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PENETRATION OF *SALMONELLA SPP.* INTO WHOLE MUSCLE TURKEY BREASTS DURING VACUUM TUMBLING MARINATION

presented by

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Major Professor's Signature

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PENETRATION OF *SALMONELLA SPP.* INTO WHOLE MUSCLE TURKEY BREASTS DURING VACUUM TUMBLING MARINATION

By

Christopher R. Warsow

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ABSTRACT

PENETRATION OF *SALMONELLA SPP.* INTO WHOLE MUSCLE TURKEY BREASTS DURING VACUUM TUMBLING MARINATION

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Irradiated, whole muscle turkey breasts were vacuum tumbled with a marinade containing 10^8 CFU/mL of an 8-strain cocktail of *Salmonella*. Various methods were used to aseptically excise tissue from the inner portions of the muscle and then enumerate bacterial content. Effect of tumbling action, vacuum, and length of treatment were examined. An additional study was conducted to determine if exposing breast muscle portions to vacuum alone accelerates the penetration of *Salmonella* unidirectionally.

Salmonella counts of 10^3 CFU/g were recovered from the inner portions (up to 3 cm deep) of whole muscle turkey breasts after treatment. Vacuum increased unidirectional penetration of *Salmonella* ($p<0.05$) but was not significantly related to exposure time ($p>0.05$). Vacuum tumbling increased bacterial penetration when compared to controls ($p<0.05$). Vacuum tumbling time and bacterial penetration were not related ($p>0.05$). *Salmonella* penetration during vacuum tumbling with a marinade that is contaminated is reasonably likely to occur.

This thesis is dedicated to my family and friends who have supported me throughout my studies. Especially, to my Grandmother who did not get to finally see her Grandson graduate from college.

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I would like to express my ultimate gratitude to my major professor, Dr. Al Booren, who took a chance on a Food Science student. He allowed me great latitude in solving problems on my own. Also a special thank you to Dr. Alicia Orta-Ramirez who has tenaciously guided me through numerous endeavors throughout my graduate and undergraduate studies. A great amount of respect is owed to Drs. Ryser and Marks who exhibited great confidence in my abilities.

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INTRODUCTION

Salmonella is the second most common cause of food-borne illness behind *Campylobacter*, both of which are commonly found in poultry products. Salmonellosis accounts for an estimated 1.4 million cases of food-borne illness per year, of which more than 500 are fatal (CDC 2001). Pathogens, like *Salmonella*, that are present on the surface of poultry may be translocated into the product during value added processing (Boyd and others 1978, Raccach and Henrickson 1979, Phebus and others 1999). Many products sold in the retail meat case today are marinated or enhanced through value added processing. Vacuum tumbling, a value added process, which is often used to tenderize and distribute marinade in whole muscle foods, may contribute to this translocation.

According to the Food Safety Inspection Service (FSIS, 2002a) turkey is a common source of *Salmonella* in the U.S. food supply. Between July 1999 and June 2000, 30% of ground turkey samples were positive for *Salmonella*. An average of 31.1 million metric tons of poultry was consumed in the world between 1988 and 1990, of which 8% were turkey and turkey products (Roenigk 1999). Turkey products that are potentially contaminated with *Salmonella* could pose additional risks to consumers if they are further processed with non-invasive tenderization techniques.

After gaining access to the inner portions of whole-muscle products, *Salmonella* may be more thermally resistant compared to microbes that are found on the meat surface. This would be of concern to consumers who marinate their own meat or purchase pre-marinated products and then do not adequately

cook the product. Consumption of meat products that contain viable pathogenic organisms can cause food-borne illness (Mead and others 1999).

It is the overall goal of this research to determine if vacuum tumbling contributes to the penetration of *Salmonella* into intact turkey breast muscle. Our hypothesis is *Salmonella* penetration is greater when whole muscle turkey breasts are subjected to vacuum tumbling marination. Specific objectives of this research include the following: 1) determine the unidirectional penetration depth of *Salmonella* in marinated, intact turkey breast muscle when exposed to vacuum 2) determine the multidirectional penetration depth of *Salmonella* in marinated turkey breast muscle during vacuum-tumbling marination.

LITERATURE REVIEW

Poultry Consumption

The meat and poultry industry is the single largest component of U.S. agriculture, contributing over $90 billion in annual sales to the Gross National Product (AMI 2000). Poultry products were the second most consumed animal-based protein behind pork, with an average of 31.1 million metric tons consumed in the world between 1988 and 1990 (Roenigk 1999). Overall, 8% of all poultry products consumed originated from turkey (Roenigk 1999). In 2001 the United States produced 7.15 billion pounds of turkey products valued at $2.79 billion (USDA 2002).

According to a recent Food Safety Inspection Service (FSIS) report, approximately 30% of all ground turkey contains *Salmonella* (FSIS 2002a). Food-borne illness is a major contributing factor to lost production in the American workforce. Since *Salmonella* is the second most prevalent cause of food-borne illness, a clear understanding of its fate during value added processing is necessary (CDC 2000).

Salmonellae Physiology

Salmonella is an enteric microbe commonly found in the intestinal tract of warm and cold-blooded animals. *Salmonella* has several distinctive physiological characteristics that are used for biotyping. Biotyping is a method to identify and differentiate species of microbes based on their respiratory and metabolic traits. *Salmonella* is a Gram negative, facultatively anaerobe, chemoorganotrophic bacillus. (Jay 2000). *Salmonella* metabolizes by respiration and fermentation and

grows optimally at 37°C. Typical metabolic factors used for identification include catabolism of D-glucose and other carbohydrates with the production of acid and gas, and lack catalase and oxidase activity (D' Aoust and others 2001).

Most *Salmonella* serovars are motile by peritrichous flagella and very adaptive to their environments. The bacterium can tolerate a pH range from 4.5 to 9.5 and proliferates at an optimum of 6.5 to 7.5 (D' Aoust and others 2001). Growth is normally inhibited by 3-4% NaCl, and salt tolerance is positively correlated to temperature from 10 to 30°C (D' Aoust 1989, D'Aoust 1989). *Salmonella* cells that have been preconditioned to low temperatures during incubation can proliferate at temperatures used to store commercial meat products, calling into question the efficacy of refrigerated storage for growth suppression (Airoldi and Zottola 1988).

Today, one of the most commonly used means to differentiate *Salmonella* serovars and other food-borne pathogens in outbreak investigations, is pulse-field gel electrophoresis (PFGE). This method is used for nation-wide monitoring of food-borne illness by the CDC (CDC 2002). In PFGE, the bacteria's genomic DNA is first cut using one of several rare restrictive endonucleases, after which the fragments are resolved in an agarose gel that is subjected to a pulsed electrical field. Personnel in several health department laboratories around the country are trained to perform this test identically to ensure consistency, with the results sent to CDC for analysis through the Pulsenet system (Swaminathan and others 2001, CDC 2002).

Salmonellosis

Salmonella is responsible for an estimated 1.4 million cases of food-borne illness each year in the United States, of which 40,000 are culture-confirmed and reported to the Center for Disease Control (CDC 2001). Symptoms of salmonellosis, namely nausea, abdominal cramps, diarrhea and vomiting are usually evident 12-72 h after exposure and normally last 4-7 days (CDC 2001). Salmonellosis accounts for over 500 deaths per year, while 2% of all cases are complicated by chronic arthritis (CDC 2001).

An infective dose can be as little as 10 to 100 cells in susceptible populations, which include newborns, the elderly, or people with weakened immune systems (D'Aoust and others 2001). A low infectious dose has also been connected to the type of food in which *Salmonella* is found. High fat foods like chocolate, cheese, and meat pass through the stomach faster leading to increased survival of the pathogen (D'Aoust and others 2001). In addition, Salmonella can likely become be trapped within hydrophobic lipid micelles, and therefore be protected from the acidic environment (D'Aoust and others 2001).

Salmonella is an entero-invasive organism, which means it can infect epithelial cells of the intestines. Invasion begins by attachment to the intestinal wall by proteinaceous appendages. Bacterial motility ensures that an appropriate attachment site is located. The invasion gene locus of the bacteria 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).

Salmonella produces two toxins, a diarrheagenic enterotoxin and a thermolabile cytotoxin. The diarrheal toxin is composed of an A (active) subunit and a B (binding) subunit. This enterotoxin produces diarrhea by activating adenylcyclase, which leads to increased cAMP levels, decreased absorption of Na^+ and release of Cl^- into the intestine with water being drawn into the intestine because of this electrolyte imbalance. The cytotoxin is an outer membrane protein that inhibits protein synthesis and lyses the host cells, thereby providing nutrients for further bacterial growth (D'Aoust and others 2001).

There have been numerous changes in the nomenclature regarding *Salmonella*. Many microbiologists still give each serovar of *Salmonella* species status. However, the latest methodology places all salmonellae into two species *S. enterica* and *S. bongori.* Five subspecies are recognized within these two species, most of which belong to *S.* enterica (Jay 2000). For example, *S. typhimurium* in now *S. enterica* subsp. *enterica* serovar Typhimurium, or shortened to *S.* Typhimurium (Jay 2000).

Half of all salmonellosis cases are caused by two serovars: *S.* Enteriditis (SE) and *S.* Typhimurium (ST) (CDC 2001). Although the reported cases of SE have always been proportionally high, the incidence of emerging pathogenic strains has also been increasing. Serovars that were previously of little concern to widespread public health, such as *S.* Newport, are becoming increasingly

prevalent. Greater concern lies in the increasing number of antibiotic-resistant strains like ST DT104. Definitive type (DT) 104 is resistant to at least five commonly used antibiotics (Glynn and others 1998). Salmonellosis has been an economic burden not only to the food service and manufacturing industries, which can be held financially liable for confirmed cases, but also to employers whose employees are unable to work. The best possible way to prevent infection is to eliminate or reduce the spread of pathogens during food manufacturing by implementing the Hazard Analysis Critical Control Point (HACCP) systems and Good Manufacturing Practices (GMPs).

Turkey is a common source of *Salmonella* in the U.S. food supply. According to one FSIS survey of small and large production facilities, 30% of ground turkey sampled from July 1999 to June 2000 was positive for *Salmonella* (FSIS 2002b).

Regulatory Environment

FSIS, a division of the USDA, adopted the HACCP system in 1996 was to better "ensure that meat, poultry and egg products are safe, wholesome, and properly marked, labeled, and packaged" (Federal Register 1996). Early in the 20th century, the first meat inspectors relied on organoleptic evaluation of both carcasses and the meat products to judge wholesomeness. At the time, the main concern for food-borne illness arose from diseased animals, and they believed this system worked well. However, they were unaware of unseen hazards that arose from pathogenic bacteria that infected the meat post-harvest. Today's animals are healthier and the threats now come from microbial sources that are

undetectable by organoleptic methods (FSIS 1998). It is now the focus of food producers to prevent contamination.

In July of 1996, major legislation was passed stating that all USDA inspected meat production facilities were required to implement a pathogen reduction system. HACCP is a "farm to fork" approach to reducing the likelihood of food-borne disease, based on established science instead of a simple inspection of the raw and finished product (Federal Register 1996).

Several directives have been handed down from the FSIS since the inception of HACCP, including testing for *Salmonella* in the facility and its products for effectiveness (FSIS 2002a, b). Plants that are found to be in non-compliance, either through higher than allowable contamination levels or unsatisfactory HACCP plans can be immediately shut down by suspending their inspection certification and their products may be recalled if the transgression is severe enough.

The HACCP concept has shifted the role of the USDA inspector. Instead of inspectors examining the process on a daily basis and looking for critical violations on the production floor, they are now guiding the producer in developing a method to ensure that violations do not occur. Some USDA inspectors have argued that the HACCP system is equal to allowing the industry to self regulate itself and that the U.S. government, by enacting the FSIS HACCP plan, is endangering the safety of the U.S. food supply (Osburn 2002). However, it is entirely in the best interest of the producer to minimize the possibility of

contamination because of the high costs of recalls and litigation by harmed consumers.

Mechanical Tenderization

Mechanical tenderization is used throughout the meat industry to increase palatability of lesser quality cuts of meat (Johnson 1978). Several methods can be used to achieve the desired result. Blade tenderization utilizes several rows of sharp blades that penetrate the meat approximately every 2 cm. When the blades repeatedly pierce the meat, they cut the connective tissue, making the meat more palatable. Blade tenderization has recently come under scrutiny because of concern that this procedure could transport bacteria from the meat surface into the interior (Phebus and others 1999). When this method is used for beefsteaks, which are commonly eaten undercooked, *Escherichia coli* O157:H7 that may now be present inside the muscle because of blade tenderization can cause serious health problems. Sporing (1999) stated that 3-4% of *E. coli* present on the surface can be translocated to the inside of the meat. A recall involving marinated and tenderized steaks was issued on June 29, 2003 due to a several state outbreak of *E. coli* O157:H7 (FSIS 2003).

Vacuum tumbling is also a popular method of tenderizing and infusing flavor into whole-muscle roasts. During vacuum tumbling, the whole muscles are placed in a large vacuum chamber with paddles on the inner walls, with or without a marinade; a vacuum is drawn, and the vessel is rotated on its axis. The impact of the muscles with the wall of the vessel, as well as with each other, tenderizes the meat. Tenderization is thus accomplished by disrupting the

structure of the muscle. It also helps distribute the marinade (Barbut 2002). Muscle pieces are also vacuum-tumbled to extract soluble proteins that will later act as glue to stick the meat together when heat is applied. During thermal processing, a protein matrix is formed that binds the muscles trapping fat and moisture (Barbut 2002).

Marinades are usually liquid solutions drawn into meats by chemical (osmosis) or mechanical (vacuum-tumbling) means. Bacteria present on the surface of the roast prior to marination could be drawn into the meat. Johnston (1978) proposed a ban on mechanical methods of tenderization because of the risk posed by bacteria reaching the muscle interior. This risk is increased if the meat is vacuum packaged because purge from the meat can also enhance the environment for bacterial growth (Johnston 1978).

Needle injection is also a common operation before vacuum tumbling. Needle injection involves pumping a whole muscle with a solution that may contain flavorings, phosphates, and/or nitrites, depending on the application (Barbut 2002). The risk of bacterial penetration may be further complicated if the excess pump exuded from the muscle is collected and introduced into the next muscle. Vacuum tumbling is often coupled with this process to evenly distribute the pump (Barbut 2002), which could push surface bacteria further into the product.

Muscle Food Microbiology and Bacterial Penetration

Bacterial contamination on the surface of meat can be either intrinsic or extrinsic (Gill 1980). Intrinsic contamination occurs before processing on the in-

coming animal whereas extrinsic contamination occurs during or after slaughter from sources such as a food handler or the chill bath. Bacteria on one carcass can also be easily spread to others by several mechanical means, including the chill bath and insects (Jones and others 1991). Minimal research has been done to determine the fate of these surface bacteria as the meat products undergo further value added processing.

It is a commonly held belief that the interior of intact, unpenetrated whole muscle foods from a healthy carcass is sterile and free from external bacteria (Elmossalami and Wasef 1971). Possible migration of bacteria into meat from the surface could occur through several mechanisms: proteolytic activity (Gill and Penney 1977; Gill and Penney 1982; Gupta and others 1983; Thomas and others 1987), bacterial motility (Gill and Penney 1977; Thomas et al 1987), water availability in the muscle (Sikes and Maxey 1980; Maxey 1981), and muscle fiber orientation (Sikes and Maxey 1980; Maxey 1981). Bacteria could also penetrate the gap areas between the fibers after post-rigor changes in the muscle orientation (Sikes and Maxey 1980; Maxey 1981).

