

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Dissertations, Theses, & Student Research in
Food Science and Technology

Food Science and Technology Department

5-2012

VALIDATION OF THERMAL PROCESSING TO CONTROL *SALMONELLA* SPP. AND *CLOSTRIDIUM PERFRINGENS* DURING PRIME RIB PREPARATION FROM INTACT AND NON-INTACT MEAT

Maria A. Calle

University of Nebraska-Lincoln, alexandra.calle1@gmail.com

Follow this and additional works at: <https://digitalcommons.unl.edu/foodscidiss>



Part of the [Food Microbiology Commons](#), and the [Food Processing Commons](#)

Calle, Maria A., "VALIDATION OF THERMAL PROCESSING TO CONTROL *SALMONELLA* SPP. AND *CLOSTRIDIUM PERFRINGENS* DURING PRIME RIB PREPARATION FROM INTACT AND NON-INTACT MEAT" (2012). *Dissertations, Theses, & Student Research in Food Science and Technology*. 22.
<https://digitalcommons.unl.edu/foodscidiss/22>

This Article is brought to you for free and open access by the Food Science and Technology Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Dissertations, Theses, & Student Research in Food Science and Technology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

VALIDATION OF THERMAL PROCESSING TO CONTROL *SALMONELLA* SPP.
AND *CLOSTRIDIUM PERFRINGENS* DURING PRIME RIB PREPARATION
FROM INTACT AND NON-INTACT MEAT

By

Maria Alexandra Calle-Madrid

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science
Major: Food Science and Technology

Under the supervision of Professor Harshavardhan Thippareddi

Lincoln, Nebraska

May, 2012

VALIDATION OF THERMAL PROCESSING TO CONTROL *SALMONELLA* SPP.
AND *CLOSTRIDIUM PERFRINGENS* DURING PRIME RIB PREPARATION
FROM INTACT AND NON-INTACT MEAT

Maria Alexandra Calle-Madrid, MS

University of Nebraska, 2012

Advisor: Harshavardhan Thippareddi

Beef prime rib is a delicacy cooked at low temperatures for long period and extended hot holding prior to consumption. Due to the potential survival and outgrowth of foodborne pathogens, this process may pose a public health risk. Considering an increase in the availability of non-intact beef, the microbial safety of the preparation of prime rib from intact and mechanically tenderized beef was evaluated.

In the first study, lethality of the prime rib (from intact and non-intact, blade tenderized beef) cooking process to *Salmonella* spp. was evaluated. Boneless beef rib eye was surface inoculated with a five-strain cocktail of *Salmonella* spp. to obtain ca. 5.9 log CFU/g. The inoculated blade tenderized subprimals were passed with the fat side facing the blades to prepare non-intact beef rib eye. The subprimals were seared for 15 min at 260°C, cooked at 121°C to internal temperatures of either 37.8 or 48.9°C and held at 60°C for up to 8 h. The searing of blade tenderized rib eye resulted in 0.63 log CFU/g reduction in *Salmonella* spp. and cooking to internal temperatures of 37.8

and 48.9°C resulted in 2.86 and 3.58 log CFU/g reduction in *Salmonella* spp., respectively. Subsequent holding of the blade tenderized prime rib at an oven temperature of 60°C for 8 h resulted in an increase of 1.99 log CFU/g in *Salmonella* spp. population. Thermal processing of intact rib eye resulted in 5.22 and 5.54 log CFU/g reduction of *Salmonella* spp. population after cooking to 37.8 and 48.9°C respectively. Subsequent holding at 60°C during 8 h resulted in an increase in *Salmonella* spp. population of 1.07 and 0.44 log CFU/g, respectively.

In the second study, the growth of *C. perfringens* during hot holding and cooling was evaluated. Ground rib eye was dispensed in 5-g pouches, inoculated to obtain 2.5 spores/g of *C. perfringens*, and vacuum-packaged. *C. perfringens* spores were heat activated and the pouches with the inoculated meat were held at 43 or 49°C. Hot holding of inoculated rib eye resulted in germination and outgrowth of *C. perfringens* spores by 6.5 and 6.05 log CFU/g. Abusive cooling of cooked prime rib from 57.2 to 5°C within 6, 9, 12, and 15 h resulted in *C. perfringens* spore germination and outgrowth by 0.2, 0.4, 0.8, and 2.2 log CFU/g respectively.

Use of non-intact rib eye can pose an enhanced risk of foodborne illness when prime rib is cooked to low end point temperatures, held for extended periods of times at these low temperatures, and cooled at slow rates.

DEDICATION

To my children Jose Alejandro and Juan David, my most valuable treasure.

ACKNOWLEDGMENTS

I would like to express special gratitude to my academic advisor, Dr. Harsharvardan Thippareddi for the opportunity to work in his laboratory. I gained valuable experience and foundations that allowed me to start my career in food microbiology.

My deepest gratitude to Dr. John Rupnow for opening the doors of the University of Nebraska-Lincoln. I will be forever thankful to him because this was the starting of a new stage in my life, and a dream that became true.

I would like to thank and give special recognition to all my professors, who shared their knowledge and contributed to my professional training. I feel very fortunate and proud to have learned from some of the best food scientists in the United States.

I would like to give my gratitude to my friends who always reminded me that I was capable of completing further education in spite of the difficulties. Also, thanks to my closest friends who gave me emotional support just in the right moment and helped me to see the path when everything seems to be cloudy.

To my children, who were able to tolerate me during times of stress. Your love, but mostly having both of you present in my life were the best encouragement during my Masters.

Also to my family, who supported and helped me to achieve my goals without expecting anything in return. Thanks to you all for trusting.

And finally, I want to acknowledge to those willing to look after my children during long work journeys; without them I would have not been able to complete my experiments.

TABLE OF CONTENTS

GENERAL ABSTRACT.....	ii
DEDICATION.....	iii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	ix
LIST OF FIGURES	x
 1. INTRODUCTION	 1
 2. LITERATURE REVIEW	 4
2.1 <i>Clostridium perfringens</i>	4
2.1.1 Characteristics of the organism.....	4
2.1.2 Distribution.....	5
2.1.3 <i>C. perfringens</i> food poisoning.....	6
2.1.4 Outbreaks	8
2.2 <i>Salmonella</i> spp.	9
2.2.1 Characteristics of the microorganism.....	9
2.2.2 Distribution.....	10
2.2.3 Outbreaks	11
2.2.4 Salmonellosis infection	12
2.3 Prime Rib Processing.....	14
2.3.1 Meat raw material.....	14

	vi
2.3.2 Process.....	15
2.4 Mechanical tenderization of meats.....	16
2.5 Food Safety Regulations	18
3. MATERIALS AND METHODS	20
3.1 Survey in restaurants	20
3.2 STUDY ONE. Survival of <i>Salmonella</i> spp. during thermal processing steps used for the preparation of prime rib	20
3.2.1 Effect of searing process on the destruction of <i>Salmonella</i> spp. on surface of intact subprimals	20
Bacterial cocktail	21
Meat inoculation	21
Searing of prime rib	22
Microbial analysis.....	23
3.2.2 Survival of <i>Salmonella</i> spp. during the thermal process of intact and non- intact prime rib	24
Bacterial cocktail	24
Meat inoculation	24
Mechanical tenderization	25
Effect of translocation.....	26
Searing, cooking, and holding of intact and non-intact subprimals.....	26
Microbial analysis.....	29

3.3	STUDY TWO. Germination and outgrowth of <i>C. perfringens</i> spores during hot holding and cooling of prime rib.....	31
3.3.1	Bacterial cocktail.....	31
3.3.2	Sample preparation and inoculation.....	31
3.3.3	Evaluation of hot holding.....	32
3.3.4	Evaluation of cooling during storage of prime rib	32
3.3.5	<i>C. perfringens</i> enumeration.....	33
3.4	Statistical analysis.....	34
4.	RESULTS AND DISCUSSION	35
4.1	Survey of restaurants.....	35
4.2	STUDY ONE. Survival of <i>Salmonella</i> spp. during thermal processing steps used for the preparation of prime rib	46
4.2.1	Effect of searing process for reduction of <i>Salmonella</i> spp. on surface of intact subprimals	46
4.2.2	Survival of <i>Salmonella</i> spp. during the thermal process of intact and non-intact prime rib	51
	Thermal Processing of Intact Prime Rib.....	51
	Effect of Translocation on subprimals.....	54
	Thermal processing of Non-Intact Prime Rib.....	60
4.3	STUDY TWO. Germination and outgrowth of <i>C. perfringens</i> spores during hot holding and cooling of prime rib.....	64
4.3.1	Evaluation of hot holding.....	64

	viii
4.3.2 Evaluation of cooling during storage of prime rib	68
5. CONCLUSIONS	72
REFERENCES	74

LIST OF TABLES

Table 1. Parameters followed by restaurants to prepare Prime Rib.....	38
Table 2. Statistical comparisons based on P-values for steaks of prime rib cooked to 37.8°C. Means (log CFU/g of <i>Salmonella</i> spp.) at each processing step	52
Table 3. Statistical comparisons based on P-values for steaks cooked to 48.9°C. Means (log CFU/g of <i>Salmonella</i> spp.) at each processing step	52
Table 4. Level of <i>Salmonella</i> spp. recovered at different segments of the core samples .	59
Table 5. Statistical comparisons based on P-values for steaks cooked at 37.8°C. Means (log CFU/g of <i>Salmonella</i> spp.) at each processing step	62
Table 6. Statistical comparisons based on P-values for steaks cooked at 48.9°C. Means (log CFU/g of <i>Salmonella</i> spp.) at each processing step	62
Table 7. Increase of <i>C. perfringens</i> during 10 h of holding at 43 and 49°C. Log CFU/g at different sampling time	68

LIST OF FIGURES

Figure 1. Number of infections caused by <i>Salmonella</i> . Adapted from CDC- FoodNet, Facts and Figures. April, 2010.....	12
Figure 2. Prime rib process preparation flow chart.....	17
Figure 3. Scheme of the surface samples taken every 15 min during searing.....	23
Figure 4. Inoculation of meat.....	25
Figure 5. Mechanical tenderization.....	27
Figure 6. Subprimal after mechanical tenderization.....	27
Figure 7. Scheme of the coring sample.....	28
Figure 8. Thermal process and sampling.....	30
Figure 9. Cooling profiles used to evaluate <i>C. perfringens</i> growth.....	33
Figure 10. Temperature profile during preparation of prime rib - Restaurant A.....	39
Figure 11. Temperature profile during preparation of prime rib - Restaurant B.....	40
Figure 12. Temperature profile during preparation of prime rib - Restaurant C.....	41
Figure 13. Temperature profile during preparation of prime rib - Restaurant D.....	42
Figure 14. Temperature profile during preparation of prime rib - Restaurant E.....	43
Figure 15. Temperature profile during preparation of prime rib – Restaurant F.....	44
Figure 16. Cooling profile of prime rib leftovers at three different restaurant.....	45
Figure 17. Survival of <i>Salmonella</i> spp. Comparison between two temperatures during searing of subprimals.....	48

Figure 18. Survival of <i>Salmonella</i> spp. during searing at 260°C for 60 min. Level of recovering of the microorganism from both, lean and fat side.	49
Figure 19. Survival of <i>Salmonella</i> spp. during searing at 204°C for 60 min. Level of recovering of the microorganism from both, lean and fat side.	50
Figure 20. Survival of <i>Salmonella</i> spp. during the steps used for thermal processing of intact rib eye when samples where cooked to 37.9°C.....	55
Figure 21. Survival of <i>Salmonella</i> spp. during the steps used for thermal processing of intact rib eye when samples where cooked to 48.9°C	56
Figure 22. Survival of <i>Salmonella</i> spp. in intact meat during the steps used for thermal processing. Comparison between both set of samples, cooked to 37.8 °C and 48.9 °C	57
Figure 23. Degree of translocation of <i>Salmonella</i> spp. through the interior of the meat in non-intact blade tenderized rib eye	60
Figure 24. Survival of <i>Salmonella</i> spp. during the steps used for thermal processing of non-intact rib eye when samples where cooked to 37.9°C.	65
Figure 25. Survival of <i>Salmonella</i> spp. during the steps used for thermal processing of non-intact rib eye when samples where cooked to 48.9°C	66
Figure 26. Survival of <i>Salmonella</i> spp. in non-intact meat during the steps used for thermal processing. Comparison between both set of samples, cooked to 37.8 °C and 48.9 °C	67
Figure 27. Growth of <i>C. perfringens</i> during hot holding of rib eye at 43°C and 49°C ..	69

Figure 28. Growth of <i>C. perfringens</i> during cooling of rib eye at four different exponential profiles	71
--	----

1. INTRODUCTION

Prime rib is a beef roast and is considered a high quality meal characterized for its richness in flavor and juicy texture. Prime rib is typically cooked to rare to medium rare temperatures. Traditionally, it is prepared by rubbing the meat with spices, followed by a slow-cooking process with extended hot holding periods until it is served.

Meat and meat products such as prime rib, roast beef, corned beef, and similar products are considered “potentially hazardous foods”. The Food and Drug Administration of the United States (FDA) defines those as “foods that requires time/temperature control for safety to limit pathogenic microorganism growth or toxin formation (FDA 2009)”.

Meat from healthy animals is normally considered sterile. However, the external surfaces of meat can be contaminated with foodborne pathogens and spoilage microorganisms during slaughter and fabrication (Gill 1998, Gill 1979). *Salmonella* spp. and *Clostridium perfringens* are foodborne pathogens that are normally associated with meat and meat products and have been implicated in numerous foodborne illnesses. In addition, if the meat is manipulated by mechanical tenderization, the surface microorganisms can be translocated to the interior of the meat, requiring meat to be cooked to higher end-point temperatures to assure safety.

While meat processors employ several antimicrobial interventions to reduce the presence and/or levels of foodborne pathogens, safety of meat products prepared at foodservice establishments relies on cooking as the primary method for control of

pathogens and spoilage organisms (Boles 2010, Marks 2010). Regardless of the time-temperature schedule used during slow cooking, hot holding and cooling may present a public health risk.

Subsequent to numerous salmonellosis outbreaks implicating roast beef, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) issued guidance for proper cooking of roast beef to reduce the risk of *Salmonella* spp. in such products. However, these measures did not address the potential hazard from *C. perfringens* (Roy et al. 1981).

Foodborne illness outbreaks implicating prime rib and similar products have been reported since 1977 (Pace et al. 1976, Shapiro et al. 1999). Smith et al. (2006) reported that between 1990 and 2003, 163 outbreaks and 6,111 foodborne illness cases have been linked to beef products such as prime rib, roast beef and ground beef, in which *E. coli* O157:H7, *Campylobacter* spp., *Salmonella* spp., *Bacillus cereus*, *C. perfringens*, and *Staphylococcus aureus* have been implicated. Inappropriate hot holding of food is the cause for a majority of these foodborne illness outbreaks.

Holding of food at temperatures of $\geq 60^{\circ}\text{C}$ in warming units usually results in reduction of vegetative foodborne pathogens such as *Salmonella* spp. However, if the temperature is lowered to below 50°C for a few hours, potential germination and outgrowth of spore formers such as *C. perfringens* can occur (ICMSF 1998).

