Risk factors influencing the growth and survival of Campylobacter jejuni and Salmonella enterica on moisture enhanced pork

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Risk factors influencing the growth and survival of *Campylobacter jejuni* and *Salmonella enterica* on moisture enhanced pork

By

Xuesong Wen

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Microbiology

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Iowa State University
Ames, Iowa
2010

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ABSTRACT

*E.coli* Biotype I, *Campylobacter jejuni*, or *Salmonella enterica* were inoculated into the surface of fresh pork loins and moisture enhanced with brine. After moisture enhancement, each pork loin was sliced into 1cm thick slices. All slices were randomly vacuum packed, stored at 4°C and 10°C and finally prepared using grilling practices. Studies were conducted to evaluate the potential microbiological concern presented by moisture enhanced pork (1) translocation of bacteria from the surface into the interior of the meat (2) effects of moisture enhancement on survival of bacteria in meat during storage (3) impact of moisture enhancement on survival of food borne pathogens during cooking. Our results showed that inoculated bacteria were translocated from the surface into the deep tissues in the boneless pork following moisture enhancement and slicing. Vacuum packing under chilled conditions can prevent the growth of *Campylobacter jejuni* and *Salmonella enterica* in enhanced pork. But it alone was not a substitute for safe handling and proper cooking because there were many numbers of *Campylobacter jejuni* and *Salmonella enterica* in enhanced pork during storage. The USDA recommended 160 °F as the safe minimum internal temperature for intact pork maybe also adequate for assuring the microbiological safety of moisture enhanced pork that is prepared without excessive contamination of interior tissues. Results were generally agreed that *Campylobacter jejuni* has more fastidious growth requirements and are more sensitive to various environment stresses than *Salmonella enterica*, such as vacuum packing, high cooking temperature. Compared to intact pork, moisture enhanced pork does not present a greater risk to consumers than otherwise similar meat that is intact, provided that the meat is properly cooked.
CHAPTER 1: GENERAL INTRODUCTION

It is common commercial process to inject fresh pork subprimal cuts with brine solutions to obtain a product with more consistent eating quality for the consumer and increasing profits and sale of the branded product for the processor. However, enhancement can have negative effects on the microbiological quality of meat, for example, the numbers of spoilage and pathogenic bacteria counts increase during re-circulation of brine solutions. Perhaps more importantly, from a food safety perspective, the surface contaminated pathogens can be translocated into the center of the muscle, which was initially sterile. Concerns about the microbiological safety of such non-intact meats have been raised because consumers preparing dishes with these meats may regard them as intact product, for example, grilling without raising all deep tissues to temperatures that will certainly destroy pathogenic bacteria that may be present.

Recently there has been a developing interest in the moisture enhancement by USDA-FSIS, in part due to the relative lack of information on the subject. Published data on the microbiological condition of the deep tissues of moisture enhanced pork products during production, distribution, consumption are few. There is also uncertainty about both the health risks that may be posed by pathogenic bacteria in the center of meat and the temperature of cooking required to assure safety. Hence, the overall objective of this study was to evaluate the potential microbiological concern presented by moisture enhanced pork (1) translocation of bacteria from the surface into the interior of the meat (2) effects of moisture enhancement on survival of bacteria in meat during storage (3) impact of moisture enhancement on survival of food borne pathogens during cooking. Moisture enhanced pork may also require assured control or additional protections, the second objective of this study was to evaluate
the effectiveness of current interventions in reducing the occurrence and extent of food borne pathogen contamination on moisture enhanced pork.

**Dissertation Organization**

This dissertation is organized into five chapters. The first chapter is a general introduction. The second chapter is a general literature review that contains information relevant to this research project. Chapter 3 is a manuscript entitled “Translocation of surface-inoculated *E.coli* Biotype I into pork subprimals following moisture enhancement and slicing”, Chapter 4 is a manuscript entitled “Survival and growth of *Campylobacter jejuni* and *Salmonella enterica* in moisture enhanced pork during vacuum storage.” Chapter 5 is a manuscript entitled “Effects of moisture, storage and subsequent cooking on inactivation of *Salmonella enterica* and *Campylobacter jejuni* in moisture-enhanced pork meat.” All tables and graphs in the papers appear at the end of each respective paper. The sixth chapter gives a general summary of this research.
CHAPTER 2. LITERATURE REVIEW

Production of Moisture Enhanced Pork

The Moisture Enhancement Process

Recently the moisture enhancing process of injecting water, salt and other flavoring agents into meats has been well developed in poultry and pork. According to a 2004 nationwide Retail survey, 45% of the retail fresh pork in the United States was labeled as “enhanced” with products defined as moisture added and could be valued added (flavored and/or contained additional ingredients) (4).

Moisture-enhanced meat is produced through multi-needle injection of brines or marinades that contain ingredients such as phosphates, salt, and potassium or sodium lactate (Figure 1, 2). The moisture enhancement process has been shown to have a beneficial impact on the juiciness, tenderness and sensory quality (10), while increasing profitability and sales of brand products for the processor.

Figure 1 (A) P-10 Pokomat Injector (B) Rows of needles or blades in a large mechanical tenderizer.
The level of addition of the added ingredients is monitored either through the speed of passage of the meat through the injector machine or by changing the pressure used during injection. Needle size and number, are important factors to consider, since by increasing the number of needles and using smaller needles, injectors can deliver a more uniformly distributed injection solution into the final product. Uniformity of injection is an important factor when injecting fresh pork products that would not be tumbled. Lack of uniformity in injection usually produces a two-toned meat product. The injection of solutions with higher pH values increases the final pH of the injected pork, which results in a darker color in the lean meat. The pattern of the color variation in the lean follows the path of the needles (52).

Moisture enhancing process is similar in some respects to mechanical tenderization of beef. The tenderizing treatment is usually applied at central cutting or retail store facilities by piercing prime cuts from vacuum packs with banks of thin blades before the cuts are fabricated into roasts or steaks (16). Beef tenderness also is improved mechanically during processes such as injection (enhancement) and tumbling. However, for these processes, mechanical improvement in tenderness is secondary to moisture or flavor addition (65).

**Non-Meat Ingredients**

The United States Department of Agriculture (USDA) requires that any pork product that contains added ingredients must carry an ingredient label on the front of the package and a statement noting the "addition of a _% solution of [specific ingredient names]". Several companies market their product with a branded name indicating the moist, tender or juicy characteristics.
Phosphates

The use of alkaline phosphates is widely used in meat industry. Many forms of phosphates are approved for use in the United States; however, sodium tripolyphosphate (STP) is the predominant phosphate in phosphate blends that are used in meat systems (52). Sodium tripolyphosphate has an alkaline pH and even though meat is a very good buffer, the addition of phosphates increases its pH. It was reported that the highest pH in pork loins was found in loins injected with a phosphate blend compared to control loins, or loins containing potassium sorbate, or sodium acetate, and pork chops treated with phosphate blend spoiled faster, within 2-4 weeks, than chops treated with other ingredients (51). By increasing the pH, water holding capacity improves by moving the meat pH further from the meat’s protein isoelectric point. As the pH moves further from the isoelectric point (approximately 5.2 to 5.3 in red meat), the water-holding capacity increases due to an increase in the amount of negative charges on the proteins that can bind water. Therefore, the net result is an increase in the amount of water that can be bound to the meat proteins to improve yield, juiciness, and texture (52, 25, 59). As the pH of meat increases with the addition of STP, meat becomes darker in color due to the increase in bound water, leaving less free water to reflect light (52).

With increased water holding capacity (WHC), meat with added STP has been shown to be juicier, have improved tenderness and a change in flavor. Sutton et al. (73) showed that when STP levels increased from 0 to 0.2 to 0.4% in the final product, pork roasts were juicier and had incrementally higher percentage of moisture, but the addition of 0.4% STP resulted in lower flavor intensity scores and higher levels of salt intensity when compared with pork roast that did not contain STP. The lower flavor intensity may be due to an increased dilution
effect due to higher amounts of water in the final product. In addition, the addition of STP alone is commonly associated with increasing off-flavors such as soapy and sour.

The increase in water holding capacity and pH of meat treated with sodium tripolyphosphate creates a more favorable environment for microbial growth; aerobic plate counts (APCs) were higher in pork loin slices that had been injected with brine containing sodium tripolyphosphate than in pork samples with no sodium tripolyphosphate in the brine (85).

**Sodium Chloride**

It has been an ancient practice to incorporate sodium chloride into meat to increase shelf-life and enhance flavor. The addition of sodium chloride causes dehydration and altering of the osmotic pressure so that it inhibits bacterial growth and subsequent spoilage. Sodium chloride is also used to improve water holding capacity by lowering the isoelectric point of meat proteins without changing the meat pH, resulting in subsequent improvements in purge loss and cook yields (52).

Sodium chloride is often added in combination with SP to maximize water holding capacity; however, it is important to add them in a balanced way to avoid getting too high of a salt flavor or altering the texture (52).

**Packaging**

The main objectives of meat packaging technologies are: shelf-life extension, enhanced appearance and presentation quality and reducing the need for added preservatives, etc. All these objectives must conform to the main prerequisite: packaging should provide users with portioned product in a safe and wholesome condition (43).
Typically fresh red meats are placed on trays and over-wrapped with an oxygen permeable film, or stored in modified atmosphere packaging and vacuum packaging. Self-service stores sell fresh meat packaged in rigid plastic or foam trays with a film such as polyvinyl chloride as an overwrap. Trays provide strength to the package, and the overwrap regulates the gaseous environment in the package. Overwraps are designed to provide an abundant amount of oxygen at meat surfaces to form oxymyoglobin, the bright red pigment. However, such overwrap facilitates the growth of psychrophilic aerobic bacteria, which compete for available oxygen and thereby shorten the time that oxymyoglobin persists (1). Jensen et al (42) found that after 72 hrs of retail display, pork chops enhanced with sodium tripolyphosphate and salt and packaged in polyvinyl chloride as an overwrap, reached spoilage ($10^6$ CFU/cm$^2$) levels of bacteria.

To extend the shelf-life of meat, modified atmosphere packaging is always considered. This technique is invariably applied with refrigerated storage to improve quality and safety of meat and meat products.

Modified atmosphere can be defined as one that is created by altering the natural composition of air to provide an alternative atmosphere for increasing storage time and quality of food (63). Oxygen, nitrogen and carbon dioxide are mainly used in Modified Atmosphere Packaging (MAP) preservation of meat. These gases can be applied individually or in combination in order to obtain the cumulative effect of these gases, which have different purposes in food preservation (64).

The vacuum packaging of pork seems to result in less color change than in beef. Interest in vacuum packaged pork has been increasing in the United States. In vacuum packaging, the product is placed in a bag from which the air is evacuated, causing the bag to
collapse around the product before it is sealed. Residual oxygen in the pack is absorbed through chemical reactions with components in the product and any residual respiratory activity in the product and its microflora. Vacuum packaging, could also be considered as a type of MAP because of the modification of the atmosphere produced by the removal of air from the environment (64).

Modified atmospheres exert their effect principally through the inhibition of fast-growing aerobes that would otherwise quickly spoil perishable products. Obligate and facultative anaerobes such as Clostridia and the Enterobacteriaceae are less affected. Thus shelf life is extended but there is generally little effect on pathogens, if present. The major pathogens of concern, which could survive and grow even at refrigeration temperatures and anaerobic environment, such as Aeromonas hydrophila, Listeria monocytogenes, Yersinia enterocolitica, Escherichia coli O157:H7, etc. The bactericidal and bacteriostatic effects of carbon dioxide are temperature dependent. During storage at low temperature, carbon dioxide in modified atmospheres packaging is capable of inhibiting some microorganisms, however, the lack of refrigeration at any time could allow or stimulate the growth of such organisms (83). This situation should be concerned for some mesophilic pathogens, such as Salmonella, Salmonella which can’t grow in modified atmospheres at refrigerated temperatures may constitute a risk when a temperature abuse occurs in the commercial chain of food handling (36). In experiments performed on poultry having different pH (breast-low pH and thigh-high pH) inoculated with S. enteritidis and stored in several atmospheres (vacuum, 100% carbon dioxide, 100% nitrogen and 20% carbon dioxide/80 % air), this bacterium survived, but did not grow at 3°C (36). At 10°C, the numbers of S. enteritidis
increased rapidly in samples flushed with 100% nitrogen or with CO, /air (20:80) and to a lesser extent in vacuum-packaged samples.