Sikes and Maxey (1980) studied proteolytic penetration of *Serratia marcescens* in pork and beef muscles. Whole-muscle pork and beef roasts were aseptically trimmed 5 cm on all sides, then dipped in colloidon. One side of the colloidon was then removed and bacteria were applied. Inoculated samples were incubated at 23-25 °C in a high humidity environment to retain a high a_w for up to 4 days. To measure bacterial penetration, the frozen blocks were cut into 5-mm thick slices, swabbed with sterile cotton-tipped applicator at various depths, and

streaked on plate count agar. Results showed that bacterial penetration was greatest parallel to the muscle fiber and not dependent on the bacterial proteolytic activity. They also noted that the penetration rate was highly influenced by the extent of meat protein hydration and the availability of pores and canals. Cooking and drying affects the hydration level of meat (Sikes and Maxey 1980). Vacuum tumbling, which disrupts the structure of the muscle and exposes the meat to a high water environment, could accelerate access of both proteolytic and non-proteolytic bacteria into the muscle.

Thomas and others (1987) used microscopy to demonstrate that bacteria could penetrate whole muscle foods under static conditions. They demonstrated that proteolytic bacteria and motile bacteria gained access to the inner portions of whole-muscle chicken breast faster than non-proteolytic or non-motile bacteria. However, non-proteolytic bacteria migrated into the muscle interior. Using a mixed culture of proteolytic and non-proteolytic bacteria, the non-proteolytic bacteria were able to penetrate more rapidly because of the proteolytic activity of the other bacteria. Since meat products are often contaminated with various bacteria, a non-pathogenic, proteolytic strain (*Serratia marcescens*) could facilitate quicker penetration of a pathogenic, non-proteolytic strain (*S. enteriditis*).

Thomas and others (1987) also determined that motile bacteria rapidly penetrated muscle most rapidly when applied in a pure culture. Non-motile bacteria could not penetrate the muscle regardless of whether they could produce extracellular proteases or not, even after 5 to 7 days of incubation at 15

°C. However, these experiments were conducted under static, atmospheric conditions, not taking into account any mechanical tenderization methods, which could disrupt the integrity of the muscle, and allow increased penetration.

In another experiment Elmossalami and Wassef (1971) sterilized the outer surface of a 2 kg piece of whole-muscle beef with a hot knife, wrapped it in sterile gauze, and dipped it in hot paraffin. The top surface was then aseptically removed and smeared with a pure culture of *Salmonella enteriditis*. Slices were then removed longitudinally, starting with the non-inoculated surface and slicing upward through the inoculated surface to prevent mechanical transmission of the bacteria. The exposed surface was dipped in paraffin to prevent contamination. When the procedure was repeated every 12 h up to 60 h. SE penetrated to a depth of 15 cm after 48 h (30 °C).

All of these methods worked very well for these researchers under static environmental conditions. However, none of these methods are suitable to determine bacterial penetration during a rigorous tenderization operation, like vacuum tumbling marination. For this reason, a new methodology needed to be developed.

Electrosurgery

A novel method to aseptically remove tissue from the inner portions of inoculated meat was developed during this research. The risk of mechanically transferring bacteria from the outside of the muscle to the inner portions is always a factor when using a conventional knife. However, a cauterizing knife

that utilizes high-energy radio frequencies to dissect the tissue greatly reduces this risk.

Electrosurgical generators are commonly used in hospital operating rooms to dissect and cauterize tissue simultaneously. The electrosurgical unit (ESU) consists of a generating device, a hand piece, and a grounding plate. The generating unit produces high frequency radio waves that 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 causes the water inside the cells to vaporize, rupturing the cell, and therefore cutting the tissue (Ulmer 2001).

In surgery, the electrosurgery electrode sterilizes the surgical incision as it cuts, decreasing the chance for a wound infection (Malone 1974). Available literature on viability of microbes on the activated electrode tip indicate that the tip will self-sterilize at the level (175 W) used in this procedure. Since the electrode is already sterile when the first cut is made, sterility is maintained. Shaw and others (1988) indicated that any energy level over 200 J (J = Watts x seconds) would sterilize the electrode tip when inoculated. Since we were using 175 W of constant power, the electrode tip would self sterilize in 1.1 s, limiting or eliminating, mechanical transfer of bacteria.

CHAPTER 1. PRELIMINARY STUDIES

INTRODUCTION

Initially a dye-tracing study was conducted to understand how marinades penetrate different muscles (chicken and beef). Subsequent studies observed the penetration of an 8-strain *Salmonella* cocktail suspended in a salt and phosphate marinade into intact turkey breast muscle. The "meat print" method qualitatively observed bacterial penetration into the muscle and the later slicing method quantitatively enumerated bacterial levels inside turkey breast muscle.

Dye Tracing Study

Xiong and Kupski (1999) used a thin slicing, dye tracing method to determine optimal levels of phosphate and water to add to a marinade to increase water uptake from a marinade. A similar method was used to qualitatively observe if the marinade was able to penetrate beef and chicken tissue. If the colored dye was able to penetrate the muscle during vacuum tumbling marination, so too could bacteria that may become suspended in the marinade from the exterior in the muscle. Though the dye molecule is many times smaller than a bacterial cell, a conclusion could be drawn in respect to marinade penetration.

Burn and Core Method

A study was conducted where two cores were removed from the center of an irradiated turkey breast that had been vacuum tumbled in a *Salmonella* inoculated marinade. Penetration depth was determined by sectioning the cores into 1-cm segments and enumerating *Salmonella*.

Turkey Block Slicing Study

This study was designed to determine the 3-dimensional pattern of *Salmonella* penetration into a turkey breast block that occurs during vacuum tumbling with an inoculated marinade. After vacuum tumbling, the outer portions of the block were removed, the outside flamed with ethanol, and then sliced aseptically. The slices were then further subdivided, macerated, and plated to enumerate *Salmonella*.

Salmonella Viability Study

When determining the penetration of *Salmonella* into whole muscle turkey breast, we wanted to confirm that the marinade was not bacteriocidal. This study examined the survival of the 8-strain inoculum during storage at 4°C and 37°C.

DYE TRACING STUDY

ABSTRACT

The purpose of this study was to qualitatively determine the extent a commercial marinade could penetrate boneless whole muscle chicken breasts and beef roasts. Justification was based on the hypothesis that if a marinade could visually penetrate the intact muscle, then so could bacteria suspended in the marinade or on the surface of the muscle.

Bromophenol blue was added to color a commercial marinade so that penetration could be seen in the tissue. The meat was vacuum tumbled in the dye colored marinade using a laboratory scale vacuum tumbler (8 RPM, 100 kPa). Thinly slicing the vacuum tumbled muscles revealed dye penetration of the muscles in both species, although in different patterns. Marinade uptake in the beef roasts was non-uniform and occurred along separations and fissures in the muscle fibers as well as along seam fat and marbling. In contrast, the marinade even penetrated through the bone and skin sides of chicken breasts all the way around the muscle.

MATERIALS AND METHODS

Meat Preparation

Fresh boneless chicken breasts and beef roasts were obtained from a local retailer and held at 4°C. The beef roasts were further processed by cutting into blocks measuring 32 x 32 x 127 mm with a knife.

Marinade Preparation

A commercial marinade was prepared from a recipe found in a popular formulary (Pearson and Dutson 1987). The marinade consisted of 80.7% water (filtered and deionized), 12.6% NaCl (J.T. Baker, Philipsburg, N.J.), and 6.65% mixed phosphate solution (w/w) (Butcher and Packer Supply Co., Detroit, Mich.). The salt was incorporated into the water before adding the phosphate solution to ensure dispersal. Bromophenol blue (Fisher Scientific, Pittsburgh, Penn., U.S.A.) was added to the marinade at a ratio of 0.3757 g per 500 ml of marinade and mixed by hand until dissolved.

Several other dyes were evaluated for this study, including crystal violet (not soluble in marinade), green food coloring (FD & C Yellow 5 and Blue 1) (6 mL / 500 mL), purple edible dye used for USDA inspection stamps (4 mL/500 mL), and brilliant blue (2 g / 500 mL).

Dye Treatment

For both chicken and beef, the dye-tinted marinade was added to a stomacher bag (Seward Medical, London, UK) in the proportion of 108 g of marinade per 1100 g of meat, according to Pearson and Dutson (1987). After sealing, the stomacher bag was and placed in a laboratory size tumbler (Model

T-15, D.C. Curtis Ltd., Ill.), subjected to a vacuum (100 kPa) and tumbled for 20 min at 8 rpm.

Observing Penetration

The meat was removed from the bag and sliced. Beef samples were sliced in two directions, one with the grain, and one against. Two millimeter slices were taken across the entire meat block and lined up in progressive order on a clean surface to compare marinade progression. Photographs were taken for comparison.

Four chicken breasts were treated in the same manner, except for slicing. Two breasts were laid flat and sliced perpendicular to the long axis of the muscle to reveal the penetration pattern. Two other breasts were marinated, and circular cores were taken from the thickest portion of the breast. The cores were sliced into 1-2 mm slices, perpendicular to the direction of penetration, and the color observed.

RESULTS AND DISCUSSION

In preliminary work, several different dyes were rejected. Rejection was based on solubility in the marinade and ability to penetrate the muscle. The ideal dye should be soluble in the water-based marinade, yet able to penetrate the tissue at the same rate as the marinade or bacteria that would later be suspended in the colored marinade. The original thought was to spike the marinade with both the *Salmonella* cocktail and the dye so both bacterial penetration and dye penetration could be quantified and compared. In preliminary studies, *Salmonella* died quickly when added to the marinade that contained the dye. We chose not to add the dye to the inoculated marinade for this reason.

The composition of the marinade was chosen because it appeared to represent what is commonly used in the meat processing industry. The basic dry components of a marinade are salt and phosphate, which both facilitate the uptake of water into the muscle (Chen 1982; Xiong and Kupski 1999). When the water was drawn into the muscle, so too was the dye. This study was conducted under the assumption that bacteria present in a similar marinade could penetrate the muscle in the same manner.

Dye penetration in the beef blocks appears to be along the interstitial spaces between the muscle fibers and along fat striations in the meat. Areas of dye penetration appeared dark blue, as shown in Figure 1. Dye also penetrated to the center of the meat in some cases.

Beef carcasses are often electrically stimulated (ES) to increase tenderness. If the beef muscle came from a carcass that was electrically stimulated post mortem causing cracks or fissures in the ultrastructure of the muscle (Babiker and Lawrie 1983), bacterial penetration could be greater through these fissures. The increased tenderness is attributed to a reduction in cold shortening, acceleration of the aging process, increased activity of acid proteases, and disruption of the myofibrillar structure (Babiker and Lawrie 1983). The last mentioned reason could increase bacterial penetration in ES beef by causing fractures in the muscle structure.

Dye penetration in the poultry experiment occurred differently than in beef. With more even penetration seen throughout the chicken breast (Figure 2). The dye traveled approximately 2-3 mm through both the skin and bone side of the muscle.

Images in this thesis are presented in color.

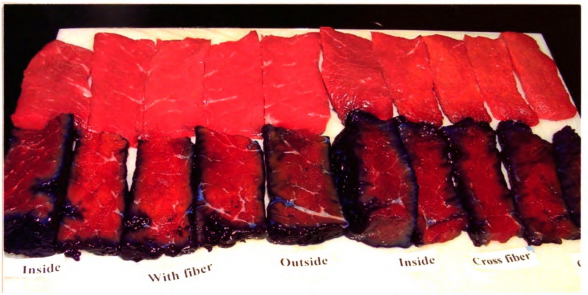

Figure 1. Penetration of bromophenol blue colored marinade into beef roast portions after vacuum tumbling marination

Figure 2. Penetration of bromophenol blue colored marinade into chicken breast after vacuum tumbling marination

CONCLUSIONS

Using the dye tracing method, we found that the marinade penetration pattern was species dependent. If bacterial penetration is similar to dye penetration, we could expect penetration in beef roasts to be along fissures in the muscle that could have been formed by electrically stimulating the muscle *post mortem* or created by aging. Penetration in poultry tissue should be more even throughout the muscle.

TURKEY BLOCK SLICING STUDY

ABSTRACT

Penetration of an 8-strain *Salmonella* cocktail suspended in a salt/phosphate marinade into turkey breast was determined. A method was developed to remove samples from the interior of intact breast muscle while minimizing the potential for mechanical carry through of surface bacteria. Repeated flaming with ethanol followed by trimming the outer layers with a sterile knife before slicing on a bench top gravity fed slicer was used. Results suggested that bacteria penetrated throughout the entire muscle portion.

MATERIALS AND METHODS

Preparation of Turkey Breast Samples

Fresh, whole-muscle, boneless, skinless turkey breasts were obtained from a local packer within 12 h of harvest in a single large lot to eliminate lot-to-lot variability. The muscles transported to M.S.U. where they individually vacuum packaged and frozen at -20°F.

To eliminate indigenous microflora, the frozen turkey breasts were irradiated (Iowa State Linear Accelerator Facility, Ames, Iowa, U.S.A.) at an average dose of 11.95 kGy. Low bacterial counts was later confirmed by taking 1 g samples from three breasts, serial diluting, and plating on Petrifilm™ aerobic count plates (3M Corp., St. Paul, Minn., U.S.A.) followed by incubation at 37°C for 24 hr.

Marinade Preparation

The marinade was a generic formula (Pearson and Dutson 1987) containing 95.8% water (filtered and deionized), 3.2% NaCl (J.T. Baker, Philipsburg, N.J., U.S.A.), and 1% mixed phosphate solution (w/w) (Butcher and Packer Supply Co., Detroit, Mich., U.S.A). 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 and autoclaved for 15 min at 121°C to ensure sterility.

Bacteria Preparation

The following eight serovars of *Salmonella* were obtained from Dr. V.K Juneja (Agricultural Research Service, Eastern Regional Research Center,

USDA-ARS, Philidelphia, Penn., U.S.A.): *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteriditis H3527 and H3502 (clinical isolates phage types 13A and 4, respectively), *S.* Typhimurium DT 104 H3380 (human isolate), *S.* Hadar MF60404 (turkey), *S.* Copenhagen 8457 (pork), *S.* Montevideo FSIS 051 (beef), and *S.* Heidelberg F5038BG1 (human isolate). All strains were stored frozen at -80°C in a tryptic soy broth (TSB) (Difco, Detroit, Mich., U.S.A) containing 10% glycerol. The cultures were propagated by transferring one loopful of frozen culture to 9 mL of TSB in a 20 mL culture tube. 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 prior to use. All strains were maintained separately and combined as needed.

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. The supernatant was poured off and the bacterial pellet was resuspended in 520 ml of sterile marinade to give a final concentration of ~10^8 CFU/ml. Concentration was confirmed by serial dilution in 0.1% peptone water followed by duplicate plating on Aerobic Petrifilm™ Plates (3M, St. Paul, Minn., U.S.A) in duplicate.

Exposure to Inoculated Marinade

Frozen turkey breasts were thawed one day prior to the experiment at 4°C. Two blocks measuring 13 x 6.5 x 6.5 cm were cut aseptically from each breast and placed individually in a sterile stomacher bag (Seward Medical, London, UK) and weighed. Inoculated marinade (50 ml) was added for every 125 g of breast muscle. The bag was then tied, placed inside an identical stomacher

bag, and tied again to form a waterproof seal. All treatments were handled in the same manner.

