This is to certify that the
thesis entitled

Thermal Resistance and Migration of *Salmonella* spp. into Marinated Pork Products

presented by

Adriana Velásquez

has been accepted towards fulfillment
of the requirements for the

M. S. degree in Food Science and Human Nutrition

Elliot T. Ryser
Major Professor's Signature

10/23/06
Date

MSU is an Affirmative Action/Equal Opportunity Institution

THERMAL RESISTANCE AND MIGRATION OF *SALMONELLA SPP.* INTO MARINATED PORK PRODUCTS

By

Adriana Velásquez

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

2006

ABSTRACT

THERMAL RESISTANCE AND MIGRATION OF *SALMONELLA SPP.* INTO MARINATED PORK PRODUCTS

By

Adriana Velásquez

Irradiated, whole muscle pork roasts were treated with a marinade containing an 8-strain cocktail of *Salmonella.* Effects of vacuum, tumbling, inoculum level, and marination time on penetration of *Salmonella* into the product were examined. Various methods were used to aseptically excise tissue from the interior of the whole muscle and then enumerate *Salmonella.* Thermal resistance of *Salmonella* was also assessed in ground and whole-muscle pork to determine the effect of the product's physical attributes on bacterial lethality. Heating temperature and physical state of the meat affected thermal inactivation, with *Salmonella* exhibiting significantly greater ($P < 0.05$) thermal resistance in whole muscle as opposed to ground pork. Different marination times for whole muscle samples did not significantly affect the numbers of salmonellae recovered ($P > 0.05$). Vacuum and tumbling enhanced absorption and retention of marinade and further facilitated the migration of *Salmonella* into pork roasts. Similar trends in pathogen migration were seen using marinades inoculated at 10^8 and 10^4 CFU/mL, suggesting that any salmonellae in marinade are likely to penetrate to the product during marination. Given these findings, the potential presence of internalized *Salmonella* should be considered when developing thermal processing guidelines for marinated meat.

This thesis is dedicated to God who has always been by my side and keeps blessing me every day.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my committee members, Drs. Elliot Ryser, Alden Booren, Bradley Marks, and Alicia Orta-Ramirez for your expertise, guidance and encouragement.

My most sincere thanks go to my not only professor but good friend Alicia for sharing time, energy, and knowledge with me. Your patience and support helped me to stay motivated when problems seemed unsolvable and deadlines seemed impossible to meet. To my fellow graduate students I extend my most heartfelt thanks.

Last but definitely not least, I would like to thank my family and friends all over the world for your support during all the years I have been away from home. Through you I have learned how important genuine love and friendships are. I am blessed to have you all in my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 LITERATURE REVIEW

Pork Consumption

The United States is the world's third largest producer and second largest consumer, exporter, and importer of pork and pork products. Pork accounts for approximately 50 percent of the daily meat protein intake worldwide and it ranks third in annual U.S. meat consumption, behind beef and chicken (USDA, ERS, 2005). In 1960, per capita pork consumption averaged approximately 59 lbs. Each American consumed an average of 51 lbs of pork per year, retail product, during 1994 - 1996 and 1998 (USDA, ERS, 2004). In 2003, pork accounted for almost 42 percent of red meats (beef, pork, lamb, and veal) consumed in the United States. Pork consumption has fluctuated slightly in the United States, with per capita consumption declining by 10 percent between 1960 and 2003. As the seventh-largest U.S. farm commodity in cash receipts, the pork sector continues to produce large quantities of pork products, estimated at a record 20.5 billion lbs in 2004 (USDA, WASDE, 2004). Total pork consumption has increased over the past years and is expected to continue increasing as the U.S. population grows.

Enhancement of meat products

Production of safe and wholesome whole-muscle meat products with acceptable tenderness and reduced aging time has been an objective for the muscle foods industry for decades (Lorca and others 2002). Due to demographic changes in the work force a significant trend towards convenience in cooking has been seen in recent years.

Nowadays a larger number of households have two working adults instead of one, allowing for less time spent preparing meals - a fact that has helped direct the shift toward convenience foods. This trend is probably best illustrated by the growth of heat-and-eat meals, such as frozen dinners with meat as the main contributor to the meal (Carmel 2000).

In order to meet the quality expectations of consumers, researchers have explored the use of many technologies for improving tenderness of meat products, including the application of enzymes, acids, blade tenderization, and hydrostatic pressure. Alternatively, meat products can be enhanced through the use of marinades that also improve the tenderness of the meat and compensate for moisture loss due to overcooking. Marinades are usually composed of a pickling or savory solution that is used to preserve, season and/or tenderize meats. Marination can improve pork quality by improving tenderness, juiciness, shelf-life, color stability, and water holding capacity. Enhancing meats by marination can have positive results for both producers and consumers. The processor benefits by increased margin/profitability due to better yields, extended shelf-life, and better product consistency while the consumer enjoys a variety of products that are convenient, sensory appealing and offer cooking flexibility.

The primary ingredients in a typical marinade include water, salt, and phosphates. Sodium or potassium phosphates are added at levels up to 0.5% and their main function is to bind and hold water while enhancing textural properties by partial extraction/solubilization of meat proteins. Salt is added at low levels (up to 0.5%) due to its water-binding properties. Secondary ingredients such as flavor extracts, spices and/or sweeteners can be added to affect appearance, flavor, texture and odor of the meat.

Mechanical tenderization is a widely utilized technique that improves the tenderness of meat while ensuring uniform distribution of the marinade. Blade and needle tenderization cut and puncture some of the connective tissue contained in lean meats improving tenderness. However, these types of mechanical tenderization can transfer surface bacteria to the interior of the muscle, where the bulk of the muscle protects bacteria from thermal inactivation during traditional cooking (Phebus and others 1999). In a previous study, Lorca and others (2002) reported that a hydrodynamic shock wave treatment, a non-invasive process that nevertheless produces a non-intact product, resulted in only minimal movement (less than 0.3 mm) of *E. coli* into the interior of surface-inoculated beef round steaks. Unsanitary conditions at the processing plant might further worsen the problem. Once introduced into the meat, these bacterial contaminants, may proliferate inside whole muscle, (provided that adequate extrinsic and intrinsic factors prevail) and shorten product shelf-life (Raccach and Henrickson 1979) with the increased availability of nutrients in juices and debris released during mechanically tenderization potentially contributing to microbial growth. More recently, various research studies have shown that bacteria, particularly *Salmonella,* can migrate into and survive inside whole-muscle products (Warsow 2003; Orta-Ramirez and others 2003). Warsow (2003) demonstrated that exposure to a vacuum significantly enhanced bacterial migration during marination of turkey breasts.

Vacuum packaging is further used to extend the shelf-life of meat products and as an aide in the distribution of marinade but at the same time it may enable facultative anaerobic bacteria to proliferate (Sutherland and others 1975). Presence of pathogens among these bacteria will reduce wholesomeness of the product (Raccach and others

1979). Several reports suggest that mechanically tenderization has been at least partly responsible for outbreaks related to *E. coli* O157:H7. Among reported *E. coli* O157:H7 infections from identified sources, two incidents were associated with steaks (Canada and Michigan), one with roasts in Canada and one associated with roast beef (Wisconsin) (FSIS 2002). In the Michigan outbreak (two individuals with illness), blade-tenderized beef steak was identified as the most likely vehicle of infection. In the Canadian incidents, two sporadic cases were associated with consumption of roast beef (FSIS 2002).

Pathogens of importance

In the US, enterohemorrhagic *Escherichia coli* and *Salmonella* are the principal pathogens of concern in pork meat. Contamination by these pathogens often occurs during pre-slaughter and is mainly due to contact with contaminated feed, water, and the environment.

The presence and numbers of *Salmonella* on carcasses after processing largely determine the likelihood of salmonellosis. Human factors have a significant effect on preventing contamination of meat products with *Salmonella* and other pathogens while in the plant. Inadequate hand washing and cross-contamination between raw meat and ready-to-eat foods are among the most frequent causes of *Salmonella* outbreaks.

In addition to *Salmonella*, several strains of *Escherichia coli* are among the most serious and prevalent pathogens infecting the U.S. meat supply. The Center for Disease Control and Prevention estimates that 73,000 cases involving *E. coli* O157:H7 and other pathogenic serotypes of *E. coli* occur annually in the United States. Virulence of *E. coli* O157:H7 is a result of its ability to produce Shiga-like toxins, or verotoxins. Shiga-like

toxins inhibit protein synthesis in eukaryotic cells and play a role in hemorrhagic colitis and hemolytic uremic syndrome by causing damage to endothelial cells in the kidneys, pancreas, brain, and other organs, thus inhibiting those organs' ability to function. While most foodborne illness outbreaks associated with *E. coli* O157:H7 have involved ground beef, such outbreaks have also been traced to unpasteurized apple and orange juice, unpasteurized milk, alfalfa sprouts, lettuce, and water (Friedman 1999). Meat typically becomes contaminated with *E. coli* O157:H7 during the slaughtering process, when the contents of an animal's intestines and feces are allowed to come into contact with the carcass. Bacteria on the carcass surface are then mixed into the meat during the grinding process.

A 2003 study on the prevalence of *E. coli* O157:H7 in livestock in 29 counties and 3 large state agricultural fairs in the United States found that *E. coli* O157:H7 could be isolated from 13.8% of beef cattle, 5.9% of dairy cattle, 3.6% of pigs, 5.2% of sheep, and 2.8% of goats (Keen 2003). FSIS currently samples and tests various raw ground beef products, including veal products, for *E. coli* O157:H7 at inspected establishments and retail stores. If the agency confirms the presence of *E. coli* O157:H7 in a sample of raw ground beef taken as part of this program, the appropriate regulatory action is taken (FSIS 1999).

Relevant Regulations in the Meat Industry

The meat and poultry industry comprise an important portion of the agricultural economy of the United States and the impact of microbial contamination of these products could have detrimental effects with significant consequences. The United States

has been inspecting meat and poultry products since 1891 in response to European concerns of trichinosis. Inspection and regulations expanded at the federal levels throughout the next century. Major milestones included the 1967 Wholesome Meat Act and the 1968 Wholesome Poultry Act requiring that all carcasses and meat products be inspected. By the mid-1990s the Food Safety and Inspection Services had over 7,400 inspectors in 6,200 slaughter and processing plants. The system under which these inspectors were operating, however, did not adequately target and reduce microbial pathogens (Crutchfield et al. 1997). To help remedy this situation, in 1996 FSIS began phasing in sanitation standard operating procedures (SSOPs), Hazard Analysis and Critical Control Points (HACCP) procedures, and microbial testing (Crutchfield et al. 1997). The USDA and Food and Drug Administration have adopted this food safety program developed nearly 30 years ago for the space program and are applying it to meats, seafood and juice. This program for astronauts focuses on preventing hazards that could cause food-borne illnesses by applying science-based controls, from raw material to finished products.

FSIS has recently finalized new regulations for the production of certain fully and partially cooked meat and poultry products (FSIS 1999). Similar regulations have been proposed for ready-to-eat and all partially heat-treated meat and poultry products (FSIS 2001). These regulations are in the form of lethality, stabilization and handling performance standards, rather than stating step-by-step processing methods with prescribed endpoint temperatures. Pathogen reduction levels are established through lethality levels as well as limits for pathogen growth. The term lethality is defined as the required reduction in the number of specific pathogenic microorganisms. The final

internal temperature and dwell time of the product must reduce *Salmonella* populations at least 6.5 logs in beef and 7.0 logs units in poultry.

Except for thermally processed, commercially sterile products, the lethality performance standards are based on *Salmonella*. Rigney and others (2004) evaluated *Salmonella* incidence on pork carcasses and found that 7.0% of 8,483 cooled, market hog carcasses sampled between January 1998 and December 2000 yielded *Salmonella*.

The Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) rule, put into practice beginning 1996, established *Salmonella* performance standards for the first time in seven categories of raw meat and poultry products: broilers, market hogs; cows/bulls; steers/heifers; ground beef; ground chicken; and ground turkey. FSIS collects and analyzes *Salmonella* samples as one part of and extensive science-based food safety verification system and publishes the data annually in aggregate form. Additionally, the US Department of Agriculture's Food Safety and Inspection Service announced a comprehensive initiative to reduce the presence of *Salmonella* in raw meat and poultry products (USDA 2003). The initiative will include concentrating resources at establishments with higher levels of *Salmonella* and change the reporting and utilization of FSIS *Salmonella* verification test results. This program follows similar patterns to the successful initiative to reduce the incidence of *E. coli* O157:H7 in ground beef. The new strategy will provide the results of its *Salmonella* performance standard testing to establishments as soon as they become available on a sample-by-sample basis. *Salmonella* contamination on pork carcasses is now slowly decreasing after hazard analysis critical control point systems (HACCP) were mandated for all meat and poultry

processors (USDA 2003) with the Healthy People 2010 national health objective for *Salmonella* now set at 6.8 cases per 100,000 people (CDC 2002).

Meat products may become contaminated with *Salmonella* during slaughter, and numerous procedures have been devised for preventing or removing these bacteria. Certain serotypes of *Salmonella* are notorious for resistance to thermal treatments, the most prominent being *Salmonella* Senftenberg 775W (Ng 1969). Although this organism is not an important foodborne pathogen, it is often used as a test organism. The implication is that if a particular thermal treatment destroys *Salmonella* Senftenberg 775W, it will also be effective against more common salmonellae in foods. FSIS advises the use of a *Salmonella* cocktail composed of relatively heat resistant serotypes or strains as well as those involved in outbreaks in order to verify compliance with performance standards (e.g. challenge studies). *Salmonella* was chosen as the target organism because it is associated with raw meat (beef, pork and poultry) and is responsible for a high incidence of foodborne illness, which can frequently be severe. Additionally, the required conditions for a suitable reduction in *Salmonella* will simultaneously reduce most other vegetative pathogenic microorganisms.

