic acid bacteria and 0% potassium lactate treatments. No statistical differences in consumer purchase intent scores among treatments on day 1 of display. At day 3, steaks treated with 1.5% lactate were more likely to be purchased than control steaks and those treated with lactic acid bacteria. Purchase intent scores were highest for 1.5 and

2.5% potassium lactate treated steaks on day 7, and were significantly more desirable than control, lactic acid bacteria and 0% potassium lactate treated steaks.

Consumer rankings of lean beef color and purchase intent for steaks packaged in low-oxygen modified atmosphere packaging (0.4%CO/30%CO_2/69.6%N_2 MAP) are presented in Tables 4 – 5. The use of this packaging treatment had a stabilizing effect on lean color scores as evidence by the treatment means. Statistical differences in lean color scores were observed on day 3 of display; showing lactic acid bacteria treated steaks had more desirable lean color than control steaks. On day 1 of display, purchase intent scores were highest for 0% potassium lactate treated samples and lowest for samples treated with 2.5% potassium lactate. By day 3 of display, samples treated with lactic acid bacteria were similar to samples treated with potassium lactate, but significantly more desirable than control steaks. At day 7, there were no differences in consumer purchase intent scores for any treatment.

Non-pathogenic aerobic plate counts on APC petrifilm from steaks packaged in high-oxygen MAP reviled 1.5 and 2.5% potassium lactate treated steaks had statistical difference with lower counts on day 1 of display compared to other treatments. On day 3 and 7, 0% potassium lactate produced the highest bacterial count of all treatments. 1.5% potassium lactate on day 7 had the least microbial load compared to all other treatments with 1.02 CFU/ml. Statistical

differences occurred over time by day 3 of lighted display for 0% and control steaks.

Steaks packaged in low-oxygen packages on day 1 of non-pathogenic microbial sampling were lowest for 1.5 and 2.5% potassium lactate compared to other treatments. On day 3 these treatments were significantly different from the control, 0% potassium lactate and LAB which all had higher microbial counts. By day 7 0% potassium lactate had the highest log count (4.31 CFU/ml) while 1.5% potassium lactate had the lowest (0.59 CFU/ml). Significant differences occurred for all treatments over time by day 7 except for 1.5% potassium lactate which had no statistical differences over time. LAB treated steaks showed statistical difference over time each day (1, 3, and 7) of lighted display.

Psychrophilic bacteria counts for steaks packaged in high oxygen packages were varying for all treatments for days 1 and 3. For all treatments, there was significant difference in bacterial counts over time until day 7. 2.5% potassium lactate treated steaks maintained lower psychrophilic counts for days 1 and 3 as compared to other treatments, however by day 7 there were no significant differences between all treatments.

For psychrophilic counts recovered from steaks packaged in low-oxygen packages both the control and lactic acid bacteria treated steaks showed significant difference in log counts each day (0, 3 and 7) over time. All potassium lactate treated steaks (0, 1.5 and 2.5%) were similar on days 1 and 3 but were statistically different on day 7. Comparing within treatment groups on day one,

control steaks and lactic acid bacteria treated steaks were significantly different from potassium lactate treated steaks (0, 1.5 and 2.5%) which were all significantly different. By day 7, control steaks, 1.5% potassium lactate and lactic acid bacteria treated steaks were all similar, being significantly different from other treatments.

No differences occurred in LAB log counts in low-oxygen or high-oxygen package types and in low-oxygen packages over day of lighted display. High-oxygen MAP packaged steaks were statistically different over day of lighted display with d 3 being different from d 1 and 7. Bacterial counts to enumerate coliform and generic *E. coli* on *E. coli* petrifilm for both packaged types were all too few to count and therefore not presented.

Quality summary. Data indicate the treatments evaluated in this study have no detrimental impact on the palatability of enhanced beef strip steaks. Consumer acceptances researched by Carr et al. (6) found that consumers scored calcium chloride marinated steaks for tenderness almost one full score higher ($P < 0.05$) than control steaks. In addition, consumers scored enhanced steaks higher for juiciness, beef flavor and overall quality than non-enhanced control steaks. Panelist indicated that they were able to differentiate tenderness levels in the meat, and were willing to pay a premium for calcium chloride marinated product for improved quality. Mechanical enhancement may be used by retailers to capture additional value in their beef products.

Enhanced strip steaks packaged in high-oxygen MAP packaging and treated with 1.5 or 2.5% potassium lactate maintained more desirable lean color scores throughout retail display and were more likely to be purchased by consumers. Steaks treated with lactic acid bacteria and packaged in high-oxygen MAP exhibited lean color scores and purchase intent scores similar to control steaks and steaks treated with 0% potassium lactate. Control and treated steaks packaged in low-oxygen MAP maintained desirable lean color scores throughout the 7 day display period (scores ranged from 1.95 to 2.83 on a scale of 1 to 6). Steaks treated with 2.5% potassium lactate and packaged in low-oxygen MAP had low purchase intent and lean color scores initially, but were similar to all treatments on day 7 of display. Jensen et al. (2003) concluded potassium lactate and potassium lactate/potassium diacetate when used to enhance pork loins produced chops which suffered less discoloration after 3 and 4 days of retail display as compared to lactates or acetates alone and indicated potassium lactate can be an effective brine ingredient to increase shelf life and consumer acceptability under retail display.

Safety and non-pathogen microbial analysis. Two separate media were used to recover *E. coli* O157:H7 in high-inoculated samples. The MacConkey with a TSA overlay allowed us to recover injured cells of the pathogen while the MacConkey agar would not recover injured cells. Similar results have been concluded by Wu et al. (2001) showing that no difference ($P >$

0.05) occurred between heat injured *E. coli* O157:H7 recovery on TSA and TAL, however both methods produced higher numbers of injured cells than the comparative selective media. Figure 2 illustrates that at low and high inoculation levels there were no significant differences among treatments at the $P < 0.05$ level of significance. Food safety interventions containing 1.5 and 2.5% potassium lactate tended to have lower pathogen counts in solution than the lactic acid bacteria and 0% potassium lactate treatments for both inoculums. For both inoculation levels, the lactic acid bacteria and 0% potassium lactate behaved similarly while 1.5 and 2.5% potassium lactate treatments had similar tendencies (Figure 2). The data however, indicates the pathogen was injected into the interior of the product (0.5 to 1.5 log CFU/g recovered) regardless of treatment.