Vacuum tumbling was conducted in a laboratory-scale tumbler (Model T-15, D.C Curtis Ltd., Ill., U.S.A.) under a vacuum of approximately 100 kPa at 4°C. The tumbler rotated at 8 rpm and was turned off for vacuum-only marination. All tumbling and holding treatments were conducted at 4°C. Still marinated (no tumbling and no vacuum) treatments were simply bagged as described above and refrigerated (4°C). To ensure procedural sterility, three breasts were sectioned and plated as described without exposure to the marinade.

Sampling

Each meat block was dipped in 80% ethanol and thoroughly flamed with a propane torch (Figure 3) after which the outer portions of the block were excised with a sterile knife (shaded areas, Figure 4a). After flaming, the freshly exposed areas were re-flamed, and the block was then sliced into 3-5 mm thick slices (Figure 2b) using a sterile bench top gravity fed meat slicer (Model 220F, FMA OMCAN, Toronto, Canada) (Figure 5). All slicer surfaces were sterilized by scrubbing with 80% ethanol between slices. Sterility was ensured by swabbing the dried slicer surfaces with sterile cotton tipped applicators and streaking on tryptic soy agar (TSA, Difco, Detroit, Mich., U.S.A.). If after incubation at 37°C for 24 hr contamination was evident, the corresponding data was discarded.

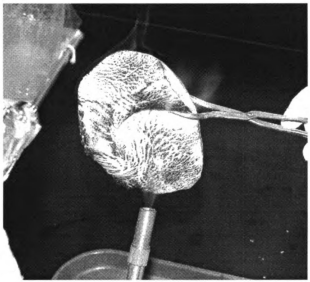

Figure 3. Flaming the turkey breast block prior to slicing

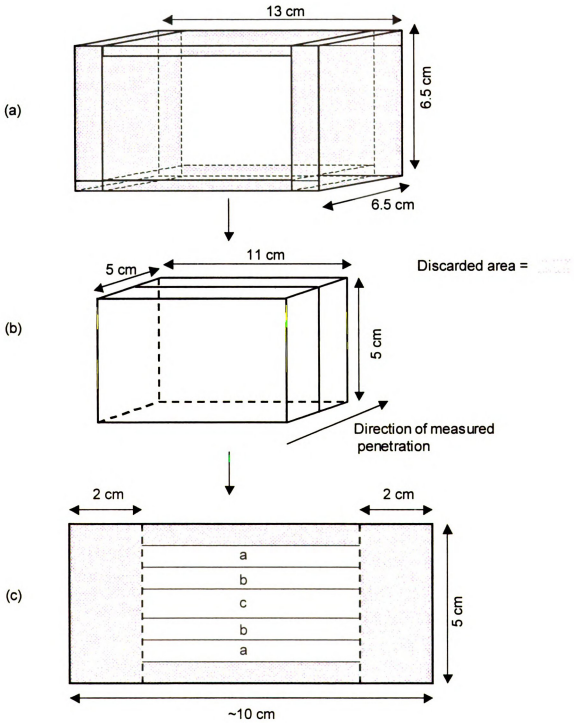

Figure 4. Schematic of sample collection for determining Salmonella penetration into intact turkey breast muscle portion

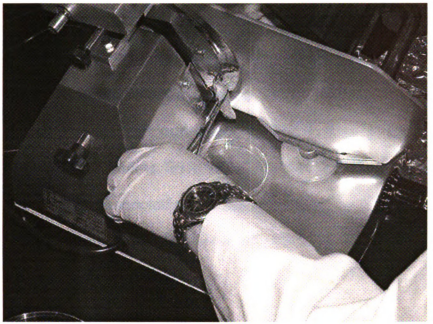

Figure 5. Removing individual slices from treated turkey breast portions to determine *Salmonella* penetration

Initially, the whole slice was macerated in peptone water to determine bacterial load. In later studies, three slices (every other slice) from each sample were subdivided further to develop a 3-D pattern of bacterial penetration into the muscle. Areas on the outer edge of the slice were discarded due to possible heat damage from flaming. Each symmetrical segment was measured with a micrometer and pooled according to location. The measured subsegments were shredded with a sterile blade, weighed and stomached in sterile 0.1% peptone water for 90 s.

Recovery

Salmonella counts were determined by serially diluting slice sections in sterile 0.1% peptone water and plating on Petrifilm™ Aerobic Count Plates. Plates were incubated at 37°C for 24 h before counting.

RESULTS AND DISCUSSION

The main problem in determining bacterial penetration into whole muscle foods is to ensure that bacteria are not transferred to the inner portions of the muscle by the experimenter during sampling. When visually comparing *Salmonella* penetration in turkey breast blocks that have undergone vacuum tumbling marination with inoculated marinade, penetration was not noticeably greater in any of the time treatments (Figure 6). The outer points on the curves (0 mm and ~50 mm) do not reflect the number of bacteria recovered from the muscle portion, but rather the number of *Salmonella* found in the inoculated marinade. We used this count because the outside of the muscle was treated with the propane torch and immersed in 80% ethanol thereby making an actual bacterial count impossible.

The lack of data points in Figures 6-9 from 30 mm to 50 mm was caused by the slicers' inability to slice the turkey breast block at that thickness. When the block became thinner that 20 mm, the product would not slice evenly and adhered to the blade. It was impossible to get an even slice at this point, so slicing was discontinued.

Data presented in Figure 7 show that the mechanical action of tumbling combined with vacuum causes greater bacterial penetration into turkey breast blocks when compared to blocks that were allowed to marinate without vacuum or tumbling. The curve for the treatment that was tumbled without vacuum fell between the two. Figure 8 supports the hypothesis that subjecting the meat to vacuum during tumbling increases bacterial penetration. When tumbled for 2 min

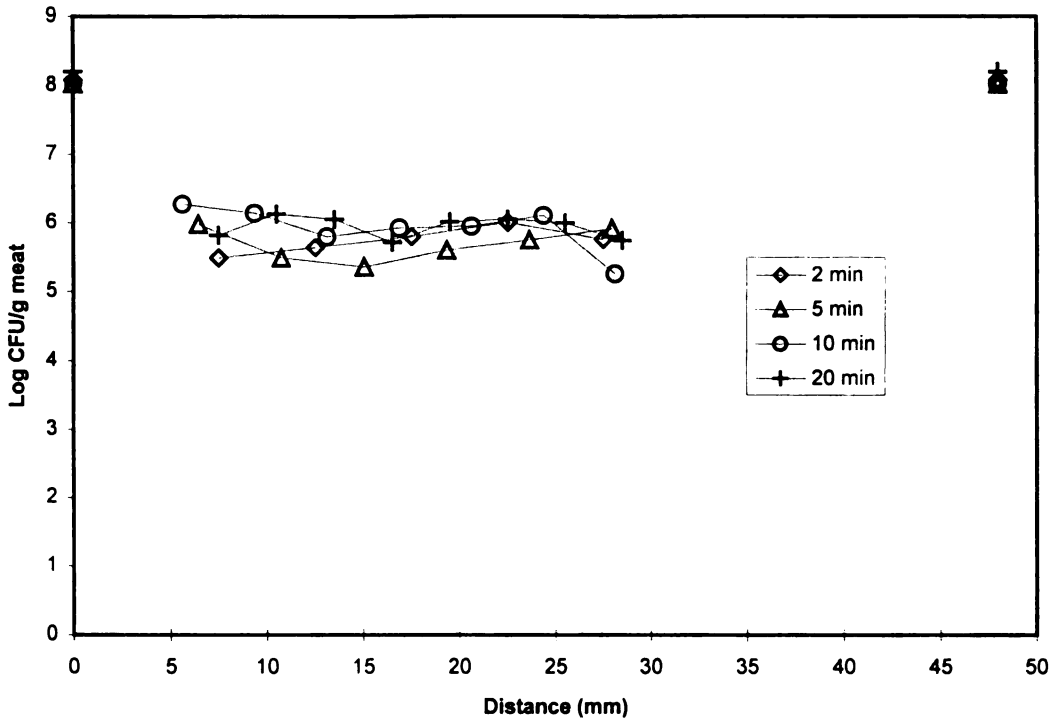

Figure 6. *Salmonella* penetration into intact turkey breast muscle portions after vacuum tumbling for various times in a marinade inoculated to contain 10^8 CFU/mL. Outer most sets of data points represent *Salmonella* present in marinade.

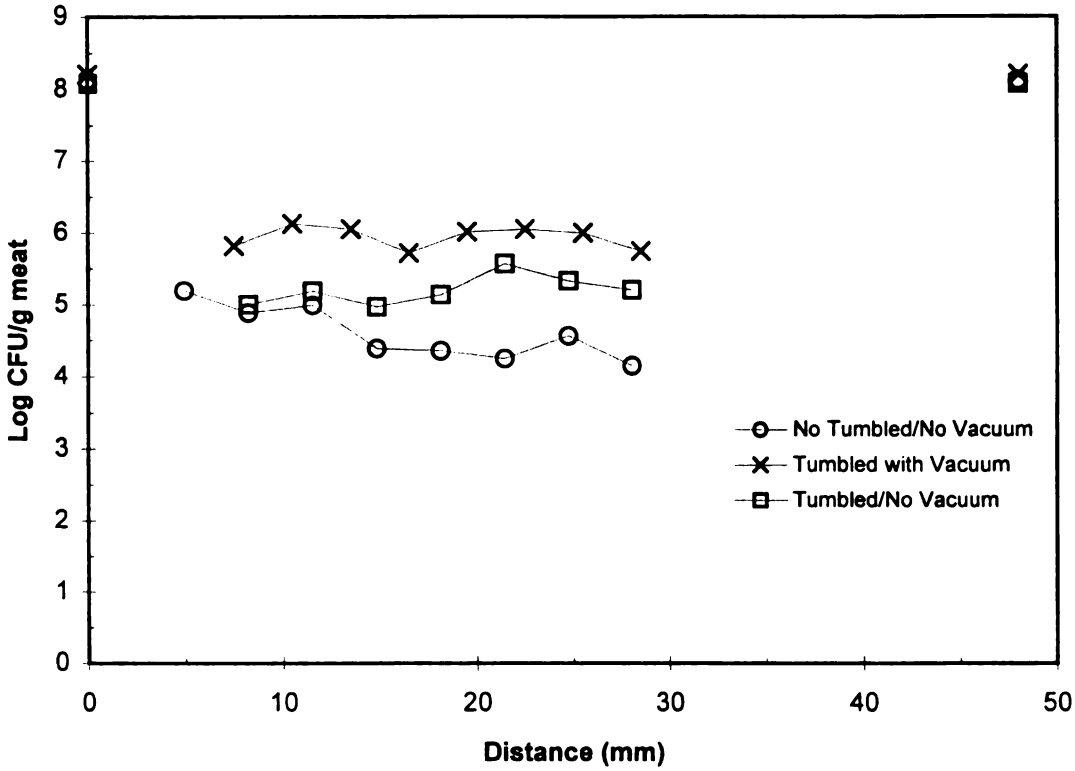

Figure 7. *Salmonella* penetration into intact turkey breast muscle after 20 min using various marination treatments

with and without vacuum, the vacuum treatment had greater bacterial penetration. The same held true when the turkey breast portions were tumbled with and without vacuum for 10 min (Figure 9).

There were mixed results when the individual slices were subsectioned (Figures 10 and 11). Every other slice taken from the block in this study was further dissected into subsections. Subsections denoted as "a" were on the outside of the slice while "c" sections were the inner section. Subsections "b" were between "a" and "c". Ideally a pattern of penetration should have been evident where a > b > c.

The lines on the graph should have resembled upward facing concave curves, where bacterial loads were lowest in the center and highest near the edges (Figure 10). This pattern held true for Slice 6 only. Bacterial loads should have been lower in Slices 6 and 4 when compared to Slices 2 and 8 because they were closer to the center where *Salmonella* would have to penetrate a greater amount of tissue.

Data presented in Figure 11 show the number of bacteria recovered from each subsection from every other slice after 20 min of vacuum tumbling with the inoculated marinade. These curves should also resemble "U" shapes with bacterial penetration highest in slices near the outside of the block. However, almost all of the subsections in Slice 8 were lower than those from Slice 2. Bacterial levels could have been lower in Slice 8 because it could have been exposed to residual ethanol that did not burn off while flaming, causing greater

bacterial death than what was found in Slice 2 that was immediately removed, macerated, and diluted.

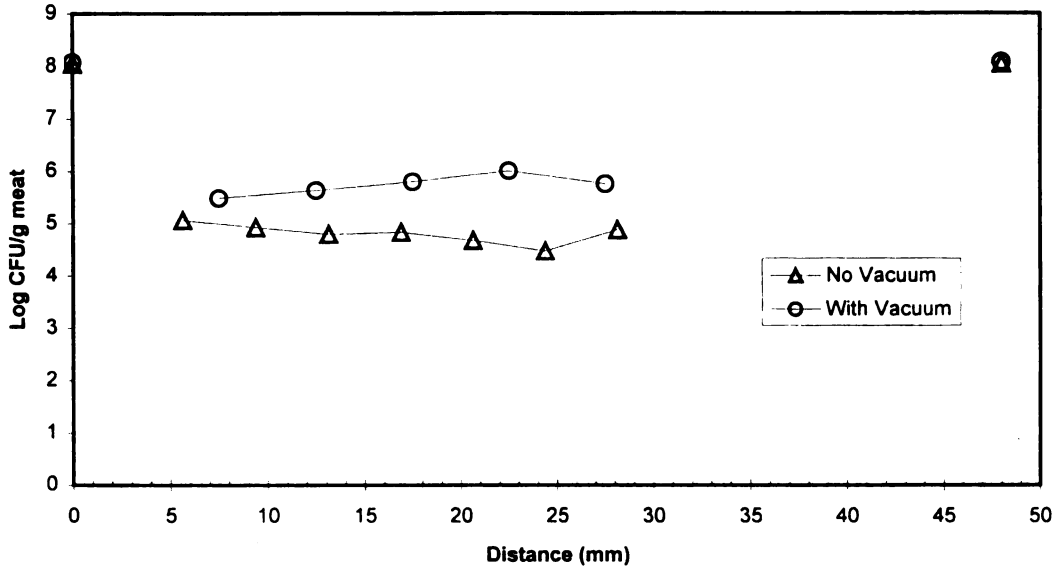

Figure 8. *Salmonella* penetration into intact turkey breast muscle after tumbling with and without vacuum for 2 min

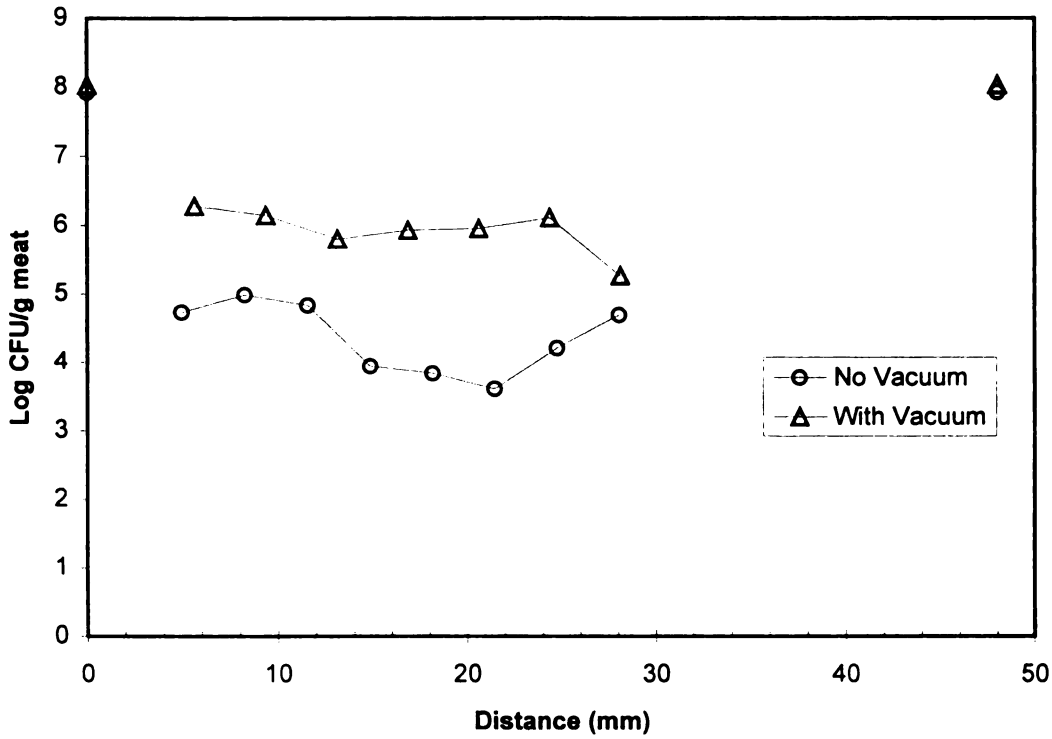

Figure 9. *Salmonella* penetration into intact turkey breast muscle after tumbling for 10 min with and without vacuum