The objectives of the research are to (i) characterize the searing, cooking, holding and cooling of prime rib in restaurants; (ii) evaluate the destruction and/or survival of *Salmonella* spp. during the thermal process (searing, cooking and holding); and (iii)

evaluate the potential germination and outgrowth of *C. perfringens* during hot holding and cooling of prime rib.

2. LITERATURE REVIEW

2.1 *Clostridium perfringens*

2.1.1 Characteristics of the organism

The genus *Clostridium* consists of a diverse group of bacteria that do not grow in the presence of oxygen and have the ability to form heat-resistant endospores. Many of these anaerobes are pathogenic to both humans and animals. *Clostridium perfringens* is a prolific toxin-producing species within the clostridial group (Heredia and Labbe 2001).

C. perfringens is a Gram-positive, non-motile bacillus. Although it is considered an anaerobe, it can tolerate moderate exposure to air (Montville and Matthews 2005). The organism is mesophilic, with an optimum temperature for growth between 37°C and 45°C and a minimum and maximum temperature for growth of 15°C and 50°C, respectively (Jay et al. 2005, Juneja et al. 2007, Labbe 1989). Spores are cold resistant; food poisoning can result if viable spores present in refrigerated or frozen foods germinate, outgrowth and multiply when that food is warmed for serving, and if temperature abuse occurs during holding or during slow cooking of such foods (McLane 2001). Optimal pH value for *C. perfringens* growth is between 6.0 and 7.0, the pH range as many meat and poultry products (Labbe 1992). The microorganism does not grow well below a water activity of 0.95 and 0.97 (Labbe 1992, Kang et al. 1969).

The bacterium is divided into five different types (A-E) according to the range of major toxins produced (α , β , ϵ , and ι) (Mead et al. 1999, Juneja et al. 2010). *C. perfringens* associated food poisoning and non-foodborne gastrointestinal diseases in humans are typically caused by type A isolates capable of producing a 35-KDa protein called *C. perfringens* enterotoxin or CPE (Huang et al. 2007). *C. perfringens* types C and D also produce enterotoxin; however, there is no data implicating these strains in foodborne illness (Heredia and Labbe 2001).

C. perfringens possesses attributes that contribute significantly to its ability to cause foodborne illness (Heredia and Labbe 2001). These attributes include (1) a ubiquitous distribution throughout the natural environment, giving it ample opportunity to contaminate food; (2) the ability to form heat-resistant spores, allowing it to survive incomplete cooking of food or improper sterilization; (3) the ability to grow quickly in foods, allowing it to reach high levels necessary for food poisoning; and (4) the ability to produce intestinally active enterotoxin, responsible for the characteristic gastrointestinal symptoms of *C. perfringens* food poisoning.

2.1.2 Distribution

C. perfringens is ubiquitous in the environment and food-poisoning strains are found in the soil, dust, raw ingredients such as spices used in food processing, and in intestines of humans and animals (ICMSF 1996, Jay et al. 2005). The widespread distribution of *C. perfringens* has been considered an important factor in its frequent occurrence as an agent of foodborne illness (McLane 2001). Surveys of asymptomatic

individuals show that this bacterium can be isolated from almost any stool sample, and is usually present at population of ≤ 4.0 log CFU/g except in the elderly, where it frequently exceeds 4.0 log CFU/g (Armstrong et al. 1998).

Raw protein foods of animal origin are frequently contaminated with *C. perfringens*. However, a large proportion of *C. perfringens* strains found in raw foods are enterotoxin negative (Saito 1990, Labbe 1992).

C. perfringens is a common surface contaminant of beef; the organism is found frequently in meats, generally resulting from fecal contamination of carcasses, from other ingredients such as spices, or post-processing contamination (Juneja et al. 2010). According to the U.S. Centers for Disease Control (CDC), the most common vehicles for *C. perfringens* foodborne disease in the United States are meats (beef and poultry) (Olsen et al. 2000).

In most instances, the actual cause of poisoning by *C. perfringens* is temperature abuse of prepared foods. Small numbers of *C. perfringens* spores are often present after cooking with germination, outgrowth, and multiplication to levels that can cause food poisoning during improper or abusive cooling and storage of prepared foods.

2.1.3 *C. perfringens* food poisoning

C. perfringens is a common cause of foodborne disease in the United States (Shandera et al. 1983, Labbe 1989). It is estimated that *C. perfringens* is the third most frequent pathogen responsible for about one million of foodborne illnesses a year in the U.S. (Scallan et al. 2011).

Illnesses caused by *C. perfringens* are considered under-diagnosed (McLauchlin and Grant 2007). Foodborne illness cases from *C. perfringens* are not routinely reported because they usually appear as a mild illness. The number of reported cases is assumed to be 10 times the average annual number of outbreak-related cases reported to Centers for Disease Control and Prevention (Mead 1999, Scallan et al. 2011).

C. perfringens food poisoning occurs typically from the ingestion of $>10^6$ viable vegetative cells of the organism (Labbe and Juneja 2002). The symptoms include diarrhea, severe abdominal pain, and occasional vomiting (Heredia and Labbe 2001), and begin 8-22 h after consumption of contaminated food. The illness is usually over within 24 h but less severe symptoms may persist in some individuals for 1 to 2 weeks (FDA 1992). Death is uncommon but has occurred in debilitated or sensitive populations, such as young and the elderly (Heredia and Labbe 2001, Crouch and Golden 2005).

C. perfringens enterotoxin (CPE) has been shown to be the virulence factor for *C. perfringens* type A food poisoning (Brynstad and Granum 2002). CPE is a single polypeptide chain with a molecular weight of 3.5 kDa that binds to receptors on the target epithelial cells (Brynstad and Granum 2002).

The enterotoxin is the only known *C. perfringens* toxin that is not secreted from vegetative bacterial cells but is released during sporulation. Improved sporulation has been demonstrated when cells are exposed to acidic environment such as in the human stomach (Wrigley et al. 1995). Due to the acidic environment in the stomach many cells die during the passage to the small intestine, thus large numbers of cells ($>10^6$) need to be ingested to cause illness (Labbe 1995). The spores of the organism survive initial cooking

and strains that produce relatively heat resistant spores may survive in greater numbers. The spores germinate and multiply during cooling and may produce illness after ingestion, unless the food is re-heated to a temperature adequate to destroy vegetative cells before serving (Shandera et al. 1983).

2.1.4 Outbreaks

Outbreaks of *C. perfringens* are associated with products that have been exposed to inadequate temperatures to destroy the spores (Crouch and Golden 2005) and subsequently temperature abused either during cooking or subsequent serving. Incomplete cooking of food may not only fail to destroy *C. perfringens* spores in foods, but can induce germination of *C. perfringens* spores (McClane 2001). Spores are activated during heating resulting in germination and subsequent multiplication to high levels if food containing the cells is (1) hot held for extended periods at insufficiently hot temperatures, (2) improperly cooled, or (3) improperly stored (Crouch and Golden 2005). Episodes of *C. perfringens* food poisoning occur throughout the year with no particular seasonal prevalence. Outbreaks have followed meals prepared for large numbers of people in schools, hospitals, factories, and restaurants (ICMSF 1996).

A typical example of an outbreak is presented by McLauchlin and Grant (2007). In a psychiatric hospital, 170 patients were affected with diarrhea, resulting in three deaths. Patients had eaten roast lamb, which had been cooked, stored at room temperature for 20 h and served cold. The same type of *C. perfringens* was recovered from the lamb dish, patient's feces, and post-mortem samples.

In the period between 1976 and 1980 the CDC received reports of 62 outbreaks of foodborne disease due to *C. perfringens* in the United States, involving 4,093 individuals (Shandera et al. 1983). In 2006, the largest outbreak with a known etiology and single food commodity were attributed to baked chicken contaminated with *C. perfringens* (741 cases). In addition, prime rib served at a banquet facility was confirmed to be contaminated with *C. perfringens* in which 32 people were affected (CDC 2006).

2.2 *Salmonella* spp.

2.2.1 Characteristics of the microorganism

Salmonella is a rod shaped, Gram negative, non-spore-forming bacterium that belongs to the family *Enterobacteriaceae*. It is a facultative anaerobe; it produces acid and gas from glucose, produces hydrogen sulfide, decarboxylates lysine, and reduces nitrate to nitrite. *Salmonella* is also oxidase negative, catalase positive with an optimal growth temperature of 37°C (ICMSF 1996, Montville and Matthews 2005).

The genus *Salmonella* is composed of two species, *S. enterica* and *S. bongori* (Anderson and Ziprin 2001, Montville and Matthews 2005). *S. enterica* is divided into six subspecies differentiated by the genomic and biochemical relatedness, which are normally referred to by using a Roman numeral and a name: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* (I, II, IIIa, IIIb, IV, and VI respectively) (Montville and Matthews 2005, Cooke et al. 2007).

Salmonella has the ability to survive and adapt to extreme conditions such as low levels of nutrients, and a wide spectrum of temperatures and pH values (D'Aoust 2001). Some *Salmonella* serovars can grow at elevated temperatures of up to 54°C. *Salmonella* spp. is not considered a psychrotroph; however, D'Aoust (1991) reported potential growth of the microorganism at temperatures < 10°C. Hence, its ability to survive at refrigeration temperatures is a possible concern during processing.

Although literature indicates that salmonellae are heat sensitive, several studies have shown its resistance to high temperatures (McDonough 1968, Mackey and Derrick 1986, Podolak et al. 2010). Research indicates that thermal resistance of *Salmonella* increases when the microorganism has been exposed to temperatures $\leq 50^{\circ}\text{C}$ for 15 to 30 min (Mackey 1986).

2.2.2 Distribution

Salmonella are distributed worldwide and is associated with a broad variety of foods. Its primary habitat is the intestinal tract of humans and animals, although it can be found in other parts of animals. Due to their reservoirs and the intensive animal husbandry, *Salmonella* can be easily spread and be present in a variety of environments. Polluted water and raw materials of animal origin have been identified as vehicles to spread these pathogens to processing environment and transmitting them to humans (ICMSF 1996, D'Aoust 2001, Montville and Matthews 2005). *Salmonella* serotypes are often reported in fresh produce, eggs, poultry products, and products from aquaculture (Bell 2002).

Meat and meat products have been identified as sources of contamination with *Salmonella* (Alban et al. 2002; Abdunaser et al. 2009). Contamination occurs during slaughter and failure of intervention measures can result in the transfer of microorganisms to processed meats. Typically the occurrence of this bacterium indicates lack of hygienic practices in the production and processing facilities.

2.2.3 Outbreaks

Over the years, *Salmonella* has caused the highest numbers of outbreaks and illnesses. *Salmonella* is responsible for 3 billion human illnesses a year on a global scale (Cooke et al. 2007). The CDC (2010) estimated that 1.4 million cases of salmonellosis occur annually in the United States, of which approximately 40,000 are laboratory confirmed and 400 leading to death. FoodNet statistics indicate that from 1996 to 2009, the confirmed foodborne illness caused by *Salmonella* increased more than three times (CDC 2010) (Fig. 1).

Typically, *Salmonella* outbreaks are associated with the consumption of undercooked eggs, dairy products, raw produce, and vegetables products. In 1994, an outbreak of *Salmonella* serotype Typhimurium occurred in Wisconsin (CDC 1995) associated with eating contaminated undercooked ground beef. In addition, the CDC reported in 2002 an outbreak with multidrug-resistant *Salmonella* Newport. In this outbreak, ground beef was the vehicle for a confirmed multistate outbreak of *Salmonella* Typhimurium in 2004 (CDC 2002 and 2006).

Two of the largest recorded outbreaks of salmonellosis occurred under unusual conditions (Jay et al. 2005). The first one in 1994 was associated with ice cream produced with milk that was cross-contaminated during transportation in a vehicle that previously contained liquid eggs. This outbreak involved more than 224,000 individuals. The second one, which occurred in 1985, affected approximately 200,000 people and was caused by contaminated milk.

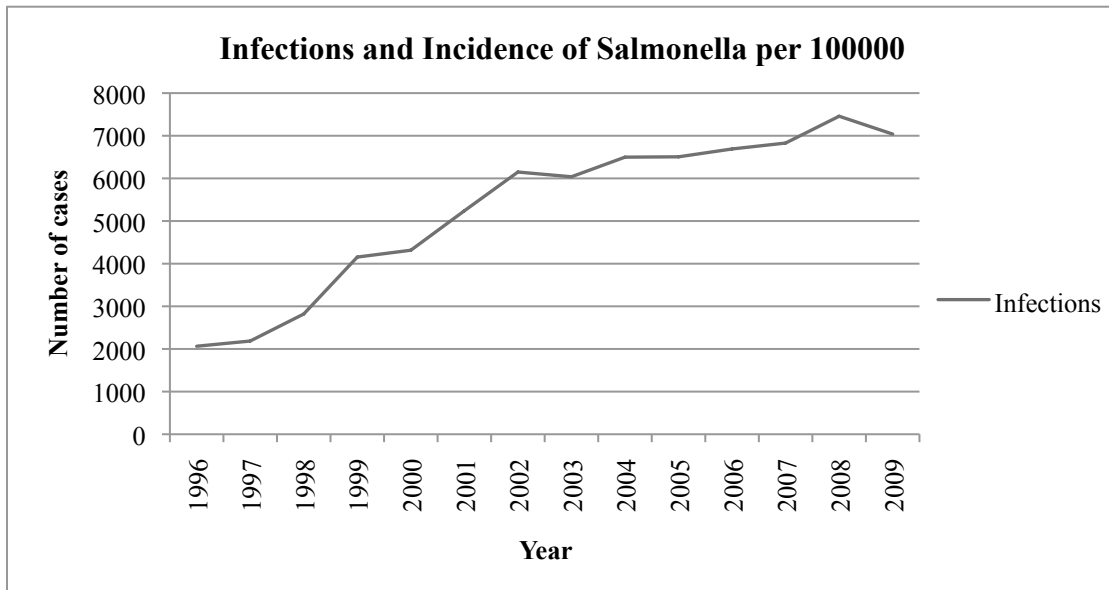


Figure 1. Number of infections caused by *Salmonella*. Adapted from CDC- FoodNet, Facts and Figures. April, 2010.

2.2.4 Salmonellosis infection

Salmonellosis is associated with a wide variety of foods. Typically, products of animal origin have been implicated as the main source of transmission. The disease is

normally transmitted *via* oral route through the ingestion of contaminated food. According to Bhunia (2008) 10^5 to 10^{10} CFU/g are necessary to cause disease in humans; although the infectious dose increases if consumed with liquid food that traverses the stomach rapidly.

S. enterica serovar Typhi is responsible for causing typhoid fever. Common symptoms include fever, flu-like symptoms, headaches, and chills. They may appear after 8 to 14 days after ingestion of the bacteria resulting in a clinical disease. The illness may last for a few months if no antibiotic treatment is provided (Parry 2006). However, *Salmonella* Typhi infections occur primarily through human-human and not zoonotic.

Non-typhoidal *Salmonella* serovars (NTS) are responsible for most foodborne illness associated with *Salmonella*, causing self-limiting gastroenteritis (Molbak et al. 2006). The incubation period may depend on the dose of pathogen ingested and may range from 5 h to 5 days with an onset of symptoms from 12 to 36 h (ICMSF 1996, Jay et al. 2005). Symptoms usually include nausea, diarrhea, headache, fever, chills, and myalgia that last 2 to 5 days. Some cases may lead to bacterimia, septicemia and death (Ziprin and Hume 2001, ICMSF 1996).