While we recovered around 1.0 log CFU/g from steaks, not all steaks were positive in the interior surfaces for *E. coli* O157:H7 (Figure 2). Treatment means presented in Figure 4 include steaks from all cooking endpoints. The lactic acid bacteria treatment had the lowest percentage of internal *E. coli* O157:H7 samples (23.2%), while 0% potassium lactate treatment had the highest (91.4%). 1.5 and 2.5% potassium lactate treatments had percent positives for *E. coli* O157:H7 of 31.3 and 59.2% respectively. Therefore, approximately 51.3% of the steaks tested positive for the pathogen, depending on the treatment, with significant difference occurring among the various treatments.

A similar trend was observed in steaks enhanced with a brine solution containing low levels (log 10^1 CFU/g) of *E. coli* O157:H7 (Figure 6). There were no statistical differences between treatments for the percentage of positive *E. coli* O157:H7 samples, but data indicate 97.9% of samples were positive on average at the low inoculation level.

The amount of *E. coli* O157:H7 recovered from steaks after cooking varied depending on the final endpoint cooking temperature (Figure 3 and 5) and the initial inoculation level. Temperature was significant at the high and low inoculation levels (P = 0.0066 and 0.0164 respectively) in reducing the percent positive internal steak samples for *E. coli* O157:H7. For steaks inoculated with 1 x 10^4 CFU/g *E. coli* O157:H7, at 0 and 50 °C, 100.0% of the samples tested positive for the pathogen. As the temperature increased, the total number of steaks testing positive decreased as expected. At 55 and 60 °C, 95.9% and 52.0% of the samples tested positive with none of the samples testing positive after cooking to an endpoint temperature of 70 and 75 °C. A similar trend was observed with steaks inoculated with *E. coli* O157:H7 at 1 x 10^1 CFU/g, but the kill rate was faster. After reaching final endpoint temperatures of 60, 65, 70, and 75°C, there were virtually no steaks that tested positive (Figure 5). At endpoint temperatures of 55°C, 4.5% of the samples tested positive while at 0 and 50 °C, a total of 100.0% and 99.9% of the samples tested positive, respectively. Gill et al. (2008) with a similar study concluded an internal temperature greater than 60

but less than 65°C was sufficient to kill all bacteria internalized in beef steaks *(16).*

Microbial analysis was also conducted on the brine solution 30 minutes post inoculation with low and high levels of *E. coli* O157:H7 (Figure 1). Data analysis within inoculation level revealed no significant treatment differences between interventions for the high inoculums (P = 0.4111). The lactic acid bacteria treatment tended to have the least amount of pathogens present in solution that other treatments (0, 1.5 and 2.5% potassium lactate). However at the low inoculum, there was a significant difference between treatments (P = 0.0276). The 2.5% potassium lactate food safety intervention was significantly different from all other treatments (Figure 1).

Microbial analysis summary. The food safety interventions tested in this study had no effect on the amount or prevalence of *E. coli* O157:H7 injected into steaks during enhancement at low and high inoculation levels. Approximately one-half (51.2%) of samples enhanced with a brine solution containing log 10^4 CFU/g *E. coli* O157:H7 and a food safety intervention tested positive for *E. coli* O157:H7. Approximately one-half (51.3%) of samples enhanced with a brine solution containing log 10^1 CFU/g *E. coli* O157:H7 and a food safety intervention tested positive for *E. coli* O157:H7. Peak internal steak temperature during cooking significantly reduced *E. coli* O157:H7 counts and percent positives. A minimum peak internal temperature of 70°C was required to reduce the percentage of positive samples to 0% in steaks enhanced with a brine solution

containing log 10^4 CFU/g *E. coli* O157:H7. Finally, these data indicate the presence of a food safety intervention in brine solutions does not cause *E. coli* O157:H7 to become more susceptible to heat and die at lower temperatures during cooking.

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Table 1. Consumer Sensory Scores for Each Treatment

Brine Treatment	Tenderness[1]	Juiciness[2]	Flavor[3]	Overall[4]
Control – no brine	3.73[a]	4.70[a]	5.04[a]	4.27[a]
0% potassium lactate	6.42[b]	6.27[b]	6.46[b]	6.43[b]
1.5% potassium lactate	6.61[b]	6.40[b]	6.46[b]	6.43[b]
2.5% potassium lactate	6.63[b]	6.35[b]	6.29[b]	6.24[b]
Lactic acid bacteria	6.70[b]	6.29[b]	6.46[b]	6.44[b]
SEM	0.27	0.23	0.20	0.32

[1] Tenderness scores: 1 = extremely tough; 8 = extremely tender
[2] Juiciness scores: 1 = extremely dry; 8 = extremely juicy
[3] Flavor scores: 1 = extremely bland; 8 = extremely beef-like
[4] Overall liking scores: 1 = dislike extremely; 8 = like extremely
[a,b] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$)

Table 2. Consumer **Color Scores**[1] of Beef Strip Loin Steaks Packaged in **High-Oxygen** (80% O_2 / 20%CO_2) Modified Atmosphere Packages

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	2.65	4.15[c]	5.27[b]	0.41
0% potassium lactate	3.31	3.80[abc]	5.14[b]	0.41
1.5% potassium lactate	2.37	2.69[a]	3.72[a]	0.41
2.5% potassium lactate	3.02	2.95[ab]	3.94[a]	0.41
Lactic acid bacteria	2.93	4.03[bc]	5.57[b]	0.41

[1] Color: Consumer agreement to the statement "The meat in this package has good color". 1 = very strongly agree; 2 = strongly agree; 3 = slightly agree; 4 = slightly disagree; 5 = strongly disagree; 6 = very strongly disagree.
[a,b,c] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$).

Table 3. Consumer **Purchase Intent Scores**[1] of Beef Strip Loin Steaks Packaged in **High-Oxygen** (80% O_2 / 20%CO_2) Modified Atmosphere Packages

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	2.67	4.41[b]	5.38[bc]	0.46
0% potassium lactate	3.34	3.95[ab]	5.22[bc]	0.46
1.5% potassium lactate	2.28	2.93[a]	3.88[a]	0.46
2.5% potassium lactate	2.96	3.17[ab]	4.11[ab]	0.46
Lactic acid bacteria	2.85	4.30[b]	5.56[c]	0.46

[1] Purchase intent: Consumer response when asked how likely they would be to purchase the meat in the package based on its color. 1 = definitely would purchase; 2 = probably would purchase; 3 = may purchase; 4 = may not purchase; 5 = probably would not purchase; 6 = definitely would not purchase.