**Microbiological Aspects of Moisture Enhanced Pork**

Although moisture enhanced products have been available for some time, relatively little is available within the public domain regarding the microbiological properties of the product. In a survey of retailed pork conducted in 2000, four types of retail pork products in six continental U.S. cities were collected, they were (a) whole-muscle, store-packaged pork retail cuts; (b) fresh, store-ground, store-packaged pork and/or pork sausage; (c) prepackaged (at the processing plant) ground pork and/or pork sausage; and (d) whole-muscle, enhanced (injected and marinated) pork cuts. Enhanced pork products were not statistically (P>0.05) different from other products on almost every category of microbiological analysis: aerobic plate counts, total coliform counts, *Escherichia coli* counts, and incidences of *Salmonella spp.*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Campylobacter coli*. The only marked difference was that the incidence of *Yersinia spp* in enhanced products was significantly lower in the moisture enhanced products than in the store-packaged products (28).

Greer *et al* (38) found the moisture enhancing process resulted in an increase in spoilage bacteria in enhanced pork and enhanced pork had larger bacterial numbers than non-enhanced products at each storage time compared to non enhanced pork, but the shelf life was the same as that for non enhanced pork. They also observed there were no consistent injection treatment effects on bacterial growth.
The Challenges of Foodborne Pathogens in Moisture Enhanced Pork

Concern Associated with Processing

In 1997, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) stated that “Due to the low probability of pathogenic organisms being present in or migrating from the external surface to the interior of beef muscle, cuts of intact muscle (steaks) should be safe if the external surfaces are exposed to temperatures sufficient to effect a cooked color change.” However, if the exterior of an intact muscle is violated by mechanically tenderization or injection, contamination maybe carried out from the exterior into the interior. Since pathogens can be in the center portion of meat through these processing, failure to cook meat completely may enable pathogens that are present to survive in the meat.

Published reports have confirmed that naturally present bacteria, or surfaced-inoculated pathogens such as *Escherichia coli* O157:H7 and *Salmonella*, could be carried from the surface to the interior of beef muscle by mechanical tenderization. Wendelburg et al (82) reported that 1% to 7% and 4 to 8% of the surface-inoculated *Salmonella* were translocated into the interior of pork loin cubes due to blade tenderization and needle injection. Similar results from other published reports showed that, 3 to 4% of the surface inoculums with the level of 3 and 6 log_{10}CFU/g were translocated into the geometric center of beef top sirloin subprimals due to blade tenderization. Luchansky et al (47) found blade tenderization transferred *E coli* O157:H7 primarily into the topmost 1 cm; relatively few cells were carried into the deep tissues of beef subprimals. As such, adequate cooking targeted for the genometric center would effectively eliminate low levels of the pathogens.
The process of moisture enhancement is similar in some respects to mechanical tenderization, but there are sufficient differences to warrant further investigation. For example, the moisture enhancing solution is re-circulated during production. This is an obvious source for the contamination of enhanced products. Significant increases in the populations of spoilage bacteria were observed during the first hour of re-circulation, and a significant increase in the populations of *Listeria monocytogenes* were also observed (38). The influence of bacterial contamination in brine on the safety of moisture enhanced meat products is still unknown. Moisture enhancing also can carry bacteria into the interior of meat. But distributions of bacteria in enhanced products after moisture enhancement are necessary to determine if this is the case. Some ingredients of brine, such as lactates and diacetates, have been reported as potent antimicrobial agents in processed meat. Further research is necessary to determine the possible bacteriostatic effect of brine on the differences of bacterial growth in moisture enhanced pork.

There is no sufficient evidence on the effectiveness of cooking on the killing of pathogens that has been translocated into the interior of meat by moisture enhancing or mechanically tenderization. Some studies indicated that, a thermal inactivation of 5 log_{10} reduction of *E. coli* O157:H7 inoculum per gram was achieved by cooking blade-tenderized steaks to an internal temperature of 60°C or more (62, 71). These studies also reported that, gas grilling was least effective in achieving a 5 log reduction of *E. coli* O157:H7 populations translocated to the interior of blade-tenderized beef steaks, while oven broiling of the steaks eliminated all populations of *E. coli* O157:H7. In 2002, a comparative risk assessment for intact and non-intact beef by USDA/FSIS indicated that over boiling and cooking to an internal temperature of 60°C or more would ensure blade-tenderized beef steaks safe for
consumption (79). Mukherjee et al (7) tested some common tenderization ingredients, including organic acids, potassium and calcium salts and sodium chloride, for their influence on thermal inactivation of \textit{E.coli} O157:H7 in ground beef. Treatment with citric or acetic acid showed greater ($P<0.05$) reduction (4 to 5 $\log_{10}$CFU/g) of \textit{E.coli} O157:H7 than all other ingredients.

**Concern Associated with Foodborne Pathogens**

\textit{Salmonella, Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, Campylobacter coli} are all foodborne pathogens that are of concern to the pork industry. In particular, \textit{Salmonella enterica} and \textit{Campylobacter jejuni} are amongst the more prevalent bacterial pathogens that cause foodborne diseases and economic loss (Table 1, 23). \textit{Salmonella enterica} and \textit{Campylobacter jejuni} are also commonly recognized as emerging foodborne pathogens in the last 30 years. The emerging foodborne pathogens are defined as emerging based on the infections caused by these pathogens have newly appeared in the population, or have existed but have a rapidly increasing in incidence or geographic range (72). For examples, infections caused by nontyphoid strains of \textit{Salmonella}, which have increased significantly decade by decade since World War II (66). Along with emerging nontyphoid strains of \textit{Salmonella}, new food vehicles of transmission have also been implicated in recent years. An increase in \textit{Salmonellosis} during the 1980s was clearly observed throughout the developed world. This increase largely comprised \textit{S. enteritidis} phage type (PT) 4, which was epidemiologically and microbiologically linked to shell eggs and poultry, for centuries, the internal contents of an egg were presumed safe to eat raw, however, research has demonstrated that this \textit{Salmonella} has adapted to preferentially
colonise the avian reproductive tract, persist in the ovary and oviduct, survive in internal contents of hen's eggs and egg is a new food vehicle of Salmonella transmission (35).

Table 1 Estimated annual costs associated with selected foodborne pathogens, 2000 (23).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Cases</th>
<th>Hospitalizations</th>
<th>Deaths</th>
<th>Cost(billion 2000US $)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter species</em></td>
<td>1,963,141</td>
<td>10,539</td>
<td>99</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>1,341,873</td>
<td>15,608</td>
<td>553</td>
<td>2.4</td>
</tr>
<tr>
<td><em>E.coli O157</em></td>
<td>62,458</td>
<td>1,843</td>
<td>52</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Shiga toxin-producing (non-O157)E.coli</em></td>
<td>31,229</td>
<td>921</td>
<td>26</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>2,493</td>
<td>2,298</td>
<td>499</td>
<td>2.3</td>
</tr>
<tr>
<td>Total</td>
<td>3,401,194</td>
<td>31,209</td>
<td>1,229</td>
<td>6.9</td>
</tr>
</tbody>
</table>

*Salmonella serotype* other than *Salmonella typhi*

Unlike Salmonella, *Campylobacter jejuni* has no useful historical typing data with which to investigate changing trends in bacterial populations (26). It was known as a rare opportunistic bloodstream infection until veterinary diagnostic methods used on specimens from humans showed it was a common cause of diarrheal illness (24). Recently it has been reported an increasing proportion of human infections caused by *Campylobacter jejuni* are resistant to fluoroquinolones, which may be due to the application of these agents in animals (29).

One of potential factors that precipitate disease emergence is the change of technology in food production. Modern production technology yields increased efficiency and reduced costs but it can increase the chance of accidental contamination and amplify the
effects of such contamination. This problem is further compounded by globalization, allowing the chance to introduce agents from far away. It has been reported that more extensive handling and processing cause lower microbiological quality in pork products. More importantly, with food production practice change, there are a lot of evidences concerning continuous adaptation and development of resistance by pathogenic microorganisms to antibiotics and potentially to traditional food preservation barriers such as low pH, heat, cold temperatures, dryness or low water activity, and chemical additives \((41, 86)\). Furthermore, the development of pathogenicity has also been reported, for example, existence of strains of pathogens with enhanced ability for survival in their hosts, low infective doses, and increased virulence, sometimes after exposure to common environmental stresses \((67)\). These microorganism-associated developments make pathogens more of a threat to human health.

It is noteworthy that *Salmonella enterica* and *Campylobacter jejuni* are present on the moisture-enhanced pork samples collected from stores in six cities in United States. The reported incidence of *Salmonella enterica* and *Campylobacter jejuni* on moisture-enhanced pork were 10.4% and 1.0%, respectively \((28)\).

Recently some outbreaks of *Escherichia coli* O157:H7 have been also associated with no-intact, mechanically tenderized steaks. In an outbreak investigation by the Minnesota Department of Health (MDH) on June 2003 \((44)\), the outbreak PFGE subtype was isolated from unopened brand A bacon-wrapped fillets from five affected Minnesota household, the same PFGE subtype was also found in the interior of the partially cooked steaks from one affected household. These findings suggest that the technologies used in moisture enhanced pork create new challenge for prevention of food-borne infection. Food regulatory officials
and scientists should evaluate safety issues present by moisture enhanced pork, such as microbiological hazards of processing methods, education of the public and the commercial food establishments.

**Hazard Characterization of *Campylobacter jejuni***

**Characteristics of the Organism**

*Campylobacter jejuni* belongs to the epsilon class of proteobacteria in the order Campylobacterales. The genus *Campylobacter* comprises slender, spirally curved, Gram-negative rods. Exposure to certain unfavorable environments may result in a coccoid form. Most of species of *Campylobacter* display a characteristic corkscrew-like darting motility by means of a single polar unsheathed flagellum at one or both ends. *Campylobacter* can’t ferment or oxidize sugars and are oxygen sensitive microaerophiles, growing best in an atmosphere containing 5-10% oxygen and 3-5% carbon dioxide (50).

As a commensal organism routinely found in cattle, sheep, swine and avian species, in particular, birds, and especially poultry, are regarded as the primary reservoir, which may be due to *Campylobacter* has evolved specialized strategies that allow it to exploit the restricted ecological niche (67). For example, the optimal growth temperature for the organism (42 °C), correlates with that of the avian gut and not that of the mammalian gut (37 °C).

**Associated with foods**

*Campylobacter* inevitably were found in meat when carcasses are contaminated with intestinal contents during slaughter and evisceration. The incidence of *Campylobacter* on retail meats in several countries has been found to vary from 0-8.1% for red meats and from 23.1-84% for chicken (56). In a survey of retail pork conducted in 2000, *Campylobacter*
jejuni and Campylobacter coli were found in 1.3% of retail samples and 6.7% of plant samples.

Compared to other foodborne bacterial pathogens, the growth conditions required for the culture of Campylobacter are unusual and this places unique limitations on the range of food environments in which the species can multiply (Table 2, 40). Campylobacter are unable to grow below 30°C but display physiological activity at 4°C (39). Campylobacter are sensitive to heat and readily inactivated by pasteurization and domestic cooking process. They are also susceptible to desiccation and accordingly do not survive well on dry surface. The stress responses elicited by Campylobacter during these exposures and the regulatory mechanisms which govern these responses are not very clear. Data on strain variability regarding virulence or pathogenicity and survival during these exposures were limited.

**Dose response relationship**

Campylobacter are the leading cause of bacterial diarrhoeal disease worldwide. The infections of Campylobacter jejuni sometimes stimulate some autoimmune responses, which result serious disease, such as Guillain-Barré Syndrome (GBS), Reiter syndrome (2). The required infectious dose for disease, as determined by volunteer studies, was around $10^4$ colony forming units (9) but asymptomatic infection occurs at lower doses (a few hundred cells). It has been reported that the outcome of disease is dependent on the immune status of the host. In industrialized countries, such as the United States and United Kingdom, infection results in acute watery or bloody diarrhea. In contrast, diarrhea associated with infection is usually apparent only in children under the age of 2 years in nonindustrialized countries. Thereafter, infection appears to be asymptomatic in these countries. The reason for this
difference is not clear but may reflect differences in the immune status of individuals in the nonindustrialized world (50).

**Hazard characterization of *Salmonella enterica***

**Characteristics of the Organism**

Salmonella are members of the Enterobacteriaceae, in the Gamma-proteobacteria subdivision. Two species of Salmonella are recognized: *Salmonella bongori* and *Salmonella enterica*. *Salmonella enterica* can be further divided into seven subspecies on the basis of biochemical typing and genetic typing (68).

Salmonella are Gram negative, non-sporeforming rods which are facultatively anaerobic, catalyse-positive, oxidase-negative and generally motile with peritichous flagella. They will grow at 7–48°C with an optimum growth at 37°C and at pH 4.05–9.5 with an optimal growth at pH 6.5–7.5. Salmonella grows optimally at a water activity of 0.995 (56).

Salmonella are responsible for a number of different clinical syndromes, grouped as enteritis and systemic disease. Gastrointestinal infections are predominantly associated with those serovars which occur widely in animals and humans, such as *Salmonella enteridis* and *Salmonella typhimurium*. Host adapted serotypes, such as *Salmonella typhi* and *Salmonella.paratyphi*, which occurs only in humans and higher primates, are more invasive and tend to cause systemic disease in their hosts (56).