A more recent concern relates to the emergence of drug-resistant *Salmonella*, due to the increased use of antimicrobials in production and processing (Bailey et al. 2010). Reports indicate that the organism is resistant to at least five drugs: ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (Helms et al. 2002).

2.3 Prime Rib Processing

2.3.1 Meat raw material

Boneless rib eye (*Serratus dorsalis* and *Longissimus costarum* muscles and related intermuscular fat) is the cut traditionally used to prepare prime rib (Jones et al. 2004). Prime rib owes its name to the fact that a primal cut used has been graded USDA Prime. USDA grades are based on nationally uniform federal standards of quality. The USDA grades account for the amount and distribution of marbling in the muscle of the rib eye to determine the quality grade (Hale et al. 2010). Prime grade is produced from young, well-fed beef cattle, having abundant marbling (USDA 2008). From the 2005 National Beef Tenderness Survey, rib eye is one of the most tender beef cuts (Savell et al. 2006). Prime rib possesses an adequate amount of finely dispersed marbling within a fine textured lean.

Given the quality of the subprimal, intact meat is commonly used. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has defined intact beef steak as a cut of whole muscle(s) that has not been injected, mechanically tenderized, or reconstructed (USDA-FSIS 1999, FDA 2009). Currently, any rib roast is called “prime” regardless the USDA grade, and mechanically tenderized meat may be used for the preparation of prime rib.

Raw meat is highly susceptible to contamination and growth of foodborne pathogens. Microorganisms can contaminate the meat during production, processing, and

handling, resulting in an illness if not properly cooked. Thus, interventions need to be implemented to assure the safety of meat processed products.

2.3.2 Process

Prime rib is a delicacy prepared in full service restaurants. Prime rib preparation consists of rubbing the exterior of the meat with spices, followed and then proceeds to heat treatment. The beef is seared at high temperatures (202°C (400°F) to 260°C (500°F)) for a short time (15 to 30 min). This is a desirable step in cooking meats as it is intended to retain the meat juices and hence contributes to flavor and color formation (Brown 2008). The rib eye is subsequently cooked in a convection oven at low temperatures to achieve the desired level of doneness. The rib eye is normally cooked at temperatures between 107 and 135°C (225 to 275°F) for 1.5 to 3 h (Fig. 2), to reach an internal temperature of 38 to 60°C (100 to 140°F). The cooking time and temperature is based according to the size of the rib and the desirable degree of doneness. The prime rib is subsequently held hot before serving it to the customers. Typically, food service establishments serve prime rib for dinner, and as it is prepared with a few hours of anticipation, holding time may be extended up to 11 h. The leftovers portions are normally stored under refrigeration for use them the following day either as prime rib steaks or as the meat in prime rib sandwiches.

The low internal cooking temperatures, combined with long, low holding temperatures and slow cooling may allow survival of vegetative and spore forming

pathogens such as *Salmonella* spp. and germination, outgrowth and multiplication of spore forming pathogens such as *C. perfringens*.

2.4 Mechanical tenderization of meats

Tenderness is an important quality factor valued by consumers of red meats (Johnston et al. 1978). Over the years, several methods have been used to improve meat tenderness. Mechanical tenderization is a method used as it is cost effective and retains the nutritional value of the meat. The muscle is repeatedly penetrated with sharp needles or blades to disrupt muscle fibers, improving tenderness without changing other attributes of the meat (Hajmeer et al. 2000, Dominguez and Schaffner 2009).

Meat that has been processed using mechanical tenderization is considered as non-intact meat. According to the USDA-FSIS, “*non-intact beef products include beef that has been injected with solutions, mechanically tenderized by needling, cubing, frenching, or pounding devices, or reconstructed into formed entrees*” (USDA-FSIS 1999). Non-intact beef used for the preparation of rib roasts refers to meat that has been mechanically tenderized.

Blade or needle tenderization can translocate pathogens from the surface of intact meat to the interior, requiring longer internal cooking temperatures to destroy these microorganisms (Phebus et al. 2000, Luchansky et al. 2008, Dominguez and Schaffner 2009, Luchansky et al. 2009). Thus, application of antimicrobial interventions to subprimals intended for the production of non-intact beef is necessary to minimize the risk of microbial translocation to the interior of the meat.

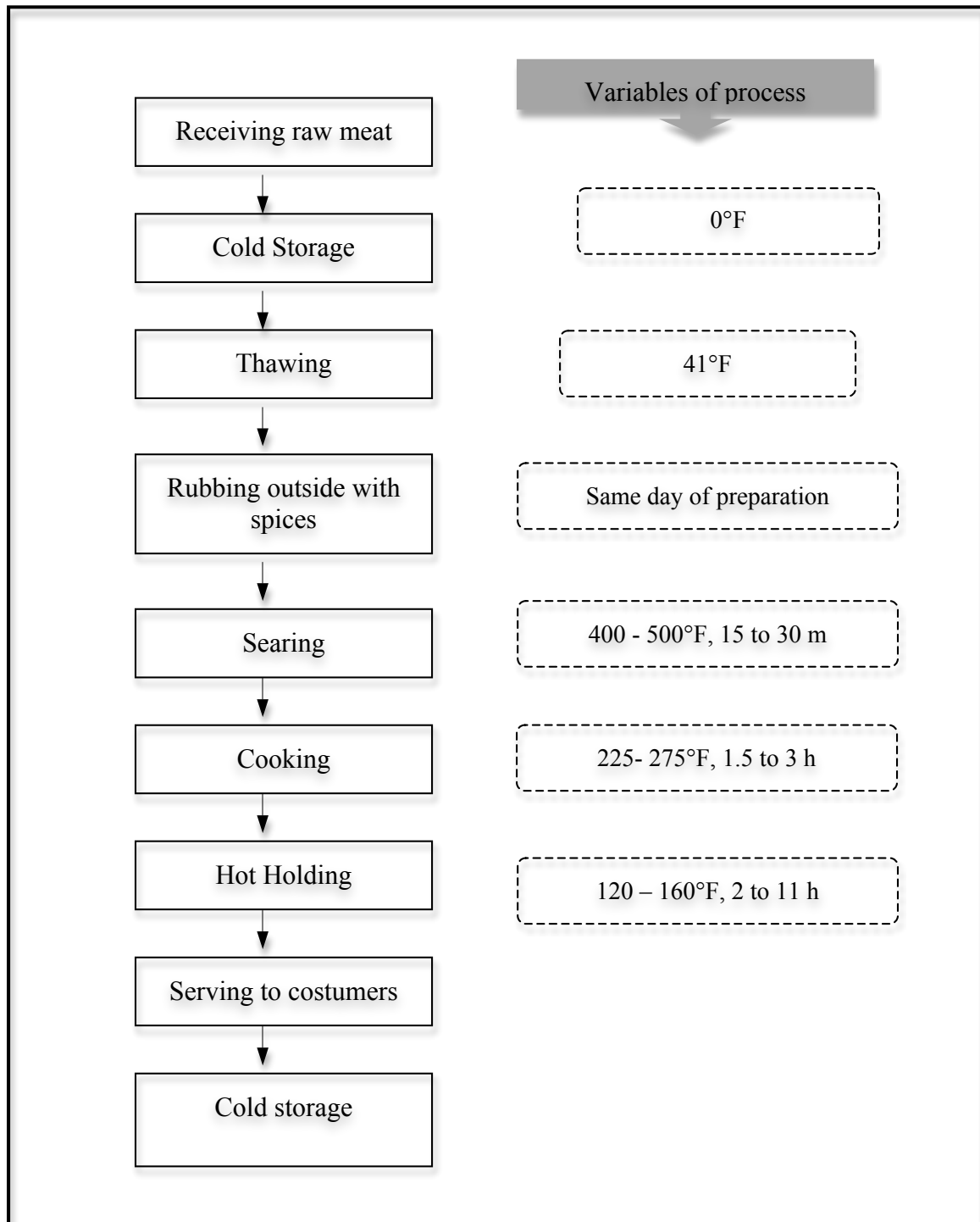


Figure 2. Prime rib process preparation flow chart.

Research has shown that after cooking, the reduction of microorganisms inside of mechanically tenderized meat is less than those on the surface when cooked at low temperatures (Marsden et al. 2004). However, if the meat is cooked to the proper temperature, destruction of pathogens and vegetative cells can be achieved (Juneja 2002, Buffo and Holley 2006).

Outbreaks associated with non-intact mechanically tenderized meat have been reported. In 2005 an outbreak associated with non-intact blade tenderized meat sold by door-to-door vendors was reported (Laine et al. 2005). Recently, in 2009 an outbreak due to consumption of mechanically tenderized beef steaks resulted in a recall of 248,000 pounds of meat (Huang and Sheen 2010).

The traditional prime rib preparation process represents a public health concern due to low internal temperatures achieved, long holding periods, and slow cooking when non-intact meat is used.

2.5 Food Safety Regulations

Outbreaks associated with pathogens such as *Salmonella* and *C. perfringens* due to inadequate cooking temperatures have been reported. The USDA-FSIS published performance standards to assure food safety during the production of cooked beef, roast beef, and cooked corned beef products. Performance standards are intended to facilitate the industry in developing thermal processing schedules without the need to adhere to safe harbors (USDA-FSIS, 1999).

Specifically, USDA-FSIS compliance guidelines (Appendix A) specify that the production of cooked beef, roast beef, corned beef, and similar ready-to-eat products should follow a specific time-temperature combination to meet 6.5- \log_{10} or 7.0- \log_{10} reduction of *Salmonella* during cooking process (USDA-FSIS 1999). Similarly, the compliance guideline for stabilization or cooling of heat-treated meat (Appendix B) establishes the time frame in which cooked products should be cooled down to avoid no more than 1-log growth of *C. perfringens* (FSIS 1999). Processors that are not able to meet the compliance guidelines should validate their customized schedule to demonstrate that they meet the performance standards.

Food service establishments are exempt from inspection under USDA-FSIS regulations, therefore local and county health departments have the regulatory authority for inspecting these establishments based on regulations from the Food Code (FDA 2010).

The objectives of the research are to (i) characterize the prime rib preparation (searing, cooking, holding and cooling) in restaurants; (ii) evaluate the destruction and/or survival of *Salmonella* spp. during the thermal process (searing, cooking and holding); and (iii) evaluate the potential germination and outgrowth of *C. perfringens* spores during holding and cooling of prime rib.

3. MATERIALS AND METHODS

3.1 Survey in restaurants

Temperatures during the processing of prime rib were monitored in six local restaurants. A type K thermocouple connected to a data logger (Model EL-USB-TC-LCD, Lascar Electronics, UK) was inserted in the geometric center of the rib eye and the recording was started once the meat was placed in the oven. At least three different temperature profiles were recorded on different days and downloaded to a computer through the data logger software. The product temperature was measured from the time the meat was placed in the searing oven until it was served to the last of the customers for the evening in the restaurant. The recorded temperature was plotted.

3.2 STUDY ONE. Survival of *Salmonella* spp. during thermal processing steps used for the preparation of prime rib

3.2.1 Effect of searing process on the destruction of *Salmonella* spp. on surface of intact subprimals

A preliminary experiment to assess the destruction of *Salmonella* spp. during searing of the prime rib was conducted.

Bacterial cocktail

Five *Salmonella* isolates MFS 248 (isolated from a pork processing plant), MFS 330 (isolated from a pork processing plant), MFS 3446 (isolated from pork), MFS 3447 (isolated from a pork sausage), and MFS 3448 (a clinical isolate), were used in the study. Stock cultures were maintained in Tryptic Soy Broth (TSB; Bacto; Sparks, MD) with 50% Glycerol at -70 °C. The five strains from the stock cultures were thawed at room temperature and 10 µL aliquots of each isolate was transferred separately to 10 ml of TSB and incubated for 18 h at 35°C. Two subsequent transfers into fresh TSB were performed and 20 ml bacterial cocktail was prepared by combining 4 ml from each strain into a sterile centrifuge tubes. The five-serovar cocktail was centrifuged (Beckman; GS – 15R; Palo Alto, CA) at 6000 x g, for 10 min at 4°C and the supernatant discarded. The cells in the pellet were resuspended to the original volume with 0.1% buffered peptone water (PW; Difco; Sparks, MD), diluted in PW and used for inoculation of the rib eye.

Meat inoculation

Fresh boneless rib eye lip-on (NAMP 112A) was purchased from a local wholesale store and stored under refrigeration at 1°C for up to 5 days. The meat was placed fat side up on a grid and placed into a biohazard plastic bag for the inoculation. The meat was surface inoculated on both fat and lean side using a spray nozzle with 10

ml of the five strains of *Salmonella* cocktail on each side. The inoculated meat was stored at 4°C for 30 min to allow the attachment.

Searing of prime rib

Two searing temperatures 260°C and 204.4°C were evaluated to assess the destruction of *Salmonella* spp. A standard consumer electric oven used for this experiment was pre-heated to the corresponding temperature. To increase the humidity inside of the oven, a stainless steel pan with water was placed in the lower part of the oven and replaced as necessary. Thermocouples were inserted to the rib eye into (i) the geometric center, (ii) surface of the fat side (ca. 0.5 cm from the surface), and (iii) surface of lean side (ca. 0.5 cm from the surface) to continuously monitor the temperature during searing (every 30 s). The meat was placed fat side up on the same grid used during the inoculation, and seared individually at the corresponding temperature for 60 min. Surface samples were obtained using a sterile, 7 cm diameter stainless-steel coring device. Two samples were obtained from opposite sides of each of the lean and fat surfaces at 15-minute intervals (Fig. 3). The samples were placed in sterile filter stomacher bags (Spiral Biothec, Bethesda, MD), and immersed in an ice water bath immediately.

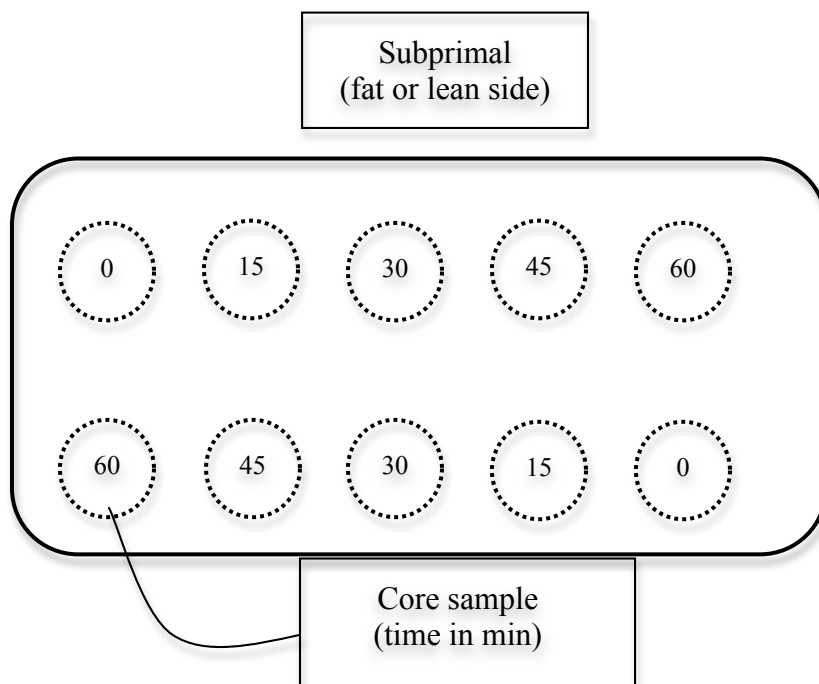


Figure 3. Scheme of the surface samples taken every 15 min during searing.