[a,b,c] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$)

Table 4. Consumer **Color Scores**[1] of Beef Strip Loin Steaks Packaged in **Low-Oxygen** (0.4%CO/30%CO_2/69.6%N_2) Modified Atmosphere Packages

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	2.56	2.67[b]	2.50	0.22
0% potassium lactate	2.28	2.09[ab]	1.98	0.22
1.5% potassium lactate	2.53	2.05[ab]	2.02	0.22
2.5% potassium lactate	2.83	2.47[ab]	2.29	0.22
Lactic acid bacteria	2.40	1.95[a]	1.97	0.22

[1] Color: Consumer agreement to the statement "The meat in this package has good color". 1 = very strongly agree; 2 = strongly agree; 3 = slightly agree; 4 = slightly disagree; 5 = strongly disagree; 6 = very strongly disagree

[a,b] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$)

Table 5. Consumer **Purchase Intent Scores**[1] of Beef Strip Loin Steaks Packaged in **Low-Oxygen** (0.4%CO/30%CO_2/69.6%N_2) Modified Atmosphere Packages

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	2.57^{ab}	2.85^{b}	2.65	0.25
0% potassium lactate	2.13^{a}	2.14^{ab}	1.94	0.25
1.5% potassium lactate	2.45^{ab}	2.22^{ab}	2.09	0.25
2.5% potassium lactate	2.86^{b}	2.61^{ab}	2.28	0.25
Lactic acid bacteria	2.34^{ab}	2.10^{a}	2.02	0.25

[1] Purchase intent: Consumer response when asked how likely they would be to purchase the meat in the package based on its color. 1 = definitely would purchase; 2 = probably would purchase; 3 = may purchase; 4 = may not purchase; 5 = probably would not purchase; 6 = definitely would not purchase

[a,b] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$)

Table 6. **APC** Non-pathogenic bacteria counts[1] of Beef Strip Loin Steaks Packaged in **High-Oxygen** (80% O_2 / 20%CO_2) Modified Atmosphere Packages ($P < 0.0001$)

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	2.25^{bx}	2.98^{by}	2.35^{bx}	0.39
0% potassium lactate	3.13^{bx}	3.91^{cy}	4.49^{cy}	0.39
1.5% potassium lactate	1.06^{axy}	1.67^{ay}	1.02^{ax}	0.39
2.5% potassium lactate	0.91^{ax}	1.04^{ax}	2.22^{by}	0.39
Lactic acid bacteria	2.41^{bx}	2.43^{bx}	3.67^{cy}	0.39

[1] Log CFU/ml

[a,b,c] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$)

[x,y] Least squares means within a row lacking a common superscript letter differ ($P < 0.05$)

Table 7. **APC** Non-pathogenic bacteria counts[1] Beef Strip Loin Steaks Packaged in **Low-Oxygen** (0.4%CO/30%CO_2/69.6%N_2) Modified Atmosphere Packages ($P < 0.0001$)

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	2.03^{bcx}	2.81^{by}	3.41^{bcy}	0.36
0% potassium lactate	3.35^{cx}	2.81^{bx}	4.31^{cy}	0.36
1.5% potassium lactate	0.92^{abx}	0.93^{ax}	0.59^{ax}	0.36
2.5% potassium lactate	0.18^{ax}	0.34^{ax}	1.74^{aby}	0.36
Lactic acid bacteria	2.00^{bcx}	2.83^{by}	3.78^{cz}	0.36

[1] Log CFU/ml

[a,b,c] Least squares means within a column lacking a common superscript letter differ ($P < 0.05$)

[x,y,z] Least squares means within a row lacking a common superscript letter differ ($P < 0.05$)

Table 8. **Psychrophilic** non-pathogenic bacteria counts[1] of Beef Strip Loin Steaks Packaged in **High-Oxygen** (80% O_2 / 20%CO_2) Modified Atmosphere Packages (P = 0.002)

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	1.96^{abx}	2.15^{abcx}	3.16^{y}	0.37
0% potassium lactate	3.85^{cx}	4.07^{cx}	4.73^{y}	0.37
1.5% potassium lactate	2.18^{abcx}	2.08^{abx}	2.86^{y}	0.37
2.5% potassium lactate	1.17^{ax}	1.13^{ax}	3.45^{y}	0.37
Lactic acid bacteria	3.04^{bcx}	3.19^{bcx}	3.96^{y}	0.37

[1] Log CFU/ml

[a,b,c] Least squares means within a column lacking a common superscript letter differ (P < 0.05

[x,y] Least squares means within a row lacking a common superscript letter differ (P < 0.05)

Table 9. **Psychrophilic** Non-pathogenic bacteria counts[1] Beef Strip Loin Steaks Packaged in **Low-Oxygen** (0.4%CO/30%CO_2/69.6%N_2) Modified Atmosphere Packages *(*P < 0.0001)

Brine Treatment	Day of Lighted Display			SEM
	1	3	7	
Control – no brine	1.70^{abx}	2.41^{bcy}	3.74^{bz}	0.39
0% potassium lactate	3.47^{cx}	3.66^{dx}	4.76^{cy}	0.39
1.5% potassium lactate	2.00^{bx}	2.19^{bx}	3.53^{by}	0.39
2.5% potassium lactate	1.29^{ax}	1.37^{ax}	2.52^{ay}	0.39
Lactic acid bacteria	1.70^{abx}	2.98^{cdy}	3.88^{bz}	0.39

[1] Log CFU/ml

[a,b,c,d] Least squares means within a column lacking a common superscript letter differ (P < 0.05)

[x,y,z] Least squares means within a row lacking a common superscript letter differ (P < 0.05)

Table 10. LAB counts[1] of LAB Treated Beef Strip Loin Steaks Packaged in **Low-Oxygen** (0.4%CO/30%CO_2/69.6%N_2) or **High-Oxygen** (80% O_2 / 20%CO_2) Modified Atmosphere Packages

Packaging Type	Day of Lighted Display			SEM
	1	3	7	
High-Oxygen	3.04^{y}	2.20^{x}	3.03^{y}	0.56
Low-Oxygen	3.06	3.05	2.66	0.56