If the given reduction for *Salmonella* is met, a safe reduction for *Staphylococcus aureus*, *E coli* O157:H7 and *Trichinella spiralis* should also be achieved, because these organisms are usually more sensitive to heat than *Salmonella*. However, organisms such as *L. monocytogenes* that are particularly heat resistant will most likely contaminate the product during post-processing, due to improper or inadequate sanitation, rather than as a result of defective processing.

Salmonella

Salmonella spp. are Gram-negative, facultative anaerobic, nonsporing rods belonging to the family of *Enterobacteriaceae*. There are currently at least 2,463 serovars (serotypes) of *Salmonella* (Brenner et al. 2000). Differences between serotypes are based on major somatic (O lipopolysaccharides of the bacterial outer membrane), flagellar (H antigens), and capsular (Vi antigens occurring in only three *Salmonella* serovars) antigens (D'Aoust 2001). Identification, confirmation, classification and diagnostics are some of the applications where bacterial antigenic structure can be utilized.

The nomenclature and taxonomy of *Salmonella* are intricate and have recently undergone various changes. The defining development in *Salmonella* taxonomy occurred in 1973 when Crosa and others (1973) demonstrated by DNA-DNA hybridization that all serotypes and subgenera I, II, and IV of *Salmonella* were related at the species level; thus, they belonged in a single species. In the United States, most local public health agencies follow the nomenclature standards of the Centers for Disease Control and Prevention (CDC). The World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* (Institut Pasteur, Paris) defines and maintains *Salmonella*'s antigenic formulae and separates the genus into two species, *S. enterica* and *S. bongori*, each containing multiple serotypes. *S. enterica* is divided into six subspecies differentiated by genomic relatedness, biochemical reactions and by the Kauffman-White serotyping scheme. The largest number of serotypes falls within *S. enterica* subsp. I (*S. enterica* subsp. *enterica*). Contained within this group, the CDC utilizes the terms for serotype (e.g. Heidelberg, Enteritidis, Typhimurium) and follows the terminology set by

the WHO Collaborating Centre. *Salmonella* is commonly found in the intestinal tract of warm-blooded animals and many of its serotypes are responsible for gastroenteritis.

Salmonella is a mesophilic organism that grows between 5.5-45°C with optimal growth occurring at 37°C. Most *Salmonella* serovars are motile by peritrichous flagella and are very adaptive to their environments. The bacterium can tolerate a pH range from 4.5 to 9.5 and proliferates at an optimum pH of 6.5 to 7.5 (D'Aoust and others 2001).

The maximum growth temperature and extent of thermal resistance varies between strains with cultures in the late log/early stationary phase typically being the most heat resistant (Doyle 1999).

The bacterium is normally inhibited by 3-4% NaCl, and salt tolerance is positively correlated with temperatures from 10 to 30°C (D'Aoust 1989). *Salmonella* metabolizes by respiration and fermentation and can be identified by its characteristic catabolism of D-glucose and other carbohydrates with the production of acid and gas, and its lack of catalase and oxidase activity (D'Aoust and others 2001).

Salmonella is an entero-invasive organism, meaning it can infect intestinal epithelial cells. Invasion begins with attachment to the intestinal wall by proteinaceous appendages. Peritrichous flagella significantly enhance cell motility and ensure that an appropriate attachment site is located. The *Salmonella* invasion gene locus causes physiological changes in the intestinal epithelial cells. The *inv*E gene causes an influx of Ca^{2+} into the intestinal lumen and a rearrangement of the cell's actin structure, which causes invagination and subsequent uptake of the invading cell by pinocytosis. *Salmonella* also possess the ability to aggregate the host's plasma membrane around itself

to avoid detection by the immune system, adding to its virulence (D'Aoust and others 2001).

Salmonellosis is the second most common cause of bacterial gastroenteritis. It is usually self-limiting and is most typically resolved within 4 to 7 days (CDC 2006). Nonetheless, acute symptoms and secondary complications such as reactive arthritis and septicemia have been reported in particularly susceptible groups. Symptoms of salmonellosis include nausea, abdominal cramps, diarrhea and vomiting 12-72 h after exposure (CDC 2006). Patients without complications are usually successfully treated with fluid and electrolyte replacement. An estimated 1.4 million cases occur annually in the United States; of these, approximately 30,000 are culture-confirmed cases reported to CDC (CDC 2006).

The costs associated with salmonellosis, which include medical costs, value of time lost from work, productivity losses, and the economic value of premature deaths, are estimated at $2.4 billion (Frenzen 2002). The magnitude of this estimated burden of disease highlights the need for effective solutions and economic evaluations of methods for decreasing this social cost in the context of *Salmonella* control in the pork supply chain.

According to CDC data (2006), half of all salmonellosis cases are caused by two serotypes: *S.* Enteritidis (SE) and *S.* Typhimurium (ST). The proportion of salmonellosis cases caused by SE increased markedly from 1980 to 1995, but has decreased 22% since 1996. The incidence of ST decreased 24% since 1996, but an increasing proportion of isolates show resistance to multiple antimicrobial agents. *S.* Newport has increased 32%

from 1996 to 2001 to become the third most frequent serotype, with many isolates resistant to >9 antibiotics.

Salmonella is a microorganism of significant resistance to heat damage compared to other similar pathogens and is commonly used as a target during process designs. Thermal resistance of *Salmonella* is impacted by many factors including the heating menstrum, phase of growth and the physiological state of the organism. Cells in the logarithmic growth phase are more susceptible to heat damage than those in the stationary phase (Humphrey 1995). Cells grown at higher temperatures or exposed to a sublethal heat shock (Bunning and others 1990, Xavier and Ingham 1997) and those grown at limiting carbohydrate levels are more heat resistant.

When attached to glass and stainless steel, *Salmonella* Enteritidis has exhibited about a two-fold increase in *D*-values at 52°C, as compared to unattached cells (Doyle 1999). This is relevant for cells attached to dishes or food-processing equipment and may be related to the increased heat resistance observed for salmonellae attached to meat (Dhir and Dodd 1995). In addition, salmonellae in foods left at room temperature for some time seem to be more heat resistant than those that have been surviving in a refrigerator (Doyle 1999). Furthermore, salmonellae attached to meat surfaces (or to surfaces such as stainless steel or glass) are generally more heat resistant than those that are unattached and dispersed throughout a food or broth (Doyle 1999). Humphrey and others (1997) reported that D-values at 58°C for *S.* Typhimurium DT104 in pork muscle tissue were >10 min for attached cells versus only 2 min for free cells. Additionally, growth and inactivation of microorganisms in food and model systems may be quite different than in liquid media. For example, Murphy and others (1999) found that a

Salmonella cocktail containing 6 strains was more heat resistant in ground chicken breast patties than in an agar-water solution. A 7-log (CFU/g) reduction of *Salmonella* in agar-peptone occurred 19% faster than in chicken meat. Moreover, Quintavalla and others (2001) found that the heat resistance of eight strains of *Salmonella* (*S.* Typhymurium, strains ATCC 14028, 133 and 1116, *S.* ser Derby B4373, *S.* ser Potsdam 1133, *S.* ser. Menston 179, *S.* ser Eppendorf 166, and *S.* Kingston I124) was 1.5 - 4 times higher in pork meat as opposed to trypticase soy broth.

The physical and chemical characteristics of foods also affect heat resistance of microorganisms. Increased solids, lower pH (greater acidity), and decreased moisture in foods increase heat resistance (Blackburn and others 1997). Some food additives, including bacteriocins, EDTA, polyphosphates, hydrogen peroxide, and the lactoperoxidase system, make salmonellae more sensitive to heat (Doyle 1999). Several of these chemicals produce transitory oxidation products that attack cellular proteins. Others including EDTA and polyphosphates chelate metal ions important for integrity of the cell wall and membranes. However, some of these compounds might be more effective in culture media than when present in complex foods where they might interact with fat and protein and have less interaction with bacterial cells.

Doyle and Mazzotta (1999) investigated the effect of competitive flora on the heat resistance of *Salmonella* and concluded that a rapid decrease in dissolved oxygen (caused by respiration of the added cells) may reduce oxidative damage to salmonellae, allowing greater survival. To verify compliance with performance standards (challenge studies), the FSIS advises the use of a *Salmonella* cocktail composed of relatively heat resistant serotypes or strains as well as those involved in outbreaks.

Certain food components such as fat offer protection against heat treatments (Ahmed and others 1995, Veeramuthu and others 1998). Maurer (2001) found that increased levels of fat in turkey increased the D-values of *S.* Senftenberg. Additionally, Juneja and Eblen (2000) observed significantly higher D-values at 58°C for an eight strain *S.* Typhimurium DT104 cocktail in ground beef containing 7, 12, 18, and 24% fat and in chicken broth containing 3% fat. They suggested that these differences could be a result of poor heat transfer through the heating matrix as a consequence of high fat levels. Thermal protection afforded by increased fat levels may be due to localized absence of moisture (reduces water activity) within bacterial cells (Juneja and Eblen 2000).

Water activity (a_w) is defined as the ratio of the water vapor pressure of a food substrate to the vapor pressure of pure water (Jay 2000). This definition can be interpreted as the moisture that is available to support the growth of bacteria. A decrease in a_w is generally associated with a corresponding increase in the thermal resistance of microorganisms (Doyle and Mazzotta 2000, Carlson 2002, Jay 2000).

Bacterial Migration

It is commonly believed that the interior of *intact*, undamaged whole muscle is sterile (Elmossalami and Wassef 1971). Contamination of outer surfaces occurs during slaughter, hide removal, and further processing. While blades, knives, and gloves can transfer gut or environmental flora to cut surfaces, the interior of the meat remains essentially sterile. Previous studies examined movement of bacteria from the surface into sterile inner tissues of muscle foods (Gill and Penney 1977; Maxcy 1981, Warsow 2003)

and have concluded that proteolytic enzymes might enhance migration towards the interior of the muscle.

During grinding of meat, various portions and cuts from different carcasses are combined to produce large quantities of product. Due to cross-contamination between surfaces and inner portions of meat, ground meat is more likely than intact meat to be contaminated with bacterial pathogens and numerous outbreaks of foodborne disease involving *Salmonella* and *E. coli* have been traced to ground beef (Doyle 1999).

The Food Safety Inspection Service (FSIS) defines intact beef cuts as steaks, roasts, briskets, and stew beef. In contrast, non-intact products are those that have been injected with solutions, mechanically tenderized by needling, cubing, or pounding devices, or reconstructed into formed entrees when establishing policies regarding *E. coli* O157:H7 contamination (FSIS, 1999). FSIS also stated, "in these intact cuts the interior remains protected from pathogens that may exist on the exterior. It is highly unlikely that pathogens would migrate below the surface" (FSIS, 1999a). However, subsequent studies have demonstrated that pathogenic bacteria, particularly *Salmonella*, can indeed migrate into and survive inside whole-muscle products (Warsow 2003; Orta-Ramirez et al. 2003).

Many whole-muscle meat products are consumed in a semi-rare state, i.e., cooking may be insufficient to raise all deep tissues to temperatures at which bacterial pathogens would be likely destroyed. This presents a concern/risk for blade-tenderized meats being served very rare with cold spots (less than 120° F internal temperature), particularly to immunocompromised individuals. Non-intact, blade tenderized beef

steaks do not present a greater risk to consumers if the meat is oven broiled and cooked to an internal temperature of 140° F or above (FSIS 1999).

Bacterial attachment to cell structures in a whole-muscle product (in contrast to the more homogeneous environment in ground product) will also affect thermal resistance. Orta-Ramirez and others (2005) concluded that *Salmonella* was significantly more heat resistant when present in whole muscle, compared to ground beef products. Studies in beef and turkey illustrate the importance of the physical arrangement of a product and the effect it has on thermal inactivation of *Salmonella*. Current lethality standards for *Salmonella* in meats are based on ground meat products. Thermal validations are needed to consider the physical state of individual products being manufactured in order to successfully design processes that ensure the microbiological safety of meat products.

Sampling methods- Electrosurgery

Methods previously used to examine penetration of bacterial cells into whole muscles have been problematic. While studying proteolytic penetration of *Serratia marcensens* into pork and beef, Sikes and Maxcy (1980) cut the muscles to the appropriate size aseptically and dipped them in colloidon. They removed one side of the colloidon and then applied the bacterial culture. Bacterial penetration was measured by freezing and then cutting the blocks into 5 mm-thick slices. Swabs were used on individual slices with sterile cotton applicators and streaked on plate count agar to qualitatively determine bacterial migration at different depths. Due to *Salmonella*'s sensitivity to freezing (Ray and others 1972) this method did not seem to be an

appropriate alternative. While others have used conventional scalpels (Raccach and Henrickson 1979), the risk of mechanically transferring bacteria on the blade to the interior during cutting is significant.

One promising alternative method involves the use of a cauterizing knife that utilizes high-energy radio frequencies to dissect tissue, thereby greatly reducing the risk of contamination during cutting. Electrosurgical generators are often used in human and animal surgical rooms to dissect and cauterize tissue simultaneously. The electrosurgical unit (ESU) used in this research consists of a generating device, a hand piece, and a grounding plate. The high frequency radio waves produced by the generating unit oscillate between negative and positive poles at over 100,000 Hz. The current travels through the blade of the hand piece, into the tissue at the dissection site and back through the grounding plate, completing the circuit. The high voltage vaporizes water inside the cell leading to cell rupture, thus cutting the tissue (Ulmer 2001).