Microbial analysis

Twenty ml of 0.1% PW was added to the samples and blended (Neutec; NR 1378 / 471, Barcelona, Spain) for 2 min. Appropriate dilutions were plated in duplicate on a non-selective medium Tryptic Soy Agar (TSA; Difco; Sparks, MD), and a selective medium Xylose Lysine Deoxycholate Agar (XLD; Difco; Sparks, MD) and the plates were incubated for 24 h at 35°C.

3.2.2 Survival of *Salmonella* spp. during the thermal process of intact and non-intact prime rib

Destruction and/or survival of *Salmonella* spp. during thermal processing for the preparation of prime rib (searing, cooking, and holding) was evaluated. The experiments were conducted at the USDA Agricultural Research Service Eastern Regional Research Center (Wyndmoor, PA) following procedures for cocktail preparation, meat inoculation, and mechanical tenderization described by Luchansky et al. (2009).

Bacterial cocktail

Salmonella spp. isolates and protocol for the cocktail preparation used for the evaluation of the effect of searing on the destruction of *Salmonella* spp. on surface of intact subprimals were used.

Meat inoculation

Boneless rib eye, lip-on (NAMP 112A) subprimals were purchased from a wholesale distributor and stored under refrigeration at 4°C for up to 5 d. For each replicate, three subprimals were inoculated, one to evaluate the translocation of *Salmonella* spp. and the other two to evaluate the thermal destruction of *Salmonella* spp. during the preparation of prime rib. Bags containing the meat were opened from one end by cutting approximately one quarter of the seal, and the meat juices were allowed to drain out. The meat was placed on a table in the original packaging fat side down until inoculation. To achieve a high inoculation level, the 50 ml-*Salmonella* spp. cocktail was applied dispensing it through the opening with a pipette to the fat side of the meat (Fig.

4). The meat was gently massaged to facilitate distribution of the inoculum while the meat remained in the same position. To allow for attachment, inoculated meat was stored refrigerated at 4°C for ca. 30 min.



Figure 4. Inoculation of meat.

Mechanical tenderization

A blade tenderizer (Series TC 7000M, Ross Industries, Midland, VA) was used for tenderizing the rib eye. The machine was equipped with two sets of blades, each one containing 7 rows of 12 stainless-steel blades. The tenderizer belt advanced 3.6 cm at a time, after which the 3 mm-wide blades descended and penetrated the meat until they were within 2 mm of the belt, after which they immediately retracted. The inoculated subprimals were passed through the machine fat side up (Fig. 5 – 6). The equipment was

immediately sanitized after the process using hot water at 88°C followed by 400 ppm quaternary ammonium detergent (BDD, Koch Supplies, Kansas City, MO). After blade tenderization, the meat was placed on trays, covered with aluminum foil, and stored overnight under refrigeration to allow bacterial attachment.

Effect of translocation

To evaluate the translocation of *Salmonella* spp. after blade tenderization, six core samples were obtained from an inoculated blade tenderized subprimal using an alcohol-sanitized, cylindrical stainless-steel coring device. The device was inserted from the surface to the bottom of the subprimal to extract a portion that represents the thickness of the subprimal (ca. 8 cm). Each core sample was divided into segments, with the first segment beginning at the surface of the meat (fat side) and the last segment ending at the bottom (lean side) (Fig. 7). Segments 1 to 4 were 1 cm thick, and segments 5 and 6, were 2 cm thick. *Salmonella* spp. population was determined on each segment as described below.

Searing, cooking, and holding of intact and non-intact subprimals

Temperatures/data collected from survey of the restaurants were used to select the time-temperature schedules during preparation of prime rib. Intact and non-intact (mechanically tenderized) subprimals were processed and evaluated independently on different days. For the evaluation of intact meat, inoculated subprimals were removed from their packages and carefully placed fat side up on a tray. The non-intact subprimals



Figure 5. Mechanical tenderization.

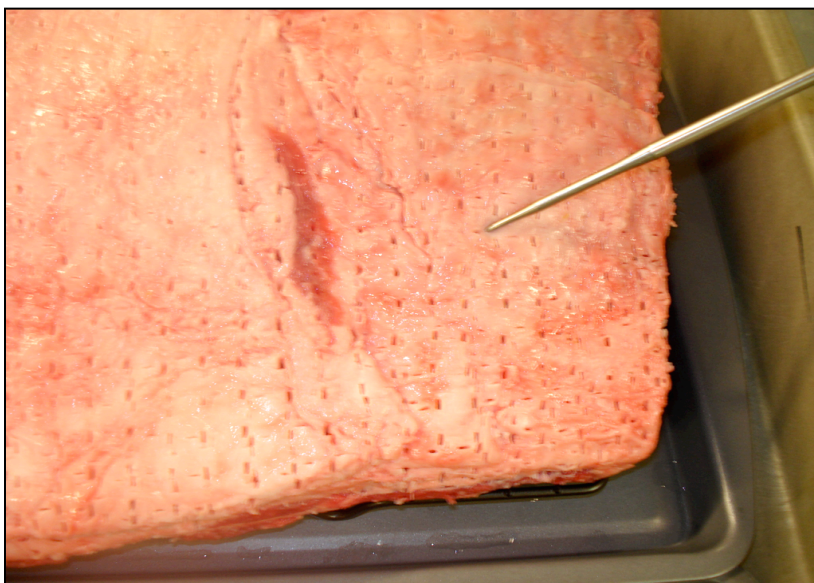


Figure 6. Subprimal after mechanical tenderization.

were left on the same tray in which they were placed after tenderization, and the aluminum foil was removed.

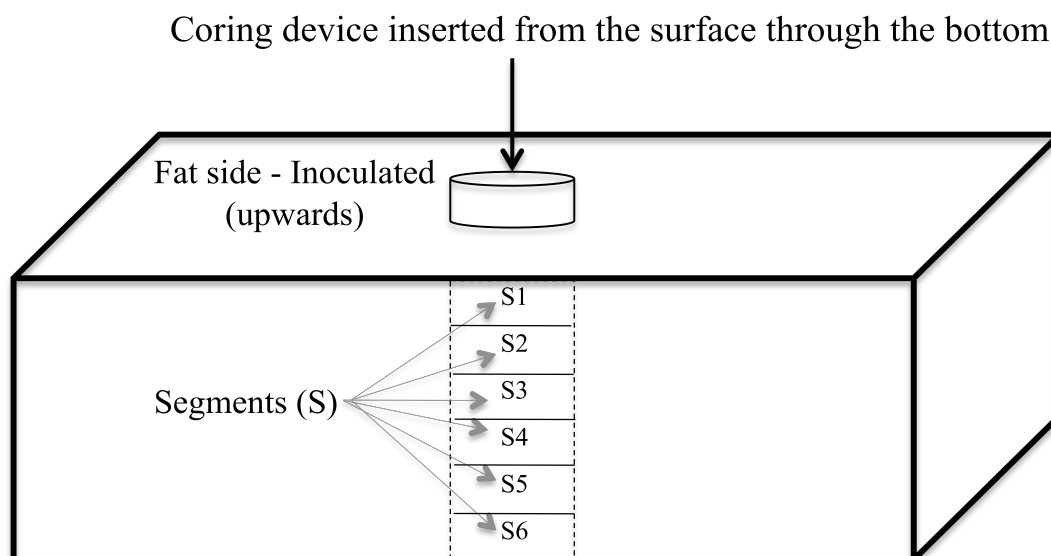


Figure 7. Scheme of the coring sample.

Internal temperatures of the meat and the oven were monitored using type J thermocouples (Omega Engineering Inc., Stamford, CT) connected individually to a six-channel digital panel temperature indicator (model 500T, Doric Instruments, VAS Engineering Inc., San Diego, CA) inserted in the geometric center of the meat, surface of fat side, and inside of the ovens.

A sequence of steps and variables obtained from the survey of the restaurants was used to evaluate *Salmonella* spp. destruction or survival during searing, cooking, and holding of the subprimals. As the cooking was conducted to attain two different internal temperatures (37.8 and 48.9°C), two subprimals were processed for each replicate of the experiment (Fig. 8). The first step, searing, was conducted on a standard kitchen electric

oven pre-heated to 260°C. Once the set temperature of the oven was attained, the two subprimals were placed in the searing oven for 15 min. After searing, the subprimals were placed in the cooking oven (Vulcan/Wolf Model VC4ED, Louisville, KY) preheated to 135°C and cooked to internal temperatures of either 48.9°C or 37.8°C. Following this, the meat was transferred to the holding oven (NFS, Ann Arbor, MI) preheated to at 60°C and held up to 8 h.

The samples were obtained from each subprimal by slicing a portion of ca. 2 cm from each end (steaks). Those steaks were divided into three strips, top, middle, and bottom (Fig. 8) and referred to as the vertical location of the steak. Each strip was weighed, and the internal temperature of each steak was recorded. After obtaining the steaks, the remaining subprimals were placed immediately in the oven to continue the corresponding thermal process.

Microbial analysis

Core or strip samples were combined with 50 ml or 200 ml of 0.1% PW, respectively, blended for ca. 30 s (Magic Bullet, Homeland Housewares, LLC, Los Angeles, CA), and transferred to sterile filter bags. Appropriate dilutions were spread-plated in duplicate onto non-selective media Tryptic Soy Agar (**TSA**; Difco) and selective media Xylose Lysine Deoxycholate Agar (**XLD**; Difco), and incubated for 24 h at 35°C. Typical black colonies were counted as *Salmonella* spp.

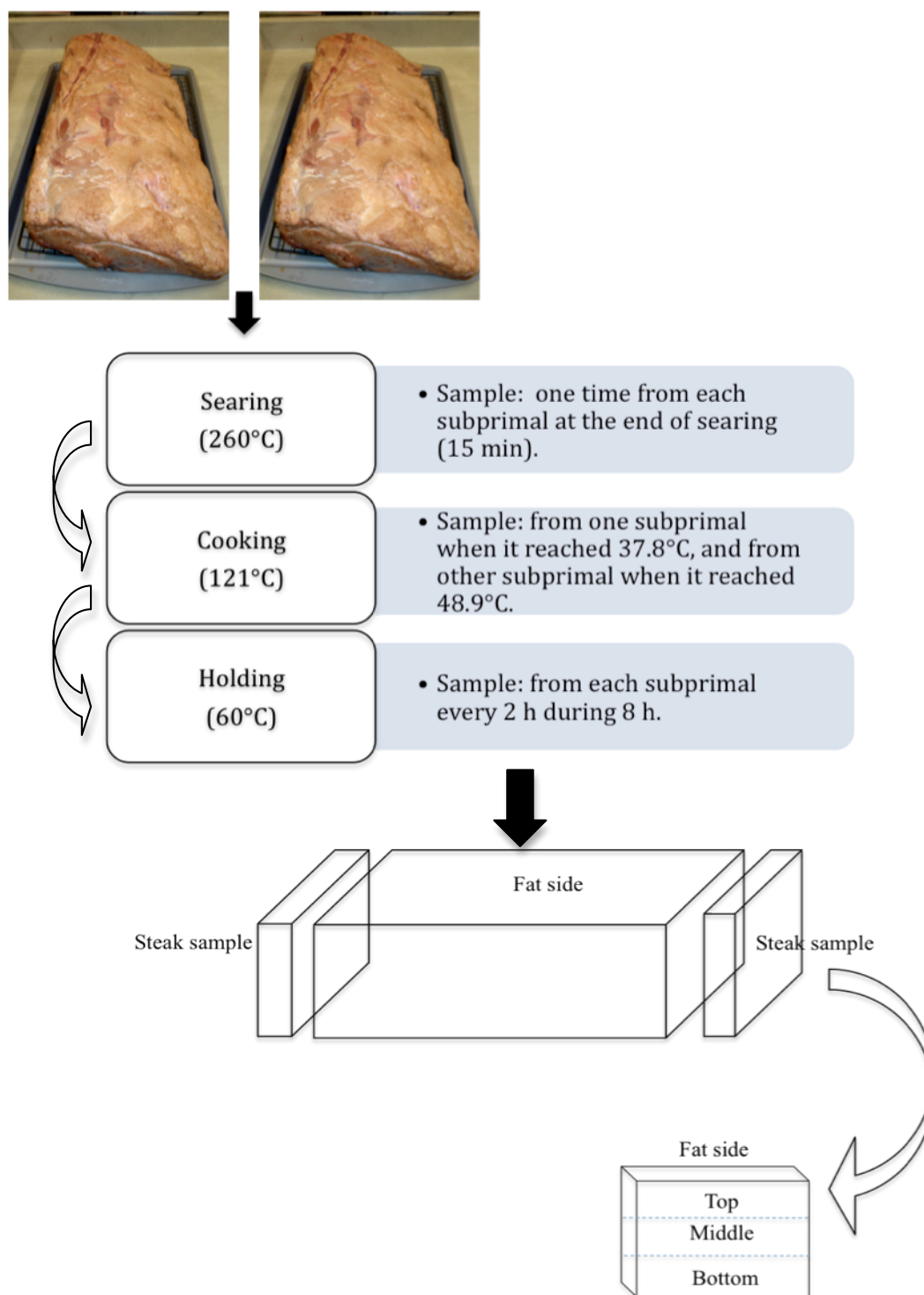


Figure 8. Thermal process and sampling.

3.3 STUDY TWO. Germination and outgrowth of *C. perfringens* spores during hot holding and cooling of prime rib

3.3.1 Bacterial cocktail

C. perfringens cocktail was prepared by combining three different enterotoxin producing strains: NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13), to yield 2.5 log spores/g. Specific information about these strains and preparation of the spores is reported elsewhere (Juneja 2004). The strains used were previously harvested and maintained in refrigeration as stock cultures, following the protocols described by Juneja et al. (1993).

3.3.2 Sample preparation and inoculation

Rib eye was obtained ground from the meat science lab at the Anima Science Department, University of Nebraska-Lincoln, and stored under refrigeration at 1°C. Five g of ground rib eye was aseptically weighed and dispensed into 2.5 by 5.0 in (6.35 by 12.7 cm) vacuum pouches (Prime Source, Kansas City, MO) (3-mil standard barrier polyethylene-nylon bag with a water vapor transmission rate of 10 g/liter/m²/24 h at 37.8°C and 100% relative humidity and an oxygen transmission rate of 3,000 cm³/liter/m²/24 h at 23°C and 1 atm). The meat was inoculated with an aliquot of 100 µL of the 3-strain *C. perfringens* spore cocktail to obtain 2.5 log spores/g, and vacuum-

sealed at 12 mbar (1.2 kPa) with a vacuum packaging machine (A300/H, Multivac, Wolfertschwenden, Germany). The spore inoculum was distributed by hand massaging and flattening the bags to obtain even distribution of the meat in the pouches. The pouches were folded and placed in plastic racks to facilitate handling and improve heat transfer. *C. perfringens* spores in the meat samples were activated at 75°C for 20 min in a water bath (Isotemp 3013H, Fisher Scientific) and immersed in an ice-water bath for ca. 1 min.