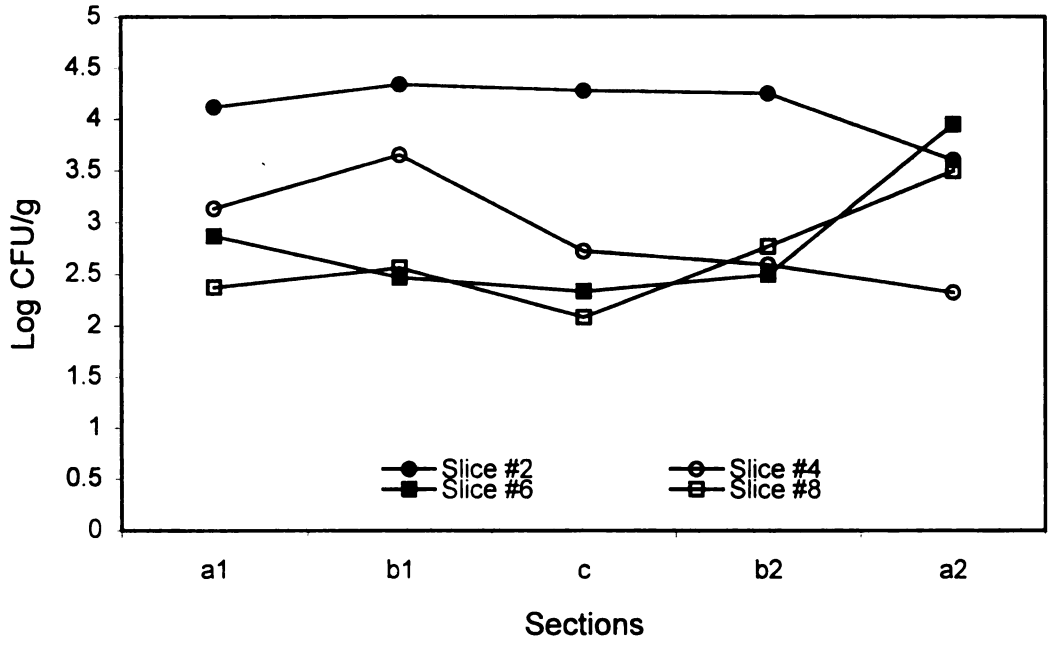

Figure 10. *Salmonella* population recovered from subsections of intact turkey breast muscle after vacuum tumbling for 20 min

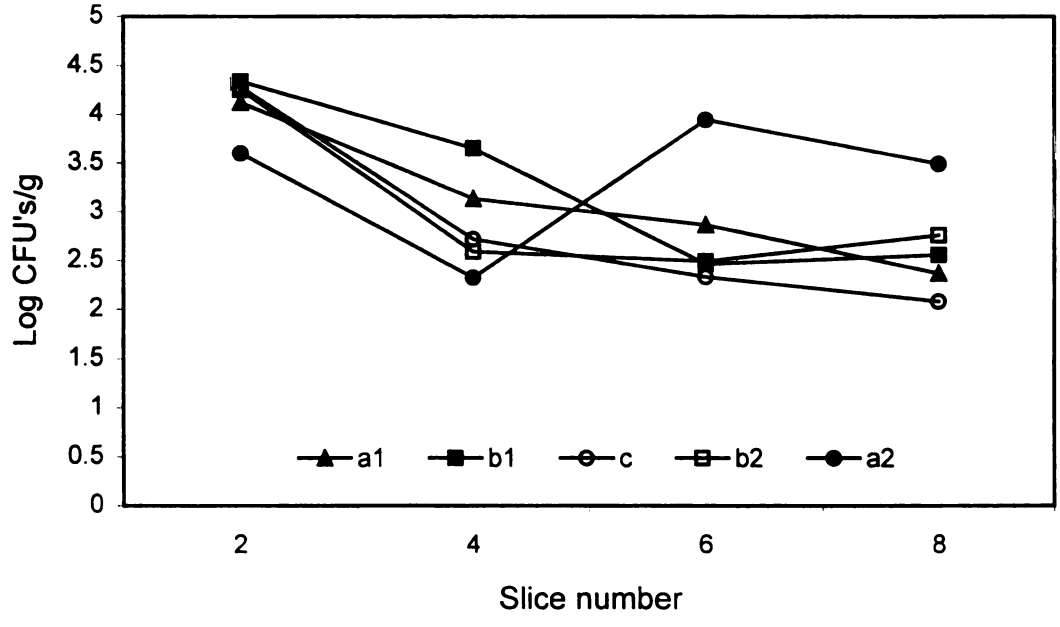

Figure 11. *Salmonella* populations recovered from subsections of intact turkey breast muscle slices after vacuum tumbling for 20 min

CONCLUSIONS

Even though the turkey breast was tumbled for as little as 2 min, obtaining a zero count inside the muscle was difficult. Sanitation practices during slicing were very high. However, mechanical transfer of bacteria from slice to slice may still be occurring. Only two of more than 140 TSA streak plates were positive. These experiments provided great insight into how best to enumerate bacteria that have penetrated whole muscle turkey breast. However, the study was not completed due to non-uniformity of the data and restrictions in both labor and resources. A steady, decreasing gradient of bacterial load should have been evident from outside to inside as tumbling time decreased. However, internal bacterial loads remained almost constant for all times. These patterns lead us to believe that there may be a problem with bacteria being mechanically transferred by the blade. Better methods can be developed to examine bacterial penetration during vacuum tumbling marination that do not involve burning or singeing the outside of the meat which could cause bacterial death and inaccuracy in bacterial counts.

DETERMINING THE PENETRATION OF *SALMONELLA* SPP. INTO WHOLE MUSCLE TURKEY BREASTS BY SAMPLING CORES FROM THE CENTER OF THE BREAST

ABSTRACT

When bacteria that are suspended in a marinade penetrate whole muscles, penetration occurs in all directions. In this study which considers all possible directions of penetration. Irradiated (~11 kGy), whole muscle turkey breasts were vacuum tumbled in a marinade containing eight pathogenic strains of *Salmonella* and evaluated for bacterial penetration. The objective of this study was to determine how far *Salmonella* can penetrate into intact turkey breast muscle after vacuum tumbling.

Six marination methods were used: (1) vacuum tumbling for 20 min, (2) tumbling without vacuum for 20 min, (3) vacuum without tumbling for 20 min, (4) no vacuum or tumbling for 20 min, (5) drawing a vacuum followed by immediate release, and (6) dipping. To determine the amount of penetration after treatment, the turkey breast was laid flat with the cut side (bone side) down, then aseptically cut parallel to the long axis of the muscle. The coring device was plunged into the newly exposed cut wall. One core was removed from each half and subdivided into five 1-cm pieces. Core segments were macerated, diluted, and plated to determine total number of bacteria.

All treatments showed bacterial penetration up to 2 cm. However, vacuum tumbling for 20 min yielded the greatest degree of penetration, followed by

tumbling without vacuum for 20 min ($p<0.05$). No differences ($p>0.05$) were found between samples that were cored immediately after drawing a vacuum and releasing or dipping the turkey breasts into the *Salmonella* cocktail-spiked marinade.

INTRODUCTION

The interior of intact, undamaged muscle is believed to be sterile (Elmossalami and Wassef 1971). However, bacterial penetration into muscle can occur (Gill and Penney 1982, Gupta and others 1981, Maxcy 1981, Sikes and Maxcy 1980). Some studies have examined the role of extra cellular proteases in bacterial penetration into muscle (Gill and Penney 1977, Gill and Penney 1982, Gupta and others 1983, Thomas and others 1987). Others have concluded that other factors including bacterial motility, (Gill and Penney 1977, Thomas and others 1987), water availability within the tissue (Sikes and Maxcy 1980, Maxcy 1980, Thomas and others 1987) or fiber orientation (Sikes and Maxcy 1980, Maxcy 1981) are important in bacterial penetration. Mechanical means of bacterial transfer through invasive techniques, like blade tenderization have also been studied (Raccach and Henrickson 1979, Boyd and others 1978, Sporing 1999). Johnston (1978) even suggested a ban on invasive meat tenderization techniques because of the risk of bacterial penetration. However, no research to date has been done to study bacterial penetration facilitated by non-invasive techniques, like vacuum tumbling marination.

Bacteria that penetrate whole muscle foods during marination may pose a problem if the product is not adequately cooked. Bacteria present below the surface of the meat may not be as susceptible to heat as those found on or near the surface due to the protective effect of the tissue. Extra cooking time may be needed for sufficient heat transfer to inactivate pathogens below the surface. If bacteria are present on the surface of a whole muscle food that is destined to be

vacuum-tumbled with a marinade, transfer into the muscle via the marinade becomes a possibility. The purpose of this study was to determine if bacteria present in a marinade are able to penetrate whole muscle turkey breasts during vacuum-tumbling marination.

Tenderization and marinade uptake are facilitated by mechanically impacting the muscle against the sides of the tumbling vessel, disrupting the structure of the muscle with a vacuum, and changing osmotic pressures (Xiong and Kupski 1999). Each of these principles was considered in designing the following experiment by isolating the effects of each. Tumbling turkey breast without a vacuum isolated the migratory effect of the impact against the side of the vessel. Breasts were also subjected to a vacuum without tumbling to isolate the effects of structure disruption on bacterial penetration. Controls included (1) marination without tumbling for 20 min, (2) subjecting the breast to a vacuum that was immediately released, and (3) dipping the product into the spiked marinade. These controls were selected to ensure that vacuum tumbling was the mechanism by which the *Salmonella* penetrated the muscle and not simply by diffusion or the bacteria's motile characteristics.

MATERIALS AND METHODS

Preparation of Turkey Breast Samples

Fresh, whole-muscle, boneless, skinless turkey breasts were obtained from a local packer within 12 h of harvest. A single large lot was purchased to eliminate lot-to-lot variability. The muscles were individually vacuum packaged and frozen at -20°F at M.S.U.

To eliminate indigenous microflora, the muscles were transported frozen to the Iowa State Linear Accelerator Facility where they received an average dose of 11.95 kGy. Low bacterial counts were later confirmed using 1-g samples from three breasts that were serial diluted and plated on Petrifilm™ Aerobic Count Plates (3M, St. Paul, Minn., U.S.A) and incubated at 37°C for 24 h.

Marinade Preparation

The marinade was a generic formula that contained 95.8% water (filtered and deionized), 3.2% NaCl (J.T. Baker, Philipsburg, N.J., U.S.A.), and 1% mixed phosphate solution (w/w) (Butcher and Packer Supply Co., Detroit, Mich, U.S.A.). Salt was incorporated into the water before adding the phosphate solution to ensure total dispersal. Marinade (520 mL) was poured into 650 mL glass bottles with plastic screw caps and autoclaved for 15 min at 121°C to ensure sterility.

Inoculum Preparation

The following eight serovars of *Salmonella* were obtained from Dr. V.K. Juneja (Agricultural Research Service, Eastern Regional research Center, USDA-ARS, Philidelphia, Pa., U.S.A.): *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteriditis H3527 and H3502 (clinical isolates, phage types 13A and 4,

respectively), *S.* Typhimurium DDT104 H3380 (human isolate), *S.* Hadar MF60404 (turkey), *S.* Copenhagen 8457 (pork), *S.* Montevideo FSIS 051 (beef), and *S.* Heidelberg F5038BG1 (human isolate). All strains were stored frozen at -80° C in a solution of tryptic soy broth (TSB) (Difco, Detroit, Mich, U.S.A.) containing 10% glycerol. The cultures were propagated by transferring one loopful of frozen culture to 9 mL of TSB in a 20 mL culture tube. The cultures were maintained by transferring to fresh TSB media every 18-24 h and incubating at 37°C, with a minimum of two consecutive transfers prior to use. All of the strains were maintained separately and combined as needed.

On the day of the experiment, 9 ml of each of the eight serovars grown in TSB were combined equally and centrifuging at 6,000 x g for 20 min at 4°C. The supernatant was poured off and the pellet resuspended in 520 ml of sterile marinade to give a final concentration of ~10^8 CFU/mL. Concentration was confirmed by serial dilution in 0.1% peptone water followed by duplicate plating Aerobic Petrifilm™ Count Plates (3M, St. Paul, Minn., U.S.A.).

Exposure to Inoculated Marinade

Whole turkey breast previously thawed for 24 hr at 4°C was placed in a sterile 38 x 51 cm stomacher bag (Fisher Scientific, Pittsburgh, Pa., U.S.A.) and weighed. Inoculated marinade (50 ml) was added for every 250 g of breast muscle. The bag was then tied, placed inside an identical stomacher bag, and tied again to form a waterproof seal.

Vacuum tumbling was conducted in a laboratory-scale tumbler (Model T-15, D.C Curtis Ltd., Ill., U.S.A.) under a vacuum of 100 kPa at 4°C. The tumbler

rotated at 8 rpm during vacuum tumbling and was turned off for vacuum-only marination. Product marinated without tumbling or vacuum was simply bagged as described above and refrigerated (4°C).

Sampling

Treated turkey breasts were removed from the bags and placed fascia side down on a sterile metal tray. A hand-held propane torch was used to flame a strip (~3-4 cm) on top of the turkey breast axially. A large, sterile knife was then used to make a single longitudinal cut through the singed area (Figure 12). A Warner-Bratzler hand-coring device (G.R. Electrical Mfg. Co., Manhattan, Kans., U.S.A.) was used to remove a core longitudinally from each turkey breast half (Figure 13). Cores were removed from the non-cutting end of the coring device to avoid contaminating the inner regions of the core (Figure 14). The coring device was sterilized between cores by scrubbing in an alcohol solution (75% ethanol) followed by flaming.