When used, the ESU electrode heats very rapidly and self-sterilizes as it cuts, thus decreasing the risk of infection (Malone 1974). The electrode is assumed to be sterile when the first cut is made and sterility is maintained during subsequent cuts. Existing literature on the viability of microbes on the activated blade indicate that the tip will self-sterilize at a power level of 175 W as used in this study. Shaw and others (1988) demonstrated that $\geq$ 200 L (J Watts x seconds) was sufficient to sterilize the electrode blade. When using the ESU at a constant power level of 175 W, the electrode tip is assumed to self-sterilize in 1.1 s, thus significantly reducing or eliminating the risk of mechanically transferring bacteria to the inside of the muscle.

Objectives of Study

The overall objective of the study being reported here was to determine the numbers of *Salmonella* that migrate into whole-muscle pork roasts during marination, as well as the effects of vacuum, tumbling, inoculum level, and marination time on migration of *Salmonella* into whole muscle pork. Additionally, thermal resistance of *Salmonella* in ground vs. whole-muscle pork was assessed to determine the effect of the product's physical attributes on bacterial lethality.

In the industry, predictive microbial models for thermal inactivation are utilized to mathematically represent response of microorganisms under different environmental conditions. In order to be of practical value, predictive models must consider the effects of time and the various intrinsic and extrinsic factors affecting the microbial response (Carlson 2002).

Primary models describe the response of a microorganism with time to a single set of conditions. Each population vs. time curve can be described by a set of specific values for each of the parameters in the model (Whiting and Buchanan 1993). The information generated from this study will be used to further refine a predictive model for thermal inactivation of *Salmonella* in different species and various environmental conditions.

CHAPTER 2 ENHANCED THERMAL RESISTANCE OF *SALMONELLA* IN WHOLE MUSCLE COMPARED TO GROUND PORK

ABSTRACT

The internal muscle environment may enhance thermal resistance of bacterial pathogens. Because application of marinade assists pathogen migration into whole muscle, the validity of current thermal inactivation models in whole- vs. ground muscle products needs to be verified to ensure product safety. The objective of this work was to compare thermal resistance of *Salmonella* in whole pork muscle vs. ground pork after marination. Irradiated, intact (whole pork loin) and ground pork loin (5.5-7.5 g) was exposed to a *Salmonella*-inoculated (10^8 CFU/mL) marinade for 20 min. All samples were aseptically placed in sterile brass tubes (12.7-mm dia.), sealed, and heated isothermally at 55, 60, or 62.5°C for predetermined times. Surviving salmonellae were enumerated by plating serial dilutions on Petrifilm™ aerobic count plates. The thermal lag times and initial bacterial counts were similar for both whole-muscle and ground samples ($P > 0.05$), with all samples having equivalent compositions, inoculums, and thermal histories. Assuming log-linear inactivation kinetics, the inactivation rates (k values) were 0.12/min and 0.23/min at 55°C, 1.68/min and 2.13/min at 60°C and 3.37/min and 4.37/min at 62.5°C in whole- and ground-muscle, respectively. Heating temperature and physical state of the meat (whole vs. ground muscle) affected *Salmonella* inactivation, with greater thermal resistance observed in whole as compared to ground muscle ($P < 0.05$). Therefore, thermal process validations for meat and poultry products may also need to account for the physical arrangement of the muscle fibers to ensure product safety.

INTRODUCTION

Foodborne illness is a significant public health concern with *Salmonella* being the second most common cause of food-borne illness in the US (CDC 2006). The meat industry is particularly concerned with compromised product safety as a result of bacterial contamination. Various heat treatments utilized by the food-processing industry and the cooking of foods at home are generally effective at destroying vegetative, foodborne bacterial pathogens. However, occasionally there are salmonellae that will survive these food-processing techniques. This may result from changes in the formulation or characteristics of foods and alterations in total solids, acidity, or water activity (a_w) that affect the thermal tolerance of salmonellae (Doyle 1999) or defects in the process itself.

Mechanical tenderization is a widely used to improve the tenderness of marinated meat while ensuring uniform distribution of the marinade. Blade and needle tenderization cut and puncture some of the connective tissue contained in lean meats, improving tenderness. However, mechanical tenderization can transfer surface bacteria to the interior of the muscle, where the bulk of the muscle protects bacteria from thermal inactivation during traditional cooking (Phebus and others 1999). Once introduced into the meat, these bacterial contaminants may proliferate inside the whole muscle (provided that adequate extrinsic and intrinsic factors prevail), and shorten the shelf-life of the product (Raccach and Henrickson 1979). More recently, several studies have shown that bacteria, particularly *Salmonella,* can indeed migrate into and survive inside whole-muscle products (Warsow 2003; Orta-Ramirez and others 2003), with Warsow (2003)

showing that exposure to a vacuum significantly enhanced pathogen migration during marination of turkey breasts.

Several reports suggest that mechanical tenderization has been at least partly responsible for outbreaks related to *E. coli* O157:H7 (Sporing 1996). This could be a concern for consumers who purchase commercially marinaded products or marinate meat at home, because consumption of undercooked meat products is a well-known cause of foodborne illness (Mead and others, 1999). While current USDA-FSIS performance standards dictate a 6.5-log reduction for *Salmonella* during cooking of pork and other meats, these standards do not account for bacterial migration into roasts during marination, with potential survival after cooking.

Certain food components, such as fat, can protect pathogens against thermal inactivation (Ahmed and others 1995, Veeramuthu and others 1998). Juneja and Eblen (2000) suggested that differences in thermal sensitivity could result from poor heat transfer through the heating matrix as a consequence of high fat levels. Thermal protection afforded by increased fat levels may be due to localized absence of moisture (reduces water activity) within bacterial cells (Juneja and Eblen 2000).

The objective of this study was to quantify the effects of product structure (whole-muscle vs. ground) on thermal resistance of *Salmonella* in pork products.

MATERIALS AND METHODS

Experimental design

A lot of nine pork roasts (*longissimus dorsi* muscle) was utilized for this study. Ground and whole-muscle samples were subjected in triplicate to each temperature, 55, 60, and 62.5°C.

Salmonella strains

Eight serovars of *Salmonella*: *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteritidis H3527 and H3502 (chicken isolates phage 13A and 4, respectively), *S.* Typhimurium DT104 H3380 (human isolate), *S.* Hadar MF60404 (turkey isolate), *S.* Copenhagen 8457 (pork isolate), *S.* Montevideo FSIS 051 (beef isolate), and *S.* Heidelberg F5038BG1 (human isolate), previously shown to have moderate to high thermal resistance (Juneja and others 2001), were obtained from Dr. V.K. Juneja (USDA-ARS, Eastern Regional Research Center, Wyndmoor, PA) and maintained at -80°C in tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) containing 20% glycerol. The cultures were grown by transferring one loopful of each frozen stock culture to 9 mL of TSB. The cultures were maintained by daily transfer to fresh TSB followed by 18 - 24 h of incubation at 37°C, with a minimum of two consecutive transfers to obtain cells in late log phase (Smith and others, 2001).

On the day of the experiment, 9 mL of each of the eight serovars grown separately in TSB were combined and centrifuged at 6,000 x *g* for 20 min at 4°C, after which the resulting cell pellet was resuspended in 500 mL of sterile marinade to give a final concentration of ~10^8 CFU/mL ($1.45 \times 10^8 \pm 3.77 \times 10^7$ CFU/mL). Concentration was

confirmed by serial dilution in 0.1% peptone water followed by duplicate plating on Aerobic Petrifilm™ Count Plates (3M, St. Paul, MN).

Marinade

A typical aqueous marinade solution for whole muscle foods containing 96.0% water (filtered and deionized), 3.2% NaCl, and 0.8% potassium phosphate solution (Butcher and Packer Supply Co. Detroit, MI) was prepared according to Pearson and Dutson (1987), and salt was incorporated into the water before adding the phosphate solution to ensure total dispersal. Aliquots (520 mL) of the marinade were poured into glass bottles with plastic screw caps, autoclaved for 15 min at 121°C to ensure sterility, and stored at room temperature (~22°C) for no longer than 30 days until use.

Meat preparation

Nine non-enhanced pork loins (*longissimus dorsi* muscle) (~0.75 kg each) were obtained from a local supplier and randomly divided into two equal lots. One lot was ground twice through a 4-mm diameter plate using a Kitchen Aid grinder (Model k5-A, Hobart, Troy, OH,), vacuum packaged in 100-g portions and frozen at –12°C. In the second lot, muscle samples were manually removed using a coring device (1.27-cm diameter, G.R. Electrical Mfg. Co., Manhattan, KS.) to produce whole muscle plugs (5.5 - 7.0 g) measuring 1.27 cm x 6.0 – 8.0 cm, with the muscle fibers running parallel to the length. Whole muscle cores and ground samples were vacuum packed and frozen at –20°C at MSU. Both lots were transported on dry ice to CFC Logistics, Quakertown, PA, irradiated (~10kGy), returned frozen to MSU and stored at –20°C. After irradiation,

numbers of any potentially interfering background organisms were assessed by diluting the pork 1:5 in peptone water (Becton Dickinson) followed by homogenization in a masticator (Model 0410, IUL Instruments USA, Inc. Cincinnati, Ohio,) and plating on Petrifilm^{TM} aerobic count plates followed by incubation for 48 h at 37°C. Moisture, fat, and protein levels were determined using AOAC methods 950.46B, 991.36, and 981.1, respectively (AOAC, 1996). To determine the pH, 10-g samples of ground pork were added to 90 ml of distilled water and homogenized for 30 s in a Polytron homogenizer (model PT 10/35, Brinkman Instruments, Westbury, NJ.) at speed setting 3 (Smith and others 2001). The pH of the mixture was then measured using a combination electrode (Model 145, Corning, Medfield, MA). All tests were done in triplicate on portions randomly selected from the original lots.

Inoculation

Immediately before meat inoculation, equal volumes (9 mL of TSB) of each culture were combined, centrifuged (6,000 x *g*, 20 min at 4°C), and resuspended in 500 mL of sterile marinade to a concentration of ~10^8 CFU/mL. All marinades were used within 10 min of inoculation, with no loss in *Salmonella* viability, as previously reported by Warsow (2003). Whole muscle cores were thawed in a cooler for 24 h before the experiment, and then immersed in the inoculated marinade for 20 min at 4°C (Fig. 2.1). To determine marinade uptake, each core was weighed before and after marination. Average marinade uptake (~0.15 g/g of meat) was then used to calculate the volume needed for the ground product targeting levels of 0.42% salt and 0.10% phosphate. Inoculated marinade was aseptically added dropwise to ground samples that were then

hand-mixed in a sterile plastic container using sterile gloves to ensure uniform distribution of *Salmonella.* Unheated marinated samples of whole-muscle and ground pork were randomly selected and tested for uniformity of inoculation. All samples (whole and ground muscle, 5–5 - 7.0 g) were aseptically packed into sterile brass tubes (1.27 cm dia., 10 cm length) (Fig 2.2). After sealing both ends with sterile rubber stoppers and Teflon tape, the tubes of meat were stored at 4°C and heated within 2 h.

Thermal Inactivation

All samples of tubed meat were placed in a plastic rack and immersed in an agitated, temperature-controlled water bath (NESLAB Instruments Inc., Newington, NH) for isothermal heating at 55, 60, or 62.5°C with the temperature of the water bath set 0.5°C above the target temperature (Fig. 2.3). A thermocouple (Type T, 1.0 mm, Omega Engineering, Stamford, CT) was inserted into the center one of the samples in each replicate and attached to a data logger (DuaLogR Thermocouple Thermometer, Model 91100-50, Cole Parmer Instrument Company, Vernon Hills, IL) to monitor the internal temperature. At and beyond the thermal lag time (defined as the time when the sample core temperature is within 0.5°C of the setpoint), tubes were removed from the water bath at predetermined intervals and immediately placed in an ice-water bath. Whole muscle and ground pork samples were tested in triplicate.

***Salmonella* recovery**

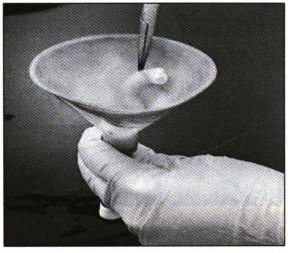
Salmonella counts in the marinade were determined before sample inoculation. The target level of 10^8 CFU/mL (8.1± 0.1 Log CFU/mL) was consistently achieved for

all experiments. No differences ($P > 0.05$) in initial *Salmonella* counts were observed between whole and ground muscle following marination. After being heated and cooled, the samples were diluted 1:10 in 0.1% peptone water and homogenized for 90 s in a masticator (Model 0410, IUL Instruments USA, Inc. Cincinnati, OH). Surviving salmonellae were enumerated by plating serial dilutions in duplicate on Petrifilm™ aerobic count plates followed by incubation at 37°C for 48 h.

Analysis of variance (ANOVA) was run to evaluate log (N/N_0) of the *Salmonella* cocktail as a function of thermal resistance in whole muscle vs. ground pork.

Fig. 2.1. Whole-muscle cores inoculated by immersion in marinade for 20 min.

Fig. 2.2. Whole muscle (a) and ground (b) pork samples packed into sterile brass tubes.



(a)

(b)

Fig. 2.3. Samples heated isothermally in water bath at 55, 60 or 62.5°C.

RESULTS

Proximate analyses

Raw pork had a pH of 5.7 and contained 13.5 ± 0.2% fat, 64 ± 0.7% moisture, and 20.3 ± 2.8% protein.