3.3.3 Evaluation of hot holding

The pouches containing the inoculated rib eye were immersed in a water bath (RTE 740, Thermo Neslab, Portsmouth, NH) previously set at 43°C or 49°C. Hot holding evaluation was conducted separately at each temperature, for 10 h. Samples were obtained at 2 h intervals from 0 to 10 h of holding and *C. perfringens* populations were enumerated as described below.

3.3.4 Evaluation of cooling during storage of prime rib

Inoculated rib eye contained in pouches containing the heat activated *C. perfringens* spores were exponentially cooled from 57.2°C to 5°C within 6, 9, 12, and 15 h (Fig. 9). Samples were obtained at: (i) start of the process (control samples); (ii) when the temperature reached 27°C; (iii) after 6, 9 and 12 hours; and (iv) at the end of each profile.

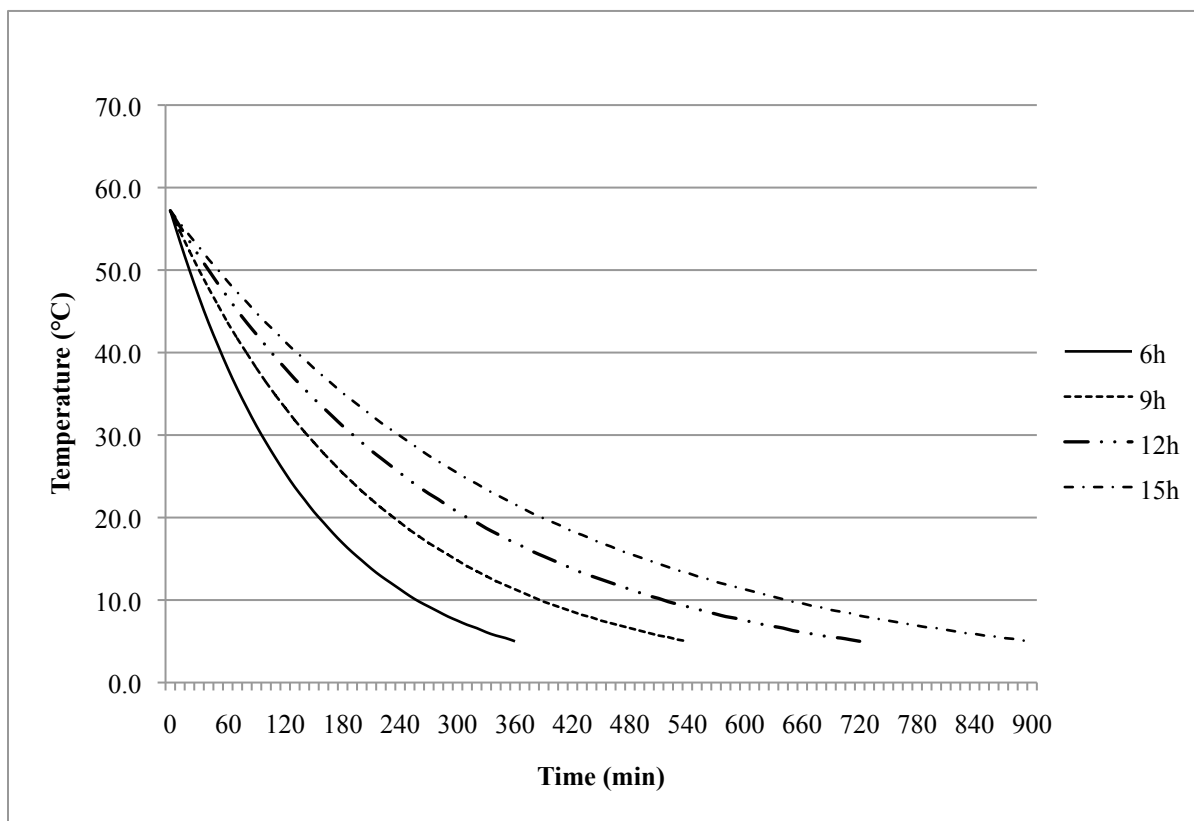


Figure 9. Cooling profiles used to evaluate *C. perfringens* growth.

3.3.5 *C. perfringens* enumeration

The meat was aseptically transferred to filter stomacher bags (Spiral Biotec, Bethesda, MD). Twenty ml of 0.1% buffered peptone water (**PW**; Difco; Sparks, MD), was added and the samples were stomached (Neutec; NR 1378 / 471; Barcelona, Spain) for 2 min. Serial decimal dilutions of the sample were prepared in 9 ml of PW. Duplicate sets were pour plated using Tryptose Sulfite Cycloserine (**TSC**; Oxoid, Ltd., Basingstoke, UK) without egg yolk. Once the agar was solidified, it was overlaid with 8 to 10 ml of TSC and allowed to solidify. Plates were anaerobically incubated at 35°C for 24 h in an anaerobic chamber (Bactron IV; Sheldon Laboratories, Cornelius, OR).

3.4 *Statistical analysis*

A complete randomized split plot design was used. The preliminary experiment (searing) and thermal processing evaluation of searing, cooking, and holding were carried out in duplicate and a set of two independent samples was obtained each time. Hot holding and cooling was conducted in triplicate with a sample for each time point. The microbial data was log-transformed and analyzed using the Statistical Analysis System SAS (SAS Institute, Inc., Cary, NY) version 9.2 (SAS, 2003). The significant differences were determined at $\alpha = 0.05$. All variables were analyzed using PROC MIXED of SAS for analysis of variance (ANOVA), and least squares means were separated (F test, $p \leq 0.05$) by using least significant differences generated by the PDIFF option.

4. RESULTS AND DISCUSSION

4.1 *Survey of restaurants*

All the restaurants surveyed used rib eye, boneless, lip-on subprimal, of ca. 4.3 kg weight (NAMP Foodservice cut 112A) for preparing prime rib. The rib eye portion contains the *longissimus dorsi*, *spinalis dorsi*, *complexus*, and *multifidus dorsi* muscles, and the lip consists of the *serratus dorsalis* and *longissimus costarum* muscles and related intermuscular fat on the short plate side (NAMP 2007).

All restaurants followed similar steps of searing, cooking and holding (Table 1), and cooked to rare and medium rare temperatures. Variations in time-temperature schedule were observed, and were mostly related to differing in-house standards. Factors such as equipment type, meat size, and personnel training also contributed to these variations.

Searing is the first step in the preparation of prime rib; it is normally conducted at an oven temperature between 204.4°C and 260°C for a period of 15 to 40 min. Refrigerated subprimals are rubbed with spices and seasonings and placed in a preheated convection or rotisserie oven. The main purpose was to quickly seal the surface of the meat to retain the juices and keep the moisture of the meat inside. A minimal increase in the internal temperature of the meat (1 to 12°C) was observed during the searing process.

The seared rib eye was transferred to a preheated convection oven, and cooked at a low temperature for a long period of time. Cooking temperature varied by restaurant,

from 107°C to 135°C and the rib eyes were cooked for 1.5 to 3 h. During this step, it was common for all surveyed restaurants to stop the cooking process when the meat reached an internal temperature of 48.9°C.

The pre-cooked meat was transferred to a convection holding oven before serving to the consumers. Holding oven temperatures were between 49°C and 71°C, depending on the restaurant. Changes in the doneness of the meat were minimal during this step. Holding times were not related to the preparation of the prime rib, but rather associated with the planning process for each restaurant. Prime rib is typically served for dinner, and in spite of that some restaurants prefer to start the preparation early in the morning. In such cases, holding times could last up to 11 h (Figs. 10 to 15)

As in any food service operation, leftovers remain at the end of the day and are stored under refrigeration at ca. 5°C, and used following day. The risk factor during this activity is the cooling time from 48°C to 6.7°C, which can be up to 14 h (Fig. 16).

Potentially hazardous food relies on thermal processing treatments as a means to control biological hazards when antimicrobial interventions intended to reduce the risk of foodborne pathogens are not applied. Regulatory agencies (FDA and USDA) have developed guidance that helps processors to establish thermal processing standards to assure food safety. The FDA Food Code (2009) serves as a guide for food service establishments on proper cooking and subsequent handling and recommends cooking intact meat to a minimal internal temperature of 54.4°C for about 2 h, which is higher than the established restaurant practices. In addition, the Food Code allows serving undercooked intact beef if it has been cooked to a surface temperature of 63°C and color

change is achieved (FDA 2009). Searing process achieved 63°C on the surface of the rib eye but was only maintained for ca. 10 min at 204.4°C. On the other hand, holding temperatures of 54.4°C are recommended for food served hot. A maximum of 4 h is allowed for hot holding if the temperature of the meat is maintained at $\geq 57^{\circ}\text{C}$ (FDA 2009).

The meat processors should follow compliance guidelines for cooking and stabilization or cooling (Appendix A and Appendix B), which cautions them to avoid the use of slow-cooking methods (Ingham et al. 2004), and long cooling periods. The USDA FSIS Compliance Guidelines For Meeting Lethality Performance Standards For Certain Meat and Poultry Products (Appendix A) recommends cooking meat to a minimum of 54.4°C for 112 min similar to the Food Code. However, a main feature of the compliance guidelines is the requirement that the cooking process needs to be able to reduce *Salmonella* spp. population by 6.5 log CFU/g (USDA 1999).

Appendix B addresses stabilization of the cooling process to prevent growth of *C. perfringens* in no more than 1-log. It requires that the product should not remain between 54.4 and 26.7°C for more than 1.5 h, nor between 27.6 and 4.4°C for more than 5 h (USDA 1999). The internal meat temperature of the surveyed restaurants indicated that 4.5 h are needed to cool from the initial temperature of 26.7°C; and more than 9 h between 27.6 and 4.4°C.

RESTAURANT	SEARING OUTSIDE		COOKING			HOLDING	
	Oven temp. (°C / °F)	Time (min)	Oven temp. (°C / °F)	Time (h)	End-point (°C / °F)	Oven temp. (°C / °F)	Time (h)
Restaurant A	218 / 425	20	107 / 225	1.5 - 2 h	48.9 / 120	60 / 140	4 - 9
Restaurant B	204 / 400	20 to 25	121 / 250	1.5 - 2 h	48.9 / 120	48.9 / 120	5 - 11
Restaurant C	260 / 500 (Rotisserie)	30 to 45	121 / 250	2.5 - 3 h	48.9 / 120	71 / 160	1 - 5
Restaurant D	No searing	No searing	121 / 250	3 h	48.9 / 120	60 / 140	1 - 4
Restaurant E	204 / 400	15 to 20	132 / 270	2 - 2.5 h	48.9 / 120	48.9 / 120	5 - 9
Restaurant F	260 / 500 (Rotisserie)	20 to 25	135 / 275	1.5 - 2 h	48.9 / 120	60 / 140	2 - 7

Table 1. Parameters followed by restaurants to prepare Prime Rib.

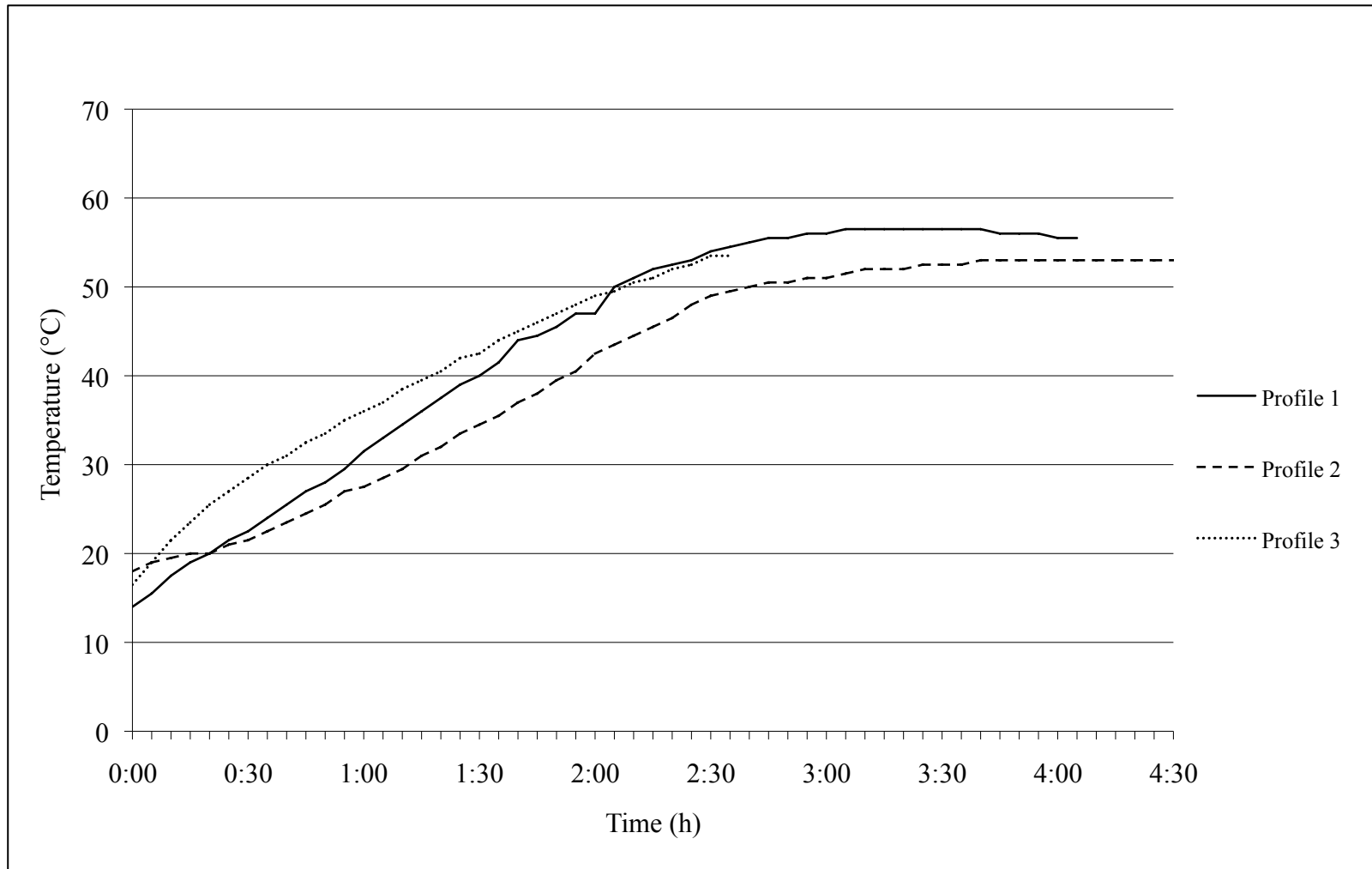


Figure 10. Temperature profile during preparation of prime rib - Restaurant A.