**Associated with foods**

The Salmonella live primarily in the intestinal tracts of animals. *Salmonella enterica* subsp. *enterica* inhabit warm-blooded animals, whereas all other *S. enterica* subspecies and *S. bongori* are commensals of cold-blood animals and only rarely infect humans (50). Meat, milk, poultry and eggs are primary vehicles for Salmonellosis. A period of temperature abuse
which allows Salmonella to grow in the food and an inadequate cooking or cross contamination with other food are major factors contributing to outbreaks (56).

Heat treatment to around 70°C will kill the organism in meat products. Salmonella is capable of surviving in frozen meat, dried foods and multiplying under both aerobic and anaerobic conditions as well as in modifies atmosphere with 20% CO₂ (34).

Salmonella have a complex regulatory system, which mediates their response to the external environment where nutrients are replete or limiting. For example, in response to starvation, changes in pH or temperature, RNA polymerase S (RpoS) is produced by Salmonella. RpoS regulates the expression of up to 50 other proteins and is also involved in the regulation of virulence plasmids, which are found in many Salmonella spp (77).

**Dose response relationship**

The infective dose for causing foodborne Salmonellosis is difficult to determine and is likely to vary with strain, source (e.g., the particular food matrix could differentially protect from stomach acid), and the underlying health of the individual (20, 21). The infective dose for in humans was believed to be very high (~ 10⁵ to 10⁶ cells) for a number of years, but there have been various outbreaks in which the infective dose was found to be as low as < 10-100 cells (8). Newborns, infants, the elderly, and immunocompromised are more susceptible to Salmonella infections as compared to healthy adults (50).
Table 2. The limits of growth and temperature sensitivity of *Campylobacter jejuni* (compared with other common foodborne bacterial pathogens)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Temperature(°C)</th>
<th>Typical D value at 55°C</th>
<th>a_w</th>
<th>pH</th>
<th>Oxygen requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>0</td>
<td>4.5</td>
<td>0.92</td>
<td>4.4</td>
<td>Facultative</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>7.0</td>
<td>3.0</td>
<td>0.83</td>
<td>4.0</td>
<td>Facultative</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>5.2</td>
<td>4.7</td>
<td>0.93</td>
<td>3.8</td>
<td>Facultative</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>7-10</td>
<td>5.5</td>
<td>0.95</td>
<td>4.4</td>
<td>Facultative</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>30</td>
<td>1.0</td>
<td>0.987</td>
<td>4.9</td>
<td>Microaerophilic 5-10% O₂</td>
</tr>
</tbody>
</table>

Data taken from International Commission on Microbiological Specifications for Foods (1996)
Risk Assessment

Historical Aspects of Food Safety Systems

Microbial risk assessment is a relatively new and emerging methodology in the quest for a better means of ensuring the production of safe food. The need to produce safe food has a long history. Thousands years ago, most food safety requirements were established in various religious taboos, which include a ban including on eating specific items, such as pork, in the Jewish and Muslim religions (76).

In the nineteenth century, Louis Pasteur observed that certain bacteria were either associated with food spoilage or caused specific disease (1854-1864), Robert Koch isolated *Bacillus anthracis* (1877), *Tuberculosis bacillus* (1882) and *Vibrio cholerae* (1883) and develop Koch's postulates, which has been accepted that microbial agents were often responsible for foodborne illness. Since then, systems for controlling the safety of the food supply began to be introduced (11). The microbiological testing of foods became widely accepted as means of assessing food safety early in the twentieth century. Testing of the product is usually an integral part of the overall control program for food safety, and the perceived risk of foodborne illness form the presence of a particular pathogen is reflected in the limit values that are set for the organism in a specific type of food. Process performing criteria to ensure safety production were also established at the start of in the twentieth century, it had already been recognized that protection of the public against foodborne hazards required proper control of heat treatments used commercially in food production (30,31, 32).

With more knowledge of foodborne disease, microbiology and epidemiology, comprehensive means of controlling food safety in production have been developed. These
included the good manufacture practice (GMP), a quality system covering the manufacture and testing of active pharmaceutical ingredients, diagnostics, foods, pharmaceutical products, and medical devices, and the hazard analysis critical control point (HACCP) system. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) defines HACCP as “a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product” (57). HACCP is being accepted throughout the farm-to-table continuum as the best available method under which to produce, process, and prepare food for consumption (75). However, HACCP still does not lead to a comprehensive design of a food process. One step in the HACCP system is to determine critical control points (CCPs) where risk management efforts can be focused. Given data gaps and the complexity of the system, determining CCPs represents a substantial analytical challenge (11). There is also no mechanism in HACCP to link the different steps to each other, to quantitatively determine how much control is necessary, or determine the impact of a critical step on the incidence of food-borne disease (27).
Table 3. Important milestones in the development of food safety systems.

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distant past</td>
<td>Use of ‘prohibition’ principle to protect special groups within society against foodborne illness</td>
</tr>
<tr>
<td>1900 to present</td>
<td>Microbial examination of food</td>
</tr>
<tr>
<td>1922</td>
<td>Introduction of process performance criteria by Esty &amp; Meyer for canned, low-acid food products</td>
</tr>
<tr>
<td>1930-1960</td>
<td>Use of risk assessment (for different pathogenic organisms) in setting process performance criteria for heat pasteurization of milk</td>
</tr>
<tr>
<td>1960</td>
<td>Introduction of good manufacturing practice</td>
</tr>
<tr>
<td>1971</td>
<td>Introduction of formal hazard analysis critical control point system</td>
</tr>
<tr>
<td>Ca 1978</td>
<td>Start of predicative modeling of bacterial growth in food</td>
</tr>
<tr>
<td>1995</td>
<td>Introduction of formal quantitative risk analysis</td>
</tr>
</tbody>
</table>

The relatively new field of food safety risk assessment provides the link by which a board base of scientific knowledge can be synthesized into a meaningful product, inform regulatory decisions, and reduce food-borne risk (75). The development of microbiological modeling in mid-1990s has helped to make possible the introduction of quantitative microbiological risk assessment. Increased computational capabilities and modeling software facilitated the development of computer models capable of complex calculations and simulations using sophisticated mathematical techniques. In 1998 the U.S Department of Agriculture (USDA) issued the first U.S microbial risk assessment for *Salmonella enteritidis* in shell eggs and egg products (78). This work was shortly followed by other quantitative microbial risk assessments.
Using Science-based Risk Assessment to Develop Food Safety Policy

Risk assessment is a structured science-based process to estimate the likelihood and severity of risk with attendant uncertainty (75). It provides information on the extent and characteristics of the risk attributed to a hazard. In many circumstances, risk assessment is intended to provide information useful for policy making, and should therefore provide insight into the factors responsible for increasing the risk and, more importantly, ways to reduce it. Risk assessment is an essential component of risk analysis, the process for gathering information, doing analysis and making decisions about risk (56). Other components in risk analysis include risk management and risk communication. Risk management is the process of deciding, in collaboration with risk assessors, which risk assessment should be undertaken and then weighing policy alternatives to accept, minimize or reduce assessed risks. Risk communication involves an exchange of information and opinion concerning risk and risk-related factors among the risk assessors, risk managers, and other interested parties (Figure 3). The development of risk assessment was strongly stimulated in 1995 by the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) that requires that countries signatory to the agreement base their laws concerned with protecting human, animal and plant health on a risk analytical basis (3,33). Thus, the SPS Agreement requires food safety legislation to be scientifically based and the process of risk assessment to be applied. For example, when introducing international microbiological criteria for controlling imported foods (Figure 3).
Figure 3. The use of risk analysis to convert a food safety policy into food safety objectives, adapted by Martyn Brown and Mike Stringer (11).

As a bridge between science and policy, risk assessment is scientific, but not pure science. A research scientist always articulates a hypothesis and then conducts tests under controlled conditions to learn about the nature world, risk assessment functions within a totally different process with a different purpose. Table 4 summarizes the different characteristics of science and risk assessment (53).
Table 4. A comparison of feature of science with those of risk assessment \cite{53}

<table>
<thead>
<tr>
<th>Features of science</th>
<th>Features of Risk assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical testing</td>
<td>Testing often impractical</td>
</tr>
<tr>
<td>Full documentation</td>
<td>Incomplete documentation</td>
</tr>
<tr>
<td>Reporting uncertainty</td>
<td>Uncertainty usually incomplete or missing</td>
</tr>
<tr>
<td>Peer review</td>
<td>Review not standard and in some case arduous</td>
</tr>
<tr>
<td>Open debated</td>
<td>Nature of process hinders debate</td>
</tr>
</tbody>
</table>

The Methodology of Microbiological Risk Assessment

There are many approaches available for risk assessment, ranging from qualitative to fully quantitative in nature. If the available data are inadequate to develop a numerical estimate of risk, a qualitative assessment maybe developed by assigning descriptive rating of probability and severity such as ‘negligible’, ‘low’, ‘medium’, ‘high’ \cite{27}.

Quantitative assessments are mathematical analyses of numerical data. Based on mathematical (and probabilistic) models \cite{11}, there are two approaches in quantitative assessments, deterministic approach or probabilistic approach. In a deterministic approach, the average values for the model’s parameters are used to make an estimate of the average rate of illness or expected number of illness. Risk estimates through this approach are insufficiently or inappropriately stringent. In a probabilistic approach, probability distributions of the model parameters are assigned based on experimental data or maybe derived from expert elicitation, Monte Carlo simulation, are used to calculate the distribution of the output. This technique involves the random sampling of each probability distribution
in the model to produce a large number of scenarios. The probabilistic approach is now becoming the preferred approach to quantitative assessments.

**Key Steps in Microbiological Risk Assessment**

As shown in figure 4, a microbiological risk assessment consists of four steps: hazard identification; exposure assessment; dose-response assessment or hazard characterization; and risk characterization (49).

**Hazard Identification.** The first step in risk assessment is hazard identification, the identification of known or potential health effects associated with a particular agent (75). Hazard identification should provide an estimate of variability in the behavior or responses between types of the same pathogen, so that the subsequent exposure assessment can take account of variations in behavior by processing. These variations in behavior may affect toxin production, growth range, thermo- resistance and survival, and provide a more certain basis for estimating the effectiveness of controls, and hence risks to consumers (11).
Exposure Assessment. Microbiological exposure assessment is overall models of the level of pathogens or toxins in foodstuff moving through the supply chain. It addresses the prevalence or likelihood of a hazard’s presence and the expected quantity of agent that might be present and consumed by an individual (75). In general, an assessment of exposure to foodborne pathogens requires two types of information: 1) The amount of food consumed and by whom 2) Where in the food chain the microbial hazard arises, and what factors affect the prevalence and concentration of the pathogen in the food at the time of consumption (54). The key desired outputs of an exposure assessment for foodborne pathogen are prevalence,
concentration, and, if possible, we need know the physiological state of a pathogen in foods at the point of consumption (27).

The risks associated with foodborne pathogens are influenced by a complex interplay of variable factors. A conceptual exposure model is used to describe the variables and their interactions that result in an exposure to foodborne pathogens (54). Typical conceptual exposure models are flow diagram containing boxes and arrows to illustrate relationship. It is a useful tool for communicating important pathway clearly and concisely and can be used to generate new questions about relationships that help formulate plausible risk analysis (81).

Typically in a conceptual exposure model, the various steps in the food production continuum are broke down to several components or steps (e.g. pre-harvest, post-harvest, transportation and storage, and preparation and food handling). The pathogen behavior can be modeled throughout each of the steps. The concentration at the conclusion of one step is the initial concentration for the next step.

In practice, data relating to frequencies of contamination and numbers of pathogen ingested are usually not available. Instead, data relating to pathogen levels and frequencies at some earlier stage in the food chain are used to infer levels and frequencies that could be expected to be present in consumption with a conceptual exposure model.

The microbial ecology of foods can be applied to the conceptual exposure model to increase the scientific credibility and utility of the risk assessment outcomes. For examples, major factors affecting the microbial ecology of foods have always been categorized four groups (55): intrinsic factors (the physicochemical properties of the food, such as structure, water activity, acidity, etc), extrinsic factors (conditions of the environment, such as temperature), implicit factors (the physiological characteristics of the microbes) and
processing factors (actions during processing and distribution that change the ecology of food). These four categories offer systematic frameworks for identification of factors that could affect the frequency and levels of contamination of pathogens in foods. As such, they can be useful aids for developing conceptual models for microbial risk assessment of foods that encompass the influence of microbial ecology and physiology (27).