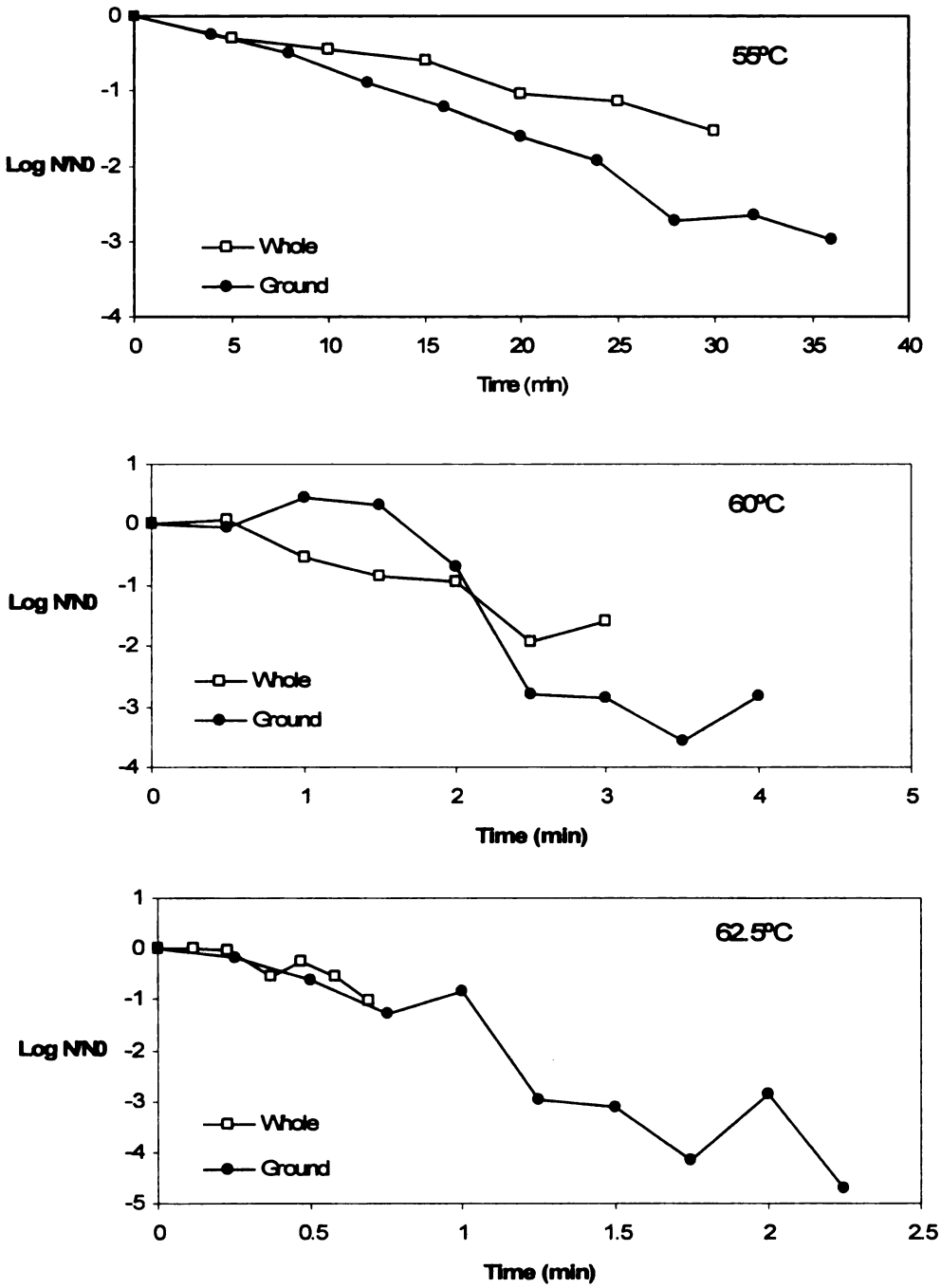
Salmonella populations in marinated whole muscle and ground pork

Before inoculation, the irradiated samples of whole muscle and ground pork contained microbial populations of < 25 CFU/g as determined using Petrifilm™ Aerobic Plate Counts. After exposing the 1.27 cm-dia core samples of whole muscle and ground pork to an inoculated marinade containing 8.1 ± 0. Log (CFU/ mL) of an 8-strain *Salmonella* cocktail, no significant differences ($P > 0.05$) in numbers of *Salmonella* were observed between whole (7.42 ± 0.1 log CFU/g) and ground muscle (7.35 ± 0.1 log CFU/g). After marination, whole muscle cores contained a mean *Salmonella* population of 10^7 CFU/g. Based on previous work by Warsow (2003), where *Salmonella* migrated to depths > 2 cm, *Salmonella* distribution in the marinated, whole muscle cores was assumed to be uniform across the sample mass.

Thermal inactivation of *Salmonella* in marinated samples

Thermal lag times ranged from 2.3 to 3.0 min for whole muscle and 2.6 to 3.1 min for ground samples. No significant differences in *Salmonella* populations ($P > 0.05$) were found between whole and ground muscle after the thermal lag time (time zero of inactivation curves) with the differences in thermal lag times between whole and ground muscle also not statistically significant ($P > 0.05$).

Figure 2.4. Thermal inactivation of *Salmonella* spp. in whole (W) vs. ground pork muscle (G) at 55, 60, and 62.5°C.



As expected, the inactivation rate for *Salmonella* increased with cooking temperature in both whole and ground muscle (Fig. 2.4). At 60°C, there was some variability in the survival of pathogenic cells during the first minutes compared to the last ones when those in whole muscle samples demonstrated to be more resistant to heat than those in ground samples. Due to a limited number of whole muscle samples we had more time points of ground samples.

Analysis of variance (ANOVA) (Table 2.1) shows the effects of time, temperature, and grinding on *Salmonella* survival.

Table 2.1. *P* values for thermal inactivation of *Salmonella* spp. in whole vs. ground pork muscle.

Test factors	*P* values
Grinding	**<0.0001**
Temperature	**0.0174**
Time	**<0.0001**
Grinding * Temperature	0.5427
Grinding * Time	0.2226
Temperature * Time	0.7838

Overall, *Salmonella* was significantly more heat resistant in whole-muscle compared to ground pork. Grinding, temperature and heating time had significant effects on thermal inactivation of *Salmonella* in pork (Table 2.1). The physical structure of the muscle (grinding) had a significant effect on lethality with *Salmonella* more readily inactivated by heat in ground pork than whole muscle. As expected, thermal inactivation also increased with both time and temperature.

As shown in Table 2.2, k values - the 1st-order kinetic rate constants, ranged from 0.12 to 4.37.

Table 2.2. First-order inactivation rate constants, k (mean ± S) for thermal inactivation calculated by linear regression of *Salmonella* survivor data (Ln CFU/g versus time) for whole vs. ground pork at 55, 60 and 62.5°C

	Temperature (°C)		
	55	**60**	**62.5**
Whole muscle	0.12 ± 0.52	1.68 ± 0.5	3.37 ± 0.52
Ground muscle	0.23 ± 0.5	2.13 ± 0.52	4.37 ± 0.52

DISCUSSION

Whole and ground muscle samples were similar in composition, bacterial load, and thermal histories, suggesting that differences in *Salmonella* thermal inactivation were due to differences in the physical structure of the meat. Destruction of the original muscle structure during grinding results in a loss of water and a more even distribution of the fat and protein within the product. These physical changes will have an effect on the exposure that bacterial cells will be subjected to during thermal processing. Thus, more liquid is likely to be retained by whole muscle than ground product due to its more rigid structure. Having more opportunities for internalization and surface attachment within whole muscle may result in differential heat resistance.

The protective effect of fat against thermal inactivation (Ahmed and others 1995; Veeramuthu and others 1998) may be partially lost after the fat has been uniformly distributed as a result of grinding. Bacteria attached to or surrounded by intramuscular fat in whole muscle tissue may be able to utilize this protective effect in a way that is not possible in more homogenous ground products (Orta-Ramirez and others 2005). During cooking, meat proteins denature and contract, releasing water and fat that changes the meat microstructure (Troutt and others 1992b).

Thermal protection afforded by increased fat levels may be due to a localized absence of moisture (lower a_w) within bacterial cells (Juneja and Eblen 2000). According to data from previous studies (Ahmed and others 1995, Line and others 1991, and Smith and others 2001), the protective effect of fat was particularly significant ($P < 0.05$) between 50° and 58°C and decreased as the temperature increased. At higher

temperatures, intramuscular fat in the meat will begin to liquefy, with the accompanying increase in heat transfer decreasing the previous thermal protective effect of fat. During grinding, the muscle structure is partially destroyed with the components distributed uniformly throughout the product. These meat matrix changes lead to a loss of moisture with the being more evenly dispersed, thus affecting the environmental conditions for bacterial growth.

The physical and chemical characteristics of a food also affect microbial heat resistance with the effect of product composition on thermal inactivation documented in the literature (Veeramuthu and others 1998, Orta-Ramirez and others 2005, Troutt and others 1992b). In our study, thermal resistance of *Salmonella* was affected by the physical structure of the meat. Because fat provides pathogenic cells with a protective effect against thermal inactivation (Ahmed and others 1995, Juneja and others 1997, Veeramuthu and others 1998, Smith and others 2001), homogeneous distribution of the fat in a ground sample, as opposed to tissue segregation in whole muscle products may decrease this protection.

The literature contains limited information on thermal inactivation of *Salmonella* in ground pork with any direct comparisons to our results complicated by differences in bacterial strains, meat species, muscle type, formulations, and other environmental factors. Juneja and others (2001) studied the heat resistance of 35 *Salmonella* strains at 58 and 60 °C using different substrates and found that the D values in meat were higher than those in broth. Salmonellae are generally more heat resistant when attached to meat surfaces (or to surfaces such as stainless steel or glass) than when unattached and dispersed throughout a food or broth (Doyle 1999).

The unrealistically high inoculation level of 10^8 CFU/mL in our study was necessary to quantify the rate of *Salmonella* migration. However, different patterns of migration will likely occur at lower levels of contamination.

So far, there have been no reported events of food poisoning related to *Salmonella* in whole-muscle "enhanced" products. The severity of current cooking standards is likely to be the reason for this to occur. These guidelines are conservative enough to be effective eliminating populations of the pathogen present in the meats. Additionally, institutions commonly cook or process meats beyond guidelines in order to further ensure their safety, lowering the likelihood of survival of *Salmonella* cells. Having this margin of variability in the settings of the thermal process of a meat product allows for a lack of consistency in the overall production of these items, possibly leading to miscalculations of the lethality of pathogenic cells, thus increasing safety risks.

In this work, *Salmonella* was more heat resistant when present in whole muscle, compared to ground pork products. Consequently, thermal process validations for meat products should consider the physical state (whole muscle versus ground) of the product. Further work is needed to better understand the mechanisms for these observed differences in thermal resistance of *Salmonella* in whole muscle as compared to ground products during cooking.

CHAPTER 3 MULTIDIRECTIONAL PENETRATION OF *SALMONELLA* INTO INTACT PORK LOIN ROASTS DURING MARINATION

ABSTRACT

The interior of any intact, whole-muscle food is typically to be sterile. However, marination - a common value-added process to improve meat quality - can introduce bacterial pathogens into roasts, with such cells likely to exhibit enhanced thermal resistance during cooking. Consequently, the objective of this study was to determine the numbers of *Salmonella* that migrate into whole-muscle pork roasts during marination. A typical marinade containing 96.0% water, 3.2% NaCl, and 0.8% potassium phosphates was inoculated with an 8-strain *Salmonella* cocktail to contain either 10^8 or 10^4 CFU/mL. Irradiated whole-muscle boneless pork loin (*longissimus dorsi*) roasts measuring 12 cm x 8.7 cm x 4.5 cm (0.75 kg each) were marinated for 15, 30, or 60 s, and subjected to combinations of vacuum and/or tumbling. After marination, a 1-cm thick center slice was cut perpendicular to the muscle fibers using an electrosurgical unit with a heated cauterizing blade. The slices were then sectioned into 1-cm cubes to assess multidirectional migration of *Salmonella*. Samples were diluted 1:10 in sterile 0.1% peptone and homogenized for 90 s in a masticator. Surviving salmonellae were enumerated by plating serial dilutions in duplicate on Petrifilm™ Aerobic Count Plates followed by incubation at 37°C for 48 h. *Salmonella* populations decreased ($P < 0.0001$) from 5.4 log CFU/g at the surface to 2.6 log CFU/g at the geometric center of the roast. Marination time (15, 30, or 60 s) did not significantly affect the numbers of salmonellae recovered. Given these findings, a presumption of interior sterility may not be valid, which could negatively affect the safety of marinated meats.

INTRODUCTION

The interior of intact, undamaged whole muscle is commonly believed to be sterile (Elmossalami and Wassef, 1971). Contamination of the outer surface can occur during slaughter, hide removal, and further processing. While blades, knives, gloves, and other product contact surfaces can transfer bacteria to cut surfaces, the interior of the meat is presumed to remain essentially sterile. Value-added processing techniques, such as blade tenderization and marination, are often used in commercial meat processing, with vacuum tumbling increasing the uptake of marinade into whole muscle meats (Chen 1982; Xiong and Kupski 1999). However, such processing techniques may compromise sterility of the muscle interior. Several early studies examined bacterial penetration into meat under static conditions (Gill and Penney 1982, Gupta and others 1983, Maxcy 1981, Sikes and Maxcy 1980). Gill and Penney (1977) concluded that bacterial invasion was facilitated by production of collagen-degrading enzymes. Additionally, with slow freezing and thawing might also disrupt the integrity of the sarcolemma due to formation of interfiber ice crystals (Sikes and Maxcy 1980). As interfiber ice crystals are formed, water is drawn across the sarcolemma, which results in large ice crystals external to the muscle fibers. These large ice crystals create stress on the sarcolemma, which produces tears in the membrane. During thawing, water is lost; creating evacuated channels that can serve as portals of entry for invading bacteria.