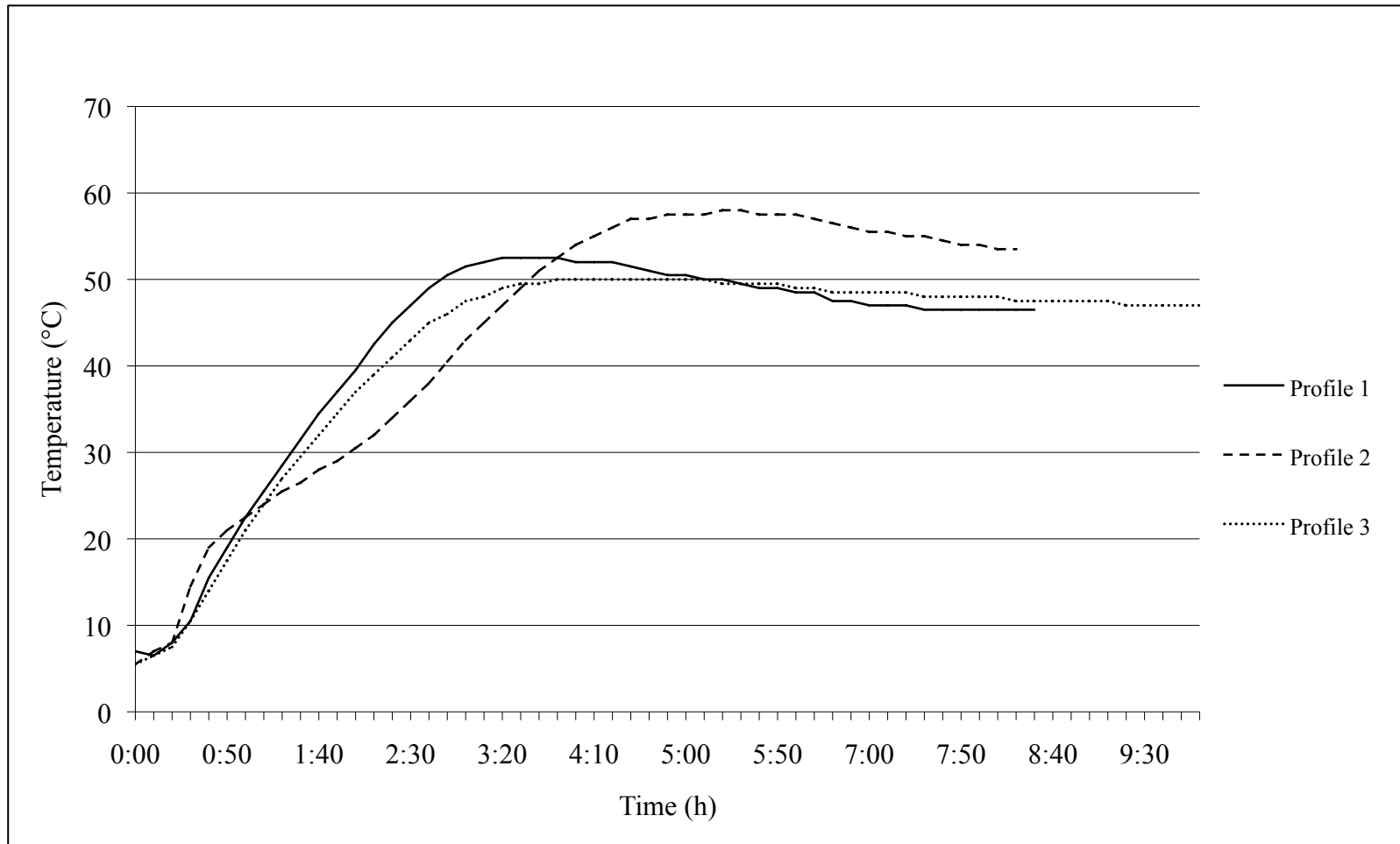


Figure 11. Temperature profile during preparation of prime rib - Restaurant B.

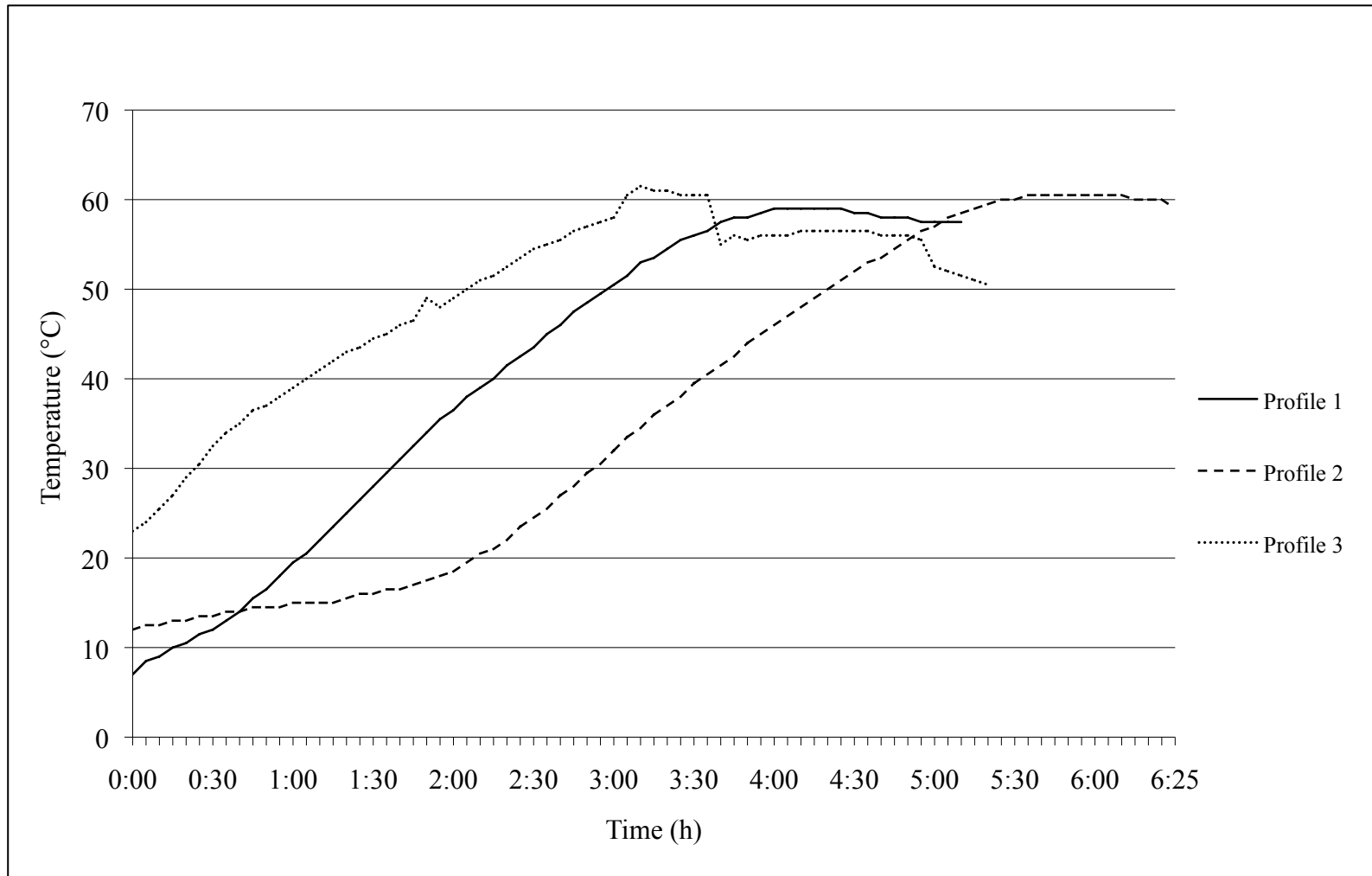


Figure 12. Temperature profile during preparation of prime rib - Restaurant C.

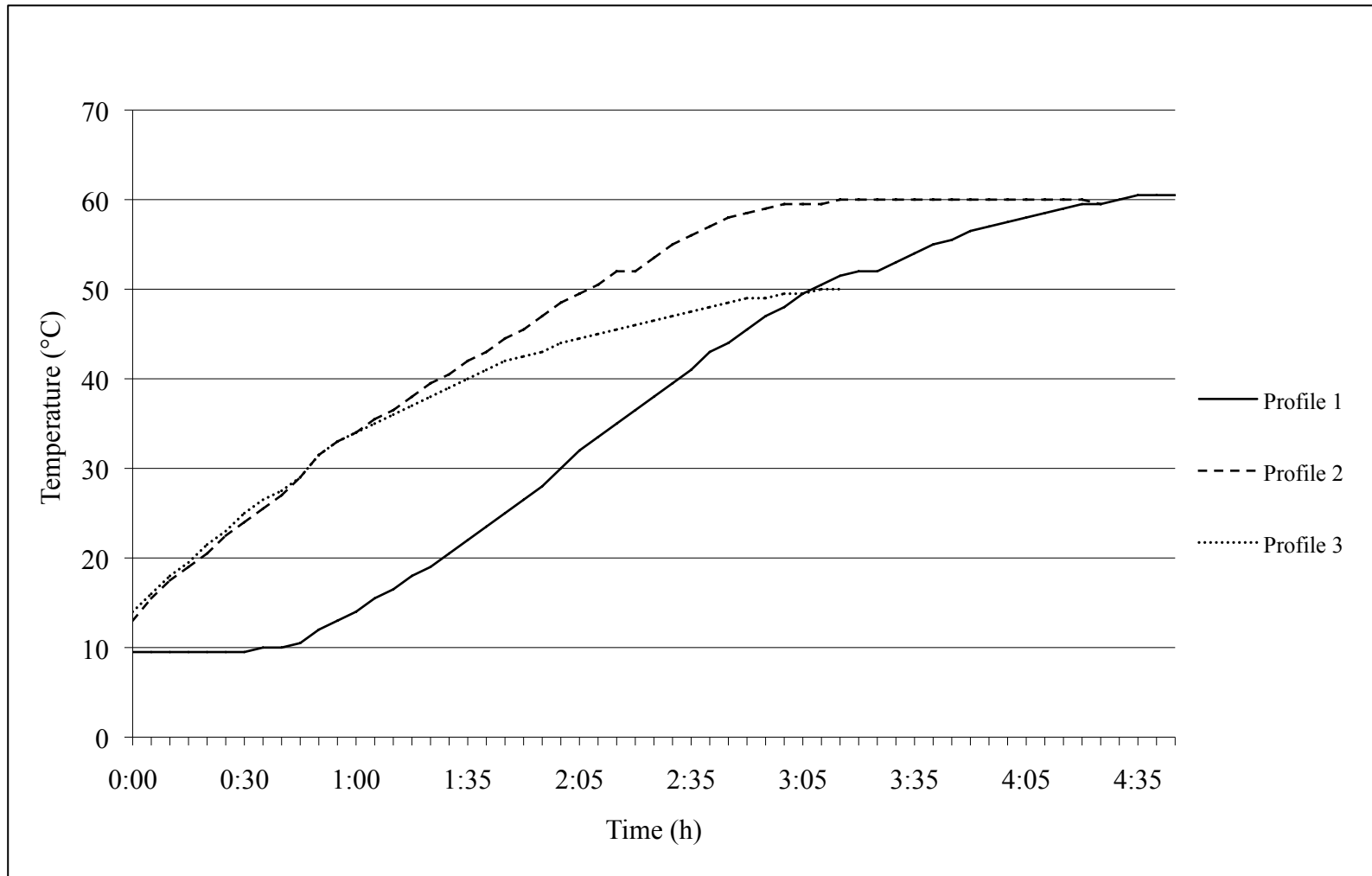


Figure 13. Temperature profile during preparation of prime rib - Restaurant D.

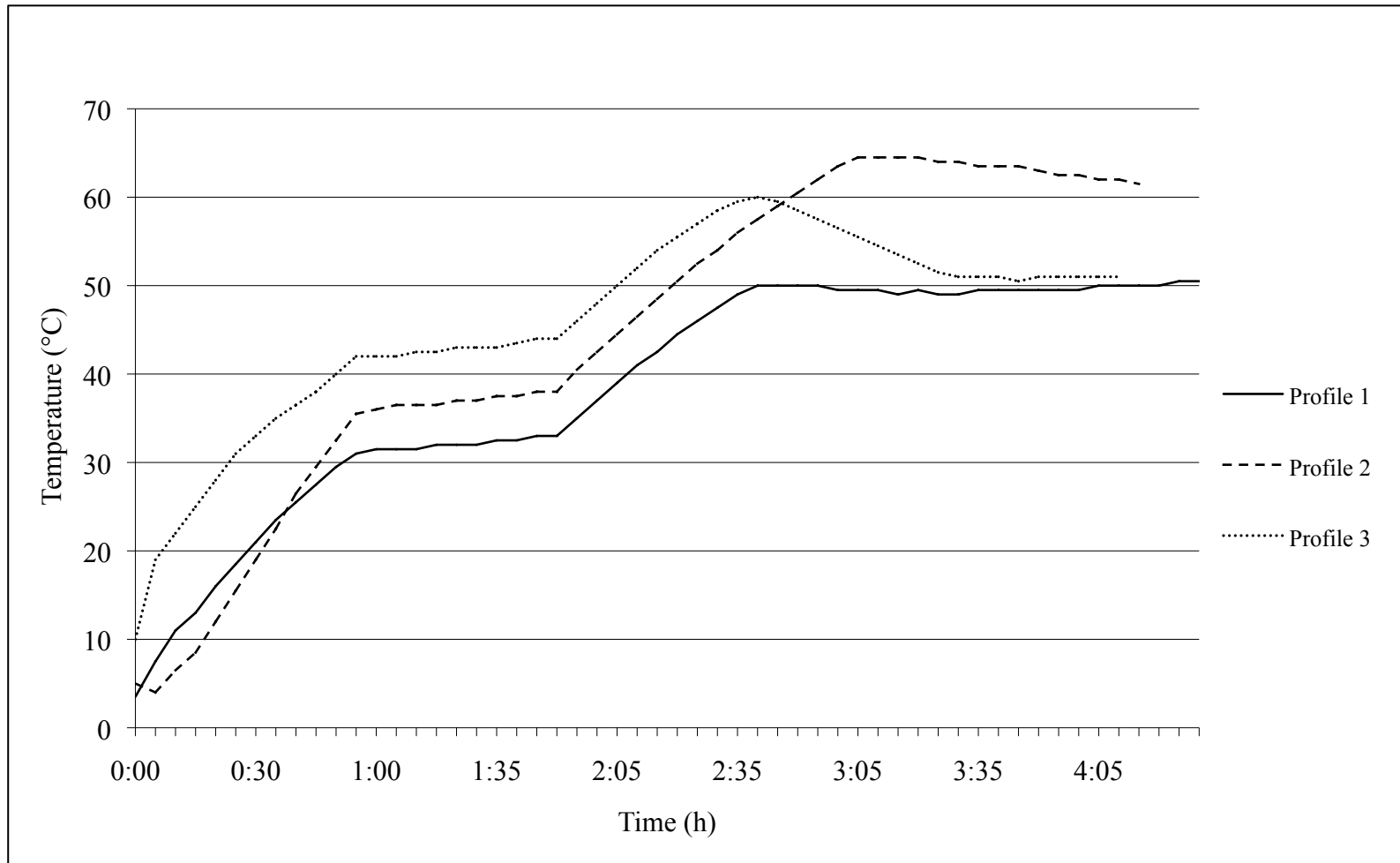


Figure 14. Temperature profile during preparation of prime rib - Restaurant E.