It was consider the microbial response in foods were highly unpredictable under temporally conditions in traditionally microbial ecology. In recent decades, mathematical approaches such as predictive microbiology models, which predict the growth, survival and inactivation responses of microorganisms to different environmental conditions, including water activity, storage temperature, pH, etc. have provided necessary information that can be developed for each step in an exposure model, from production up to preparation and prior to consumption (13). By assigning values to the variables (inputs) in the model, the equations describing the origin and amount of a pathogen in the food and its activity level can be solved to yield a numerical estimate of exposure at various points during production, storage, distribution. Models have also been developed the health impact of consuming specific numbers of microbial pathogens (12). These dose-response relationships consider the pathogenicity of the microorganism (virulence), the food matrix (fat levels, physical distribution of pathogen in food) and the susceptibility of the person consuming the pathogen. Once these are determined, a decision can be made on whether the levels are acceptable or not and what action, if any, is needed to minimize the risk to the consumer.

There are primary, secondary and tertiary models used for predictive modeling. Primary models are those that describe the change in microbial populations over time under constant environmental conditions, Secondary models describe the influence of key
environmental conditions on the parameters of the primary model (13), such as lag time, D-value or exponential growth rate. Tertiary models integrate data for all aspects of response of microbes to their environment into expert systems or decision support systems. The risk assessor’s interests center on secondary models in combination with primary model, and translate information about environmental conditions into predictions of the size of microbial populations over time under different processing scenarios.

**Hazard characterization.** It is often referred to a dose-response assessment because it describes the relationship between levels of a pathogen consumed (dose) and the probability of subsequent development and severity of illness or other adverse health outcome. Coleman *et al* (49) discussed some important topics in this dose-response relationship. For example, the probability of illness is a complex function of factors associated with the disease triangle: the host, the pathogen, and the environment including the food vehicle and indigenous microbial competitors. Two hypotheses were also used to describe dose-response relationship. The threshold model assumed that there was some level of the pathogen that particular individuals can tolerate without becoming infected. Conversely, non-threshold models assumed that a single microbial cell was capable of causing illness. Types of data used to understand dose-response relationships include clinical studies, epidemiological and active surveillance studies, animal studies, in vitro studies, biomarkers, and expert opinion (84).

**Risk characterization.** It is defined as the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a population based on hazard identification, exposure assessment, hazard characterization (22, 33). When risk estimate is quantitative, the results
are expressed as probabilities of potential adverse outcomes for an individual on per serving basis (e.g., 1 illness per 10,000 servings), individual basis (e.g., chance of foodborne illness is 1/20,000 per year), or population-based risk (e.g., 10 annual illness in the United States).

**Variability and Uncertainty.** The population variability and uncertainty of estimated parameters should be clearly and distinctly described when conducting a risk assessment. Variability refers to temporal, spatial, or inter-individuals difference (heterogeneity) in the value of an input. It is an inherent property of all physical, chemical, and biological systems. Uncertainty refers to the incompleteness of one’s knowledge or information, such as random and systematic errors, as well as reliance on models or surrogate indicators, which are all source of uncertainty. Often uncertainty exists when assumptions have to be made about the range of an unknown quantity and their probabilities of occurrence. The degree of uncertainty can be reduced by the acquisition of new data or knowledge, whereas additional data will not decrease variability.

In a quantitative risk assessment, variability and uncertainty are always estimated quantitatively, the specific method may vary and may include the use of distributions, or scenarios (e.g., mean, worst case, etc). There is no specific way in any input parameter are retained and reflected precisely in the final risk estimate in qualitative risk assessment, variability and uncertainty are always evaluated in narrative, descriptive terms.

**Sensitivity Analysis.** The term sensitivity refers to the total effect of an input on the risk estimate and sensitivity analysis refers to the process of determining and describing those factors which most affect the results of the risk estimate. Such analysis can provide insight into how real world system is sensitive to perturbation of some of its
components or processes, assuming that such relationships are adequately represented in the model.

This analysis is important in handing uncertainty, information on distributions of factors with most effect, and especially correlations between them, is much poorer than information on typical or average values. Uncertainty of the most sensitive inputs has the largest effect on predicted risk outcomes. Such uncertainty is often mitigated by stating the sensitivity conclusions in conditional terms such as: “If input changes by x and nothing else changes then risk changes by y” (12).

Sensitivity analysis can be used to determine and describe the most influential steps in the farm-to-table chain that have most effect on the predicted risk, by inference, those steps in a food chain can be considered as a potential critical control point in HACCP system (19). The use of sensitivity analysis to identify potential critical control points (CCPs) was well illustrated in a modeling the risk associated with *Escherichia coli* O157:H7 in ground beef hamburgers (17), the factors most affecting risk were host susceptibility, the concentration of *Escherichia coli* O157:H7 in the faeces of those cattle shedding the pathogen, the cooking preference of consumers and retail storage temperature. The efficacies of three risk mitigation strategies were evaluated by modifying the values of the predictive factors and comparing the new predicted risk. The per meal illness was predicted to be reduced by 80% by lowering storage temperature. This strategy was predicted to be more effective than a hypothetical intervention which estimated a plausible reduction in the concentration of E. coli O157:H7 in the feces of cattle shedding the pathogen and one aimed at convincing consumers to cook hamburgers more thoroughly.
The results of sensitivity analysis are subjected to the structure and assumptions inherent in the model (17). It also should be noted, that sensitivity analysis is based upon the correlation between the variability in the output and the variability in the input factors (87). For example, if a model predicting the extent of microbial growth included the assumption that temperature were controlled throughout the production within a very narrow interval, the output might not be sensitive to temperature even through temperature is known to have a significant influence on the growth of microorganisms.
Summary of Literature

Over the last ten years, quantitative risk assessment has emerged as an accepted, science-based approach to making choices between options for managing the microbiological safety of food. A risk assessment typically aims at describing the complex dynamics of a pathogen in a single food commodity during food processing, and estimate the relative public-health effect of different interventions strategies-alone and in combination. Within the risk assessment, exposure assessment provides an estimate of the occurrence and levels of the pathogen in a specified portion of food at the time of consumption.

In a comparative risk assessment for Non-tenderized and tenderized beef by USDA and FSIS, March 2002, the risk of illness per serving from intact versus non-intact beef steaks and roasts prepared using traditional cooking practices (grilling, broiling, and frying) were evaluated. The results found non-intact beef does not pose a greater risk of illness than intact beef. On February 1, 2010, the American Meat Institute (AMI) urged USDA’s Food Safety and Inspection Service to revise the agency’s “Comparative Risk Assessment for Intact (Non-Tenderized) and Non-Intact (Tenderized) Beef, March 2002” in light of a new analysis of foodborne illness outbreaks linked to tenderized products. Marinated or enhancement solution-added products were also not differentiated in the 2002 risk assessment.

To evaluate the microbiological hazards of technologies used in the production of moisture enhanced pork and understand the behavior of Salmonella enterica and Campylobacter jejuni in the entire food system from production to consumption so that interventions could be identified, we defined the process of moisture enhanced pork as beginning with the moisture enhancement of the raw pork through the final cooking prior to
consumption (Figure 5) and studied the microbiology of this process from a quantitative standpoint by analyzing the effects of intrinsic, extrinsic, implicit and processing factors that may influence on the survival throughout each of the steps in the process. The results provided can be used in exposure assessments of *Salmonella enterica* and *Campylobacter jejuni* in moisture enhanced pork.

Figure 5. A conceptual process for the production, preparation of moisture enhanced pork.
References


CHAPTER 3. TRANSLOCATION OF SURFACE-INOCULATED *E.COLI* BIOTYPE I INTO PORK SUBPRIMALS FOLLOWING MOISTURE ENHANCEMENT AND SLICING

A manuscript to be submitted to *Journal of Food Protection*

Xuesong Wen, James S Dickson

Abstract

Pork loins were surfaced inoculated with *Escherichia coli* Biotype I and pumped to 10%, 20% and 30% added moisture, through a needle injector. After injection, pork loins were sliced into 1cm thick slices. *Escherichia coli* Biotype I bacteria were recovered from both the surface and interior tissue of the sliced meat. Before injection, the surface and interior tissue of each pork loin was found no *Escherichia coli* Biotype I bacteria. *Escherichia coli* Biotype I were recovered from both the surface and interior tissue of the sliced meat. The mean number of *Escherichia coli* biotype I recovered from the interior of sliced meat with 10%, 20% and 30% were 4.19±0.07, 4.07±0.23 and 4.16±0.19 log_{10}CFU/g, respectively. The mean number of *Escherichia coli* biotype I recovered from surface of sliced meats with 10%, 20% and 30% were 5.45±0.11, 5.51±0.13 and 4.76±0.01 log_{10}CFU/g, respectively. Thus, *Escherichia coli* Biotype I were translocated from the surface to the depth of approximately 1 cm in the boneless pork following moisture enhancement and slicing.

Introduction

Pork processors are currently using the moisture enhancement process to produce both boneless and bone-in pork products for the retail market. Fresh pork loins are injected with brine solutions containing alkaline phosphates, sodium chloride, potassium or sodium lactate and other flavors. The addition of these ingredients has been shown to improve
juiciness, tenderness, and flavor (1). Moisture enhanced pork products has become a common practice in meat industry and well received by consumers.

Mechanical tenderization is another process to improve meat tenderness, it is usually applied at central cutting or retail store facilities by piercing prime cuts are fabricated into roasts or steaks (2). Both mechanical tenderization and moisture enhancement may introduce bacteria normally are present only on meat surfaces into the interior of muscle (6, 10). But bacteria transfer maybe different between the process of mechanical tenderization and moisture enhancement. It was reported that most of the bacteria carried into the tissues from the surface by tenderizing blades are found near the incised surface during mechanical tenderizing of beef (3, 7). In moisture enhancement process, large numbers of bacteria can accumulate in brines which are recirculated in equipment used for injecting brines into raw meats (4), while brine is distributed throughout the product, albeit unevenly (12). However reports on the distribution of bacteria in meats injected with brine appear to be lacking.

During the production of enhanced pork products, slicing is always followed by enhancement to produce pork products with desired thickness to consumer, but this process also can introduce bacteria normally are present only on meat surfaces into other parts of meat, that were previous sterile. The introduction of bacteria into the interior or other parts of sliced meat has potential to reduce storage life and to increase the risk of foodborne illness for the consumer.

*Escherichia coli* Biotype I maybe useful as indicators for *Escherichia coli* O157:H7 (8) and *Salmonella enterica* (9), as a result of various meat processes, such as storage, fermentation etc. To study the microbiological contamination of the deep tissue, we inoculated *Escherichia coli* Biotype I onto the lean–side surface of top subprimal pork loins
and assessed the contamination in the interior and surface of sliced pork after moisture enhancement and slicing and the effect of the added moisture on the transfer of bacteria into the interior muscle.

**Materials and methods**

**Bacterial Cultures.** A mixed culture of five strains of non-pathogenic, *Escherichia coli* Biotype I was prepared. The five surrogate bacteria are all nonpathogenic *E. coli* strains, previously described by Marshall et al (8), which were isolated from cattle hides. The strains were cultured separately in tryptic soy broth (TSB) at 37°C, and then mixed in equal volumes. The strains were previously characterized and identified as suitable surrogates for both *Escherichia coli* O157:H7 (8) and *Salmonella enterica* (8). Table 1 shows the ATCC strain designations that were assigned to these isolates, as well as the internal designation that was used by Marshall et al. in 2005 (8).

**Inoculation and moisture enhancing of pork loins.** The mixed culture of *Escherichia coli* Biotype I was inoculated on to the surface of whole boneless pork loins at a target population of $10^6$ colony forming units/cm$^2$ using a foam paint brush. The pork was then subjected to a single pass through a needle injector moisture enhancement process, with a target injection of 10%, 20%, 30% (wt/wt; P-10 Pokomat Injector, Quality Food Equipment, El Monte CA). Each pork loins was weighed before and after injection to determine the percentage of solution added to the meat by weight. A brine solution that was composed of water plus sodium tripolyphosphate and sodium chloride, the moisture enhancement resulted in 0.2% sodium chloride and 0.3% sodium tripolyphosphate in the enhanced loins.
Sample analysis. Before injection, the populations of *Escherichia coli* biotype I were enumerated on both the surface and internally at a depth of approximately 1 cm in each boneless pork loin as controls. After injection and slicing, the populations of *Escherichia coli* biotype I were enumerated on both the surface and internally at a depth of approximately 1 cm in the boneless pork at three locations on each pork loin: approximately 6 cm from the leading edge, 6 cm from the trailing edge and the approximate geometric center of the loin. Samples were collected aseptically using a sterile scalpel and forceps. Samples were homogenized 1:10 in sterile buffered peptone water with a Tekmar Stomacher 400 Mk. II for 2 min (Tekmar, Cincinnati, OH). Bacterial populations were enumerated by surface plating on Violet Red Bile Glucose Agar (VRBA). The plates were incubated at 37°C for 24 hours, and then manually counted. The minimum detection limit was 10 cfu/g.