Vacuum tumble marination is a mechanical method of meat tenderization that helps to ensure uniform infusion of the brine and marinade (Barbut 2002). Vacuum tumbling also extracts myofibrilar proteins and reduces the number and size of air

bubbles formed by proteins (Barbut 2002). During vacuum processing, the muscle structure is disrupted, leaving available space for potentially contaminated marinade. Given that vacuum tumbling enhances marinade penetration, pathogen penetration into the interior of muscle products could also be potentially accelerated during this process.

Previous research has examined the effect of invasive tenderization techniques on bacterial penetration. Needle (Boyd and others 1978), mechanical (Raccach and Henrickson 1979), and blade tenderization (Phebus and others 1999) can all introduce bacteria into the interior of whole-muscle products. In contrast, in one recent study by Warsow (2003), non-invasive tenderization by vacuum and/or tumbling also enhanced penetration of *Salmonella* into turkey breast muscle. Such internalized salmonellae pose a potential public health, as evidenced from a recent outbreak involving steaks that were tenderized by vacuum tumbling (FSIS 2001).

In response to these concerns, the objective of this study was to determine the numbers of *Salmonella* that migrate multi-directionally into whole-muscle pork roasts during immersion marination, as well as the effect, of vacuum, tumbling, inoculum level, and marination time on penetration of *Salmonella* into whole muscle pork.

MATERIALS AND METHODS

Experimental design

A lot of thirty-nine pork roasts (*longissimus dorsi* muscle) was used for this study. Samples were subjected in triplicate to each of four treatments (Vacuum-Only, Tumbling-Only, Vacuum and Tumbling, and Still Marination).

Salmonella strains

Eight serovars of *Salmonella*: *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteritidis H3527 and H3502 (chicken isolates phage 13A and 4, respectively), *S.* Typhimurium DT104 H3380 (human isolate), *S.* Hadar MF60404 (turkey isolate), *S.* Copenhagen 8457 (pork isolate), *S.* Montevideo FSIS 051 (beef isolate), and *S.* Heidelberg F5038BG1 (human isolate), previously shown to have moderate to high thermal resistance (Juneja and others 2001), were obtained from Dr. V.K. Juneja (USDA-ARS, Eastern Regional Research Center, Wyndmoor, PA) and maintained at -80°C in tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD) containing 20% glycerol. The strains were propagated by transferring one loopful of each frozen stock culture to 9 mL of TSB. The cultures were maintained by daily transfer to fresh TSB, followed by 18 - 24 h of incubation at 37°C, with a minimum of two consecutive transfers to obtain cells in late log phase (Smith and others, 2001). Each set of tubes was used as a culture source for a maximum of 6 days.

On the day of the experiment, 9 mL of each of the eight serovars grown separately in TSB were combined and centrifuged at 6,000 x *g* for 20 min at 4°C, with the resulting

cell pellet resuspended in 500 mL of sterile marinade to yield a final concentration of 8.1± 0.1 Log CFU/mL which was confirmed by serial dilution in 0.1% peptone water followed by duplicate plating on Aerobic Petrifilm™ Count Plates (3M, St. Paul, Minn., USA). In order to obtain 10^4 CFU/mL marinade, the procedures were followed as previously described but 1 mL of the 10^8 CFU/mL marinade was diluted into 500 mL of sterile marinade (10^6 CFU/mL). Then, 1 mL of the 10^6 CFU/mL marinade was diluted into 500 mL of fresh sterile marinade, giving a final concentration of 10^4 CFU/mL.

Marinade

A typical commercial aqueous marinade for ready-to-eat whole muscle foods containing 96.0% water (filtered and deionized), 3.2% NaCl, and 0.8% potassium phosphate solution (Butcher and Packer Supply Co. Detroit, MI) was prepared according to Pearson and Dutson (1987). Salt was incorporated into the water before adding the phosphate solution to ensure complete dispersion. Aliquots (500 mL) of the marinade were poured into glass bottles with plastic screw caps, autoclaved for 15 min at 121°C to ensure sterility, and stored at room temperature (~22°C) for no longer than 30 days until use.

Meat preparation

Thirty-nine pork loins (*longissimus dorsi* muscle) (~0.75 kg each) were obtained from a local supplier in a single large lot to eliminate lot-to-lot variability. All muscle samples were individually vacuum packaged and frozen at -20°C at MSU. To eliminate indigenous flora, the meat was transported frozen on dry ice to CFC Logistics,

Quakertown, PA, irradiated (~10 kGy), and then returned frozen to MSU for storage at -20°C. After irradiation, presence of any interfering background microflora was assessed by diluting a 1.0-g sample of pork 1:5 in peptone water (Becton Dickinson) followed by plating on Petrifilm™ Aerobic Plate Counts. Moisture and fat levels were determined using AOAC methods 950.46B, and 991.36, respectively (AOAC, 1996). To determine pH, 10-g samples of ground pork were added to 90 ml of distilled water and homogenized for 30 s in a Polytron homogenizer (model PT 10/35, Brinkman Instruments, Westbury, NJ.) at speed setting 3 (Smith and others 2001). The pH of the mixture was then measured using a combination electrode (Model 145, Corning, Medfield, MA). All tests were done in triplicate on randomly selected portions from the original lot.

Exposure to inoculated marinade

Whole, roughly cylindrically-shaped, irradiated, whole-muscle pork loin (*longissimus dorsi*) roasts measuring 12 cm x 8.7 cm x 4.5 cm (0.75 kg each), previously thawed for 48 h at 4°C, were double-bagged in sterile 38 x 51 cm stomacher bags (Fisher Scientific, Pittsburgh, PA) and then submerged in 500 mL of inoculated marinade for 15, 30, or 60 s. To examine the effects of tumbling time on bacterial penetration, pork roasts were subjected to "Vacuum Only", "Tumble Only", and "Still Marination" for 20 min each. Vacuum and tumbling were conducted in a laboratory scale tumbler (Model T-15, DC, Curtis Ltd. Westfield, WI) at 4°C. The tumbler rotated at 8 rpm under a vacuum of 100 kPa, and each function was turned on and off according to the experiment being

performed. Samples marinated without vacuum or tumbling were bagged as described above and refrigerated at 4°C.

Marination times only included those periods during which the pork roasts were immersed in the marinade. After marination, the marinade was discarded into a biohazard bag, and the pork roast was placed on the electrode pad before being sectioned with the ESU. However, 5 and 10 min were needed to obtain the sample cubes from the different slices of the product. Therefore, the *Salmonella* were free to move within the roast until the time each cube was placed in a plastic bad prior to the preparation of serial dilutions and plating. This extended time of exposure to the inoculated marinade during sampling could have allowed further migration compared to exclusively the periods of immersion.

Sampling

Salmonella populations in the marinade were determined before sample inoculation. The target level of 8.1± 0.1 Log CFU/mL was consistently achieved in all experiments. After marination, three slices (cranial, middle, and caudal sections) were selected longitudinal to the muscle fibers (Fig. 3.1). By taking slices at different points in the z direction (i.e., at different points down the axis parallel to the muscle fibers) and excising the 1-cm3 segments in the x and y directions (both perpendicular to the fiber orientation) the sampling plan was designed to generate representative data to estimate the parameters of the actual migration of bacterial cells. Within these three slices, 1-cm^3 cubes were excised using an electrosurgical unit (ESU), Surgistat™ II-20, Valleylab™ Boulder, CO) with the high temperature generated from steam immediately next to the

heated cauterizing blade and ohmic heating lethal to any bacteria, thus preventing cross-contamination during cutting (Fig. 3.2). The 1-cm^3 cubes of meat weighing ~2.0 g were then individually placed in an 8 oz. sterile Whirl-Pak™ bag (Nasco, Fort Atkinson, WI), diluted 1:10 in 0.1% peptone water, and homogenized for 90 s in a masticator (Model 0410, IUL Instruments USA, Inc. Cincinnati, Ohio, USA). To ensure sterility, samples from all pork roasts were also sectioned and plated as was done for the non-marinated samples.

Recovered salmonellae in each cube were enumerated by plating serial dilutions in duplicate on Petrifilm™ aerobic count plates followed by incubation at 37°C for 48 h.

Analysis of variance (ANOVA) was run to evaluate log (N/No) of the cocktail as a function of the main effects of vacuum, tumbling, and marination time. All tests were done in triplicate on portions randomly selected from the original lots.

Figure 3.1. Sampling templates for excising samples from marinated/treated whole-muscle roast samples. (a) Removal of 5 slices, (approximately perpendicular to the fiber orientation) symmetrically around the sample midpoint, (b) Segments (1 cm^3) to excise from slices labeled as "C" and (c) Segments (1 cm^3) to excise from slices labeled "A" and "A*".

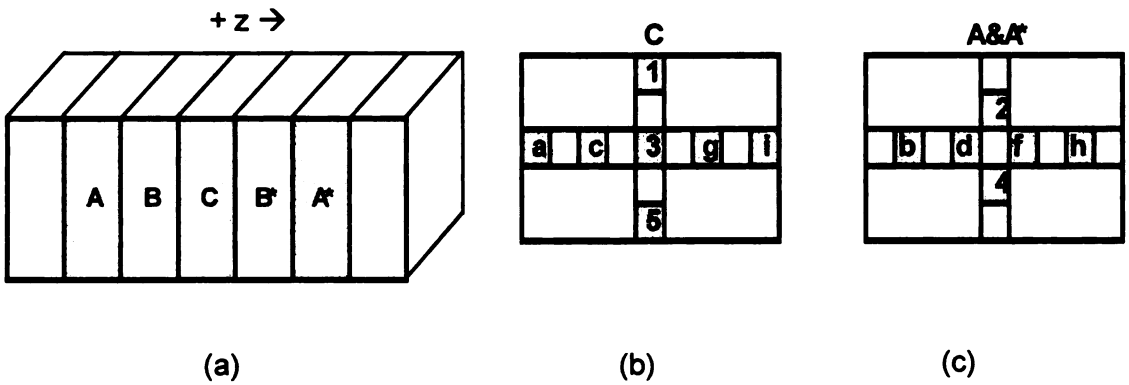
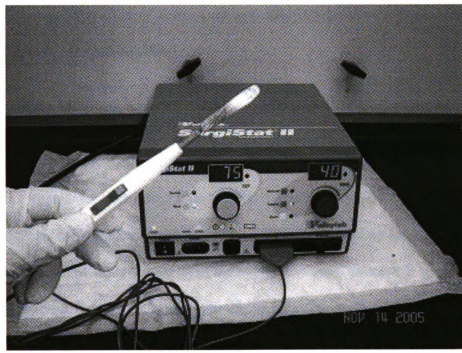


Fig. 3.2. Electrosurgical Unit utilized during sampling.



RESULTS

Multidirectional Migration of *Salmonella* spp. into Whole-muscle Pork Roasts During Various Marination Times

Salmonella populations decreased significantly ($P < 0.0001$) from 5.4 log CFU/g at the surface to 2.6 log CFU/g at the geometric center of the roast (Table 1), but marination time did not significantly affect ($P > 0.05$) the numbers of salmonellae recovered. Results for both slices from the side extremes of the roast (A and A*) were not significantly different from each other ($P > 0.05$) and showed no specific pattern of bacterial migration towards the center of the roast. However, the central slice yielded decreasing salmonellae vertically closer to the center of the roast as shown in Fig. 3.3. Horizontal samples of the central slice also show a trend of decreasing counts of bacterial counts closer to the center (Fig. 3.4). However, due to the greater distance from the extremes on the horizontal plane compared to the vertical one, the counts in the center segment (C3e) were higher than those between the center and surface (C3e > Cc and Cg) but lower than those on the surface (C3e < Ca and Ci).

Figure 3.3. Vertical migration of *Salmonella* into the central slice of whole-muscle samples. Surface samples (C1, C5) center sample (C3e)

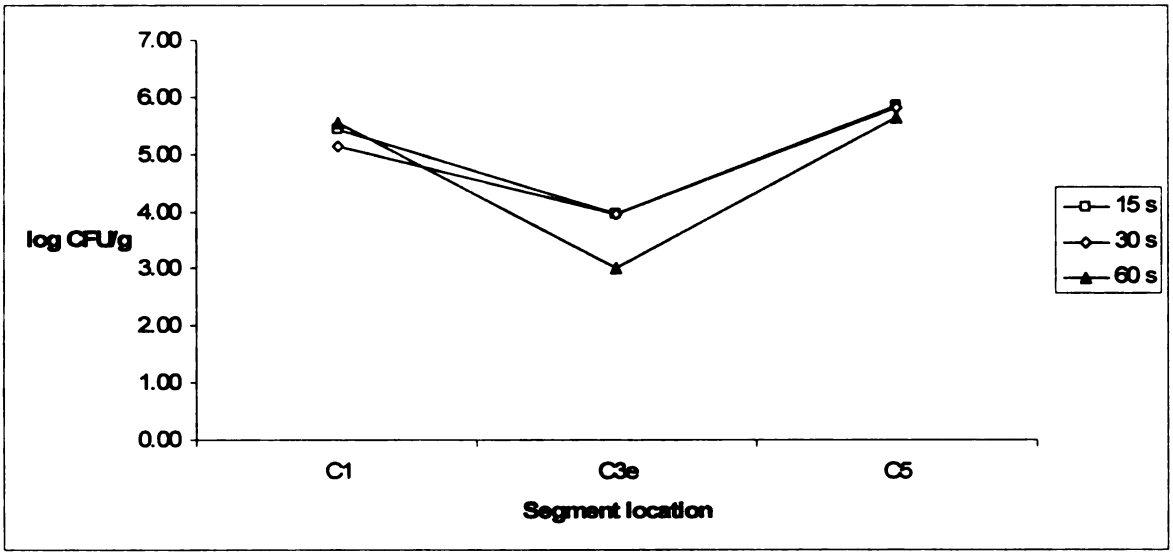


Figure 3.4. Horizontal migration of *Salmonella* into the central slice of whole-muscle samples. Surface samples Ca, Ci, center samples (Ca, Cc, C3e, Cg)