[1] Log CFU/ml

[x,y] Least squares means within a row lacking a common superscript letter differ (P < 0.05)

Table 11. *E. coli* O157:H7 Low Inoculation[1] Brine Average Percent Pump Enhancement of Strip Loin

Brine Treatment	%	SD
Control – no *E. coli* O157:H7	10.78	1.30
0% potassium lactate	10.46	0.25
1.5% potassium lactate	11.06	0.42
2.5% potassium lactate	9.96	0.22
Lactic acid bacteria	10.80	0.91

[1] (10^1 CFU/ml)

Table 12. *E. coli* O157:H7 High Inoculation[1] Brine Average Percent Pump Enhancement of Strip Loin

Brine Treatment	%	SD
Control – water only	9.93	0.24
0% potassium lactate	8.74	1.25
1.5% potassium lactate	9.92	0.44
2.5% potassium lactate	8.94	0.57
Lactic acid bacteria	9.90	0.58

[1] (10^4 CFU/ml)

Table 13. Average Brine pH 30 min. after *E. coli* O157:H7 Low Inoculation[1]

Brine Treatment	pH	SD
Control – no *E. coli* O157:H7	7.4	0.1
0% potassium lactate	7.3	0.3
1.5% potassium lactate	6.9	0.1
2.5% potassium lactate	6.7	0.1
Lactic acid bacteria	7.4	0.4

[1] (10^1 CFU/ml)

Table 14. Average Brine pH 30 min. after *E. coli* O157:H7 High Inoculation[1]

Brine Treatment	pH	SD
Control – water only	6.0	0.0
0% potassium lactate	7.3	0.1
1.5% potassium lactate	6.8	0.1
2.5% potassium lactate	6.7	0.1
Lactic acid bacteria	7.3	0.3

[1] (10^4 CFU/ml)

Table 15. Demographic Consist of Consumer Panelists

Characteristic	Frequency	Percentage
Gender		
Male	32	36.36
Female	56	63.64
Household Size		
1 person	11	11.34
2 people	34	35.05
3 people	18	18.56
4 people	20	20.62
5 people	11	11.34
6 people	3	3.09
7 or more people	0	0
Household Income		
Single Income	32	33.68
Dual Income	63	66.32
Age		
Under 18	0	0
18-34	22	22.68
35-50	27	27.84
Over 50	48	49.48
Ethnic Origin		
African-American	0	0
Caucasian/White	89	92.71
Native American	1	1.04
Hispanic	5	5.21
Asian	1	1.04
Other	0	0
Income		
Under $20,000	8	8.51
$20,000 - $29,999	7	7.45
$30,000 - $49,999	26	27.66
$50,000 - $69,999	31	32.98
$70,000 - $100,000	12	12.77
More than $100,000	10	10.64
Education		
Non-High School Graduate	3	3.06
High School Graduate	7	7.14
Some College/Technical School	48	48.98
College Graduate	24	24.49
Post Graduate	16	16.33
Beef Consumption / Week		
None	2	2.04
1 to 2 times	27	27.55
3 to 4 times	49	50.00
5 to 6 times	15	15.31
7 or more times	5	5.10

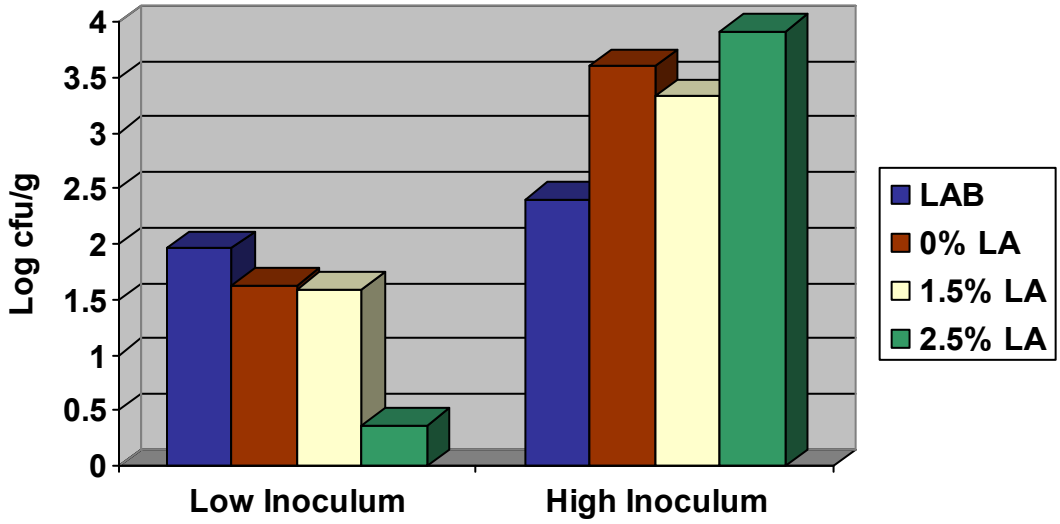

Figure 1. *E. coli* O157:H7 counts (log CFU/g) in brine solutions with intervention treatments 30 minutes after inoculation at low (log 10^1 CFU/g) and high (log 10^4 CFU/g) levels. (Treatment *P* = 0.0276 and 0.4111 for low and high inoculums respectively)