Statistical design and analysis. The experiment was independently replicated three times using a split plot design, with loin as whole-plot and slices as split-plot. The bacterial populations were transformed to log$_{10}$ cfu/g, significant levels were determined at P<0.05. Data was analyzed using procedure of the Statistical analysis System software program, version 9.2 (SAS Institute Inc., Cary, N.C.).

Results and Discussion

No *E. coli* biotype I were recovered from the deep tissues of each pork loin before injection. The mean number of *Escherichia coli* biotype I recovered from the interior of sliced meats with 10%, 20% and 30% were 4.19±0.07, 4.07±0.23 and 4.16±0.19 log$_{10}$CFU/g, respectively. There were significantly (P<0.05) more cells of *E.coli* biotype I on the surface than that in the interior for each sliced meat. The mean number of *Escherichia coli* biotype I recovered from surface of sliced meats with 10%, 20% and 30% were 5.45±0.11, 5.51±0.13
and 4.76±0.01 log_{10}CFU/g, respectively (Table 2). Results indicated that moisture enhancement and slicing carried bacteria from the surface into the depth of approximately 1 cm in the boneless pork. Previous research by Sporing (11) had suggested that that relatively few bacteria (2-3%) were transferred from inoculated to un-inoculated beef when the beef was mechanically tenderized. It was also reported that blade tenderization transferred *E. coli* O157:H7 primarily into the topmost 1 cm of beef subprimals (7). In our study we found much higher percentages (~70%) transferred by moisture enhancement.

Some studies (4, 5) investigated the microbiological conditions (aerobic bacteria, coliform, etc) of moisture enhanced meat prepared at packing plants. Those results indicated that the numbers of bacteria on the surface of pork or chicken before and after injection were similar. In this study, after injection and slicing, the number of *Escherichia coli* biotype I recovered from deep tissues after injection there was no significant ($P>0.05$) affected by the number on the surface of enhanced pork subprimals. The number of *Escherichia coli* biotype I recovered from deep tissues after injection and slicing was also not significantly affected ($P>0.05$) by moisture enhancement level and different locations in pork loins (approximately 6 cm from the leading edge, 6 cm from the trailing edge and the approximate geometric center of the loin). J.B.Luchansky *et al* (7) observed that, translocation was not also appreciably influenced by whether the inoculum was applied to the fat or lean side of subprimal nor was it appreciably influenced by the number of times the subprimal was passed through the tenderizer.

Moisture enhancement, followed by slicing and then vacuum packing is a common practice for the production of enhanced pork products. It provides the sliced meat with desired thickness to consumer. However, bacteria can be transferred from the surface to
the deep tissue of sliced pork subprimals following by moisture enhancement and slicing. Since the behaviors of pathogen in the deep tissue of sliced pork subprimals are unknown during production and preparation, concerns about the microbiological safety of such products should be addressed because people may regard enhanced products as intact meats and only thoroughly cook surface tissue (6), for example, grilling without raising all deep tissues to temperatures that will certainly destroy pathogenic bacteria that may be present, further studies are in process to evaluate the survival of pathogenic bacteria during storage and cooking.

**Acknowledgements**

The authors acknowledge Mrs. Jing Li (Department of Statistics, Iowa State University) for her statistical advice and Steven Niebuhr (Department of Animal Sciences, Iowa State University) for his technical laboratory assistance.

**References**


Table 1 ATCC accession numbers that correspond to the internal designators used in the study of bacteria translocation.

<table>
<thead>
<tr>
<th>E. coli strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ATCC accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>BAA-1427</td>
</tr>
<tr>
<td>P3</td>
<td>BAA-1428</td>
</tr>
<tr>
<td>P8</td>
<td>BAA-1429</td>
</tr>
<tr>
<td>P14</td>
<td>BAA-1430</td>
</tr>
<tr>
<td>P68</td>
<td>BAA-1431</td>
</tr>
</tbody>
</table>

<sup>a</sup> As noted in Marshall et al (8).
Table 2. The numbers *Escherichia coli* Biotype I recovered from the surface and interior of meat after moisture enhancement.

<table>
<thead>
<tr>
<th>Moisture enhancing Levels (%)</th>
<th>Location</th>
<th>The populations of <em>E. coli</em> Biotype I (log(_{10})CFU/g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exterior(^d^)</td>
<td>Interior(^e^)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Front(^a^)</td>
<td>5.54±0.25</td>
<td>4.22±0.06</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Mid(^b^)</td>
<td>5.38±0.16</td>
<td>4.37±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Back(^c^)</td>
<td>5.54±0.22</td>
<td>4.00±0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>5.45±0.11</td>
<td>4.19±0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Front(^a^)</td>
<td>5.39±0.05</td>
<td>4.03±0.38</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Mid(^b^)</td>
<td>5.80±0.35</td>
<td>3.91±0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Back(^c^)</td>
<td>5.34±0.15</td>
<td>4.28±0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>5.51±0.13</td>
<td>4.07±0.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Front(^a^)</td>
<td>4.96±0.11</td>
<td>4.40±0.40</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Mid(^b^)</td>
<td>4.73±0.22</td>
<td>3.87±0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Back(^c^)</td>
<td>4.60±0.15</td>
<td>4.20±0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>4.76±0.01</td>
<td>4.16±0.19</td>
<td></td>
</tr>
</tbody>
</table>

Note. Counts are expressed as the mean (±standard error) log counts

\(^a^\) Front represents 6 cm from the leading edge

\(^b^\) Mid represents the geometric centre of pork loin

\(^c^\) Back represents 6 cm from the trailing edge

\(^d^\) Exterior represents the surface of the boneless pork loin

\(^e^\) Interior represents the depth of approximately 1 cm in the boneless pork loin
CHAPTER 4. SURVIVAL AND GROWTH OF CAMPYLOBACTER JEJUNI AND SALMONELLA ENTERICA IN MOISTURE ENHANCED PORK DURING VACUUM STORAGE

A manuscript to be submitted to Journal of Food Protection

Xuesong Wen, James S Dickson

Abstract

Translocation of bacterial pathogens into the interior tissue of pork meat through moisture enhancement may be of concern if enhanced pork is undercooked. Little data exists in the public domain on the microbiological safety of the product and the quality of the process with pork. The present study was conducted to determine the extent of bacteria translocation through moisture enhancement and the effects of refrigeration, abuse temperature and moisture enhancing on the survival of Campylobacter jejuni and Salmonella Enterica in moisture enhanced pork during vacuum storage. In the moisture enhancement process, pork loins were surface inoculated with E.coli biotype I, bacteria translocated from the surface into the deeper tissue of pork following moisture enhancement. In a storage study, pork loins were surface inoculated with either Campylobacter jejuni or Salmonella enterica and then subjected to a single pass through a needle injector moisture enhancement process, with a target injection volume of 10% and 20%. The moisture enhanced pork loins were sliced into 1 cm thick slices and vacuum packaged. Samples were collected, plated and the populations of survival organisms were analyzed periodically during storage at 4°C and 10°C. There was no significant effect of moisture enhancement on the populations of Campylobacter jejuni and Salmonella enterica in samples (P>0.05). After 28 days, the populations of Campylobacter jejuni and Salmonella enterica in samples were significantly lower (P<0.05) than those of day 0. Mean populations of Campylobacter jejuni and
*Salmonella enterica* in samples at day 28 were 4.24, 4.78 log_{10} CFU/g, respectively. No significant differences in *Campylobacter jejuni* counts were observed between samples at abuse temperature (10°C) and those at the refrigerated temperature (4°C). In contrast, the population size of *Salmonella enterica* in samples at abuse temperature (10°C) was significantly (P<0.05) higher than those at refrigerated temperature (4°C). This study indicates that, vacuum packing under chilled conditions alone is not a substitute for safe handling and proper cooking. The event of temperature abuse during handling should be avoided. Further studies are necessary to evaluate the survival of these pathogens in moisture enhanced pork following cooking.

**Introduction**

The process of injecting brine solutions into meats has been well developed in pork. Brines containing various ingredients such as salt, phosphates, sodium lactate and lemon juice have been reported to improve pork juiciness, tenderness, and flavor (2).

Although moisture enhanced products have been available for some time, relatively little is available within the public domain regarding the microbiological properties of the product. It was reported that brine injection did not affect color or odor deterioration, and the storage life for vacuum packaged loins was the same as that for non injected loins. From a food safety perspective, moisture enhancement process may introduce bacteria into the interior meat from the meat surface or re-circulating brines, which may increase the risk of foodborne illness for the consumer (14, 17, 18, 23).

Vacuum packaging under chilled conditions has been proved to be very effective for preventing the growth of spoilage bacteria, in extending the shelf of red meat, and preventing the growth of some food-borne pathogens (4). However, some pathogenic bacteria, such as
facultative anaerobic, psychrotrophic pathogens, especially *Listeria monocytogenes*, like low-oxygen environments and reproduce well in vacuum-packaged foods. Without competition from spoilage bacteria, such pathogens reproduce even more rapidly. Such food may become unsafe from pathogenic bacterial growth with no indicators caused by spoilage bacteria to warn the consumer. Another potential concern is *Salmonella*, which may constitute a risk when a temperature abuse occurs in the commercial chain of food handling. This situation may also occur with other mesophilic pathogenic bacteria (8).

*Campylobacter jejuni* is among the most frequency reported causes of bacterial gastroenteritis in humans. *Campylobacter* have an optimal growth temperature range of 37 to 42°C and do not grow below 30°C, but *Campylobacter jejuni* has been shown to display physiological activity at 4°C (13) and can survive in water for several weeks (20). The infectious dose of *Campylobacter jejuni* has been reported to be very low (3) and growth may not be a prerequisite for disease. Published data on the microbiological condition of the deep tissues of moisture enhanced pork during production are few. Hence, the objectives of our study were to determine the effects of refrigeration, abuse temperature and moisture enhancement on the survival of *Campylobacter jejuni* and *Salmonella enterica* in moisture enhanced pork during vacuum storage. The information obtained will help identify conditions for the safe storage and handling of moisture enhanced pork that can be used as guideline to reduce the possibility of growth and survival of these pathogens in moisture enhanced pork.
Materials and Methods

**Bacteria cultures.** Five-strain cocktails of *Salmonella enterica* serotype Typhimurium phage type DT104 (G29, G30, G32, G33 and G34) were obtained from CDC for this study. These strains were all isolated from human clinical. Five-strain cocktails of *Campylobacter jejuni* were obtained from Dr Qijing Zhang lab in the department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa. Of these strains, three strains (CT 1:1, CT 2:2, CT 3:7) were isolated from turkeys, and two strains (Clev9100, F12469) were isolated from human.

*Salmonella Typhimurium* cultures were maintained on tryptic soy agar slants at 4°C. The *Salmonella Typhimurium* cultures were individually grown in 10 ml of Tryptcase soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) at 35°C for 24 h. A 1-ml aliquot from each individual strain culture was combined to give a 5-ml mixed culture of *Salmonella Typhimurium*, and 2 ml of this culture mixture was transferred to 98 ml of peptone water to make an inoculum concentration appropriate to achieve $5 \log_{10} \text{CFU/g}$ on the chops.

*Campylobacter jejuni* cultures were maintained on Charcoal Cephoperazone Desoxycholate Agar (CCDA). Individual cultures of *Campylobacter jejuni* were grown in 10 ml of Bolton selective enrichment Broth with 5% lysed horse blood and incubated for 24 h at 37°C under microaerophilic conditions. A 1-ml aliquot from each individual strain culture was combined to give a 5-ml mixed culture of *Campylobacter jejuni* and 2 ml of this culture mixture was transferred to 98 ml of peptone water to make an inoculum concentration appropriate to achieve $5 \log_{10} \text{CFU/g}$ on the chops.

**Inoculation and processing of pork loin.** For each experiment in storage study, six boneless pork loins were prepared. The mixed cultures of *Salmonella enterica* or
Campylobacter jejuni were inoculated on to the surface of each whole boneless pork loins at a target population of $10^6$ colony forming units/ml using a foam paint brush. The pork loins were randomly subjected to a single pass through a needle injector (P-10 Pokomat Injector, Quality Food Equipment, El Monte CA), with a target injection of 10% and 20% (wt/wt). A brine solution that was composed of water plus sodium tripolyphosphate and sodium chloride, the moisture enhancement resulted in 0.2% sodium chloride and 0.3% sodium tripolyphosphate in the enhanced loins. After moisture enhancement, each pork loin was sliced into 1cm thick slices, an inoculated pork loin was sliced without moisture enhancing as a control.

Storage and samples. All slices were randomly vacuum packed using a HencoVac 1700 machine (Howden food Equipment B.V., The Netherlands). The vacuum packaged slices were stored at 4°C and 10°C for 28 days. Samples were analyzed at 0, 3, 7, 10, 14, 21, and 28 days. On each sampling day, individual packages were opened, and single slices were randomly selected for analysis.