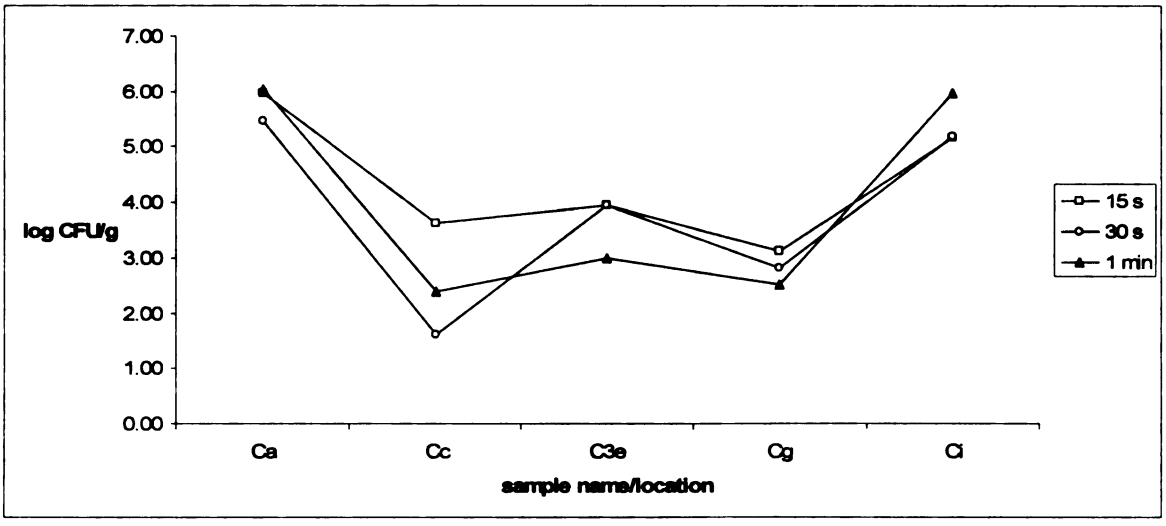


Table 3.1. *P* values from analysis of variance for counts of *Salmonella* spp. in all pork roast slices for all marination times.

Test factors	*P* values		
	A*	C	A
Segment	0.3204	**<0.0001**	0.006
Time	0.565	0.4104	0.7485
Segment * Time	0.6288	0.8651	0.7854

In the two extreme pork roast slices (A & A*), neither time nor the interaction of segment with time significantly affected *Salmonella* recovery (Table 3.1). However, both A & A* slices were statistically different from the C slice ($P < 0.0001$). Segment location had a significant effect on the migration of *Salmonella* into the muscle sample for two of three slices.

Multidirectional Migration of *Salmonella* spp. into Whole-muscle Pork Roasts During Vacuum and Tumbling Marination

For all slices, both the segment and treatment had significant effects ($P < 0.0001$) on migration of *Salmonella* into whole-muscle samples (Table 3.2), thus illustrating the population differences between surface and interior samples.

Comparing the log counts from the segments from the central slice subjected to all treatments, Vacuum Only had the strongest effect on the migration of pathogenic cells compared to Vacuum and Tumbling, Tumbling Only and Still Marination (Table 3.3). As expected, with decreased distance from the center segment, fewer salmonellae were recovered, thus samples closer to the surface had higher counts for all treatments. When exposed to vacuum only, no differences were found within samples segments of the central slice. For tumbling only, still marination and vacuum tumbling, and the sample obtained from the geometric center of the roast (C3e) and the other two internal segments from the central slice (Cc and Cg) were statistically different ($P < 0.0001$) when compared to all surface samples (C1, Ca, Ci, and C5). Within individual segments of the

central slice, the center segments C3e and C5 were the only ones that had significant differences ($P < 0.0001$) in *Salmonella* numbers due to different treatments.

Table 3.2. *P* values from analysis of variance of the migration of *Salmonella* spp. into pork roast samples subjected to various process treatments (Still Marination, Vacuum Only, Tumbling Only, and Vacuum and Tumbling).

Test factors	*P* values
Segment	**<0.0001**
Treatment	**<0.0001**
Segment * Treatment	0.0129

Table 3.3. Comparison of means of the migration of *Salmonella* spp. of segments from the central slice of pork roast samples subjected to various process treatments (Still Marination, Vacuum Only, Tumbling Only, and Vacuum and Tumbling).

Level	Mean (CFU/g)
Vacuum + Tumbling C5	6.67^{a}
Vacuum ONLY C5	6.62^{a}
Vacuum + Tumbling Ci	6.50^{a}
Vacuum + Tumbling Ca	6.45^{a}
Tumbling ONLY Ca	6.41^{a}
Tumbling ONLY C5	6.40^{a}
Vacuum ONLY Ci	$6.30^{a,b}$
Vacuum + Tumbling C1	$6.25^{a,b}$
Tumbling ONLY Ci	$6.23^{a,b}$
Still marination Ca	$6.05^{a,b,c}$
Tumbling ONLY C1	$5.91^{a,b,c}$
Vacuum ONLY Ca	$5.89^{a,b,c}$
Still marination C5	$5.86^{a,b,c}$
Vacuum ONLY C1	$5.82^{a,b,c}$
Still marination Ci	$5.55^{a,b,c}$
Still marination C1	$5.51^{a,b,c}$
Vacuum ONLY Cc	$4.90^{a,b,c,d}$
Vacuum ONLY Cg	$4.45^{b,c,d,e}$
Vacuum ONLY C3e	$3.97^{b,c,d,e,f}$
Vacuum + Tumbling Cc	$3.67^{c,d,e,f}$
Vacuum + Tumbling Cg	$2.99^{d,e,f}$
Tumbling ONLY Cg	$2.51^{e,f}$
Tumbling ONLY Cc	$2.41^{e,f}$
Tumbling ONLY C3e	$2.34^{e,f}$
Vacuum + Tumbling C3e	1.86^{f}
Still marination Cg	1.75^{f}
Still marination Cc	1.65^{f}
Still marination C3e	1.62^{f}

Levels not connected by same letter are significantly different.

Table 3.4. Comparison of means of the segments of the central slice of marinated pork roasts subjected to Vacuum Only.

Segment	Mean (CFU/g)
C5	6.61[a]
Ci	6.30[a]
C1	6.07[a]
Ca	5.89[a]
Cc	4.9[a]
Cg	4.45[a]
C3e	3.97[a]

Levels not connected by same letter are significantly different.

Table 3.5. Comparison of means of the segments of the central slice of marinated pork roasts subjected to Tumbling Only.

Level	Mean
Ca	6.40[a]
C5	6.40[a]
Ci	6.22[a]
C1	5.91[a]
Cg	2.51[b]
Cc	2.41[b]
C3e	2.34[b]

Levels not connected by same letter are significantly different.

Table 3.6. Comparison of means of the segments of the central slice of marinated pork roasts subjected to Still Marination.

Level	Mean
Ca	6.05[a]
C5	5.86[a]
C1	5.57[a]
Ci	5.54[a]
Cg	1.75[b]
Cc	1.6[b]
C3e	1.62[b]

Levels not connected by same letter are significantly different.

Table 3.7. Comparison of means of the segments of the central slice of marinated pork roasts subjected to Vacuum and Tumbling.

Level	Mean
Ci	6.85[a]
C5	6.67[a]
Ca	6.45[a]
C1	6.25[a]
Cc	3.67[b]
Cg	2.99[b]
C3e	1.86[b]

Levels not connected by same letter are significantly different.

Multidirectional Migration of *Salmonella* spp. into whole-muscle pork roasts using two inoculum levels

Samples submerged in marinade containing 10^4 CFU/mL were subjected to two treatments: Still Marination and Vacuum and Tumbling for 20 min. Treatment and segment location had significant effects ($P < 0.0001$) on recovery of *Salmonella* after marination (Table 3.4).

Since no differences were found in previous experiments between slices A and A*, only slices A and C were analyzed. Within individual segments subjected to each treatment, the inoculum level (high vs. low) had a stronger effect on the amount of recovered cells compared to the effect of the treatments (Table 3.5).

Table 3.4. *P* values from analysis of variance for the migration of *Salmonella* spp. into all pork roast samples subjected to various treatments (Vacuum Only, Tumbling Only, and Vacuum and Tumbling) using a 10^4 CFU/mL marinade.

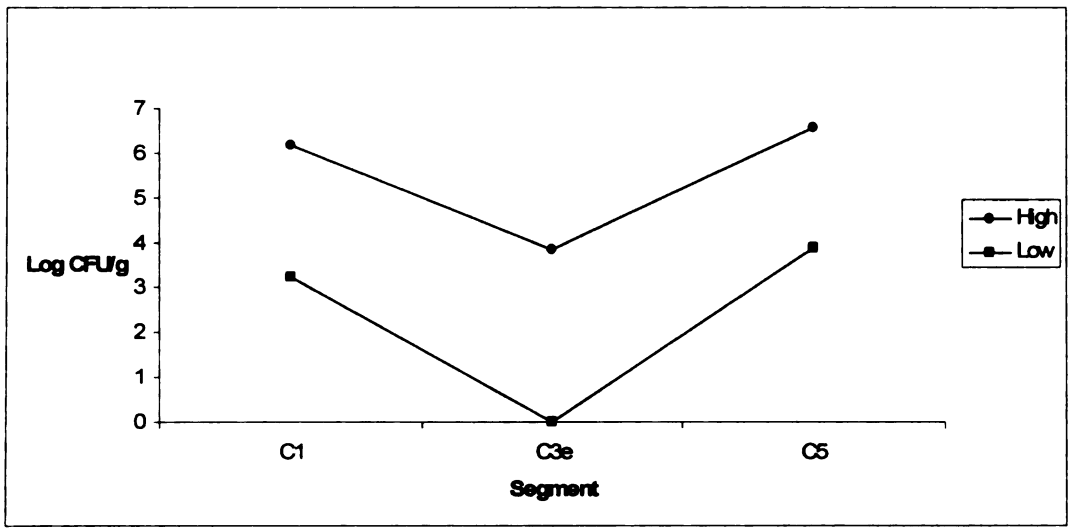
Test factors	*P* values
Segment	**<0.0001**
Treatment	**<0.0001**
Segment * Treatment	0.0197

Table 3.5. Average Log CFU/g of *Salmonella* spp. recovered from segments of the central slice of marinated pork roasts subjected to Still Marination or Vacuum and Tumbling) using a high (10^8 CFU/mL) and a low inoculum level (10^4 CFU/mL) marinade.

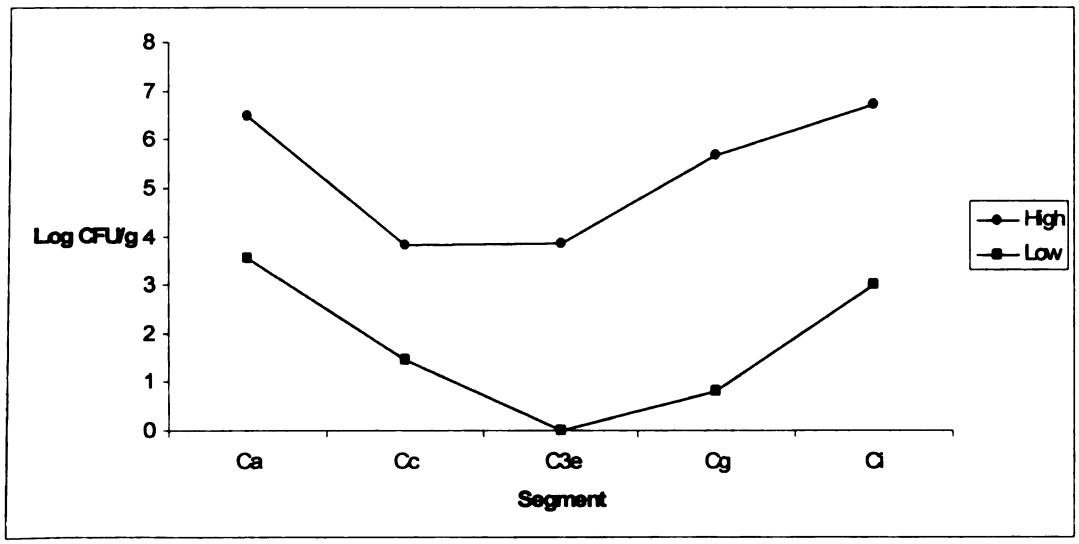
Segment	Treatment	Inoc. Level	Log CFU/g
C1	Still Marination	High	5.51[a]
		Low	2.76[b]
	Vacuum and Tumbling	High	6.25[a]
		Low	3.29[b]
C3e	Still Marination	High	1.62[a]
		Low	1.48[a]
	Vacuum and Tumbling	High	1.86[a]
		Low	1.30[a]
C5	Still Marination	High	5.86[b]
		Low	3.84[c]
	Vacuum and Tumbling	High	6.67[a]
		Low	3.92[c]
Ca	Still Marination	High	6.04[a]
		Low	3.18[b]
	Vacuum and Tumbling	High	6.45[a]
		Low	3.92[b]
Cc	Still Marination	High	1.65[b]
		Low	1.60[a,b]
	Vacuum and Tumbling	High	3.67[a]
		Low	1[b]
Cg	Still Marination	High	1.75[a]
		Low	0.96[a]
	Vacuum and Tumbling	High	2.99[a]
		Low	1.93[a]
Ci	Still Marination	High	5.55[a]
		Low	2.97[b]
	Vacuum and Tumbling	High	6.49[a]
		Low	3.37[b]

Increased migration of *Salmonella* was observed both vertically and horizontally in samples subjected to Vacuum and Tumbling previously marinated with the high inoculum compared to the low inoculum marinade (Fig 3.5). A similar pattern was observed in samples subjected to Still Marination marinated with both low and high inoculum levels (Fig. 3.6). In both treatments, when comparing the segments horizontally, the central segment (C3e) was significantly lower than the surface segments (Ca and Ci) but not different from the Cc, or Cg segments.