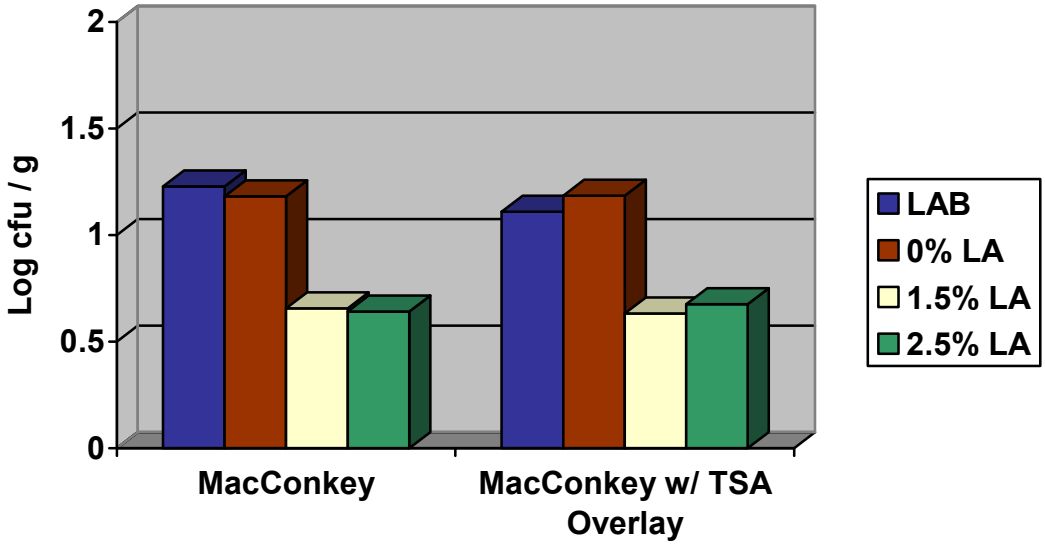

Figure 2. Internal *E. coli* O157:H7 counts (log CFU/g) in strip loin steaks injected with a brine solution containing Log 10^4 CFU/g and various food safety intervention treatments for different medias. (Treatment *P* = 0.1345 and 0.1193 for MacConkey and MacConkey with TSA overlay respectively.)

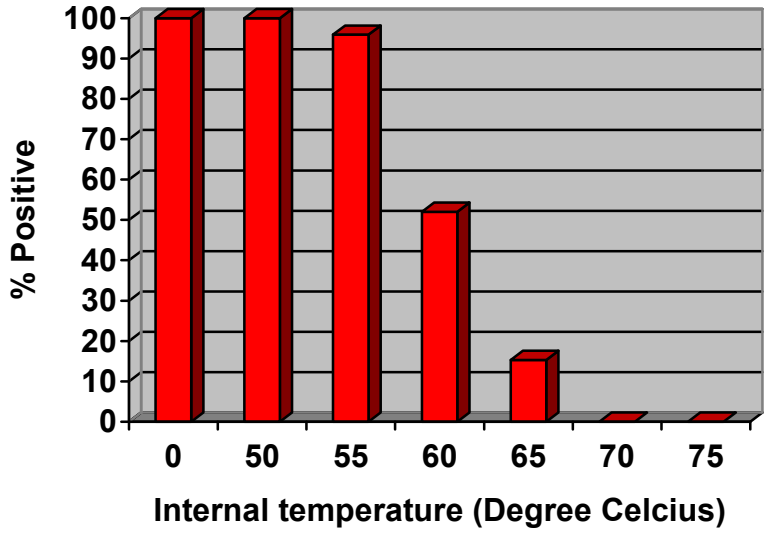

Figure 3. The percentage of *E. coli* O157:H7-positive internal steak samples for strip loins enhanced with a brine solution containing log 10^4 CFU/g *E. coli* O157:H7 and cooked to various internal temperatures. (Treatment *P* = 0.0066)

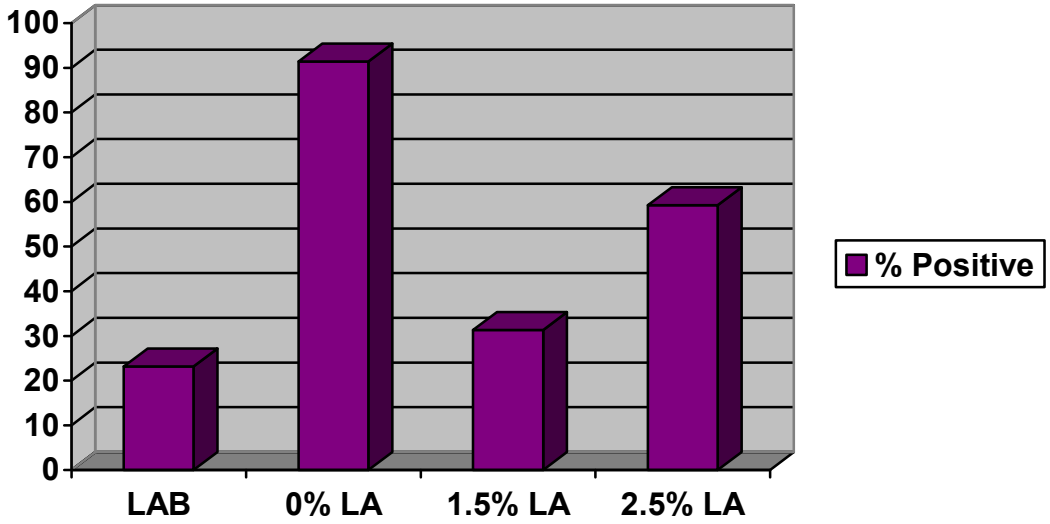

Figure 4. The effect of food safety intervention treatments on the percentage of *E. coli* O157:H7 positive samples from the internal portion of steaks enhanced with brine solution inoculated with log 10^4 CFU/g *E. coli* O157:H7 (Treatment *P* = 0.0472)

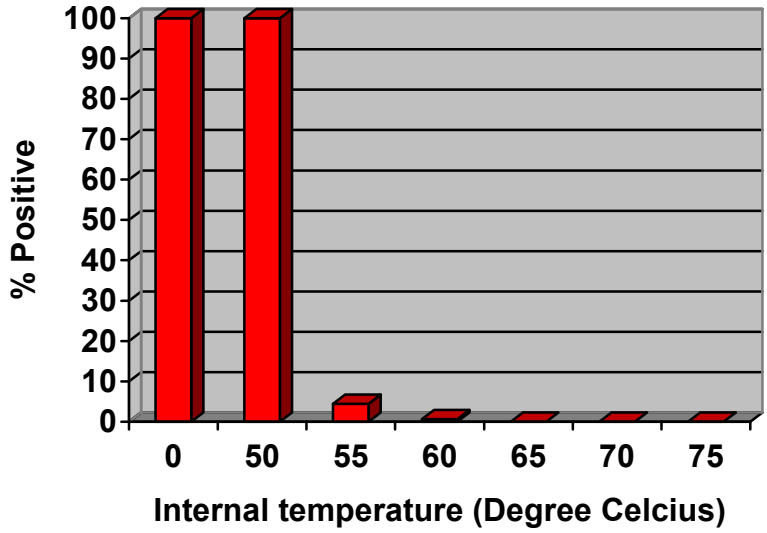

Figure 5. The percentage of *E. coli* O157:H7-positive internal steak samples for strip loins enhanced with a brine solution containing log 10^1 CFU/g *E. coli* O157:H7 and cooked to various internal temperatures. (Temperature *P* = 0.0164)