Microbial analysis of enhanced meats during storage. Two gram portions were aseptically excised from the interior of the meat with a sterile scalpel and forceps, serially diluted as necessary in 0.1% peptone water and stomached for 60 s. Salmonella enterica populations were enumerated with the DW Scientific Whitley Automatic Spiral Plater (West Yorkshire, England) onto Xylose Lysine Desoxycholate (XLD) medium for 24h at 37°C. Campylobacter jejuni populations were also enumerated by spiral plating onto Columbia Blood agar with CCDA Selective Supplement under microaerophilic conditions for 48h at 37°C. The population counts obtained from each analysis were converted to $\log_{10}$ CFU/g. The experiments were independently replicated three times with each sample being assayed
in duplicate. The minimum detection limit was 1.30 cfu/g, based on plating 50 μl of the sample on each of two replicate plates.

**Statistical design and analysis.** The experiment in translocation study was independently replicated three times using a split plot design, with loin as whole-plot and slices as split-plot. The experiment in storage study was also independently replicated three times using a split plot design, with bacteria inoculation and moisture treatment as whole plot factors and storage treatments as a sub plot factor. All data were compiled and statistically analyzed by ANOVA using the PROC MIXED procedure in Statistical Analysis System software, version 9.2 (SAS Institute Inc., Cary, N.C.). Significance was established at p <0.05 for ANOVA and mean separation using Tukey's Range test.

**Results and Discussion**

Bohaychuk *et al* (22) found there were no consistent injection treatment effects on the growth of some spoilage bacteria, such as pseudomonad, in moisture enhanced pork during storage at 2°C or 5°C. No significant (*P*>0.05) effects of moisture enhancement on the population sizes of *Campylobacter jejuni* and *Salmonella enterica* in samples were also found in our study (Table 2,Table 3). It is tempting to speculate that the brine injected was bacteriostatic and that may have accounted for differences in bacterial growth. But the hypothesis was not supported.

There were no significant (*P*>0.05) differences in the population sizes of *Campylobacter jejuni* between samples at the abuse temperature (10°C) and those at refrigerated temperature (4°C ; Table 2). It has been reported that the minimal growth temperature of *Campylobacter jejuni* is in the range of 31 to 36°C (11, 13). The refrigeration temperature and storage temperature prevent from the growth of *Campylobacter jejuni* in
moisture enhanced pork. Results were different for *Salmonella*, the population size of *Salmonella enterica* in samples at 10°C was significantly (*P*<0.05) larger than those at 4°C (Table 3). The least square means for *Salmonella enteritidis* growth pooled over time reveal that temperature abuse results in pathogen increase of 0.41 log<sub>10</sub> cfu/g. *Salmonella* is considered mesophilic with an optimal growth range between 30 and 45°C, can grow within a few days (6 to 10 days) at 2 to 7°C (6). This finding is consistent with those of other studies for the growth of *Salmonella* under different packaging methods. Nychas et al (8) reported that no growth of *Salmonella enteritidis* were observed in modified atmospheres at refrigeration temperatures (e.g.3°C), but the numbers of *Salmonella enteritidis* increased rapidly in samples flushed with 100% nitrogen,CO<sub>2</sub>/air (20:80) and in vacuum-packaged samples at 10°C.

Temperature abuse is common throughout distribution and retail markets. Some commercial equipment is incapable of maintaining foods below 7.2°C because of refrigeration capacity, insufficient refrigerating medium or poor maintenance. Most warehouses and transport vehicles in distribution chains maintain temperatures in the 0-3.3°C range. It must be assumed, however, for the purposes of assessing risk, that occasionally temperature of 10°C or higher may occur for extended periods (5). Our results indicate temperature abuse should be avoid for the supply chain for moisture enhanced pork.

Vacuum storage at 4°C and 10°C for 28 days resulted in a small decrease in the mean populations of *Campylobacter jejuni* and *Salmonella enterica* count (Figure 1 and Figure 2). Significant differences (*P*<0.05) were observed for *Campylobacter jejuni*, with counts at day 0 higher than those after 3 days (Table 4). Compare to *Campylobacter jejuni*, *Salmonella enterica* was observed more resistant to adverse conditions provided by vacuum packing.
There were no significant differences in the population sizes of *Salmonella enterica* from 0 days to 14th day \((P >0.05)\). Significant \((P<0.05)\) reductions in mean populations of *Salmonella enterica* were observed at day 21 and 28 (Table 4). Vacuum storage for 28 days did not result in dramatic reductions in the populations of *Campylobacter jejuni* and *Salmonella enterica*. The least square means for *Campylobacter jejuni* and *Salmonella enterica* in samples at 28th day pooled over temperature were 4.24, 4.78 log\(_{10}\)CFU/g, respectively (Table 4). Overall, vacuum packing at 4°C and 10°C can prevent the growth of *Campylobacter jejuni* and *Salmonella enterica* in pork meat. But vacuum packing alone can’t add a significant margin of safety with respect to these pathogens in moisture enhanced pork and cannot replace sanitary production and handling. This is the first known report about the changes in levels of contamination of moisture-enhanced pork during vacuum storage. Further studies are in progress to evaluate the survival of these pathogens in moisture enhanced pork and non moisture enhanced pork following cooking.

Over the last ten years, risk assessment has emerged as an accepted, science-based approach to making choices between options for managing the microbiological safety of food \((15, 17)\). It is a systematic process based on four inputs, hazard identification, exposure assessment, hazard characterization and risk characterization. The microbial ecology of foods can be applied to the conceptual exposure model to increase the scientific credibility and utility of the risk assessment outcomes. For example, major factors affecting the microbial ecology of foods have always been categorized into four groups: intrinsic factors, extrinsic factors, implicit factors and processing factors \((20)\). These four categories offer systematic frameworks for identification of factors that could affect the frequency and levels of contamination of pathogens in foods. As such, they can be useful aids for developing
conceptual models for microbial risk assessment of foods that encompass the influence of microbial ecology and physiology (7). In this study, the influences of moisture enhancement, refrigeration, abuse temperature and moisture enhancement on the survival of bacteria in moisture enhanced pork during production can be useful information for developing the microbial risk assessment of moisture enhanced pork.

Acknowledgements

The authors acknowledge Mrs. Jing Li (Department of Statistics, Iowa State University) for her statistical advice and Steven Niebuhr (Department of Animal Sciences, Iowa State University) for his technical laboratory assistance.

References


Figure 1. Effects of moisture enhancement processing on the growth of *Campylobacter jejuni* on boneless pork loins during vacuum storage at 4°C (A) and 10°C (B). The bars represent the mean ± standard error.
Figure 2. Effect of moisture enhancement processing on the growth of *Salmonella enterica* on boneless pork loins during vacuum storage 4°C (A) and 10°C (B). The bars represent the mean ± standard error.
TABLE 2. Summary of ANOVA on the numbers of *Campylobacter jejuni* in pork slices during vacuum storage.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>2</td>
<td>6</td>
<td>1.72</td>
<td>0.2569</td>
</tr>
<tr>
<td>Temperature</td>
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<td>78</td>
<td>0.01</td>
<td>0.9403</td>
</tr>
<tr>
<td>Time</td>
<td>6</td>
<td>78</td>
<td>7.69</td>
<td>&lt;0.0001 *</td>
</tr>
<tr>
<td>Moisture*temperature</td>
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<td>78</td>
<td>2.64</td>
<td>0.6908</td>
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<tr>
<td>Moisture*time</td>
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<td>78</td>
<td>0.66</td>
<td>0.2548</td>
</tr>
<tr>
<td>Temperature*time</td>
<td>6</td>
<td>78</td>
<td>2.03</td>
<td>0.9866</td>
</tr>
<tr>
<td>Moisture<em>temperature</em>time</td>
<td>12</td>
<td>78</td>
<td>0.22</td>
<td>0.9925</td>
</tr>
</tbody>
</table>

Note: Mixed models with moisture, temperature, time and their interactions as fixed effects, replication of experiment as a random factor. Factor abbreviations are: moisture=marture enhancing level, temperature=storage temperature, time=storage time (days). Significant effects (p<0.05) are marked with asterisks (*).
### TABLE 3. Summary of ANOVA on the numbers of *Salmonella enterica* in pork slices during vacuum storage.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Numerator df</th>
<th>Denominator df</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>Temperature</td>
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<td>78</td>
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<td>&lt;0.0001 *</td>
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<td>0.66</td>
<td>0.7851</td>
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<tr>
<td>Temperature*time</td>
<td>6</td>
<td>78</td>
<td>2.03</td>
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<tr>
<td>Moisture<em>temperature</em>time</td>
<td>12</td>
<td>78</td>
<td>0.22</td>
<td>0.9969</td>
</tr>
</tbody>
</table>

Note: Mixed models with moisture, temperature, time and their interactions as fixed effects, replication of experiment as a random factor. Factor abbreviations are: moisture=moisture enhancing level, temperature=storage temperature, time=storage time (days). Significant effects (P<0.05) are marked with asterisks (*).
TABLE 4. Least square mean populations of *Campylobacter jejuni*\(^a\) and *Salmonella enterica* \(^b\) in boneless pork during vacuum storage.

<table>
<thead>
<tr>
<th>Time(days)</th>
<th>Campylobacter jejuni Mean(^a)</th>
<th>Salmonella enterica Mean(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.11 A</td>
<td>5.36 D</td>
</tr>
<tr>
<td>3</td>
<td>4.73 B</td>
<td>5.22 D</td>
</tr>
<tr>
<td>7</td>
<td>4.62 B</td>
<td>5.27 D</td>
</tr>
<tr>
<td>10</td>
<td>4.64 B</td>
<td>5.20 D</td>
</tr>
<tr>
<td>14</td>
<td>4.41 C</td>
<td>5.12 D</td>
</tr>
<tr>
<td>21</td>
<td>4.39 C</td>
<td>4.93 E</td>
</tr>
<tr>
<td>28</td>
<td>4.24 C</td>
<td>4.78 E</td>
</tr>
</tbody>
</table>

\(^a\) Means with a letter in common are not significantly different \((P>0.05)\). The Standard error of the least squares mean = 0.1455, \(n=126\).

\(^b\) Means with a letter in common are not significantly different \((P>0.05)\). The Standard error of the least squares mean = 0.1375, \(n=126\).
CHAPTER 5. EFFECTS OF MOISTURE, STORAGE AND SUBSEQUENT COOKING ON INACTIVATION OF CAMPYLOBACTER JEJUNI AND SALMONELLA ENTERICA IN MOISTURE ENHANCED PORK

A manuscript to be submitted to Food Microbiology

Xuesong Wen, Jing Li, James S Dickson

Abstract

This study was undertaken to evaluate the influence of moisture enhancing levels, storage temperatures, and cooking temperatures on inactivation of Salmonella enterica and Campylobacter jejuni on moisture enhanced pork during storage and cooking. Boneless pork loins were surfaced inoculated with Salmonella enterica or Campylobacter jejuni and injected with brine (sodium chloride, sodium phosphate) at 10% and 20%. The moisture enhanced pork loins were sliced into 1cm thick slices and vacuum packaged at 4°C and 10°C. The slices stored at 4°C for days 21 and the slices stored at 10°C for days 14 were cooked to temperatures at the centre of at 155°F (68.3°C), 160°F (71.1°C), 165°F (73.9°C), and 170°F (76.7°C), with holding for 0 min after cooking before excision of interior muscles from each slice and enumeration of Salmonella enterica or Campylobacter jejuni from interior muscles. A Generalized Linear Mixed Model (GLMM) was created to determine the influence of all possible factors on the probability of bacteria survival (the proportion of samples from which bacteria were recovered on the medium) in pork meat slices after grilling. Overall, Campylobacter jejuni cells were significantly less resistant to various treatments than cells of Salmonella enterica. Higher internal temperature for cooking is more effective for complete bacterial inactivation. Cooking above 160°F may be adequate for assuring the microbiological safety of moisture enhanced pork slices that is prepared without excessive contamination of interior tissues. Significant interactions between storage
temperatures and moisture were observed. The model indicated moisture enhanced pork meat does not present a greater risk to consumers than otherwise similar meat that is intact, provided that the meat is properly cooked.

Keywords: Moisture enhanced pork; *Campylobacter jejuni*; *Salmonella enterica*; cooking

**Introduction**

*Salmonella enterica* and *Campylobacter jejuni* are amongst the more prevalent bacterial pathogens that cause foodborne diseases. Illnesses due to *Salmonella enterica* and *Campylobacter jejuni* have been linked to a variety of sources and foods, including pork products. It is noteworthy that *Salmonella enterica* and *Campylobacter jejuni* are present on the moisture-enhanced pork samples collected from stores in six cities in United States. The reported incidence of *Salmonella enterica* and *Campylobacter jejuni* on moisture-enhanced pork were 10.4% and 1.0%, respectively (6).