Figure 3.5. Vertical (a) and horizontal (b) migration of *Salmonella* with a High Inoculum level (10^8 CFU/mL) and a Low Inoculum Level (10^4 CFU/mL) into the central slice of whole-muscle samples when subjected to Vacuum and Tumbling for 20 min. Surface samples (Ca, C1, C5, Ci), center samples (Cc, C3e, Cg).

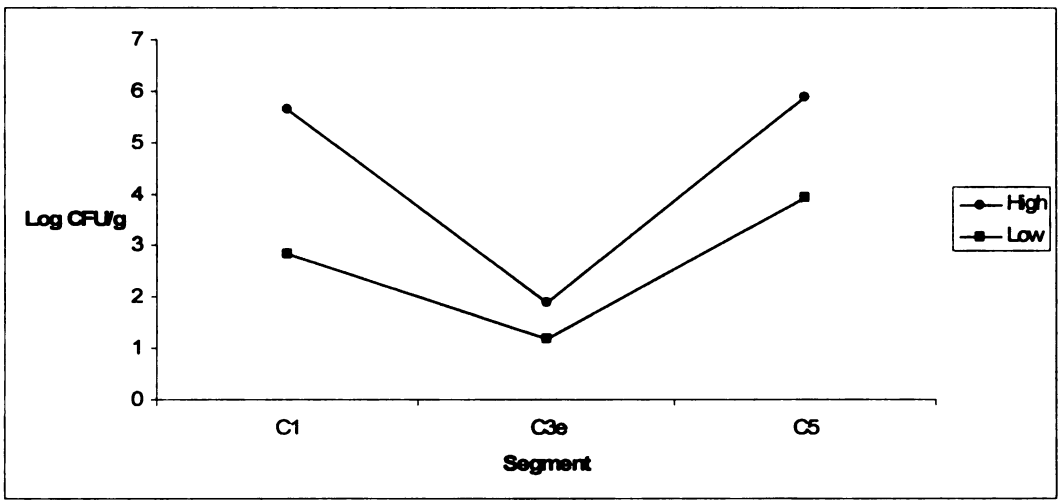


(a)

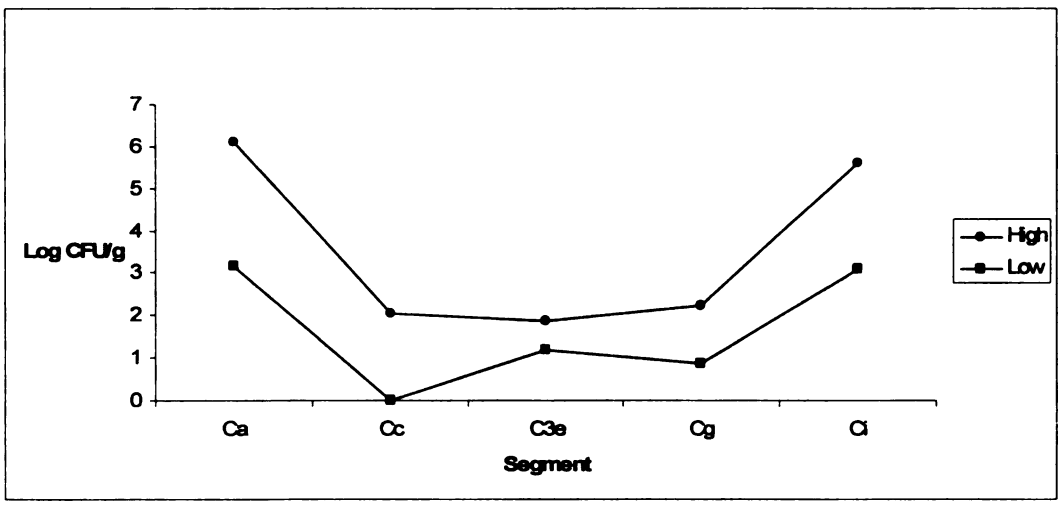


(b)

Figure 3.6. Vertical (a) and horizontal (b) migration of *Salmonella* with a High Inoculum level (10^8 CFU/mL) and a Low Inoculum Level (10^4 CFU/mL) into the central slice of whole-muscle samples when subjected to Still Marination for 20 min. Surface samples (Ca, C1, C5, Ci) center sample (Cc, C3e, Cg).



(a)



(b)

DISCUSSION

Mechanical tenderization is widely used to improve the tenderness of meat while ensuring uniform distribution of the marinade. If the marinade is contaminated, suspended bacteria can penetrate into meat during marination. Concentration gradient of bacteria and osmosis from the outside to the inside of the muscle could contribute to bacterial migration, with unsanitary conditions at the processing facility likely to further worsen the problem. Once introduced into the meat, these bacterial contaminant may proliferate inside whole muscle, (provided that adequate extrinsic and intrinsic factors prevail) and shorten the shelf-life of the product (Raccach and Henrickson 1979), with the increased availability of nutrients in the juices and debris released during mechanical tenderization potentially contributing to microbial growth.

The migration of bacteria into the interior of the meat seems to be related to the water-holding-capacity of meat proteins (Sikes and Maxcy 1980). Phosphates are added to the marinade to enhance absorption and retention of water. Potassium tripolyphosphate has the effect of increasing the water holding capacity of the altered meat proteins by increasing the ionic strength around the muscle filament (Maxcy 1981). Pyrophosphates are active muscle fluidizing agents, dissociating actin and myosin, which results in an increase in water uptake (Xiong and Kupski 1999).

Sikes and Maxcy (1980) observed that ground meat having undergone a freeze-thaw cycle was more susceptible to bacterial penetration than fresh comminuted meat. Freezing and thawing cycles cause some water molecules to be released from proteins, thus collapsing the muscle structure and producing pores and canals through which bacteria may move (Maxcy 1981). In addition, slow freezing and thawing also disrupts

the integrity of the sarcolemma due to the formation of interfiber ice crystals (Hung and others 1978). Penetration of *Salmonella* may have occurred between the muscle fibers (Gill and Penney 1977). Thomas and others (1987) described changes in poultry muscle post-slaughter where gaps between muscle fibers are created by radial shrinkage due to an increase in muscle osmolality from lactic acid formation.

Vacuum and tumbling are technologies that are used to enhance the absorption and retention of marinade by muscle foods, thus the potential of the marinade being contaminated with pathogens becomes a health concern for processors and consumers. The impact of the meat pieces against the tumbler wall and with each other further compromises the overall structure of the muscle, which could widen the gaps or openings between muscle fibers, allowing greater penetration.

In this study, both segment location and the application of treatments had a significant effect on the migration of *Salmonella* into whole-muscle products. Even though *Salmonella* is a motile microorganism, motility is not likely to be cause of penetration beyond 2 cm into the muscle after marination, in this study, cells were found in the center of the roast (~6cm deep). The action of phosphates and capillary action are likely to enhance the quick absorption and retention of marinade inside the muscle, serving as aides in the migration of pathogenic cells. In this study, *Salmonella* was potentially migrating until each sample cube was placed in the stomacher bag for microbial analysis. This extended time of exposure to the inoculated marinade during sampling could have allowed further migration. The application of Vacuum and Tumbling to samples previously marinated in both low and high inoculum marinade demonstrated to have a significant level of migration of *Salmonella* compared to those

subjected to Still Marination. However, the use of a higher inoculum level in the marinade resulted in a greater migration of *Salmonella* compared to the effect of the treatments.

While unrealistically high levels of *Salmonella* were needed in the marinade to track migration, these results nonetheless demonstrate the ability of this pathogen to migrate into meat during marination, thus raising potential safety concerns in regard to adequate cooking of marinated meats.

OVERALL CONCLUSIONS

The results of the multidirectional migration study demonstrated that migration of pathogenic cells occurs very fast and that exposure to the marinade for different marination times did not have a significant effect on bacterial penetration. The application of technologies such as vacuum and tumbling, as well as the inoculum level had an enhancing effect in the migration of *Salmonella* into the pork roasts. Vacuum only had the most significant effect among the treatments on the penetration of pathogenic cells. In all experiments of marination times, treatments, and inoculum levels, there was a significant decrease in the *Salmonella* counts recovered from the center compared to the surface in the central slice of the product. The observation of this trend is helpful to graphically map the multidirectional migration of *Salmonella* into the whole-muscle samples.

In this study, *Salmonella* was statistically more heat resistant when present in whole muscle, compared to ground pork products. Therefore, future thermal process validations for meat products should consider the physical state (whole- muscle versus ground) of the meat product being manufactured.

Although insufficient to fully elucidate the fundamental mechanisms for observed differences in thermal resistance, the results of this study provide valuable new information on the thermal resistance of *Salmonella* when internalized in meat products. Given these findings, the presence of internalized *Salmonella* should be considered when developing thermal processing guidelines for marinated meat.

FUTURE RESEARCH

This study demonstrated the potential for *Salmonella* to penetrate into the interior of pork loin roasts under different marination conditions. Additionally, *Salmonella* was more resistant to heat when present in whole muscle compared to ground products. A need exists to further elucidate and understand the mechanisms by which muscle structure/arrangements protect bacterial pathogens against inactivation during cooking.

To analyze the migration of pathogenic cells into the muscle from a different perspective, the surface of the muscle should be inoculated followed by immersion in sterile marinade. Utilizing non-irradiated and/or meat that has not been previously frozen/thawed might as well have an effect on the behavior of *Salmonella*.

The marinade was inoculated with *Salmonella*, but additional studies should examine the likelihood for similar patterns of penetration with other bacterial pathogens including *E. coli*. The specific patterns by which *Salmonella* penetrates and attaches to inner surfaces should be analyzed and compared to those of other similar pathogens.

Additionally, marinade composition is likely to impact both bacterial viability and ability to migrate into a meat product. By utilizing different types of ingredients including various salt and phosphate concentrations and/or including seasonings in the formula, the migration patterns could significantly affect both the viability of *Salmonella* and its rate/pattern of migration.

In addition, fiber orientation as well as muscle type determined by species might also result in different bacterial penetration, thus further studies need to be performed on products such as lamb, beef, and game meats.

APPENDIX

Additional experiments were conducted to prove the effectiveness of the blade sterilizing itself during cutting of the muscle samples.

Irradiated meat was used to ensure product sterility. Sets of two roasts were used during this part of the study, and only one was marinated. Two different settings and levels of energy in the cut and coagulation options were tested to determine their effect on the life of the electrode tip and its killing potential during sampling. Initially, the settings were adjusted to 75W coagulation and 40W cutting in blend mode, but due to concerns about the sterility of the blade and the results of our experiments the final set up was 120W for coagulation and 40W in pure cut mode. Segments (meat cubes) were taken from the marinated roast, and then cubes were obtained from the sterile unmarinated muscle. Samples from the intact muscle were then serially diluted and placed in a masticator for homogenization for 180 s then plated on Petrifilm™ Aerobic Count Plates and incubated at 37°C for 48 h to determine their sterility. No *Salmonella* was recovered at any dilution during the experiments done in triplicate at each energy combination.

A potential problem that could lead to internal contamination is the build up of burned material on the electrode tip. After the tip has been used for a few minutes, pieces of meat became attached to the blade and were burned due to its high temperature. Even though the muscle surfaces that come in direct contact with the blade become rapidly sterilized, surrounding materials attached to the blade could serve as insulators, decreasing the transfer of heat and electricity to the samples surface, thus decreasing its

sterilizing action during cutting. For this reason, the tip was continuously cleaned with the sponge provided by the manufacturer of the equipment. Additionally, after obtaining samples from any single slice the blade was replaced by a new one.

REFERENCES

Abdul-Raouf, UM, Beuchat, LR, Ammar, MS. 1993. Survival and growth of *Escherichia coli* O157:H7 as affected by pH, acidulants, and temperature. *Appl. Environ. Microbiol.* 59:2364-2368.

Ahmed, N.M., D.E. Conner, and D.L. Huffman. 1995. Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. *J. Food Sci.* 60:606-610.

AOAC. 1996. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Barbut S. 2002. Poultry Products Processing: An Industry Guide. 548 p. CRC Press. Boca Raton, FL.

Blackburn, C.D., L.M. Curtis, L. Humpheson, C. Billon, and P.J. McClure. 1997. Development of thermal inactivation models for *Salmonella* Enteritidis and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. *Int. J. Food Microbiol.* 38:31-44.

Carlson, T.R. 2002. Effect of water activity and humidity on the thermal inactivation of *Salmonella* during heating of meat. M.S. thesis. Michigan State University. East Lansing, MI.

Carmel, P. 2000. The Meat and Poultry Industry. October 2000 report for B2B Electronic Commerce lecture. Management of Global Information Technology, Kogod School of Business, American University. Washington, DC. http://www.American.edu/carmel/ma5901a/industry.htm

CDC. 2006. Disease Information: Salmonellosis, Centers for Disease Control, Division of Bacterial and Mycotic Diseases. Last accessed May 23 2006. http://www.cdc.gov/ncidod/diseaseinfo/salmonellosis_.htm

CDC. 2002. Preliminary FoodNet Data on the Incidence of Foodborne Illnesses- Selected Sites. United States, 2002. MMWR. 51:325-329.