The cores were sectioned on a piece of sterile gauze placed in a 150 mm diameter Petri dish. One-centimeter segments were removed from the core, starting from the end that was directly exposed to the marinade (Figure 15). The fifth and final core segment was not always the same size due to the variability between breast sizes. Core segments were aseptically transferred to sterile 2 oz Whirl-pak bags (Nasco, Fort Atkinson, Wisc., U.S.A.), weighed, diluted with 4 ml of buffered peptone water and macerated by hand. The macerated samples were diluted with buffered peptone water and 1 ml aliquots placed onto aerobic

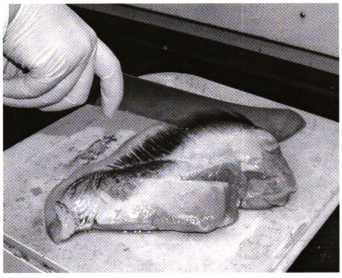

Figure 12. Transecting treated turkey breast down medial line with a sterile knife before to coring

Figure 13. Sampling split turkey breast with the Warner-Bratzler hand coring device

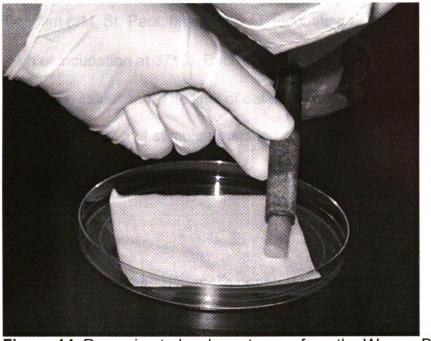

Figure 14. Removing turkey breast cores from the Warner-Bratzler hand coring device from the end opposite the cutting edge

Petrifilm (3M, St. Paul, Minn., U.S.A), incubating at 37°C and enumerated after 24 h of incubation at 37°C. Each dilution was plated in duplicate and the average of the two calculated. The limit of detection (LOD) was set at 5 CFU/ml for this experiment (0.2 g of sample deposited on each "zero" dilution plate, or 0.2 mL/1 mL). Averages of duplicate plates found below 5 CFU/g were adjusted to 5 CFU/g.

Data Analysis

To determine *Salmonella* penetration as a function of vacuum tumbling time, five replications of 5, 10, and 20 min were conducted. Statistical analysis was done using the general linear means procedure of SAS (SAS Cary, N.C., U.S.A., 1996). Individual means were compared using Tukey's multiple comparison procedure. Means were considered different at $\alpha<0.05$. The limit of detection was set at 5 CFU/g and any count below was counted as the LOD.

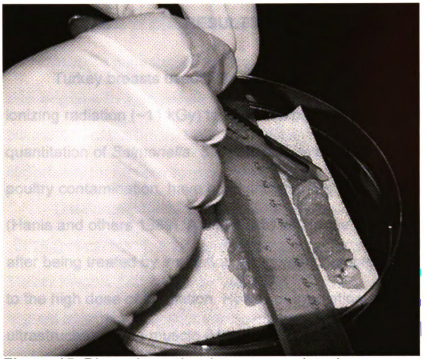

Figure 15. Dissecting turkey breast cores into 1-cm segments for subsequent maceration, dilution, and plating

RESULTS AND DISCUSSION

Turkey breasts used in this experiment were subjected to a high dose of ionizing radiation (~11 kGy) to eliminate bacteria that may interfere with the quantitation of *Salmonella*. *S.* Typhimurium and other organisms associated with poultry contamination, have been shown to be eliminated by a dose of 10 kGy (Hanis and others 1989). A noticeable difference in muscle color was observed after being treated by irradiation along with characteristic irradiation off-odors due to the high dose of irradiation. However, irradiation causes no changes in the ultrastructure of the muscle (Hanis and others 1989).

To accurately enumerate the salmonellae thet were able to migrate into the whole muscle product, a sampling method had to be developed to ensure that bacteria from the outer portions of the muscle were not mechanically transferred to the inner portions. Several methods have been used by other researchers to examine bacterial penetration under static conditions (Elmossalami and Wassef 1971, Gill and Penny 1977, Gill and Penny 1982, Gupta and others 1983, Thomas and others 1987, Maxey 1981, Sikes and Maxey 1980). However, none of these methods were suitable for the rigors of vacuum-tumbling marination. The main criterion for the new method was that it could not interfere with the tumbling process. This eliminated several earlier methods, including coating five of the six sides with a paraffin-like substance to permit bacterial entry from only one side, since such a coating would be disrupted during tumbling. The other approach would be to excise samples aseptically from different areas of the muscle after treating the product. The

inherent problem of this method is potential carry-through from the cutting instrument. To minimize carry-through, a strip on the surface of the turkey breast was singed with a propane torch before being cut with a sterile knife. Singeing the surface of the breast killed any bacteria that were present and reduced the opportunity for surface bacteria to interfere.

Data presented in Figure 16 shows the average *Salmonella* populations (CFU/g) recovered from the two cores that were removed from each breast half. Turkey breasts that had underwent 20 min of vacuum tumbling showed the highest numbers of *Salmonella* in the core ($p<0.05$), except for the treatment that was tumbled without vacuum ($p=0.13$), suggesting that the mechanical action of tumbling was a major contributor to bacterial penetration. Figure 16 also shows that subjecting the samples to only a vacuum for 20 min does not significantly increase the bacterial load when comparing it to tumbling without vacuum. It is therefore concluded that tumbling and vacuum used in conjunction with one another have a greater effect on bacterial penetration than if each are used alone.

Three negative controls were run for comparison: 20 min of marination without vacuum, drawing a vacuum and immediately releasing it, and briefly dipping the breast in the inoculated marinade. The treatments that involved drawing a vacuum and releasing it and dipping and immediately releasing it were not significantly different from each other, however letting the muscle to sit quietly in the marinade for 20 min had higher counts than the breasts that were dipped and immediately dissected. This observation shows that

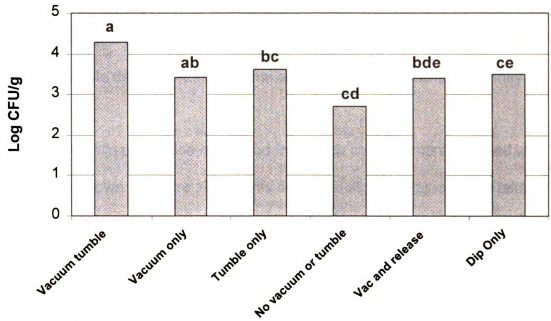

Figure 16. *Salmonella* populations recovered from cores after various treatments.
[a-e] Bars that do not share a common letter differ significantly ($p<0.05$)
SEM=0.90 log CFU/ml

bacterial migration is a function of the amount of time the muscle is in contact with the inoculated marinade. The exact mechanism by which that bacteria access the muscle is unknown. Possible explanations may be the bacteria's motility or simple diffusion (Elmossalami and Wassef 1971, Gill and Penney 1982).

Salmonella population recovered from each core segment sampled after treatment is shown in Figure 17. Counts decrease as the samples were taken closer to the center however. *Salmonella* was still found up to 4 cm from the surface of the muscle in contact with the marinade. No significant differences were seen between bacterial counts of individual segments from different treatments.

One reason for the lack of significance between individual segments may be the variability between replications. The poultry tissues used in these experiments showed visual and textural differences from breast to breast. A softer, more open texture in the muscle could lead to increased bacterial penetration as opposed to one that is firmer and more closed, which supports Gill and Penney's (1982) claim that bacteria penetrated through water filled "canals" in the tissue. In addition, harvesting practices could lead to increased penetration. Some of the muscles showed defects from cutting with several breasts exhibiting deep gouges in the bone side of the muscle, which could lead to increased penetration.

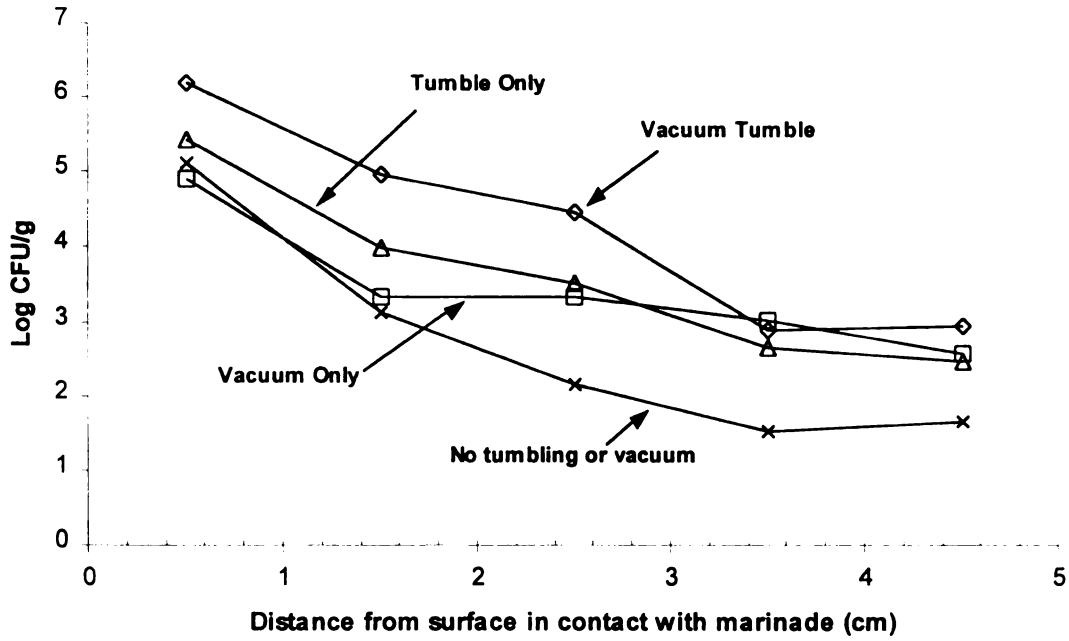

Figure 17. Penetration of *Salmonella* into whole muscle turkey breast after various 20 min treatments

CONCLUSIONS

This experiment was not completed because the true direction of penetration could not be determined with certainty. *Salmonella* could be penetrating from the top, bottom, or side of the breast. However, the results did show a decrease in the number of salmonellae recovered from cores depending on the depth of the core segment. Also, by singeing the breast with the propane torch, we could have eliminated some organisms that were near the midline of the breast where the deepest core segment was taken. Overall, this experiment provided valuable information for the development of subsequent techniques to remove samples from treated turkey breasts while limiting mechanically transferred organisms.

SALMONELLA VIABILITY IN A COMMERCIAL TYPE MARINADE

ABSTRACT

Survival of eight *Salmonella* serovars was assessed in a marinade containing 90% water, 7% salt, and 3% mixed phosphate. The marinade was inoculated with an 8-strain Salmonella cocktail (10^8 CFU/ml) and either 4°C or 37°C. Samples were taken from the 37°C treatment every 6 h during the first day then every day thereafter up to 8 d. After eight days, *Salmonella* viability had markedly decreased and sampling was discontinued in the 37°C treatment. Sampling of the 4°C marinade followed the same pattern but continued up to 61 d.

Greater viability of *Salmonella* was seen in the marinade stored at 4°C as opposed to 37°C. Salmonella population decreased 5.7 logs in marinade held at 37°C compared to only a 0.3 log when refrigerated. After 61 d of storage Salmonella populations decreased only 1 log in refrigerated marinade indicating that *Salmonella* is more likely to survive in a marinated meat product that is properly refrigerated.

MATERIALS AND METHODS

Marinade Preparation

The marinade was prepared according to a generic formula and contained 90% water (filtered and deionized), 7% NaCl (J.T. Baker, Philipsburg, N.J., U.S.A.), and 3% mixed phosphate solution (w/w) (Butcher and Packer Supply Co., Detroit, Mich., U.S.A.). Salt was incorporated into the water before the adding the phosphate solution to ensure total dispersal. Marinade (520 mL) was aliquoted into 650 mL glass bottles with plastic screw caps and autoclaved for 15 min at 121°C to ensure sterility.

Bacteria Preparation

Refer to bacterial preparation methods previously described in Whole Muscle Core Sampling Study within this chapter.

Data analysis

The *Salmonella* cocktail was deemed viable in the marinade if population decreased <1 log after 1 week of storage. Numbers of viable *Salmonella* were plotted against time on semi-log scale.

RESULTS AND DISCUSSION

Viability of the 8-strain *Salmonella* cocktail needed to be determined to ensure that the organism did not perish at the salt and phosphate concentrations used in the marinade. Marinades that contain phosphates and salt are often used to increase water-holding capacity, improve flavor, and aid in solubilizing protein in meat products (Barbut 2002). It is reported that the upper limit for salt concentration before it becomes bactericidal to *Salmonella* is 9% (Jay 2000).

The marinade used in this test contained 7% salt, 3% phosphate, and 90% water. The *Salmonella* cocktail survived well at refrigerated temperatures with populations only 1-log reduction after 61 d of storage at 4°C, as opposed to a 5.7 log reduction when stored at 35°C (Figure 18). These findings suggest that *Salmonella* could persist in a marinade at refrigerated temperatures encountered during value added processing and meat storage. *Salmonella* Typhimurium and *S.* Heidelberg reportedly grow at temperatures as low as 6.2°C and 5.3°C, respectively (Matches and Liston 1968). Airoldi and Zottola (1988) studied the survival of *S.* Typhimurium in nutrient deficient media (0.1 % peptone water) and also saw that the bacterium could survive for considerable lengths of time with populations decreasing only 1.0-1.5 log after 21 d at 7°C. Their cultures, like ours, were also propagated at 37°C in TSB, therefore supporting that *Salmonella* can survive sub-optimal growth conditions for extended periods of time at refrigeration temperatures.

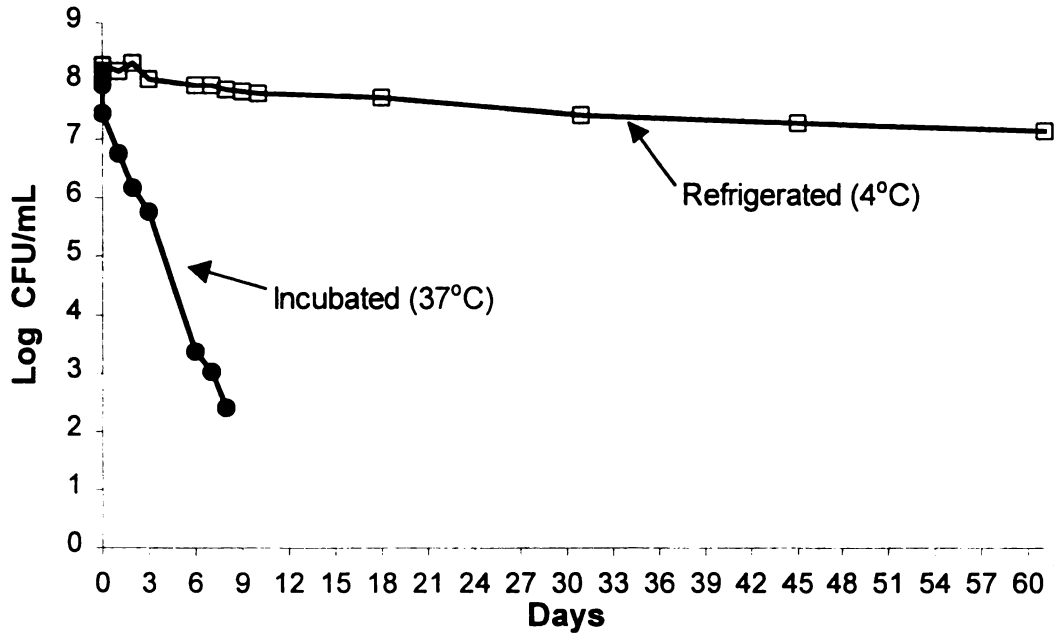

Figure 18. Viability of the 8-strain *Salmonella* cocktail suspended in a 3% phosphate, 7% NaCl, and 90% water marinade at 4°C and 37°C

CHAPTER 2. UNIDIRECTIONAL PENETRATION OF *SALMONELLA* INTO INTACT TURKEY BREAST DURING MARINATION

ABSTRACT

This study examined the effect of vacuum on migration of *Salmonella* into intact turkey breast muscle. Irradiated turkey breasts were cut into blocks and exposed on one side to a marinade inoculated with an 8-strain *Salmonella* cocktail at ~10^8 CFU/mL. Marination was conducted for durations of 5, 10, and 20 min with and without vacuum at 4°C. After treatment, cores perpendicular to the exposed surface were removed from the blocks, subdivided into 1 cm segments, macerated, diluted, and plated to quantify *Salmonella* within the segments. Bacterial penetration was greater with rather than without vacuum at 20 min ($p<0.05$). When all time levels were pooled within the vacuum and non-vacuum treatments, exposing the muscle to vacuum increased bacterial penetration ($p<0.05$). The length of time the muscle was exposed to vacuum was significant after 20 min ($p<0.05$).