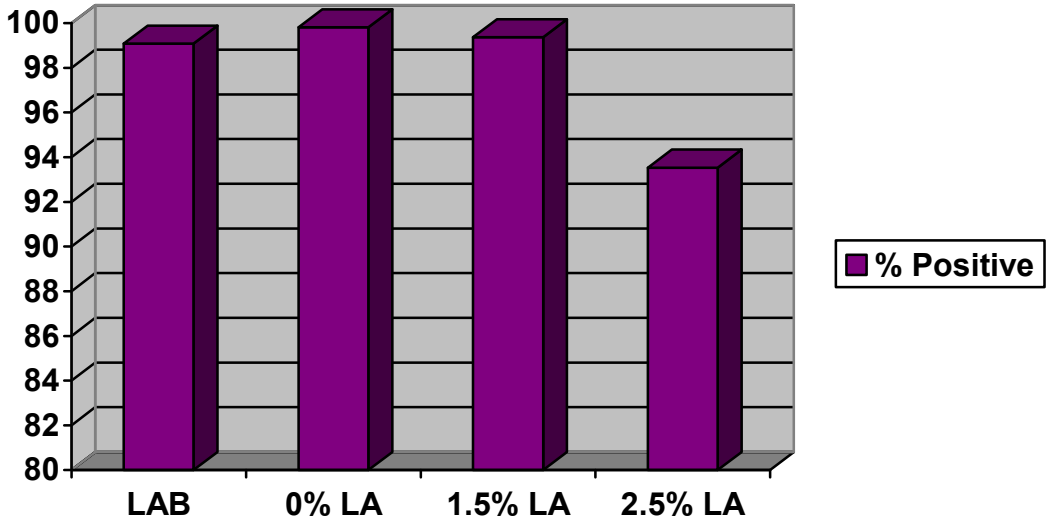

Figure 6. The effect of food safety intervention treatments on the percentage of *E. coli* O157:H7 positive samples from the internal portion of steaks enhanced with brine solution inoculated with log 10^1 CFU/g *E. coli* O157:H7 (Treatment *P* = 0.0444)

References

1. American Meat Institute. 2007. Meat matters. Consumer's guide to enhanced meats. Availible at: www.meatami.com. Accessed 25 September 2008.

2. Amezquita, A., and M.M. Brashears. 2002. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. *J. Food Prot.* 65:316-325.

3. Brashears, M.M., and W.A. Durre. 1999. Antagonistic action of *Lactobacillus lactis* toward *Salmonella* spp. and *Escherichia coli* O157:H7 during growth and refrigerated storage. *J. Food Prot.* 62:1336-1340.

4. Brooks, J.C., M. Alvarado, T.P. Stephens, J.D. Kellermeier, A.W. Tittor, M.F. Miller and M.M. Brashears. 2008. Spoilage and safety characteristics of ground beef packaged in traditional and modified atmosphere packages. *J. Food Prot.* 71:293-301.

5. Carpenter, C.E., D.P. Cornforth, and D. Whittier. 2001. Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Sci.* 57:359-363.

6. Carr, M.A., K.L. Crockett, C.B. Ramsey, and M.F. Miller. 2004. Consumer acceptance of calcium chloride-marinated top loin steaks. *J. Anim. Sci.* 82:1471-1474.

7. CDC. 2002. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating ground beef - - United States, June- -July 2002. *MMWR*, 26 July. 51:637-639. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5129a1.htm. Accessed 12 September 2008.

8. CDC. 1997. *Escherichia coli* O157:H7 infections associated with eating a nationally distributed commercial brand of frozen ground beef patties and burgers - - Colorado, 1997. *MMWR,* 22 August. 46:777-778. Available at: http://cdc.gov/mmwr/preview/mmwrhtml/00049079.htm. Accessed 12 September 2008.

9. CDC. 1993. Update: Multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers - - western United States, 1992-1993. *MMWR*, 16 April. 42:258-263. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/00020219.htm. Accessed 12 September 2008.

10. Chapman, P.A., C.A. Siddons, D.J. Wright, P. Norman, J.Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. *Epidemiol Infect.* 111:439-447.

11. Fegan, N., P. Vanderlinde, G. Higgs, and P. Desmarchelier. 2004. The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. *J. App. Microbiol.* 97:362-370.

12. Gill, C.O., L.F. Moza, and S. Barbut. 2008. Survival of bacteria in less than thorough cooked, brine-injected steaks. *Food Control*. Accepted Manuscript. Doi: 10.1016/j.foodcont.2008.07.019.

13. Gill, C.O., B. Uttaro, M. Badoni, and S. Zawadski. 2008. Distributions of brine and bacteria in beef primal cuts injected with brine without, or before or after mechanical tenderizing. *Meat Sci.* 79:181-187.

14. Gill, C.O., and J.C. McGinnis. 2005. Factors affecting the microbiological condition of the deep tissues of mechanically tenderized beef. *J. Food Prot.* 68:796-800.

15. Greer, G.G., F. Nattress, B. Dilts and L. Baker. 2004. Bacterial contamination of recirculating brine used in the commercial production of moisture-enhanced pork. *J. Food Prot.* 67:185-188.

16. Hajmeer, M.N., D.Y.C. Fung, J.L. Marsden, and G.A. Millilken. 2001. Effects of preparation method, age, and plating technique of thin agar layer media on recovery of *Escherichia coli* O157:H7 injured by sodium chloride. *J. Microbiol. Methods.* 47:249-253.

17. Jensen, J.M., K.L. Robbins, K.J. Ryan, C. Homco-Ryan, F.K. McKeith, and M.S. Brewer. 2003. Effects of lactic and acetic acid salts on quality characteristics of enhanced pork during retail display. *Meat Sci.* 63:501-508.

18. Jones, R.J., H.M. Hussein, M. Zagorec, G. Brightwell, and J.R. Tagg. 2008. Isolation of lactic acid bacteria with inhibitory activity against pathogens and spoilage organisms associated with fresh meat. *Food Microbiol.* 25:228-234.