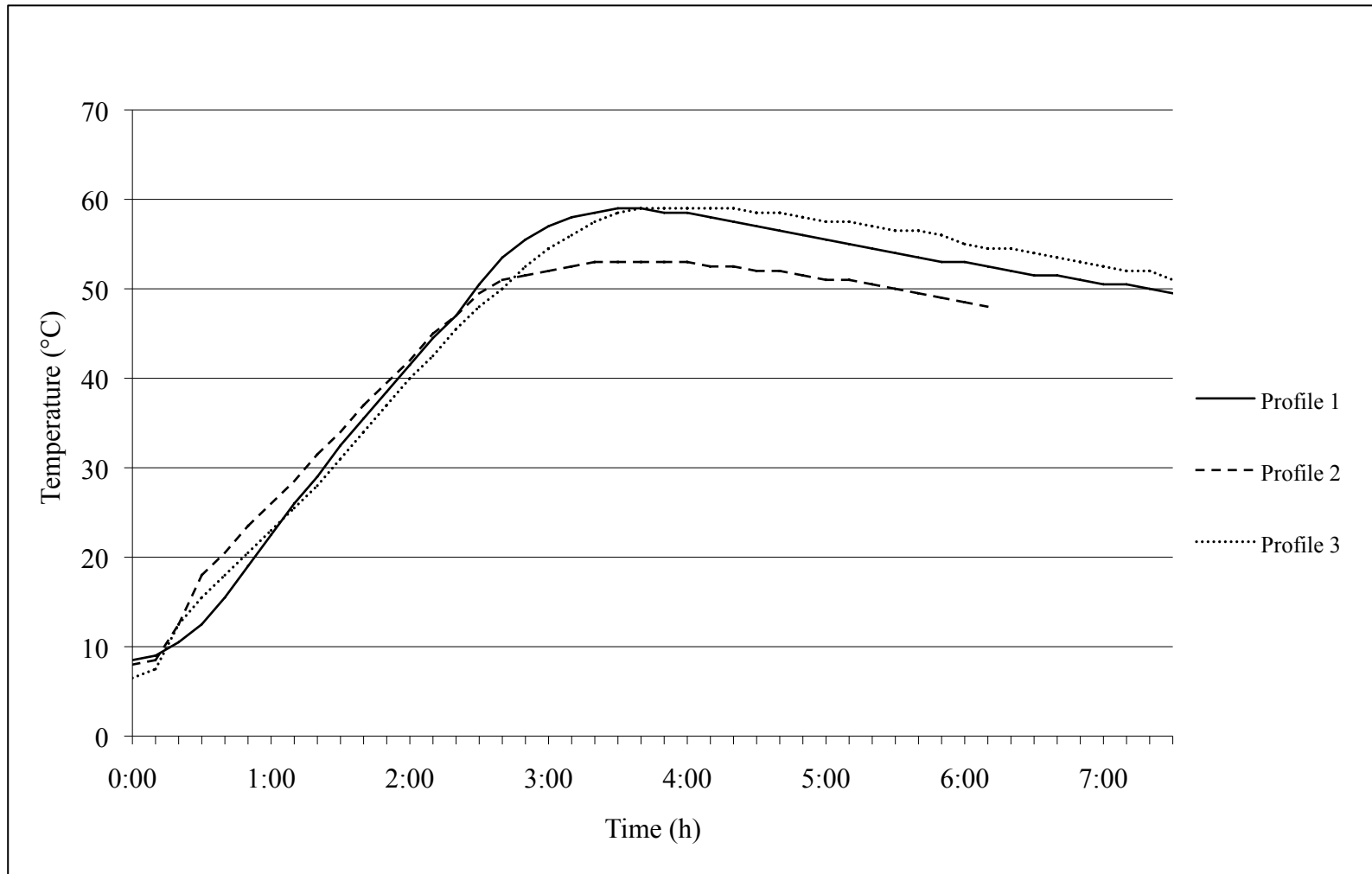


Figure 15. Temperature profile during preparation of prime rib – Restaurant F.

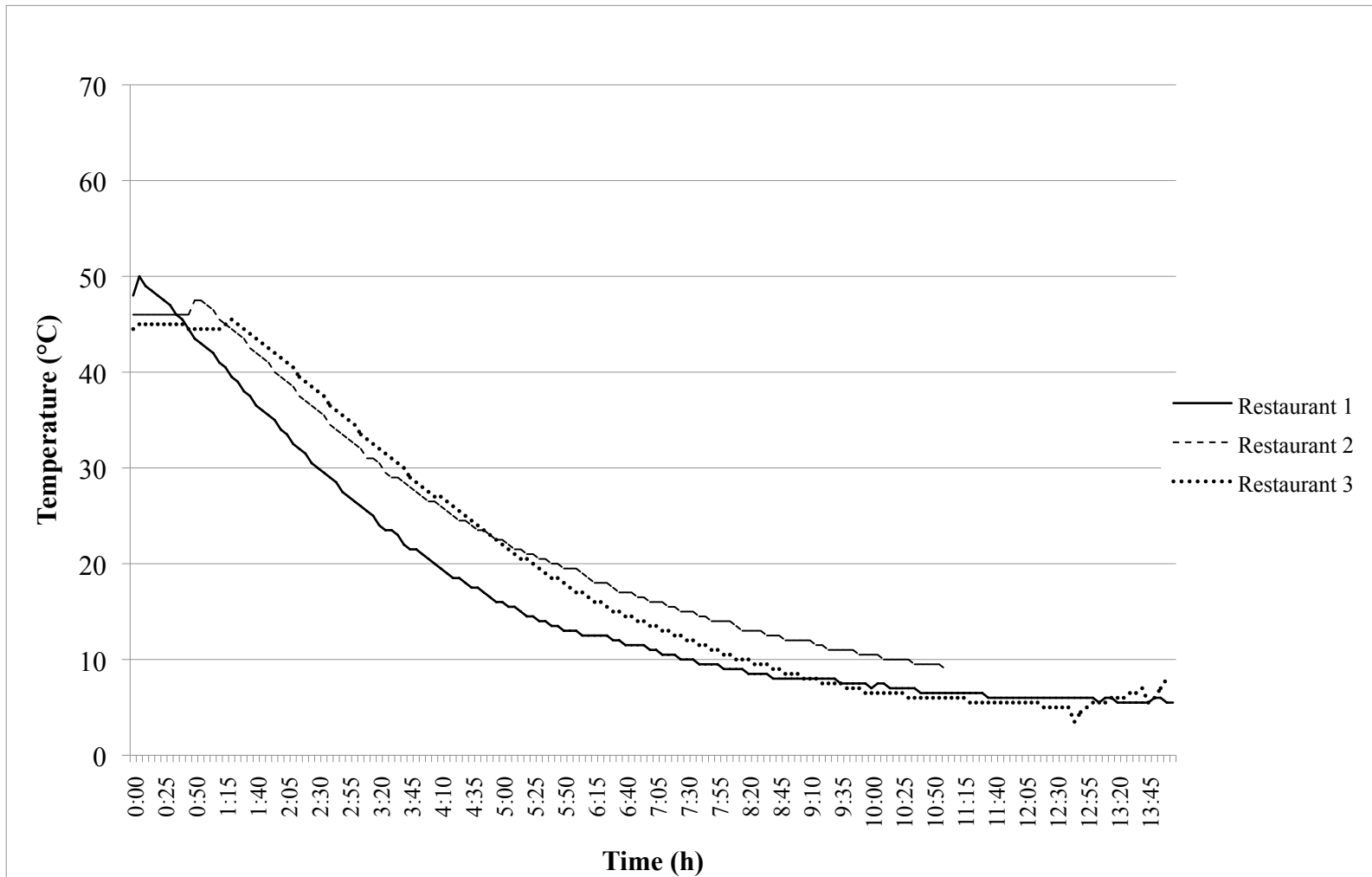


Figure 16. Cooling profile of prime rib leftovers at three different restaurant.

4.2 STUDY ONE. Survival of *Salmonella* spp. during thermal processing steps used for the preparation of prime rib

4.2.1 Effect of searing process for reduction of *Salmonella* spp. on surface of intact subprimals

Salmonella spp. population of ca. 6.54 log CFU/cm² on the surface of the subprimals was achieved subsequent to inoculation. Regardless of the side (fat or lean), searing at 260°C attained a greater reduction of *Salmonella* spp. compared to 204°C ($p \leq 0.05$) (Fig. 17). Searing rib eye at 260°C for 60 min resulted in *Salmonella* spp. reductions of 5.27 log CFU/cm², while lower reduction of 4.6 log CFU/cm² was achieved at to the lower cooking temperature of 204°C.

Greater reduction ($p \leq 0.05$) in *Salmonella* spp. was observed on the fat side at each temperature as compared to the lean side, which was in direct contact with the cooking tray (Fig. 18 – 19). *Salmonella* spp. reduction of 6.09 and 4.50 log CFU/cm² were observed on the fat and lean side respectively at an oven temperature of 260°C, whereas reductions of 5.94 and 3.38 log CFU/cm² were observed at 204°C on the fat and lean sides, respectively.

The length of cooking also had an impact ($p \leq 0.05$) on *Salmonella* spp. reductions. Regardless of the temperature and side, the destruction of *Salmonella* spp. was proportional to the searing time; the higher the temperature, the greater the reduction. In spite of the fact that after 60 min, > 6 log CFU/cm² of *Salmonella* spp.

reduction was achieved, it is important to note that the typical searing time in restaurants was ca. 30 min, which resulted in smaller reductions (3.32 log CFU/cm² at 260°C and 1.74 log CFU/cm² at 204°C) in *Salmonella* spp.

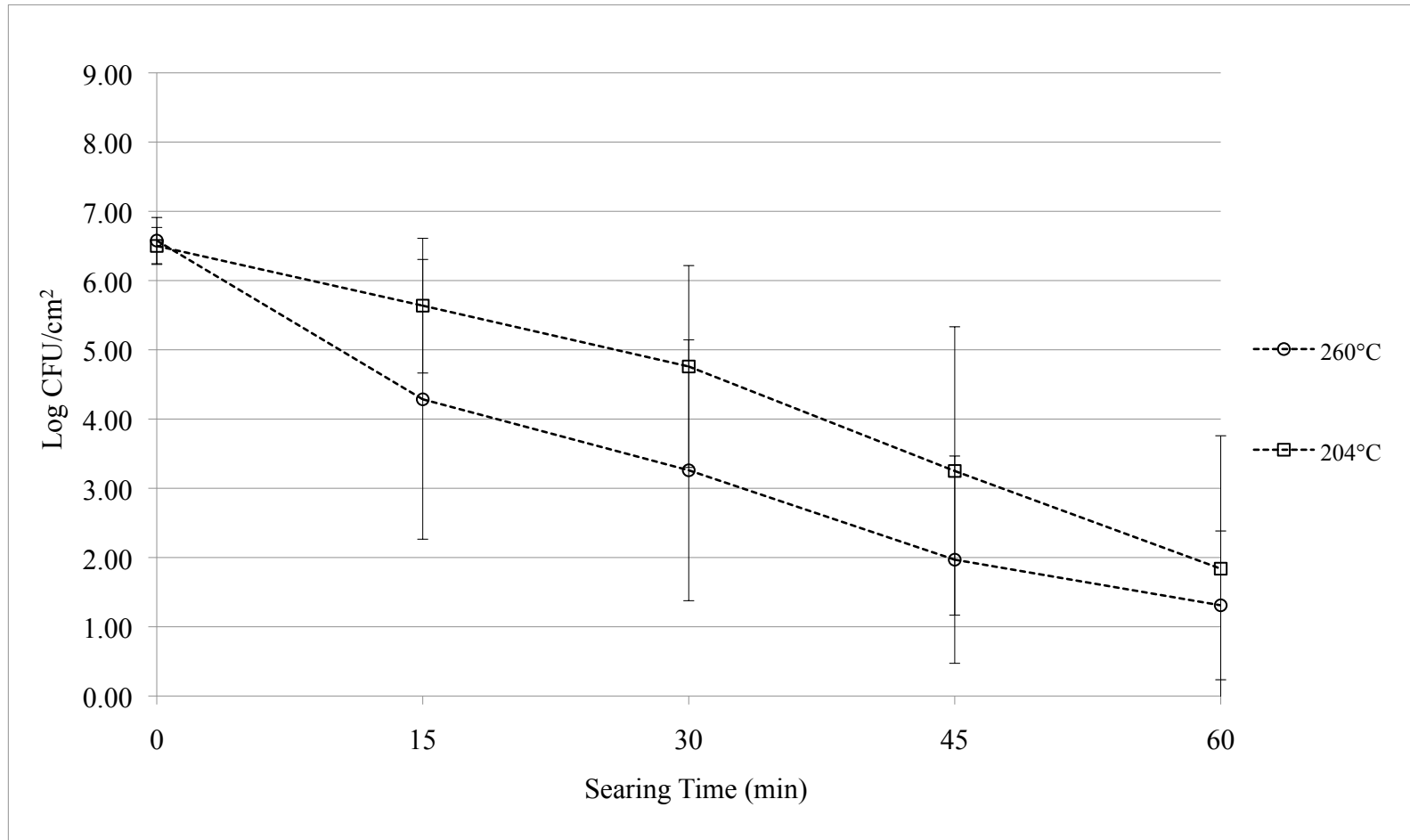


Figure 17. Survival of *Salmonella* spp. Comparison between two temperatures during searing of subprimals.