INTRODUCTION

Meals prepared outside the home and sous-vide foods often include meat that has undergone value-added processing, such as marination, to improve yield or sensory attributes. For example, vacuum tumbling is an effective way to increase the uptake of a marinade into a whole muscle meat product (Chen 1982; Xiong and Kupski 1999) and is often used in commercial meat processing. Vacuum tumbling also extracts myofibrilar proteins and the vacuum also reduces the amount of air bubbles formed by proteins (Barbut 2002). Although marination is done for quality reasons, few studies have addressed the issue of microbial safety of intact, vacuum tumbled products.

Turkey and other poultry are common sources of *Salmonella* in the U.S. food supply. *Salmonella* is responsible for an estimated 1.4 million cases of food-borne illness each year in the United States, of which 40,000 are culture-confirmed and reported to the Center for Disease Control (CDC 2000). *Salmonella* Typhimurium DT 104 is a pathogen currently of great concern because of its extreme virulence and antibiotic resistance (Glynn and others 1998).

The inner portions of intact, undamaged muscle are assumed to be sterile (Elmossalami and Wassef 1971). A few studies have examined bacterial penetration into meat under static conditions (Gill and Penney 1982, Gupta and others 1981, Maxcy 1981, Sikes and Maxcy 1980). Gill and Penney (1977) showed that *S.* Typhimurium could penetrate 3, 7, and 10 cm/h into beef muscle at 20, 30, and 37°C, respectively. However, these studies were conducted under

atmospheric conditions, and none measured the effect of vacuum on bacterial migration.

Previous research has examined the effect of invasive tenderization methods on bacterial penetration. Needle tenderization (Boyd and others 1978), mechanical tenderization (Raccach and Henrickson 1979), and blade tenderization (Phebus and others 1999) can all contaminate the interior of whole-muscle products. However, the effects of bacterial penetration when muscle tissue is subjected to vacuum have yet to be assessed.

Given that vacuum tumbling facilitates penetration of marinade, migration of pathogens into the interior of meat could also be potentially accelerated during this process. *Salmonella* present on the surface of poultry products may become suspended in an applied marinade during value added processing. Therefore, the objective of this study was to determine whether vacuum increases penetration of *Salmonella* into intact whole muscle turkey breast unidirectionally during marination.

MATERIALS AND METHODS

Sample Preparation

Fresh, whole muscle, boneless, skinless turkey breasts were obtained from a local wholesaler. A large lot was purchased to eliminate lot-to-lot variability. The muscles were individually vacuum packaged and frozen at -20°C.

To eliminate indigenous bacteria, the turkey breasts were transported frozen to a irradiation facility (Iowa State Linear Accelerator Facility, Ames, Iowa, U.S.A.), where they were irradiated. Each breast received an average dose of 11.95 kGy. Sterility was later confirmed by sampling 1-g samples from three different breasts, by plating on Petrifilm™ Aerobic Count Plates (3M Corp., St. Paul, Minn., U.S.A.), and incubating at 37°C for 24 h.

The day prior to the experiment, the frozen turkey breasts were thawed for 24 h at 4°C. On the day of the experiment, the whole turkey breasts were aseptically cut into blocks measuring 10 x 10 x 6 cm (L x W x H).

Marinade Preparation

The marinade was a generic formula containing 90% water (filtered and deionized), 7% NaCl (J.T. Baker, Philipsburg, N.J., U.S.A.), and 3% mixed phosphate solution (w/w) (Butcher and Packer Supply Co., Detroit, Mich., U.S.A.). Salt was incorporated into the water before the adding the phosphate solution to ensure total dispersal. The marinade was aliquoted in glass bottles with plastic screw caps and autoclaved for 15 min at 121°C to ensure sterility.

Bacteria Preparation

The following eight serovars of *Salmonella* were obtained from Dr. V.K. Juneja (Agricultural Research Service, Eastern Regional Research Center, USDA-ARS, Philidelphia, Penn., U.S.A.): *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteriditis H3527 and H3502 (clinical isolates, phage types 13A and 4, respectively), *S.* Typhimurium DT 104 H3380 (human isolate), *S.* Hadar MF60404 (turkey), *S.* Copenhagen 8457 (pork), *S.* Montevideo FSIS 051 (beef), and *S.* Heidelberg F5038BG1 (human isolate). All strains were stored frozen at -80°C in a tryptic soy broth (TSB) (Difco, Detroit, Mich., U.S.A) containing 10% glycerol. The cultures were propagated by transferring one loopful of frozen culture to 9 mL of TSB. The cultures were maintained by daily transfer to fresh TSB media every 18-24 h and incubated at 37°C, with a minimum of two consecutive transfers before use. All of the strains were maintained separately and combined in equal volumes as needed.

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. The supernatant was poured off and the bacterial pellet was resuspended in 520 ml of sterile marinade to give a final concentration of ~10^8 CFU/ml ($1.43 \times 10^8 \pm 3.66 \times 10^7$ 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, Minn., U.S.A) in duplicate.

Exposure to Inoculated Marinade

A full factorial experimental design was used, with three durations of exposure (5, 10, 20 min) and two vacuum levels (with and without vacuum). Additionally, a control was tested with uninoculated marinade, in order to validate aseptic procedures. All experiments were replicated six times.

Samples were exposed to inoculated marinade in a 150 mm dia. disposable Petri dish containing a custom-made round perforated stainless steel plate. Inoculated marinade (40 ml) was added to this dish so that the marinade reached approximately 5 mm up the side of the turkey breast portion (cut side down). The perforated stainless steel plate (autoclaved prior to each test) was added to ensure that the entire bottom of the meat block was evenly exposed to the marinade. All vacuum marinated samples were exposed to a vacuum of approximately 100 kPa and all experiments were performed at 4°C.

Sampling

After marination, three cores were aseptically removed from each turkey block using a Warner-Bratzler hand-coring device (G.R. Electrical Mfg. Co., Manhattan, Kans., U.S.A.) (Figure 19). Cores were removed from the coring device by pushing the core out the end opposite the cutting edge to minimize contamination from the cutting edge (Figure 20). Each core was placed in a disposable Petri dish containing a 7.6 x 7.6 cm piece of sterile cotton gauze and cut into 1-cm segments starting from the exposed side (Figure 21). All hand instruments (Coring device, scalpels, and forceps) were sterilized after every

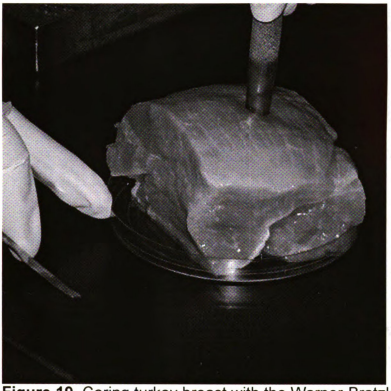

Figure 19. Coring turkey breast with the Warner-Bratzler hand-coring device.

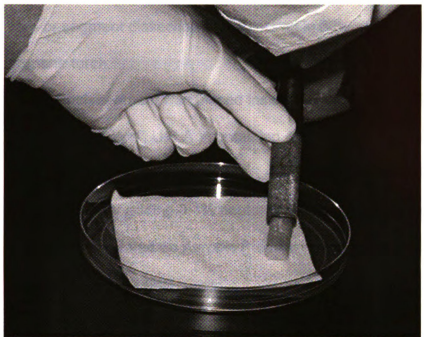

Figure 20. Removing turkey breast cores from the Warner-Bratzler hand-coring device from the end opposite the cutting edge

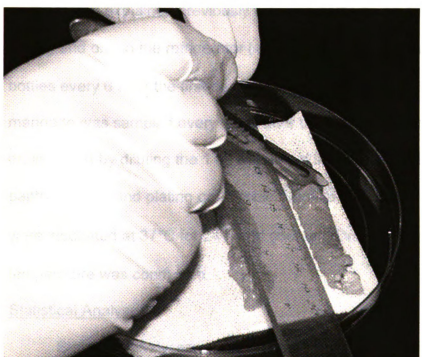

Figure 21. Dissecting turkey breast cores into 1-cm segments for subsequent maceration, dilution, and plating.

core or segment dissection by immersing in 80% ethanol and flaming with a propane torch.

Each segment was placed inside a sterile 2 oz Whirl-pak™ bag (NASCO, Fort Atkinson, Wis., U.S.A.) containing 4 mL of 0.1% peptone and manually macerated (Difco, Sparks, Md., U.S.A.). The weight of each segment was taken before and after adding 0.1% peptone. After serially diluting in 0.1% peptone, samples were plated on Aerobic Petrifilm™ Count Plates, incubated 24 h at 37°C for enumeration of *Salmonella*.

Salmonella Viability

Two bottles of inoculated marinade were prepared according to the methodology explained previously with one bottle being placed in the incubator (37°C) and one in the refrigerator (4°C). Samples (1 ml) were removed from the bottles every 6 h for the first day, then every 24 h thereafter. The refrigerated marinade was sampled every week after the first 8 d. *Salmonella* was enumerated by diluting the 1 ml aliquots of inoculated marinade in 0.1% buffered peptone water and plating on Aerobic Petrifilm™ Count Plates. The Petrifilms were incubated at 37°C for 24 h and counted. One replication at each temperature was conducted.

Statistical Analyses

Statistical analyses were conducted using the general linear means procedure (α=0.05) of SAS (SAS Cary, N.C., U.S.A.). Individual means were compared using Tukey's multiple comparison procedure. The limit of detection, based on methodology (0.2 g sample / plate on zero dilution), was 5 CFU/g.

Three cores were removed from each turkey breast block, and individual segment counts were averaged.

RESULTS AND DISCUSSION

Penetration of *Salmonella* into turkey breast meat increased significantly ($p<0.05$) under vacuum for all durations and vacuum levels. When individual segments from vacuum and non-vacuum treatments were compared (Figure 22), numbers of *Salmonella* were similar in the first, fourth, and fifth segments ($p>0.05$). However, the second and third segments were significantly different ($p<0.05$) indicating that bacterial penetration was facilitated by vacuum up to 3 cm into the turkey breast (Figure 22). Beyond 3 cm, both the vacuum and non-vacuum treatments were not significantly different, and were also near the limit of detection (5 CFU/g). Duration of the treatment was also not significant. Gupta and others (1983) concluded that *S.* Typhimurium could unidirectionally penetrate into poultry tissue at least 3 cm under atmospheric pressure after 20 h of incubation at 37°C. The present results suggest that the same degree of penetration could occur after only 20 min of vacuum treatment.

Penetration of *Salmonella* may have occurred between the muscle fibers (Gill and Penney 1977). Thomas and others (1987) describe 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. Although *Salmonella* is motile, motility alone is unlikely to explain 3 cm of penetration into the muscle after only 20 min. Bacterial penetration was parallel to the orientation of the muscle fibers. Water in the marinade may contribute to bacterial penetration by increasing the water content between muscle fibers and

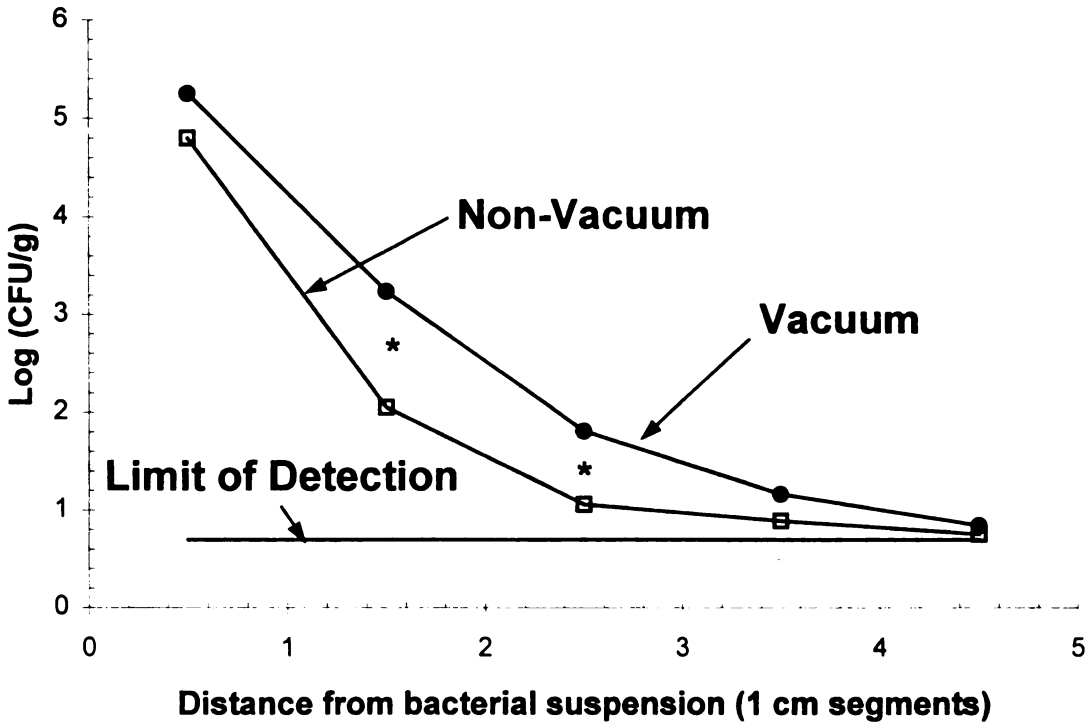

Figure 22. Unidirectional penetration of *Salmonella* into intact turkey breast muscle under vacuum (100 kPa) or non-vacuum for all time treatments combined.
* Indicates data points that are significantly different between treatments ($p<0.05$).

therefore increasing penetration, also concluded by Thomas and others (1987). Additionally, penetration was in the opposite direction from gravitational force.

Phosphates in the marinade could also contribute to increased water absorption. Pyrophosphates act as a fluidizing agent in muscle, dissociating actin and myosin, which leads to increased water uptake (Xiong and Kupski 1999). If bacteria are suspended in fluid that the muscle is absorbing, bacterial penetration could be increased.

Methods used previously to examine unidirectional penetration of bacterial into whole muscles were not appropriate for this experiment. Sikes and Maxey (1980) while studying the proteolytic penetration of *Serratia marcescens* into pork and beef, trimmed the muscles to size aseptically and dipped them in colloidon. One side of the colloidon was then removed and the bacteria applied. They measured bacterial penetration by freezing the blocks and cutting them into 5 mm thick slices. Individual slices were swabbed with sterile cotton applicators and streaked on plate count agar to qualitatively determine bacterial penetration to various depths. This method could not be used for our application, because *Salmonella* is sensitive to freezing (Ray and others 1972). Others (Raccach and Henrickson 1979) used conventional scalpels. They could mechanically transfer bacteria on the blade to the muscle interior during cutting. A new and novel method needed to be developed to determine unidirectional bacterial penetration for this case.