19. Juneja, V.K., B.S. Marmer, and B.S. Eblen. 1999. Predictive model for the combined effect of temperature, pH, sodium chloride, and sodium pyrophosphate on the heat resistance of *Escherichia coli* O157:H7. *J. Food Safety.* 19:147-160.

20. Kang, D., and D.Y.C. Fung. 2000. Application of thin agar layer method for recovery of injured *Salmonella typhimurium. International J. of Food Microbiol.* 54:127-132.

21. Kay, S. 2003. *E. coli* has cost the industry $2.8 billion. *Cattle Buyers Weekly.* Available at: http://www.cattlebuyersweekly.com/users/newsletters.php/2003 /0310.txt. Accessed 16 September 2008.

22. Kennedy, Jr., 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.

23. Marshall, V., and B. Reiter. 1980. Comparison of the antibacterial activity of hypothiocyanate anion towards *Streptococcus lactis* and *Escherichia coli. J. Gen. Microbiol*. 120:513-516.

24. Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Diseases*. 5:607-625.

25. Mead, P.S., and P.M. Griffin. 1998. *Escherichia coli* O157:H7. *The Lancet.* 352:1207-1212.

26. Miller, M.F., M.A. Carr, C.B. Ramsey, K.L. Crockett, and L.C. Hoover. 2001. Consumer thresholds for establishing the value of beef tenderness. *J. Anim. Sci.* 79:3062-3068.

27. Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England J. Medicine.* 308:681-685.

28. Smith, L., J.E. Mann, K. Karris, M.F. Miller, and M.M. Brashears. 2005. Reduction of *Escherichia coli* O157:H7 and *Salmonella* in ground beef using lactic acid bacteria and the impact on sensory properties. *J. Food Prot*. 68:1587-1592.

29. Smith, D., M. Blackford, S. Younts, R. Moxley, J. Gray, L. Hungerford, T. Milton, and T. Klopfenstein. 2001. Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pen. *J. Food Prot.* 64:1899-1903.

30. Stopforth, J.D., M. Lopes, J.E. Shultz, R.R. Miksch and M. Samadpour. 2006. Microbiological status of fresh beef cuts. *J. Food Prot.* 69:1456-1459.

31. USDA-Food Safety and Inspection Service. 2003. Illinois firm recalls beef products for possible *E. coli* O157:H7. Recall Release. FSIS-RC-028-2003. Available at: http://www.fsis.usda.gov/OA/recalls/prelease/pr028-2003.htm. Accessed 18 August 2008.

32. Vote, J.D., W.J. Platter, J.D. Tatum, G.R. Schmidt, K.E. Belk, G.C. Smith, and N.C. Speer. 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. *J. Anim. Sci.* 78:952-957.

33. Wells, J.G., B.R. Davis, K. Wachsmuth, L.W. Riley, R.S. Remis, R. Sokolow, and G.K. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clinical Microbiol*. 18:512-520.

34. Wu, V.C.H., X. Qiu, and Y.P. Hsieh. 2008. Evaluation of *Escherichia coli* O157:H7 in apply juice with *Cornus* fruit (*Cornus officinalis Sieb.et Zucc*.) extracted by conventional media and thin agar layer method. *Food Microbiol*. 25:190-195.

35. Wu, V.C.H. and D.Y.C. Fung. 2001. Evaluation of thing agar layer method for recovery of heat-injured foodborne pathogens. *J. Food Sci.* 66:580-583.

Appendix A. Beef Strip Steak Consumer Color Evaulation

Texas Tech University

BEEF STRIP STEAK

CONSUMER COLOR EVALUATION

PROJECT_____ Panelist No. _____ DATE _____

Strip Steak Color: Indicate by placing a mark in the box how strongly you agree/disagree with the statement: "***The meat in this package has good color***".

Package Purchasing: Based on the color of the meat, indicate how likely you would be to ***purchase*** this package if it were available in the retail meat case.

Strip Steak Color	Package Purchasing
6= Very Strongly Disagree	6= Definitely Would *Not* Purchase
5= Strongly Disagree	5= Probably Would *Not* Purchase
4= Slightly Disagree	4= May *Not* Purchase
3= Slightly Agree	3= May Purchase
2= Strongly Agree	2= Probably Would Purchase
1= Very Strongly Agree	1= Definitely Would Purchase

No.	Strip Steak Color	Package Purchasing		No.	Strip Steak Color	Package Purchasing
1				16		
2				17		
3				18		
4				19		
5				20		
6				21		
7				22		
8				23		
9				24		
10				25		
11				26		
12				27		
13				28		
14				29		
15				30		

Appendix B. Beef Consumer Evaluation

Instructions: Thank you for your participation in our sensory test. You will be served five USDA inspected beef steaks to taste. Thank-you!

Sample 1

Tenderness

Please indicate how much you like or dislike the ***Tenderness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Tough	Very Tough	Moderately Tough	Slightly Tough	Slightly Tough	Moderately Tough	Very Tough	Extremely Tough
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is the tenderness Acceptable? *Yes* ___ *No* ___

Juiciness

Please indicate how much you like or dislike the ***Juiciness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Dry	Very Dry	Moderately Dry	Slightly Dry	Slightly Dry	Moderately Dry	Very Dry	Extremely Dry
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Flavor

Please indicate how much you like or dislike the ***Flavor*** (all factors included) of each sample by placing a check in the appropriate box for each sample.

Extremely Unbeef like	Very Unbeef like	Moderately Unbeef like	Slightly Unbeef like	Slightly Beef like	Moderately Beef like	Very Beef like	Extremely Beef like
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Overall Liking of the Samples

Please indicate how much you ***like or dislike*** the sample by placing a check in the appropriate box for each sample.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is this Steak Acceptable? ***Yes*** ___ ***No*** ___

Sample 2

Tenderness

Please indicate how much you like or dislike the ***Tenderness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Tough	Very Tough	Moderately Tough	Slightly Tough	Slightly Tough	Moderately Tough	Very Tough	Extremely Tough
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is the tenderness Acceptable? *Yes* ___ *No* ___

Juiciness

Please indicate how much you like or dislike the ***Juiciness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Dry	Very Dry	Moderately Dry	Slightly Dry	Slightly Dry	Moderately Dry	Very Dry	Extremely Dry
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Flavor

Please indicate how much you like or dislike the ***Flavor*** (all factors included) of each sample by placing a check in the appropriate box for each sample.