Moisture-enhanced pork is produced through multi-needle injection of brines or marinades that contain ingredients such as phosphates, salt, and potassium or sodium lactate. The moisture enhancement process has been shown to have a beneficial impact on the juiciness, tenderness and sensory quality. These pork products are currently popular with the consumer. According to a 2004 nationwide retail survey, 45% of the retail fresh pork in the United States was labeled as “enhanced” with products defined as moisture added and could be valued added (flavored and/or contained additional ingredients) (1). To provide a high-quality and safe product, the information of bacterial contamination in moisture-enhanced pork should be known.
Relatively recent outbreaks due to *E.coli* O157:H7 have been occasionally and presumably linked to non-intact that is mechanically tenderized, steaks (2, 3, 15, 16). There were some studies which reported the translocation of surface-inoculated pathogen into the interior of meat muscles following moisture enhancement (9, 11, 14) and the recovery of aerobes, coliforms, and staphylococci/listeriae from the deep tissues of moisture enhanced meat at a packing plant and retail (4). In addition, recirculating brines harbor large bacterial populations and a significant increase in *Listeria monocytogenes* has been observed during recirculating (8). The potential for contamination in brines, meat surface and subsequent transfer of pathogens into in the deep tissue of moisture enhanced meat were important considerations if such products are not adequately cooked.

Over the last 10 years, the use of models to quantitatively describe the transmission of pathogens over food production is increasing in quantitative microbial risk assessment (QMRA). Such models may cover the whole “farm-to-fork” food pathway or only the part that is relevant to the problem to assess risk to consumers associated with pathogens in foods. In order to contribute to a better understanding of the risk to human health from pathogens in moisture-enhanced pork, we designed some processing steps from the production of moisture enhanced pork to preparation and consumption, aimed at evaluating (i) the effectiveness of USDA recommended safe minimum internal cooking temperatures (18) on inactivate *Campylobacter jejuni* and *Salmonella enterica* in the interior muscle of moisture enhanced and intact pork (ii) the influence of different factors associated with these steps on the survival of *Campylobacter jejuni* and *Salmonella enterica* at consumption.
**Materials and methods**

**Bacterial strains.** Five-strain cocktails of *Salmonella enterica* serotype Typhimurium phage type DT104 (G29, G30, G32, G33 and G34) were obtained from CDC for this study. These strains were all isolated from human clinical. Five-strain cocktails of *Campylobacter jejuni* were obtained from Dr Qijing Zhang lab in the department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa. Of these strains, three strains (CT 1:1, CT 2:2, CT 3:7) were isolated from turkeys, and two strains (Clev9100, F12469) were isolated from human. *Salmonella Typhimurium* cultures were maintained on tryptic soy agar slants at 4°C. The *Salmonella Typhimurium* cultures were individually grown in 10 ml of Trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) at 35°C for 24 h. A 1-ml aliquot from each individual strain culture was combined to give a 5-ml mixed culture of *Salmonella Typhimurium*, and 2 ml of this culture mixture was transferred to 98 ml of peptone water to make an inoculum concentration appropriate to achieve 5 log$_{10}$ CFU/g on the chops. *Campylobacter jejuni* cultures were maintained on Charcoal Cephoperazone Desoxycholate Agar (CCDA). Individual cultures of *Campylobacter jejuni* were grown in 10 ml of Bolton selective enrichment Broth with 5% lysed horse blood and incubated for 24 h at 37°C under microaerophilic conditions. A 1-ml aliquot from each individual strain culture was combined to give a 5-ml mixed culture of *Campylobacter jejuni* and 2 ml of this culture mixture was transferred to 98 ml of peptone water to make an inoculum concentration appropriate to achieve 5 log$_{10}$ CFU/g on the chops.

**Pork loins.** For each experiment, six boneless pork loins were prepared. The mixed cultures of *Salmonella enterica* or *Campylobacter jejuni* were inoculated onto the surface of each whole boneless pork loin at a target population of $10^6$ colony forming units/ml using a
foam paint brush. The pork loins were randomly subjected to a single pass through a needle injector moisture enhancement process, with target injections of 10% and 20% (wt/wt). A brine solution that was composed of water plus sodium tripolyphosphate and sodium chloride, the moisture enhancement resulted in 0.2% sodium chloride and 0.3% sodium tripolyphosphate in the enhanced loins. After moisture enhancement, each pork loin was sliced into 1cm thick slices; an inoculated pork loin was sliced without moisture enhancement as control.

**Storage and Cooking.** All slices were randomly vacuum packed and stored at 4°C and 10°C, the slices stored at 4 °C for up to 21 days and slices stored at 10 °C for up to 14 days were randomly selected and placed in a George Foreman grilling machine (Lake Forest, IL). An uncooked slice from each treatment was also prepared as a control. A type J thermocouple (Oakton Instruments Vernon Hills, IL) was sterilized in acid alcohol and inserted into the geometric center of each slice to monitor internal temperature. When slices reached their target endpoint temperatures at 155°F (68.3°C), 160°F (71.1°C), 165°F (73.9°C), and 170°F (76.7°C), they were immediately removed from the grill with holding 0 min, then a two gram portion of each slice was aseptically excised from the interior of meat and transferred into a sterile Whirl-Pak stomacher bag (Nasco, Ft. Atkinson, WI, USA). The samples were homogenized 1:10 in sterile buffered peptone water with a Tekmar Stomacher 400 Mk. II for 60s (Tekmar, Cincinnati, OH).

**Microbial analysis.** Appropriate dilutions of *Salmonella enterica* were spiral plated on Xylose Lysine Desoxycholate (XLD) medium with the DW Scientific Whitley Automatic Spiral Plater (West Yorkshire, England). Plates were inoculated at 37°C for 24 h before counting colonies. *Campylobacter jejuni* populations were also spiral plated on to Columbia
Blood agar with CCDA Selective Supplement. Plates were inoculated at 37°C for 48 h under microaerophilic conditions. The population counts obtained from each analysis were converted to $\log_{10}$CFU/g. The experiments were independently replicated three times with each sample being assayed in duplicate. The minimum detection limit is 1.3 cfu/g, based on plating 50 μl of the sample on each of two replicate plates.

**Model fitting.** The experiments were independently replicated three times using a split plot design, with pork loins as whole plots and individual slices as sub plots. A generalized linear mixed model was fitted to the data using multivariable logistic regression. Logistic regression modeling has proved to be useful in examining the relationship between a set of independent variables and a dependent variable that takes only two dichotomous values (7). Logistic regression uses a linear combination of independent variables to explain the variance in a dependent variable having only two states. After grilling, the results of bacteria recovery from the interior meat have two possible outcomes, the absence or presence of bacterial cells on the medium. The moisture level of pork, the storage temperature for pork, the internal cooking temperature and different inoculated bacteria in pork may be affect the results of bacteria recovery from the interior meat after grilling. Here, the dependent variable was the absence or presence of bacteria cells and the independent variables were moisture, storage temperature, cooking temperature and different inoculated bacteria. Each sample can be represented through a binary variable $Y$, which indicates whether bacteria were recovered on the medium ($Y=1$) or were not recovered ($Y=0$), and these independent variables. The GLMM model takes the following form:

$$\text{Logit}(Y) = X\beta + Zu + \varepsilon;$$
Where logit (Y) denotes the logistic link function; X is the model matrix for the fixed effects; Z is the model matrix for the random effects; \( \beta \) is the fixed effect vector; \( u \) is the random effect vector at the whole plot level; \( \epsilon \) is the independently and identically distributed random error vector at the split plot level. The final model for fitting had the following form:

\[
\text{Logit} (Y) = \beta_0 + \beta_1 (bacteria)_i + \beta_2 (moisture)_j + \gamma_{ijk} + \beta_3 (storage)_l + \beta_4 (cooking)_m + \beta_5 (bacteria*storage)_{il} + \beta_6 (bacteria*cooking)_{im} + \beta_7 (storage*moisture)_{il} + \epsilon_{ijklm}
\]

The description of the variables in this model was listed in Table 2.

Once the model was selected, the probability of bacteria survival (the proportion of samples from which bacteria were recovered on the medium) in pork meat slices after grilling was calculated using the following formula (R software 2.11.1 for windows):

\[
\ln \left( \frac{P}{1-P} \right) = \text{logit}(Y)
\]

In which the probability of bacterial survival is \( P=1/(1+e^{-\text{logit}(Y)}) \).

**Results and Discussion**

In the interior of meat slices without grilling (control), the populations of *Salmonella enterica* and *Campylobacter jejuni* were 4.08 to 5.94 and 4.12 to 5.94 \( \log_{10} \) cfu/g (not show in table), respectively. There were total 18 samples at each endpoint temperature in this study. *Salmonella enterica* were recovered from 10 samples which were cooked at 155°F, 3 samples which were cooked at 160°F and one sample from which was cooked at 165°F. When the internal temperature reached 170°F, the numbers of *Salmonella enterica* dropped below detectable levels (1.3 \( \log_{10} \) cfu/g). The recovery level ranged from 1.3 to 2.94 \( \log_{10} \) cfu/g.
Campylobacter jejuni were recovered from the interior at every endpoint temperature. Campylobacter jejuni were recovered from 6 samples which were cooked at 155°F, 4 samples which were cooked at 160°F, 4 samples which were cooked at 160°F and one sample which was cooked at 170°F, the recovery level ranged from 1.3 to 3.15 log_{10} cfu/g (Table 1).

Complete microbial destruction can’t be provided for some samples which were cooked above 160°F, due to a wide variability or uncertainty in cooking properties, such as cook time, internal temperature, etc (10) or excessive contamination. Similar results were obtained in a study which reported that E.coli O157:H7 were recovery at 170°F in moisture enhanced beef after cooking by the electric skillet (model 1876, Toastmaster, Inc., Booneville, MO) method (14).

To assure the microbiological safety of meat products, US regulators have specified cooking conditions. As specified in Food code 2009; all parts of the food should be heated to 63, 65, or 68°C for times of 180, 60 or 15s, respectively or to 70°C without the need for maintenance of that temperature (19). USDA also recommended 160°F as the safe minimum internal cooking temperatures for pork. Since the central temperature can be maintained or increased after cooking the larger cuts, such as roasts, the maintenance of a relative low temperature in these specifications can be met with such products, by ending cooking when the specified central temperature is attained and resting the meat for the specified time before serving (17) . However, thinner cuts of meat, such as steaks, are usually cooked by heating on the surface at a time. An alternative is to prolong cooking for the specified time after the specified central temperature reaches, this may result in the meat being overcooked. In this study, the minimal cooking conditions for assuring the safety of moisture enhanced pork slice...
were studied. Overall, grilling at the temperature above 160°F maybe adequate for assuring the microbiological safety of in moisture enhanced and non-moisture enhanced pork slice that is prepared without excessive contamination of interior tissues. These results are generally agreed to the USDA recommended 160 °F as the safe minimum internal temperature for pork and some other studies, for example, C.O.Gill found that aerobic bacteria were recovered at log total numbers of $1.0 \log_{10} \text{cfu} \frac{1}{25g}$ from 25 samples of injected pork cooked to a central temperature of 61°C, but no bacteria were recovered from the deep tissues of meat cooked to 70°C (4).

The presence / absence test is one of the simplest, most widely used microbiological tests for specific pathogens or indicator organisms. Though interpretation of such tests is highly dependent on the method used to determine the presence of the microorganism, particularly its lower limit of detection, this type of microbiological assay can still be used to assess the maintenance of food safety control systems. Several intrinsic (presence of brine solutions in meat and the temperature of meat), extrinsic (internal cooking temperature) factor, implicit (different characteristics between *Salmonella enterica* and *Campylobacter jejuni*) factor and processing (bacteria translocation following enhancement and slicing) factor and their interactions can influence the results of the presence / absence test for a sample from the interior pork slice after cooking. It can provide information of pathogen heat resistance in moisture enhanced pork.

To evaluate the effects of these factors and their interactions on the probability of bacteria survival (the proportion of samples from which bacteria were recovered on the medium) in pork meat slices after grilling, data were fitted to a logistic regression. The parameter estimates for the logistic regression models described the probability of bacteria
survival in pork meat slices after grilling as a function of bacteria, moisture enhancement levels, storage temperatures, and cooking temperatures. As shown in Table 3, the result of this model was summarized at the type I error level $\alpha=0.05$.

The proportion of samples in which bacteria were recovered from the interior of pork after grilling were significantly ($P<0.05$) affected by different inoculated bacteria in pork, cooking temperature, the interaction of storage temperature and the inoculated bacteria in pork, and the interaction of moisture and storage temperature. Compared to Campylobacter jejuni, Salmonella enterica was significant more resistant to various treatments. The estimated proportion of samples yielding Salmonella enterica after grilling were $2e^{+25}$ times higher than the estimated proportion of samples yielding Campylobacter jejuni after grilling if other variables were held constant.