The method developed for enumerating *Salmonella* penetration minimized the chance of mechanically transferring bacteria from the exposed surface to the

interior. By coring the breast portion with the Warner-Bratzler hand corer from the unexposed surface to the exposed surface and removing the core from the opposite end from the cutting edge, we reduced the chance for mechanically transferring *Salmonella*. The cutting edge was exposed to the inoculated side of the turkey breast block during coring.

Salmonella will reportedly survive in solutions containing up to 9% NaCl (Jay 2000). In inoculated marinade, the *Salmonella* cocktail (original concentration 10^8 CFU/ml) (Figure 18) decreased only 1-log after 61 d at 4°C, which approximates temperatures that are encountered during meat processing and storage. However, a 5.7 log reduction was seen in viable cells after 9 d when the marinade was stored at 37°C. Literature supports that *S.* Typhimurium and *S.* Heidelberg could survive at this temperature because they have been reported to grow at temperatures as low as 6.2°C and 5.3°C, respectively (Matches and Liston 1968).

CONCLUSIONS

Vacuum tumbling of meat products with a salt and phosphate marinade is commonly done to increase water and flavoring agent uptake and facilitate protein extraction. The vacuum helps disrupt the muscle structure and decrease the amount of protein foaming that results from the mechanical action of the tumbler. This study has shown that bacteria can penetrate from the surface into the intact muscle with or without the aid of vacuum. Traditional assumptions regarding the interior sterility of intact, whole muscle products that have been marinated need to be reevaluated to ensure that microbes below the surface of the meat are inactivated during subsequent cooking operations. Further examination of this phenomenon needs to be investigated in other species as well as with other pathogens.

CHAPTER 3. PENETRATION OF SALMONELLA SPP. INTO WHOLE TURKEY BREAST MUSCLE DURING VACUUM TUMBLING MARINATION

ABSTRACT

Vacuum tumbling is used in the meat processing industry to distribute injected brine, infuse marinades, and extract myofibrilar proteins. This study assessed the impact of vacuum tumbling on penetration of an 8-strain *Salmonella* cocktail into intact, irradiated (~11 kGy), whole muscle turkey breasts during marination. The effects of time, vacuum, and tumbling on bacterial penetration were examined. After treatment, interior samples were aseptically removed from the muscle using an electrosurgical device. *Salmonella* penetration was greatest in treatments that were vacuum tumbled for 10 to 20 min. Bacterial populations increased to 4.0 logs 2 cm below the surface of the muscle. Bacterial penetration was greater on the cut side of the turkey breast compared to the skin side.

INTRODUCTION

The interior of intact, undamaged muscle is typically assumed to be free from bacteria (Elmossalami and Wassef 1971). However, prior research has shown that bacterial penetration into muscle can occur (Gill and Penney 1982, Gupta and others 1981, Maxcy 1981, Sikes and Maxcy 1980). Therefore, bacteria present on the surface of meats that are vacuum tumbled could internalize in the muscle, particularly if the muscle integrity is compromised by application of mechanical energy. Blade tenderization, an invasive tenderization technique, can transfer surface bacteria to the inner portions of large roasts (Boyd and others 1978, Phebus and others 1999, Raccach and Henrickson 1979). A recent recall of tenderized beef steaks, that were also vacuum tumbled, demonstrates increased relevance of this problem (FSIS 2003). Research on the possible effects of non-invasive techniques, like vacuum tumbling marination, on microbial penetration is currently lacking.

Vacuum tumbling marination is a mechanical method of meat tenderization that is used for brine distribution, marinade infusion, and protein extraction (Barbut 2002). The mechanical energy required for this process may aid in the migration of bacteria from the meat surface of the marinade into intact muscle. The process disrupts the structure of the muscle, which could lead to bacterial penetration. Johnson (1978) went as far as to propose a ban on mechanical tenderization due to the risk of bacterial penetration. If bacteria are indeed able to penetrate intact whole muscle foods, sensitivity to thermal inactivation is decreased during cooking (Orta-Ramirez and others 2003). Thus

time and temperature relationships during thermal processing of vacuum tumbled foods may need to be modified to ensure a safe product.

The objective of this study was to determine whether vacuum tumbling marination increases the penetration of *Salmonella* into intact turkey breast muscle. Therefore, our hypothesis was that *Salmonella* can penetrate into the interior region of intact, whole muscle products during vacuum tumbling marination. The specific objectives were 1) to quantify the concentration profile of *Salmonella* in the interior of whole muscle turkey breast exposed to an inoculated marinade, and 2) to test the effects of vacuum, tumbling action, and exposure time on *Salmonella* migration into the product.

MATERIALS AND METHODS

Preparation of Turkey Breast Samples

Fresh, whole-muscle, boneless, skinless turkey breasts were obtained from a local wholesaler in a single large lot to eliminate lot-to-lot variability. The muscles were individually vacuum packaged and stored at -20°F.

To eliminate indigenous microflora, the turkey breasts were transported frozen to an irradiation facility (Iowa State Linear Accelerator Facility, Ames, Iowa, U.S.A.) where they received an average dose of 11.95 kGy. For sterility testing, 1-g samples from three breasts were serially diluted, and plated on Petrifilm™ Aerobic Count Plates (3M Corp., St. Paul, Minn., U.S.A.) with all plates incubated at 37°C for 24 h.

Marinade Preparation

The marinade was a generic formula (Pearson and Dutson 1987) containing 95.8% water (filtered and deionized), 3.2% NaCl (J.T. Baker, Philipsburg, N.J., U.S.A.), and 1% of a 50% mixed phosphate solution (w/w) (Butcher and Packer Supply Co., Detroit, Mich., U.S.A). Salt was incorporated into the water before adding the phosphate solution to ensure total dispersal. Aliquots (520 mL) of the marinade were poured into screw-capped glass bottles and autoclaved for 15 min at 121°C to ensure sterility.

Inoculum Preparation

The following eight serovars of *Salmonella* were obtained from Dr. V.K. Juneja (Agricultural Research Service, Eastern Regional research Center, USDA-ARS, Philidelphia, Pa., U.S.A.): *S.* Thompson FSIS 120 (chicken isolate),

S. Enteriditis H3527 and H3502 (clinical isolates, phage types 13A and 4, respectively), *S.* Typhimurium DDT104 H3380 (human isolate), *S.* Hadar MF60404 (turkey), *S.* Copenhagen 8457 (pork), *S.* Montevideo FSIS 051 (beef), and *S.* Heidelberg F5038BG1 (human isolate). All strains were stored frozen at -80° C in a solution of tryptic soy broth (TSB) (Difco, Detroit, Mich, U.S.A.) containing 10% glycerol. The cultures were propagated by transferring one loopful of frozen culture to 9 mL of TSB in a 20 mL culture tube. The cultures were maintained by transferring to fresh TSB media every 18-24 h and incubating at 37°C, with a minimum of two consecutive transfers prior to use. All of the strains were maintained separately and combined as needed.

On the day of the experiment, 9 ml of each of the eight serovars grown in TSB were combined equally and centrifuging at 6,000 x g for 20 min at 4°C. The supernatant was poured off and the pellet resuspended in 520 ml of sterile marinade to give a final concentration of ~10^8 CFU/mL ($1.43 \times 10^8 \pm 3.66 \times 10^7$ 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, Minn., U.S.A.).

Exposure to Inoculated Marinade

On the day before the experiment, the frozen turkey breasts were thawed for 24 h at 4°C. Each whole turkey breast was placed in a sterile 38 x 51 cm stomacher bag (Fisher Scientific, Pittsburgh, Penn., U.S.A.) and weighed. Inoculated marinade was added at a rate of 50 mL for every 250 g of breast

muscle. The bag was then tied, placed inside an identical stomacher bag, and tied again to form a waterproof seal.

Vacuum tumbling was conducted in a laboratory-scale tumbler (Model T-15, D.C Curtis Ltd., Ill., U.S.A.) under a vacuum of 100 kPa. The tumbler rotated at 8 rpm and was turned off for vacuum-only marination. All tumbling and holding treatments were conducted at 4°C. Samples marinated without tumbling or vacuum were simply bagged as described above and refrigerated at 4°C. To ensure procedural sterility, three breasts were sectioned and plated as described below without exposure to the marinade.

To examine the effect of tumbling time on bacterial penetration, turkey breasts were vacuum tumbled for 5, 10, or 20 min. Treatments described as "Vacuum Only", "Tumble Only", and "Still Marination" were also exposed to the inoculated marinade for 20 min. To test whether penetration was not "immediate" upon submersion in the marinade, two additional treatments consisted of dipping the breast in inoculated marinade for approximately 15 s ("Dip and Remove") or by drawing and immediately releasing the vacuum ("Vacuum and Immediate Release"), which took approximately 90 s.

Sampling

After treatment, the breasts were removed from the stomacher bags and placed on a sterile surface. Because dissecting and sampling the treated muscle with a conventional blade might mechanically transfer bacteria from the exterior surface to the interior, a novel method for aseptically removing samples from the inner portion of the muscle was developed. An electrosurgical generator was

used to create a high voltage electrical current that cuts by vaporizing water within the cells it contacts (Ulmer 2001). The high temperature generated from steam immediately next to the blade and ohmic heating is lethal to bacteria that come in contact with the blade, thereby preventing transfer and subsequent cross-contamination.

An electrosurgical unit (ESU)(Force 1B, Valley Lab, Boulder, Colo., U.S.A) equipped with a sterile electrode was used to cut the breast in half, perpendicular to the long axis of the muscle, where it was the thickest. The cutting electrode (E1001 Valleylab, Boulder, Colo., U.S.A.) was sterilized with 80% ethanol before every use. The ESU was set to "Blend 1" with 175 W cut and 75 W coagulation power. Five samples were then taken vertically (2 x 2 x 1 cm, except the "center" sample) on the freshly exposed cut surface (Figure 23). The size of the center sample varied due to the natural variability in breast size; however, the size of the other four sample sections remained constant.

Each sample was placed in a 2 oz. sterile Whirl-pak™ bag (NASCO, Fort Atkinson, Wis., U.S.A.) containing 4 ml of 0.1% buffered peptone water, macerated with serially diluted, and plated on Aerobic Petrifilm™ Count Plates which were counted after 24 h of incubation at 37°C.

Statistical Analyses

To determine *Salmonella* penetration as a function of vacuum tumbling time, six replications of 5, 10, and 20 min were conducted. Experimentation to determine bacterial penetration due to treatment (Vacuum tumbling 20 min,

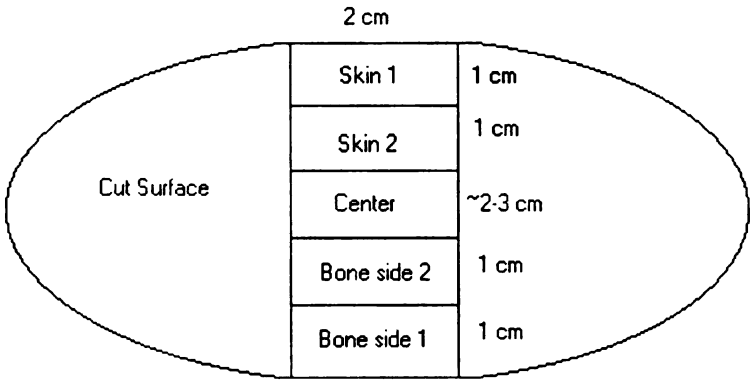

Figure 23. Schematic diagram for the aseptic removal of samples

tumbling without vacuum for 20 min, vacuum only for 20 min, still marination for 20 min, dipping and immediately removing, and vacuum and immediately releasing) six replications were also conducted. Statistical analyses were conducted using the general linear means procedure of SAS (SAS Cary, N.C., U.S.A., 1996). Individual means were compared using Tukey's multiple comparison procedure. Means were considered different at $\alpha=0.05$. The experimental limit of detection (LOD) was 5 CFU/g, and any count below the LOD was counted as the LOD. The LOD was based on the amount of sample (0.2 g) that will be plated on the zero dilution plate.

RESULTS AND DISCUSSION

Data presented in Figure 24 shows the penetration of *Salmonella* into whole muscle turkey breasts after several treatments. Penetration was greatest when the muscle was exposed to inoculated marinade for 20 min, as compared to the two controls (vacuum followed by immediate release and dipping)($p<0.05$). *Salmonella* counts were at or near the limit of detection (5 CFU/g or 0.69 $\log_{10}$ CFU/g) in the center segments in both the "Dip and Remove" and "Vacuum and Immediate Release" treatments, showing that penetration was not instantaneous when the muscle was momentarily exposed to the marinade at atmospheric pressure or when subjected to a brief vacuum. However, treating the breast with vacuum for 20 min increased penetration over both controls ($p<0.05$). If indeed bacterial penetration is based on diffusion with water into in the pores and canals in meat (Sikes and Maxey 1980, Maxey 1981), extending the time of exposure to the inoculum should increase penetration.

The effect of time on vacuum tumbling is presented in Figure 25. *Salmonella* penetration was greater in the 10 and 20 min treatments as compared to the 5 min treatment ($p<0.05$) with the 10 and 20 min treatments not significantly different from each other ($p>0.05$). The longer the meat is vacuum tumbled, the more structural damage occurs, which should increase bacterial penetration.

A penetration pattern was observed when the bacterial counts from the segments were averaged over all time treatments (Figure 26).

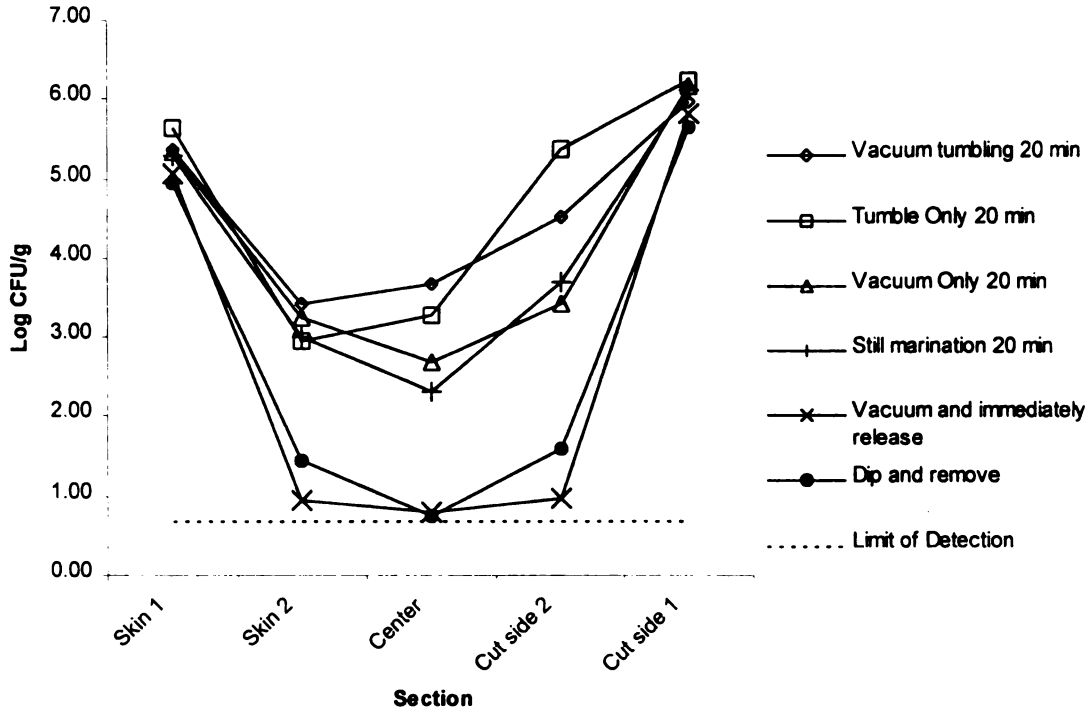

Figure 24. *Salmonella* populations recovered from different sections of turkey breast muscle after various treatments.