Extremely Unbeef like	Very Unbeef like	Moderately Unbeef like	Slightly Unbeef like	Slightly Beef like	Moderately Beef like	Very Beef like	Extremely Beef like
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Overall Liking of the Samples

Please indicate how much you ***like or dislike*** the sample by placing a check in the appropriate box for each sample.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is this Steak Acceptable? ***Yes*** ___ ***No*** ___

Sample 3

Tenderness

Please indicate how much you like or dislike the ***Tenderness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Tough	Very Tough	Moderately Tough	Slightly Tough	Slightly Tough	Moderately Tough	Very Tough	Extremely Tough
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is the tenderness Acceptable? *Yes* ___ *No* ___

Juiciness

Please indicate how much you like or dislike the ***Juiciness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Dry	Very Dry	Moderately Dry	Slightly Dry	Slightly Dry	Moderately Dry	Very Dry	Extremely Dry
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Flavor

Please indicate how much you like or dislike the ***Flavor*** (all factors included) of each sample by placing a check in the appropriate box for each sample.

Extremely Unbeef like	Very Unbeef like	Moderately Unbeef like	Slightly Unbeef like	Slightly Beef like	Moderately Beef like	Very Beef like	Extremely Beef like
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Overall Liking of the Samples

Please indicate how much you ***like or dislike*** the sample by placing a check in the appropriate box for each sample.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is this Steak Acceptable? ***Yes*** ___ ***No*** ___

Sample 4

Tenderness

Please indicate how much you like or dislike the ***Tenderness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Tough	Very Tough	Moderately Tough	Slightly Tough	Slightly Tough	Moderately Tough	Very Tough	Extremely Tough
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is the tenderness Acceptable? *Yes* ___ *No* ___

Juiciness

Please indicate how much you like or dislike the ***Juiciness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Dry	Very Dry	Moderately Dry	Slightly Dry	Slightly Dry	Moderately Dry	Very Dry	Extremely Dry
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Flavor

Please indicate how much you like or dislike the ***Flavor*** (all factors included) of each sample by placing a check in the appropriate box for each sample.

Extremely Unbeef like	Very Unbeef like	Moderately Unbeef like	Slightly Unbeef like	Slightly Beef like	Moderately Beef like	Very Beef like	Extremely Beef like
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Overall Liking of the Samples

Please indicate how much you ***like or dislike*** the sample by placing a check in the appropriate box for each sample.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is this Steak Acceptable? ***Yes*** ___ ***No*** ___

Sample 5

Tenderness

Please indicate how much you like or dislike the ***Tenderness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Tough	Very Tough	Moderately Tough	Slightly Tough	Slightly Tough	Moderately Tough	Very Tough	Extremely Tough
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is the tenderness Acceptable? *Yes* ___ *No* ___

Juiciness

Please indicate how much you like or dislike the ***Juiciness*** of each sample by placing a check in the appropriate box for each sample.

Extremely Dry	Very Dry	Moderately Dry	Slightly Dry	Slightly Dry	Moderately Dry	Very Dry	Extremely Dry
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Flavor

Please indicate how much you like or dislike the ***Flavor*** (all factors included) of each sample by placing a check in the appropriate box for each sample.

Extremely Unbeef like	Very Unbeef like	Moderately Unbeef like	Slightly Unbeef like	Slightly Beef like	Moderately Beef like	Very Beef like	Extremely Beef like
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Overall Liking of the Samples

Please indicate how much you ***like or dislike*** the sample by placing a check in the appropriate box for each sample.

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8

Is this Steak Acceptable? ***Yes*** ___ ***No*** ___

About Yourself

(Please circle the answer that applies for each item)

Gender	**Household Size**	**Household**	**Age**	**Ethnic Origin**
Male	1 person	Single Income	Under 18	African-American
Female	2 people	Dual Income	18-34	Caucasian/White
	3 people		35-50	Native American
	4 people		Over 50	Hispanic
	5 people			Asian
	6 people			Other
	7 people			
	1 or more people			

Annual Household Income (if you are a full time student indicate you parent's income)

Under $20,000	$20,000 - $29,999	$30,000 to $49,999
$50,000 - $69,999	$70,000 - $100,000	more than $100,00

Education Level

Non-high School graduate	Some College/Technical School	Post graduate
High school graduate	College graduate	

How many times a week do you consume beef?

None	3 to 4	7 or more
1 to 2	5 to 6	

What factor of beef palatability is most important?

_____ Juiciness

_____ Tenderness

_____ Flavor

Appendix C. SPIRAL BIOTECH Q COUNT™ Operating Instructions

Note: Prior to operating the Q Count™ system, you will need to be assigned a username and password.

With gloved hands, perform the following:

1. Turn the computer 'ON', remove the plastic covering from the Q Count™ hardware (plate reader) and turn the hardware 'ON'.
2. On the computer, open the Q Count™ software located on the desktop.
3. Open or create your database (file used to store data).
4. Remove plates from the incubator.
5. Place on plate in the plate template of the Q Count™ hardware.
6. The database will ask you to identify the plate (Enter your sample ID)
7. Type the dilution into the dilutions window of the database.
8. You should be able to view the plate on the screen of the computer (top portion of the database).
9. Select the 'COUNT' button. Counted colonies will appear green.
10. Carefully check to make sure larger colonies were not counted twice (one colony will have two green dots if counted as two colonies). Make sure smaller colonies were counted.
11. To edit (add countable or subtract non-countable colonies) using the mouse, right click and select 'EDIT AREA'.

12. If colonies are counted twice, using the mouse, click to deselect (green will turn to red) or to count colonies, click to select (red will turn to blue). Select 'UPDATE' and return to the main image.
13. If there is a duplicate plate of the same exact sample, press 'REP'. This feature will average duplicated plates.
14. To count plates of different samples, press 'NEW' and follow steps 5-13.
15. When finished with the Q Count™, save your file (file was previously automatically saved), close the database and exit the program.
16. Turn off the Q Count™ hardware.

Additional notations:

1. If plates are not viewable on the screen, you can adjust the shutter speed by selecting more or less light. This feature is similar the shutter speed on a camera.
2. If a large number of colonies are being counted on the outer edges of the plate, select 'REDUCE REGION'.
3. If you have mistakenly identified/counted the wrong plate, right click on the cell of the database, select 'DELETE' and then type the reason for the deletion.

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