The results of this model also demonstrated that the importance of higher cooking temperature on inactivate pathogen from 155°F to 170°F. Given the same other conditions, the proportion of samples in which bacteria were recovered from the interior of pork after grilling would decrease by 24 percent if the cooking temperature increases by one Fahrenheit from 155°F to 170°F. With other cooking results which described above, to ensure microbiological quality of pork meat, it was essential to distribute cooking temperature uniformly in the pork meat slices, and the internal temperature should be higher than 160°F.

There was no significant ($P>0.10$) effect of storage temperature on the proportion of samples in which bacteria were recovered from the interior of pork after grilling. However, the significant interaction effect between storage temperature and bacteria type indicated the effect of increasing storage temperature from refrigeration (4 °C) to abuse temperature (10°C) on the proportion of detectable Salmonella enterica was about 10.32 times higher
than if *Campylobacter jejuni*. There were also significant interaction effect between cooking temperature and bacteria. The inactivation effect of cooking temperature by increasing one Fahrenheit on the proportions of detectable *Salmonella enterica* would be 32% lower than that of Campylobacter. These results were in generally agreed that *Campylobacter jejuni* has more fastidious growth requirements and are more sensitive to environment stress, such as vacuum packing, high cooking temperature than *Salmonella enterica* (11).

Although the effects of storage temperature and moisture level on the proportion of samples in which bacteria were recovered from the interior of pork after grilling were not significant (*P > 0.05*) on their own, they had a significant interaction effect (*P <0.05*). Since the effects of moisture enhancement, storage temperature were not significant, these two terms can be ignored in the model, the resulting logistic regression model is

\[
\text{Logit} (Y) = 40.8412 + 58.3478(bacteria) + 0.2705(cooking) + 0.5355(bacteria*storage) - 0.3871(bacteria*cooking) - 0.0473(storage*moisture) + \gamma + e
\]

If the moisture enhancement level was increased and other variables were held constant, the proportion of samples in which bacteria were recovered from the interior of pork after grilling would be decreased. It seemed that, when other variables were held constant, the presence of brine soultions in sliced pork had an inactivation effect on the survival of inoculated bacteria in the interior of sliced pork after grilling. This inactivation effect by increasing 1 percent moisture was lower 5 percent lower for the meat at a certain storage temperature than if at a storage temperature that was 1 Celsius lower.

In summary, assuming that the prevalence and levels of *Campylobacter jejuni* and *Salmonella enterica* on the surface of moisture enhanced pork meat remain low and the
appropriate practice are followed for operating and monitoring, moisture enhancement was not found to significantly affect the safety of pork meats when the meat is properly cooked.

Major factors affecting the microbial ecology of foods have always been categorized four groups: intrinsic factors, extrinsic factors, implicit factors and processing factors (13). These four categories offer systematic frameworks for identification of factors that could affect the frequency and levels of contamination of pathogens in foods, As such, they can be useful aids for developing conceptual models for microbial risk assessment of foods that encompass the influence of microbial ecology and physiology (5). Our studies about the influence of different factors associated with various processing steps on the survival of pathogens at consumption and the effectiveness of USDA recommended safe minimum internal cooking temperatures on inactivate pathogens in the interior muscle will be useful in the development of QMRA for moisture enhanced pork, as well as the development of safe cooking guidelines for moisture-enhanced pork.

Acknowledgements

The authors would like to thank Dr Dixon Philip (Department of statistics, Iowa State University) for providing consultation with the statistic modeling, Steven Niebuhr (Department of Animal Sciences, Iowa State University) for his technical assistance.

References

National Cattlemen’s Beef Association and sponsored by the Cattlemen’s Beef Board, Denver.


Table 1: Means populations and frequency of detection for *Campylobacter jejuni* and *Salmonella enterica* in grilled pork meat

<table>
<thead>
<tr>
<th>Parameters</th>
<th><em>Campylobacter jejuni</em></th>
<th><em>Salmonella enterica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture enhancing levels (%)</td>
<td>Storage temperature (°C)</td>
<td>Cooking Temperature (°F)</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>control</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>4.1±0.21</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>4.04±0.34</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>4.42±0.20</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>4.51±0.47</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>4.44±0.21</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>155</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>1.52±0.86</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0.43±0.43</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>1.05±1.05</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>160</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>1.29±0.74</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0</td>
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<td>10</td>
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</tr>
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<td>20</td>
<td>4</td>
<td>0.96±0.96</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>165</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>1.29±0.74</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>0.96±0.96</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>170</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>0.73±0.73</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Counts are expressed as the mean (±standard error) log counts for three separate experiments. Frequency of detection of bacteria is indicated by the number of samples that tested positive for *Campylobacter jejuni* or *Salmonella enterica* out of the total three samples.
Table 2: Description of the variables which were used in the logistic regressions of the probability of bacteria survival (the proportion of samples from which bacteria were recovered on the medium) in pork meat slices after grilling.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>[(bacteria)_i]</td>
<td>An indicator variable equals to 1 if the inoculated bacteria is \textit{Salmonella enterica} ((i = 1)) and equals to 0 if the inoculated bacteria is \textit{Campylobacter jejuni} ((i = 2))</td>
</tr>
<tr>
<td>[(moisture)_j]</td>
<td>The moisture level applied on a pork loin: 0%, 10% or 20% when (j) equals to 1, 2, 3.</td>
</tr>
<tr>
<td>[(storage)_l]</td>
<td>The storage temperature for a slice of pork: 4(^\circ)C or 10(^\circ)C when (l) equals to 1, 2.</td>
</tr>
<tr>
<td>[(cooking)_m]</td>
<td>The target internal cooking temperature for a slice of pork: 155, 160, 165, 170(^\circ)F when (m) equals to 1, 2, 3, 4.</td>
</tr>
<tr>
<td>(\gamma_{ijk})</td>
<td>The random effect at the whole plot (pork loins) level, independently and identically normally distributed as (N(0, \sigma^2)).</td>
</tr>
<tr>
<td>(\epsilon_{ijklm})</td>
<td>The random effect at the split plot (individual slices) level, independently and identically normally distributed as (N(0, \sigma^2)).</td>
</tr>
<tr>
<td>(Y)</td>
<td>The results of bacteria recovery on the medium: presence of bacteria on the medium ((Y = 1)) or no bacteria were present on the medium ((Y = 0))</td>
</tr>
</tbody>
</table>
Table 3: Parameter estimates and associated statistics for the logistic regressions of the probability of bacteria survival (the proportion of samples from which bacteria were recovered on the medium) in pork meat slices after grilling.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>d.f</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>t-value</th>
<th>P value</th>
<th>Exponent of the Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>121</td>
<td>40.8412</td>
<td>9.3971</td>
<td>4.346142</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>15</td>
<td>58.3478</td>
<td>17.7795</td>
<td>3.281740</td>
<td>0.005*</td>
<td>2e+25</td>
</tr>
<tr>
<td>Moisture</td>
<td>15</td>
<td>0.1509</td>
<td>0.0942</td>
<td>0.1299</td>
<td>0.1299</td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td>121</td>
<td>0.1872</td>
<td>0.1078</td>
<td>1.737296</td>
<td>0.0849</td>
<td></td>
</tr>
<tr>
<td>Cooking</td>
<td>121</td>
<td>-0.2705</td>
<td>0.0588</td>
<td>-4.604246</td>
<td>&lt;0.0005*</td>
<td>0.76</td>
</tr>
<tr>
<td>Bacteria x Storage</td>
<td>121</td>
<td>0.5355</td>
<td>0.1487</td>
<td>3.601508</td>
<td>0.0005*</td>
<td>1.71</td>
</tr>
<tr>
<td>Bacteria x Cooking</td>
<td>121</td>
<td>-0.3871</td>
<td>0.1142</td>
<td>-3.390106</td>
<td>0.0009*</td>
<td>0.68</td>
</tr>
<tr>
<td>Storage x Moisture</td>
<td>121</td>
<td>-0.0473</td>
<td>0.0094</td>
<td>-5.035306</td>
<td>&lt;0.0005*</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Significant effects (P<0.05) were marked with asterisks (*).
CHAPTER 6. GENERAL CONCLUSIONS

Food product defects and food poisoning with microbiological origins have been recognized for many years and maybe ranked according to seriousness for the consumer and producers. Ensuring the microbiologically safety of food requires the identification of realistic hazards and their means of control. Moisture enhanced pork is a common non-intact meat products, the concerns about the microbiological safety of such non-intact meat products have been raised for many reasons, for examples, bacteria translocation from the exterior into the interior through moisture enhancement, people preparing dishes with these meats may regard them as intact products (only thoroughly cook surface tissue). Prior to this research, microbiological hazards associated with moisture enhanced pork were not well documented or understood. In addition, there were no available manufacturing guidelines available to processors and consumer to assure safety.

The translocation of bacteria from the surface into the interior muscle through moisture enhancement and slicing was studied in the first part of this research. The recovery of large numbers of *E. coli* biotype I from the deep tissues confirmed that bacteria were translocated from the surface into the depth of approximately 1 cm in the boneless pork following moisture enhancement and slicing. The number of inoculated *E. coli* biotype I recovered from the deep tissues after injection was not significantly affected (*P > 0.05*) by the number on the surface of enhanced meat, moisture enhancement level and different locations in pork loins (approximately 6 cm from the leading edge, 6 cm from the trailing edge and in the approximate geometric center of the loin).

In a second part of this research, the survival of *Campylobacter jejuni* and *Salmonella enterica* in moisture enhanced pork during vacuum storage was studied. There were no
significant effect of moisture enhancement on the populations of *Campylobacter jejuni* and *Salmonella enterica* in samples (*P >0.05*). After 28 days, the populations of *Campylobacter jejuni* and *Salmonella enterica* in samples were significantly lower (*P<0.05*) than those of days 0. Mean populations of *Campylobacter jejuni* and *Salmonella enterica* in samples at 28th day were 4.24, 4.78 log_{10}CFU/g, respectively. No significant differences in *Campylobacter jejuni* counts were observed between samples at abuse temperature (10°C) and those at refrigerated temperature (4°C). In contrast, the population size of *Salmonella enterica* in samples at abuse temperature (10°C) was significantly (*P<0.05*) higher than those at refrigerated temperature (4°C). This study indicates that, vacuum packing under chilled conditions alone is not a substitute for safe handling and proper cooking. The event of temperature abuse during handling should be avoided.

Finally, in a third part of this research, the survival of *Campylobacter jejuni* and *Salmonella enterica* in moisture enhanced pork, subjected to a heat treatment, conform to consumer-based grilling, was studied. Overall, *Campylobacter jejuni* cells were significantly less resistant than cells of *Salmonella enterica*. Higher internal temperature for cooking is more effective for completely bacterial inactivation. Significant interactions between storage temperatures and moisture were observed. The numbers of *Campylobacter jejuni* and *Salmonella enterica* in most of samples dropped below detectable levels with internal temperature of 160°F, the USDA recommended 160 °F as the safe minimum internal temperature for intact pork maybe also adequate for assuring the microbiological safety of moisture enhanced pork that is prepared without excessive contamination of interior tissues. Compared to intact pork, moisture enhanced pork does not present a greater risk to
consumers than otherwise similar meat that is intact, provided that the meat is properly cooked.
ACKNOWLEDGEMENTS

I would first like to thank for the heavenly father, he gives me grace, encourage, love through Christ. Self concentration, doubt, disappointment was always in my life before I knew you. Your glory helps me to overcome troubles in my study and family. I am so lucky to get a lot from you. Your greatest love is changing my life. For this, I'll run to your arms of love for ever.

I would like to thank for my major professor James Dickson, an international famous food microbiologist. Before I joined this lab, I know little about food safety. You help me to build up my career. Scientific knowledge is always neutral, hard, complicated, even boring, but I am amazing and thankful that I learn knowledge under your careful, patient, kind teaching.

I would also like to thank my graduate committee members who have supported me through the doctoral program. They are Dr Aubrey Mendonca, Dr Chris Minion, Dr Joseph Sebranek and Dr Jeffrey Wolt. A minor mistake may lead to a serious consequence. You found many small mistakes in my dissertation, even spelling, which is a great gift for me.

I would like to like to thank Steve Niebuhr, my lab manager. It is pleasure that we can study together not only about science, but also life. May the light of God shine through your lives and your good works.

I would like to thank my lab members. They are Armitra Jackson, Heather Snyder, Ginger Shipp Evan Lowry, Rachelle Carlon. I will never forget my study with your good friends.
Lastly, I would like to give a special thank to my wife, Jing Li. No matter what happened, you are always with me. I always thank God for you because of his grace given you in Christ. I will show you the same in the present and rest of my life.

From my Ph.D study in Iowa state university, there is one thing I know for sure…. Love never fails. Amen for that!