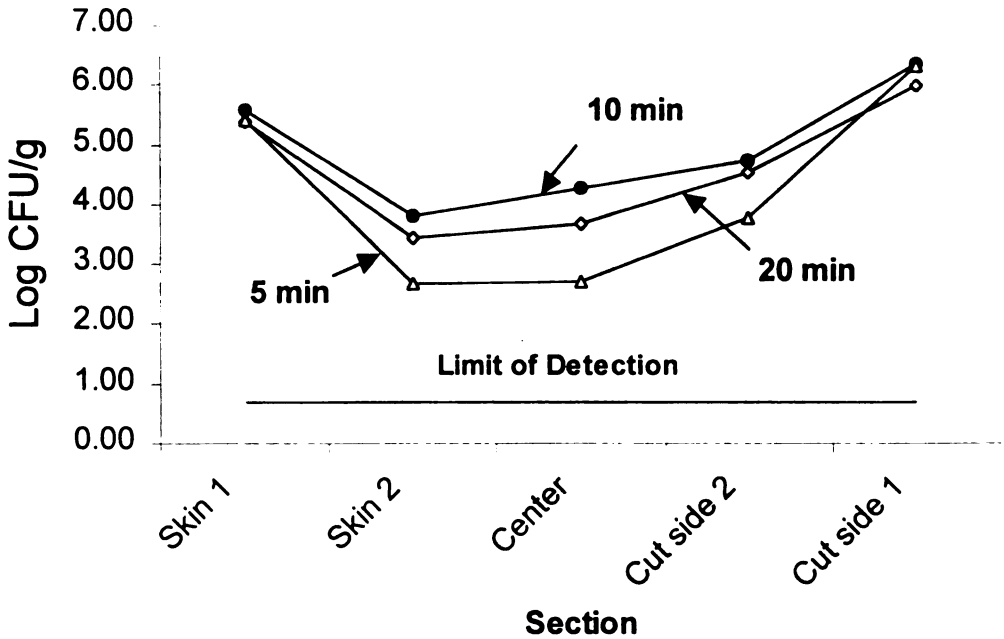

Figure 25. Penetration of *Salmonella* into whole muscle turkey breast after vacuum tumbling for 5, 10, and 20 min of vacuum tumbling with the inoculated marinade.

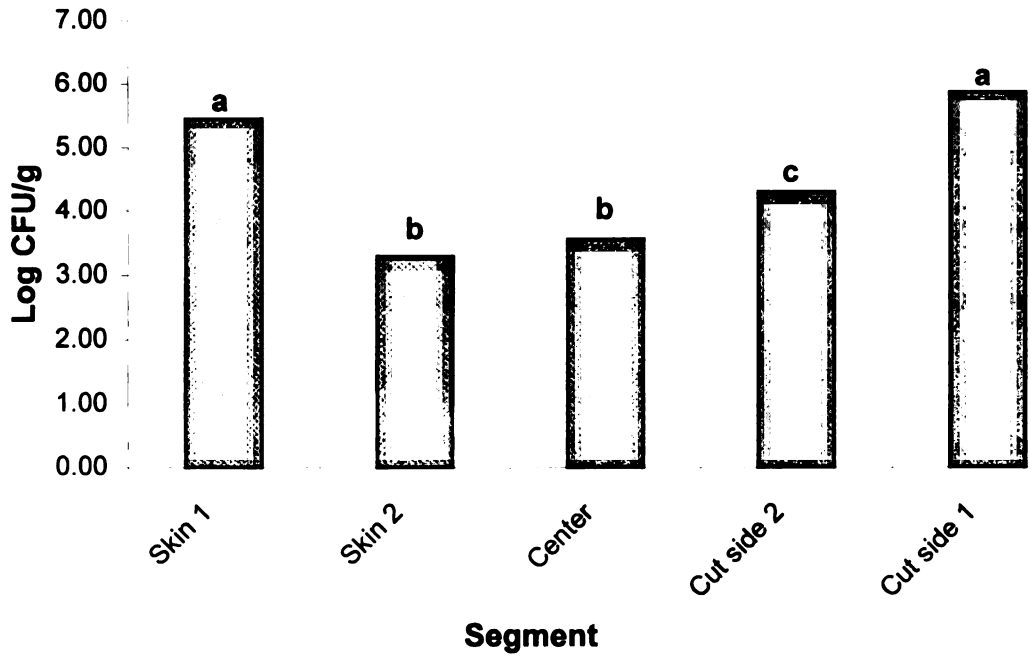

Figure 26. *Salmonella* populations in vacuum tumbled whole muscle turkey breast after excision with a cauterizing scalpel.
[a-c] Means that do not share a common letter differ significantly ($p<0.05$)

Salmonella counts were not significantly different between the two outer segments (Skin 1 and Cut side 1) ($p>0.05$). However, moving inward, the next two segments (Skin 2 and Cut side 2) were significantly different from each other ($p<0.05$), showing that the cut side allowed more bacterial penetration than the skin side. The center segment also had a significantly lower count than the two outermost segments ($p<0.05$).

Methods used previously to examine bacterial penetration in whole muscle foods were not appropriate for this experiment. Sikes and Maxey (1980), while studying the proteolytic penetration of *Serratia marcescens* into pork and beef trimmed the muscles to size aseptically and dipped them in colloidon. One side of the colloidon was then removed and the bacteria applied. They measured bacterial penetration by cutting frozen blocks and cutting them into 5 mm thick slices. The slices were swabbed at various depths, and streaked on plate count agar. This method only qualitatively determined bacterial penetration and could not be used for our application, because *Salmonella* is sensitive to freezing (Ray and others 1972).

In another study by Elmossalami and Wassef (1971) sterilized the outside of a 2 kg piece of beef with a hot knife, wrapped it in sterile gauze, and dipped it in hot paraffin. The top surface was then aseptically removed and smeared with *Salmonella*. Slices were then removed longitudinally, starting with the non-inoculated surface and slicing upward through the inoculated surface to prevent mechanical transmission of the bacteria. This methodology could not be used in our study, because the mechanical action of tumbling would disrupt the paraffin

coating. While Raccach and Henrickson (1979) used conventional scalpels that could mechanically transfer bacteria on the blade, a new method needed to be developed for our situation.

A novel method to aseptically remove tissue from the inner portions of inoculated meat was developed. The risk of mechanically transferring bacteria from the outside of the muscle into the interior is always a factor when using a conventional knife. However, a cauterizing knife that utilizes high-energy radio frequencies to dissect the tissue greatly reduces this risk.

Electrosurgical generators are commonly used in hospital operating rooms of hospitals. They are used to dissect and cauterize tissue simultaneously. The electrosurgical unit (ESU) consists of a generating device, a hand piece, electrode, and a grounding plate (Figure 27). The generating unit generates high frequency radio waves that 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 causes the water inside cells to vaporize, thereby rupturing the cell and cutting the tissue (Ulmer 2001).

In surgery, the electrosurgery electrode sterilizes the incision as it cuts, decreasing the chance for a wound infection (Malone 1974). Available literature indicates that the electrode tip will self sterilize at the levels used in this procedure (175 W)(Shaw and others 1988). Since the electrode is already sterile when the first cut is made, the concern is only maintaining sterility. Shaw and

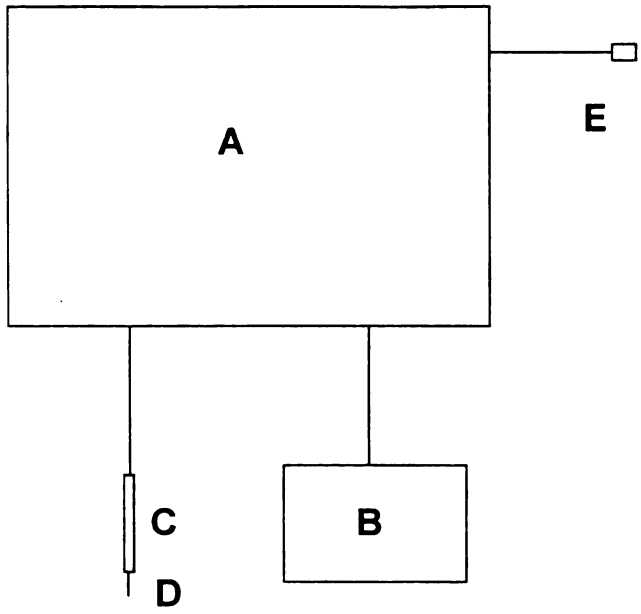

Figure 27. Schematic of electrosurgical unit and its various parts. (A) generator (B) grounding plate and return lead (C) hand unit with switch (D) cutting electrode (E) wall plug

others (1988) indicated that any energy level over 200 J (J = Watts x seconds) would sterilize the electrode tip when inoculated. Given that we were using 175 W of continuous power, the electrode tip would self-sterilize in 1.1 s, limiting or eliminating bacterial transfer. During experimentation, the electrode tip was streaked immediately on tryptic soy agar after cutting the inoculated turkey breast, and the electrode never yielded viable bacteria.

Because the tip of the electrode produces heat through ohmic heating, it was necessary to compare bacterial counts from a similar study to ensure that the heat generated was not destroying test organisms within the samples. Samples from the outer portion of turkey breasts receiving the same amount of vacuum tumbling with the inoculated marinade were dissected using a traditional scalpel and compared to samples removed from the outer portion with the ESU. After bacterial enumeration, no significant difference ($p>0.05$) was found between the traditional excision method and the ESU method indicating that the test organisms were not destroyed. Although the ESU was maintaining a sterile cutting surface, the instrument did not supply sufficient heat to inactivate bacteria inside the sample.

Salmonella likely penetrated the tissue between muscle fibers as described by Gill and Penney (1977). In poultry muscle, gaps between muscle fibers are created by radial shrinkage of the muscle fibers due to increases in muscle osmolality from lactic acid formation post-slaughter (Thomas and others 1987). Water in the marinade may contribute to bacterial penetration by increasing the water content between muscle fibers and therefore increasing

penetration. Thomas and others (1987) also concluded that higher levels of water between muscle fibers could increase penetration. The tumbling process also disrupts the overall muscle structure, leading to larger sized gaps that can increase bacterial penetration.

Because of concern for pathogens being spread in the laboratory, in this study the treated turkey breast muscle were treated with the inoculated marinade inside sterile stomacher bags, then placed in the vacuum tumbler. The bags may be limiting the effect of tenderization and tissue disruption that may occur. It is believed that bacterial penetration may be even greater that what was seen in this experiment due to greater muscle disruption.

CONCLUSIONS

Salmonella present in an inoculated marinade migrated into the interior of intact, whole muscle turkey breast during marination. Migration increased with time, and vacuum or tumbling action. Therefore, penetration during vacuum tumbling is likely to occur if *Salmonella* is present on the surface of turkey or in the marinade. Traditional microbial models for thermal inactivation for products that have undergone vacuum tumbling marination may need to be reevaluated in order to ensure that microbes below the surface of the meat are inactivated. Further examination of this phenomenon needs to be investigated in other muscle species and other bacterial pathogens.

OVERALL CONCLUSIONS

Vacuum tumbling, a non-invasive meat tenderization technique, can increase bacterial penetration and may allow pathogens such as *Salmonella* to access the inner portions of the muscle. The preliminary studies identified several key concepts that were utilized in later work. Dye-spiked marinade traveled evenly through poultry when compared to beef muscle. Beef muscle showed fissures and cracks where the dye was allowed to penetrate almost to the center of the roast. The coring method also helped develop better techniques for aseptically removing tissue from the interior of whole muscles.

In the unidirectional study, drawing a vacuum on turkey breast while in contact with a contaminated marinade increase penetration of *Salmonella*. The pathogen was internalized in tissues recovered from turkey breast portion up to 3 cm from the inoculated marinade, when compared to non-vacuum treatments. The length time the vacuum exposure did not affect bacterial penetration, which suggests that penetration is facilitated by vacuum rather than time.

The multidirectional study determined the extent of *Salmonella* penetration that occurs when whole turkey breast is vacuum tumbled with a marinade containing *Salmonella*. In this work, mechanical energy imparted from tumbling increased bacterial penetration, which was directly related to tumbling time. After vacuum tumbling whole muscle turkey breasts (8-9 cm thickness) with a marinade containing 10^7 CFU/mL, the center of the muscle contained up to 10^3 *Salmonella* CFU/mL.

The exact mechanism by which *Salmonella* penetrated was not examined in this study however, significant penetration was evident. It can be hypothesized that a number of physical and mechanical mechanisms may be factors. The impact of the meat pieces against the tumbler wall and with each other compromises the overall structure of the muscle, which could widen the gaps between muscle fibers, allowing greater penetration. Also, osmosis and a concentration gradient of bacteria from the outside to the inside of the muscle could be contributing factors increasing penetration.

FUTURE RESEARCH

The objectives of this study were to determine how a contaminated marinade could penetrate whole muscle turkey breasts during vacuum tumbling marination. We inoculated the marinade with *Salmonella*, but further studies should examine if other bacterial pathogens are able to penetrate as readily.

The results of the dye penetration studies has lead us to believe that bacterial penetration into other species of muscle may be different. Further bacterial penetration studies need to be conducted on beef, lamb, and other types of retail meats to determine if bacterial penetration is also different.

Additionally, the impact of marinade composition on bacterial penetration needs to be examined. Since alkaline phosphate is a fluidizing agent, increased concentrations could cause increased penetration. Additional ingredient concentration changes, such as salts, which when coupled with mechanical action and vacuum, have an effect of a more open muscle structure. Thus penetration of pathogens, or degree of penetration, may change depending upon the raw process conditions. These issues need to be studied.

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APPENDIX

A meat print study was conducted to qualitatively determine if *Salmonella* could penetrated turkey breasts blocks that were similar to those used in the slicing study. The turkey breast muscle that was used for this experiment was not irradiated, and could be contaminated with other bacteria. Inoculated marinade, identical to what has been used throughout these studies was added to the turkey breast blocks in a ratio of 50 mL for every 250 g of muscle.

After vacuum tumbling in the sealed stomacher bag for 5 min, the meat block was removed and placed on a tryptic soy agar (TSA) plate. The block was then cut in half down the long axis with a sterile knife. The inside surface was then put in contact with another TSA plate. The TSA plates were then incubated at 37°C for 24 h and compared visually.

The TSA plate that was put in contact with the outside of the block showed a solid colony of bacterial growth, but so too did the inside plate (Figure 28). This result showed that bacterial penetration was possible. However, the bacteria present on the inside of the muscle could have been transported there by the knife while cutting.

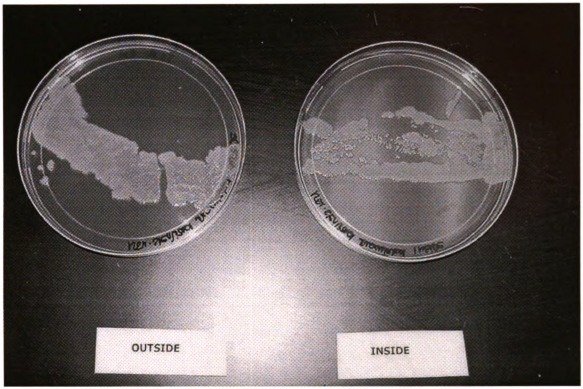

Figure 28. *Salmonella* penetration of a turkey breast block after vacuum tumbling for 5 min with an inoculated marinade