Chen T.C. 1982. Studies on the marinating of chicken part for deep fat frying. *J. Food Sci.* 47:1016-1019.

Crosa, J.H., D.J. Brenner, W.H. Ewing, and S. Falkow. 1973. Molecular relationships among the salmonellae. *J. Bacteriol.* 115:307-315.

Crutchfield, S.R., J.C. Buzby, T. Roberts, M. Ollinger, and C.-T. Jordan Lin.1997. An economic assessment of food safety regulations: the new approach to meat and poultry inspection. ERS Agricultural Economic Report No. 755, July 1997.

D'Aoust J-Y. 1997. *Salmonella* Species. In: Food Microbiology: Fundamentals and Frontiers 2nd Ed. P. 129-158. Edited by M.P. Doyle, L.R. Beuchat, and T.J. Montville. ASM Press. Washington, DC.

D'Aoust J-Y., J. Maurer, J.S. Bailey. 2001. *Salmonella* Species. In: Food Microbiology: Fundamentals and Frontiers 2nd Ed. p. 141-178. Edited by M.P. Doyle, L.R. Beuchat, and T.J. Montville. ASM Press. Washington, DC.

D'Aoust, J-Y. 1989. *Salmonella.* In M. P. Doyle, Foodborne Bacterial Pathogens. p. 816. Marcel Dekker, Inc. New York, NY.

Dhir, V.K., and Dodd. 1995. Susceptibility of suspended and surface-attached *Salmonella* Enteritidis to biocides and elevated temperatures. *Appl. Environ. Microbiol.* 61:1731-1738.

Doyle, M.E., A.S. Mazzotta. 1999. Review of Studies on the thermal resistance of salmonellae. *J. Food Prot.* 63:779-795.

ERS. Economic Research Service. Factors Affecting U.S. Pork Consumption. LDP-M 130-01. May 2005.
http://www.ers.usda.gov/Publications/LDP/may05/ldpm13001/

ERS. Economic Research Service. Briefing Room: Hogs. Last accessed June 5, 2006.
http://www.ers.usda.gov/Briefing/Hogs

FSIS. 2003. Food Safety Inspection Service. Recall notification report 028-2003. June 29, 2003.

Gill, C. O., and N. Penney. 1977. Penetration of bacteria into meat. *Appl. Environ. Microbiol.* 33:1284-1286.

Gill C.O, Penney N. 1982. Bacterial penetration of muscle tissue. *J. Food Sci.* 47:690-691.

Frenzen, P.D. 2002. ERS Briefing Room-Economics of foodborne disease: *Salmonella*. http://www.ers.usda.gov/briefing/FoodborneDisease/Salmonella/index.htm

Friedman MS. 1999. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. *Clinical Infectious Diseases*. 29:298-303.

FSIS. 1999a. FSIS policy on non-intact raw beef products contaminated with *E. coli* O157:H7. Last accessed June 7, 2006. Available at http://www.fsis.usda.gov/OA/background/O157policy.htm

FSIS. 1999b. Performance standards for the production of certain meat and poultry products. Federal Register. 64:732-749.

FSIS. 1999c. Compliance Guidelines For Meeting Lethality Performance Standards For Certain Meat And Poultry Products. Last accessed on June 18, 2006. Available at: http://www.fsis.usda.gov/oa/fr/95033F-a.htm. .

FSIS. 2001. Performance standards for the production of processed meat and poultry products. Federal Register. 66:12590-12636

Gupta L. K., M. S. Kalra, A. Singh, and B. Singh. 1983.Penetration of bacteria into muscles of goat, pork and poultry meat. *J. Food Sci. Technol.* 20:13-16.

Humphrey, T.J., E. Slater, K. McAlpine, R.J. Rowbury, and R.J. Gilbert. 1995. *Salmonella* Enteriditis phage type 4 isolates more tolerant of heat, acid, or hydrogen peroxide also survive longer on surfaces. *Appl. Environ. Microbiol.* 61:3161-3164.

Humphrey, T.J. S.J. Wilde, and R.J. Rowbury. 1997. Heat tolerance of *Salmonella typhymurium* DT104 isolates attached to muscle tissue. *Lett. Appl. Microbiol.* 25: 265-268.

Hung, C.C., E.A. Davis, J. Gordon, and H.T. Davis. 1978. Mechanisms of water loss of bovine semitendinous muscle dry cooked from the frozen state. *J. Food Sci.* 43:1191.

Jay, J.M. 2000. Modern Food Microbiology. 6th ed., USA. Aspen Publishers Inc., Gaithersburg, MD.

Juneja, V.K., and B.S. Eblen. 2000. Heat inactivation of *Salmonella* typhimurium DT104 in beef as affected by fat content. *Lett. Appl. Microbiol.* 30: 461-467.

Juneja, V.K., B.S. Eblen, and G.M. Ransom. 2001. Thermal inactivation of *Salmonella* spp. in chicken broth, beef, pork, turkey, and chicken: Determination of D and z values. *J. Food Sci.* 66: 146-152.

Keen, J.E. 2003. Occurrence of STEC O157, O111, and O26 in livestock at agricultural fairs in the United States. P. 22. Proc. 5th Int. Symp. on Shiga Toxin-Producing *Escherichia coli* Infections, Edinburgh, U.K

Line, J. E., Jr. A. R. Fain, A.B. Moran, L.M. Martin, R.V. Lechowich, J.M. Carosella, and W.L. Brown. 1991. Lethality of heat to *Escherichia coli* O157:H7: D-value and Z-value determinations in ground beef. *J. Food Prot.* 54:762-766.

Lorca, T.A., M.D. Pierson, J.R. Claus, J.D. Eifert, J.E. Marcy, and S.S. Sumner. 2002. Penetration of surface-inoculated bacteria as a result of hydrodynamic shock wave treatment of beef steaks. *J. Food Prot.* 65:616-620.

Mahrour, A., S. Caillet, J. Nketsa-Tabiri, and M. Lacroix. 2003. Microbial and sensory quality of marinated and irradiated chicken. *J. Food Prot.* 66:2156-2159.

Malone, W.F. 1974. Electrosurgery in dentistry: theory and application in clinical practice. P. 231. C.C. Thomas. Springfield, IL.

Maurer, J.L. 2001. Environmental effects on the thermal resistance of *Salmonella*, *Escherichia coli* O157:H7, and triose phosphate isomerase in ground turkey and beef. M.S. Thesis Michigan State University, East Lansing, MI.

Maxcy R. B., 1981. Surface microenvironment and penetration of bacteria into meat. *J. Food Prot.* 44:550-552.

Mead, P.S., S. Laurence, 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. Dis.* 5:607-625.

Miller, G.Y., X. Liu, P.E. McNamara, and D.A. Barber. 2005. Influence of *Salmonella* in pigs preharvest and during pork processing on human health costs and risks from pork. *J. Food Prot.* 68:1788–1798.

Murphy, R.Y., B.L. Beard, E.M. Martin, L.K. Duncan, and J.A. Marcy. 2004a. Comparative study of thermal inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ground pork. *J. Food Sci.* 69:97-101.

Murphy, R.Y., E. M. Martin, L.K. Duncan, B.L. Beard, and J.A. Marcy. 2004b. Thermal process validation for *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ground turkey and beef products. *J. Food Prot.* 67:1394–1402.

Ng, H., H.G. Bayne, and J.A. Garibaldi. 1969. Heat Resistance of *Salmonella*: the uniqueness of *Salmonella* Senftenberg 775W. *Appl. Microbiol.* 17:78-82.

Orta-Ramirez A., B.P. Marks, C.R. Warsow, A.M. Booren, and E.T. Ryser. 2005. Enhanced thermal resistance of *Salmonella* in whole muscle compared to ground beef. *J. Food Sci.* 70:359-362.

Pearson, A.M. and T.R. Dutson. 1987. Advances in Meat Research. Vol.3. Van Nostrand Reinhold Company Inc. New York, NY.

Phebus, R., H. Mardsen, H. Thippareddi. S. Sporing, and C. Kastner. 1999. Blade Tenderization and food safety. *Annu. Recip. Meat Conf. Proc.* 52:71-72.

Quintavalla, S., S. Larini, P. Mutti, and S. Barbuti. 2001. Evaluation of the thermal resistance of different *Salmonella* serotypes in pork meat containing curing additives. *Int. J. Food Microbiol.* 67:107-114.

Raccach, M., and R.L. Henrickson. 1979. Microbial aspects of mechanical tenderization of beef. *J. Food Prot.* 42:971-973.

Ray B., D.W. Janssen, F.F. Busta. 1972. Characterization of the repair of injury induced by freezing *Salmonella* anatum. *Appl. Microbiol.* 23: 803-809.

Rigney C.P, B.P. Salamone, N. Anandaraman, B.E. Rose, R.L. Umholtz, K.E. Ferris, D.R. Parham, and W. James. 2004. *Salmonella* serotypes in selected classes of food animal carcasses and raw ground products, January 1998 through December 2000. *J. Am. Vet. Med. Assoc.* 224:524-30.

Romans, R.J., W.J. Costello, C. Wendell Carlson, M.L. Greaser, and K.W. Jones. 2001. The Meat We Eat. 14th ed. Interstate Publishers, Inc. Danville, IL.

Shaw D.H., K.L. Kalkwarf, R.F. Krejci, and A.R. Edison. 1988. Self-sterilization of the elecetrosurgery electrode. *Oral Surg.* 66:290-292.

Sikes A., and R.B. Maxcy, 1980. Postmortem invasion of muscle food by a proteolytic bacterium. *J. Food Sci.* 45:293-296.

Smith S.E., J.L. Maurer, A. Orta-Ramirez, E.T. Ryser, and D.M. Smith. 2001. Thermal inactivation of *Salmonella* spp., *Salmonella* typhimurium DT104, and *Escherichia coli* O157:H7 in ground beef. *J. Food Sci.* 66:1164-1168.

Sporing, S.B. 1996. *Escherichia coli* O157:H7 risk assessment for production and cooking of blade tenderized beef steaks. MS Thesis. Kansas State University, Manhattan, KS.

Sutherland, J.P., J.T. Patterson, and J.G. Murray.1975. Changes in the microbiology of vacuum-packaged beef. *J. Appl. Bacteriol.* 39:227-237.

U.S. Dept. of Agriculture, World Agricultural Supply and Demand Estimates. WASDE-413. Aug. 12, 2004, and 1998-2002.

Orta-Ramirez A., C.R. Warsow, B.P. Marks, E.T. Ryser, and A.M. Booren. 2003. Enhanced thermal resistance of *Salmonella* in whole-muscle vs. ground beef. Abstracts of the Institute of Food Technologists Annual Meeting. Abstract No. 60C-7

Orta-Ramirez A, B.P. Marks, C.R. Warsow, A.M. Booren, and E.T. Ryser. 2005. Enhanced Thermal Resistance of *Salmonella* in Whole Muscle Compared to Ground Beef. *J. Food. Sci.* 70:359-362.

Thomas, C.J., R.D. O'Rourke, T.A. McMeekin. 1987. Bacterial Penetration of chicken breast muscle. *Food Microbiol.* 4:87-95.

Troutt, E.S., M.C. Hunt, D.E. Johnson, J.R. Claus, C.L. Kastner, and D.H. Kropf. 1992a. Characteristics of low-fat ground beef containing texture-modifying ingredients. *J. Food Sci.* 57:19-24.

Troutt, E. S., M. C. Hunt, D. E. Johnson, J. R. Claus, C. L. Kastner, D. H. Kropf, and S. Stroda. 1992b. Chemical, physical, and sensory characterization of ground beef containing 5 to 30 percent fat. *J. Food Sci.* 57:25-29.

Ulmer, B.C. 2001. The Valleylab Institute of Clinical Education Electrosurgery Continuing Education Module. http://www.valleylabeducation.org.

USDA/FSIS U.S. Dept. of Agriculture/Food Safety Inspection Service. 2001.Performance standards for the production of certain meats and poultry products. 9 CFR Parts 301, 303. Washington, D.C.: USDA/FSIS

U.S. Department of Agriculture, Food Safety and Inspection Service. 2002. *Escherichia coli* O157:H7 in Blade-tenderized, Non-intact Beef. Available at http://www.fsis.usda.gov/OPHS/NACMCF/2002/rep_blade1.htm

U.S. Department of Agriculture, Food Safety and Inspection Service. 2003. Progress report on *Salmonella* testing of raw meat and poultry products, 1998–2002. Available at: http://www.fsis.usda.gov/OPHS/haccp/salm5year.htm.

Veeramuthu, G.J., J.F. Price, C.E. Davis, A.M Booren, D.M. Smith. 1998. Thermal Inactivation of *Escherichia coli* O157:H7, *Salmonella senftenberg*, and Enzymes with Potential as Time-Temperature Indicators in Ground Turkey Thigh Meat. *J. Food Prot.* 61:171-175.

Warsow, C.R. 2003. Penetration of *Salmonella* spp. into Whole Muscle Turkey Breast During Vacuum Marination. M.S. thesis. Michigan State University. East Lansing, MI.

Warsow, C.R., B.P. Marks, E.T. Ryser, A. Orta-Ramirez, and A.M. Booren. 2002. *Salmonella* penetration into whole muscle turkey during vacuum marination. Abstracts of the Institute of Food Technologists Annual Meeting, Abstract No. 88-4.

Xavier, I.J., S.C. Ingham. 1997. Increased *D*-values for *Salmonella* Enteritidis following heat shock. *J. Food Prot.* 60:181-184.

Xiong, Y.L., D.R. Kupski. 1999. Monitoring phosphate marinade penetration in tumbled chicken filets using a thin slicing, dye tracking method. *Poultry Sci.* 78:1048-1052.