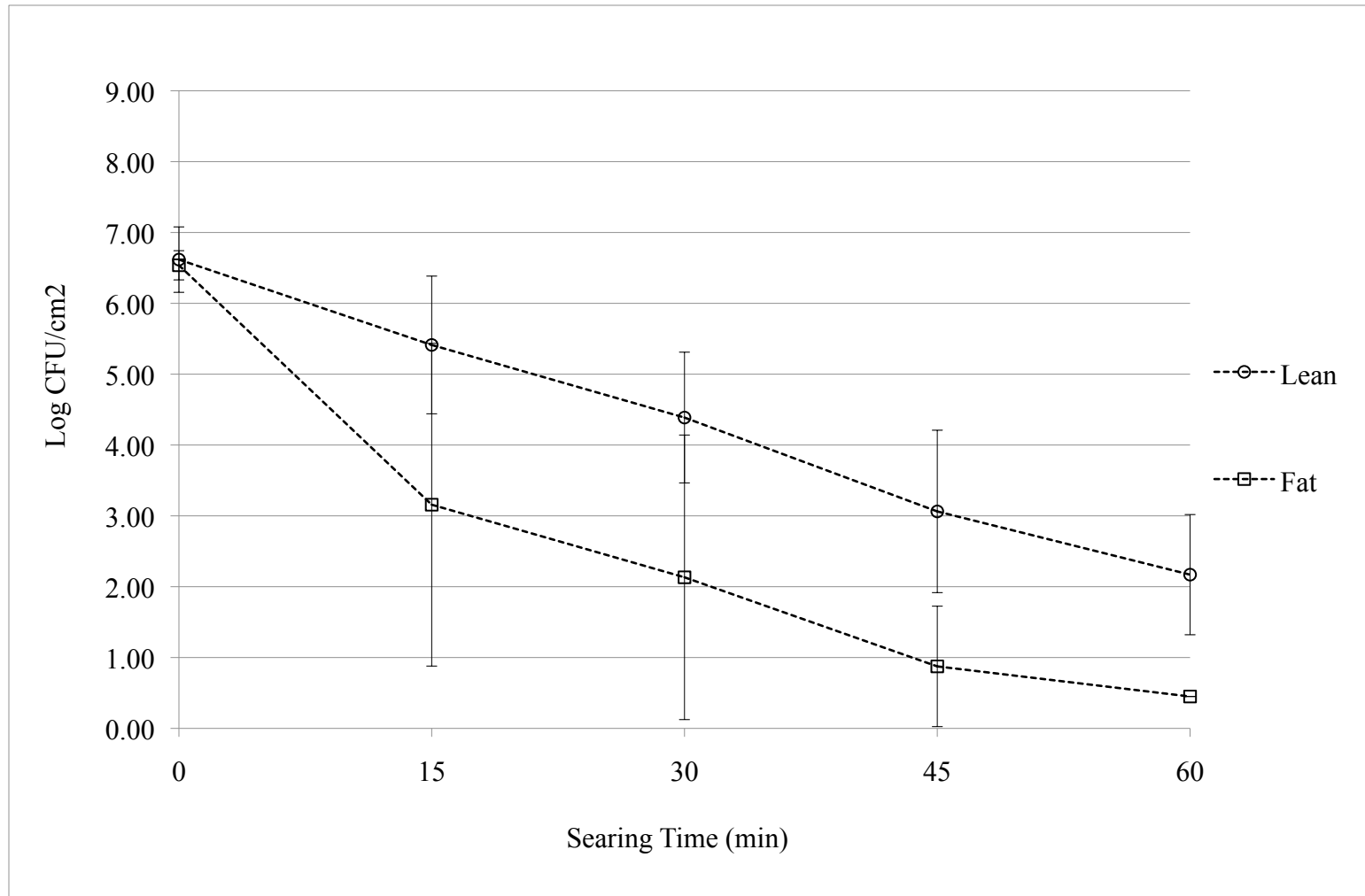


Figure 18. Survival of *Salmonella* spp. during searing at 260°C for 60 min. Level of recovering of the microorganism from both, lean and fat side.

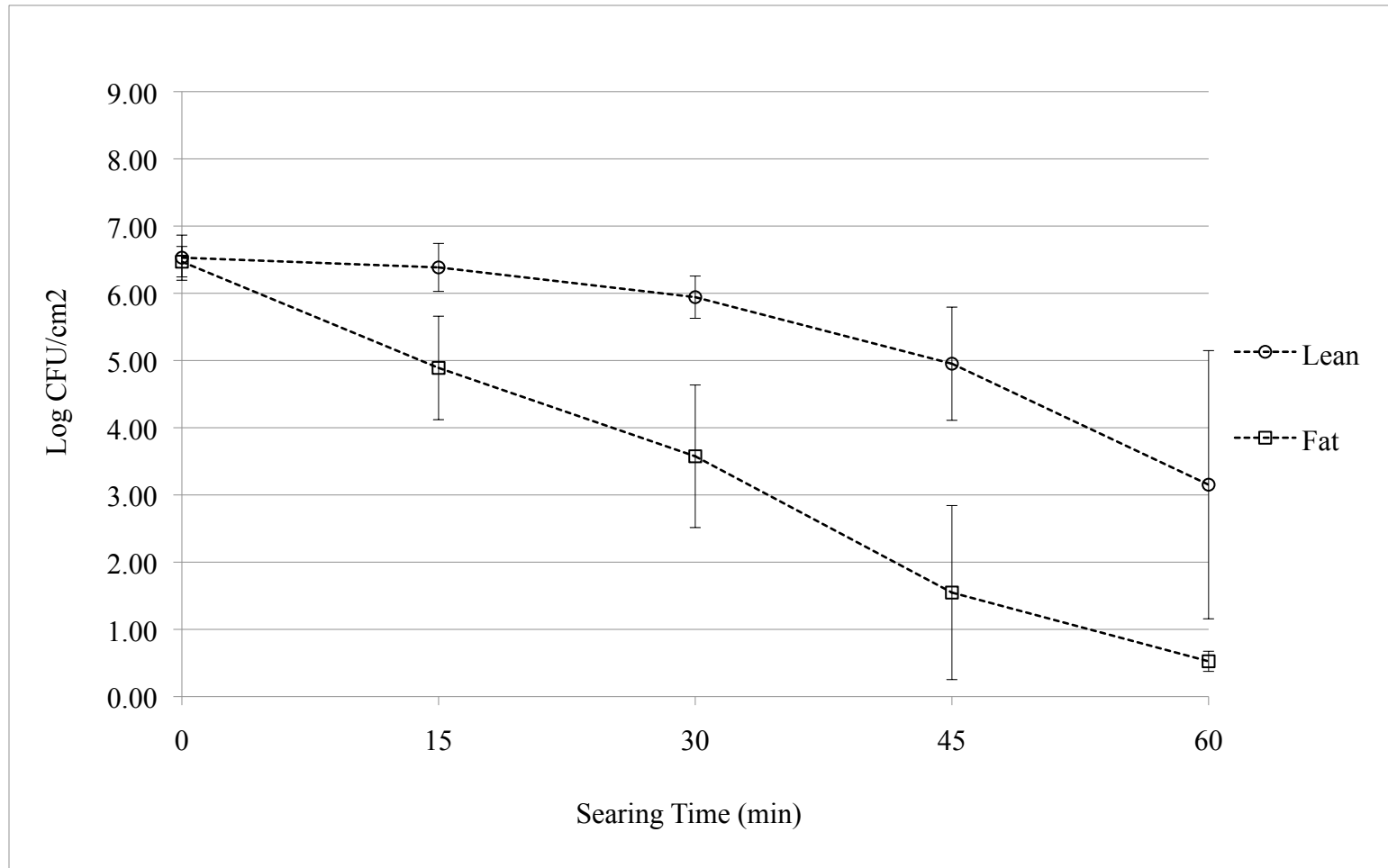


Figure 19. Survival of *Salmonella* spp. during searing at 204°C for 60 min. Level of recovering of the microorganism from both, lean and fat side.

4.2.2 Survival of *Salmonella* spp. during the thermal process of intact and non-intact prime rib

Thermal Processing of Intact Prime Rib

Salmonella spp. populations of 6.02, 5.43, and 5.54 log CFU/g were achieved on the top, middle and bottom slices of the rib eye. Since the inoculum was applied to the surface of the intact rib eye, finding such levels of microbial load in the middle and bottom strip indicates contamination of the non-inoculated surfaces by the knife from steak surfaces during slicing. The steaks were sliced and sampled to allow comparison to the non-intact rib eye and to mimic the portioning process in the restaurants.

While there is a low probability of pathogenic bacteria being present on 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 (USDA, 1999).

Analysis of the thermal processing steps (searing, cooking, and holding for 2h) revealed that in both, samples cooked to 37°C (Fig. 20), and to 48.9°C (Fig. 21) there was a treatment effect ($p \leq 0.05$) from the cooking temperature (Table 2 – 3). In addition, a difference in *Salmonella* spp. reduction between the strips (top, middle and bottom) was observed in both sets cooked to 37.8°C ($p > 0.05$), compared to those cooked to 48.9 °C ($p \leq 0.05$).

Table 2. Statistical comparisons based on P-values for steaks of prime rib cooked to 37.8°C. Means (log CFU/g of *Salmonella* spp.) at each processing step

Control	Searing	Cooking	Holding (2h)
5.54	4.31	0.32	0.67
a	a	b	b

Table 3. Statistical comparisons based on P-values for steaks cooked to 48.9°C. Means (log CFU/g of *Salmonella* spp.) at each processing step

Control	Searing	Cooking	Holding (2h)
5.54	4.19	0	0.83
a	a	b	b

Searing achieved minimal destruction of *Salmonella* spp. ($p>0.05$) (1.35 log CFU/g). Since both sets of samples (cooked to 37.8°C and 48.9°C) were seared prior cooking following the same time and temperature variables, the reduction of *Salmonella* spp. was similar. The set of samples cooked to 37°C attained reductions of 1.57, 1.00 and 1.10 log CFU/g in top, middle, and bottom strips, respectively after searing. Samples cooked to 48.9°C, showed reductions by 1.46, 1.47, and 1.12 log CFU/g in top, middle and bottom, respectively. After searing both sets of samples (cooked to 37.8°C and 48.9°C), an increase of ca. 15°C of the internal temperature of the rib eye was observed. Such increment is not sufficient to achieve destruction of the

pathogen considering the fact that the rib eye cuts were at refrigeration temperature previous to searing. *Salmonella* spp. reductions observed were most probably attained due to the high temperature of the oven on the surface of the prime rib.

Cooking rib eye to an internal temperature of 37.8°C resulted in reduction ($p \leq 0.05$) of the *Salmonella* spp. population by 5.22 log CFU/g; however, subsequent holding of the rib eye for 2 h resulted in an increase of 0.35 log CFU/g. This increase in *Salmonella* spp. population was consistent with an increase in population of the pathogen by 0.75 log CFU/g after 8 h of holding. After cooking to 48.9°C, no growth of *Salmonella* spp. was observed (< 1 log CFU/g) in the three strips. Nonetheless, after 2 h of holding, growth of *Salmonella* spp. by 0.33, 1.05, 1.10 log CFU/g in the top, middle and bottom, respectively, was observed. After the 8 h of holding, there was a small reduction ($p > 0.05$) in *Salmonella* spp.

In spite of the reductions of *Salmonella* spp. populations observed, cooking temperatures used by the restaurants surveyed may not achieve > 6.5 log CFU/g reductions in *Salmonella* spp. as required by USDA-FSIS performance standards (Appendix A). While *Salmonella* spp. reductions to below the detection limit was observed subsequent to cooking, viable cells were recovered during holding, indicating survival of a small population of the cells. Blankenship (1978) reported that *Salmonella* spp. was recovered from roast beef cooked to an internal temperature of 57.2°C. The author recommended cooking roast beef to an internal temperature of 62.8°C that has to be achieved in all parts of the roast in order to obtain a salmonellae-free product if a high inoculum is used. In addition, injection of steam either during

the beginning or ending the cooking process, also contributes to the destruction of *Salmonella* in beef roasts (Blankenship 1980).

Increase in thermal resistance of *Salmonella* after exposure to elevated temperatures (heat shock) has been reported. Ilungu and Steven (1997) observed that exposing *Salmonella* to a heat shock at 42°C for 60 min raised the D-values at 52, 54, and 56°C. Similarly, Mackey and Derrick (2008) reported that heat shock at 48°C for 30 min increases thermal resistance of *Salmonella* Thompson at a temperature of 54°C.

Holding of prime rib cooked to internal temperatures of 37.8°C and 48.9°C at 60°C for up to 8 h did not affect *Salmonella* spp. populations. In addition, when the thermal process of both sets of samples (subprimals cooked to 37.8°C and 48.9°C respectively) were compared, a similar trend in survival of *Salmonella* spp. was observed (Fig. 22); therefore, no statistical difference was found over the thermal processing steps of searing, cooking, and 2 h-holding.

Effect of Translocation on subprimals

Salmonella spp. population of ca. 5.96 log CFU/g was achieved on the surface (fat side) of the subprimals subsequent to inoculation. Translocation of the microorganism to the interior of the tenderized muscle was observed. Approximately 3.12% of the surface *Salmonella* spp. population was recovered from segments two through six of the rib eye subsequent to blade tenderization (Table 4).

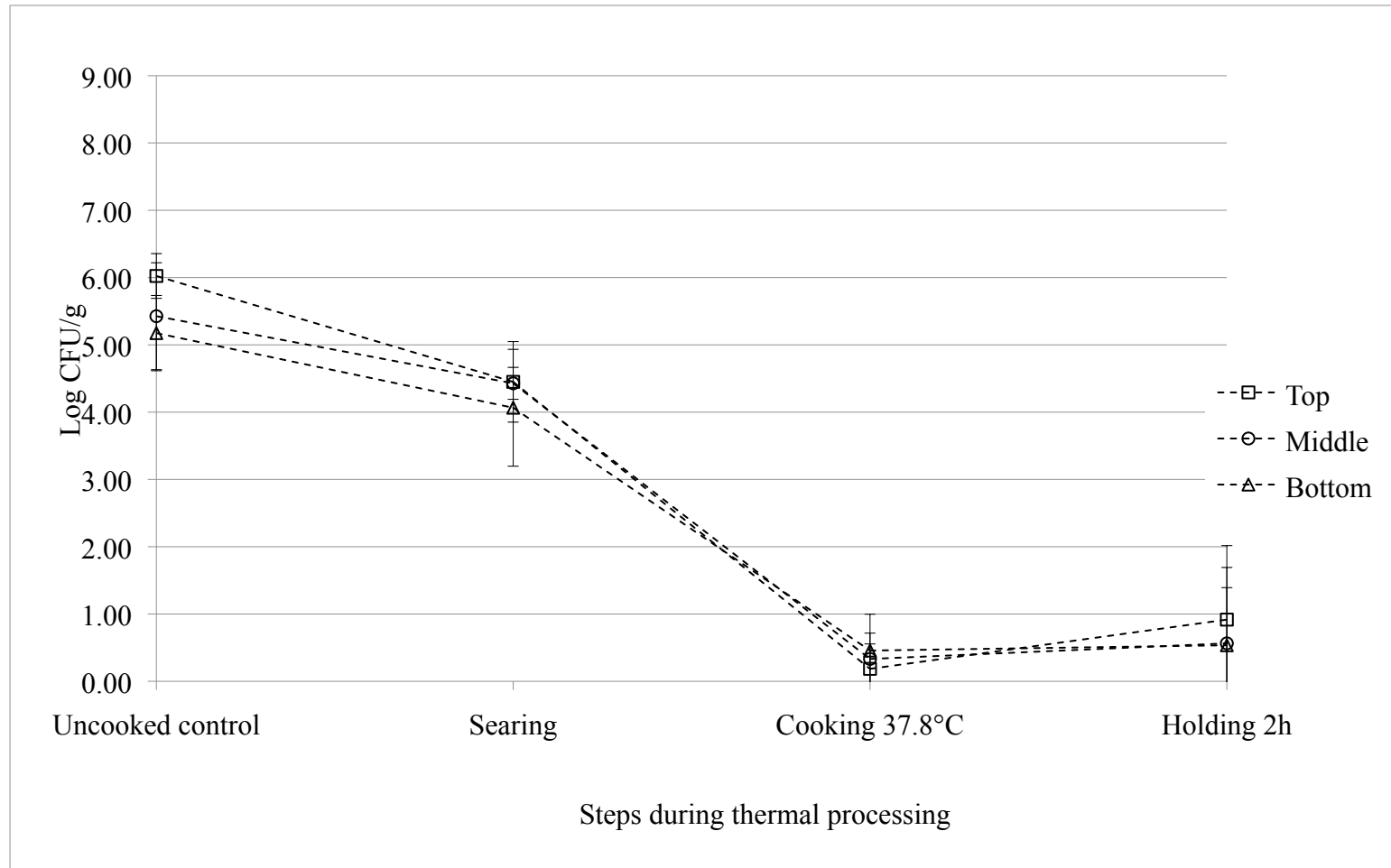


Figure 20. Survival of *Salmonella* spp. during the steps used for thermal processing of intact rib eye when samples were cooked to 37.9°C.

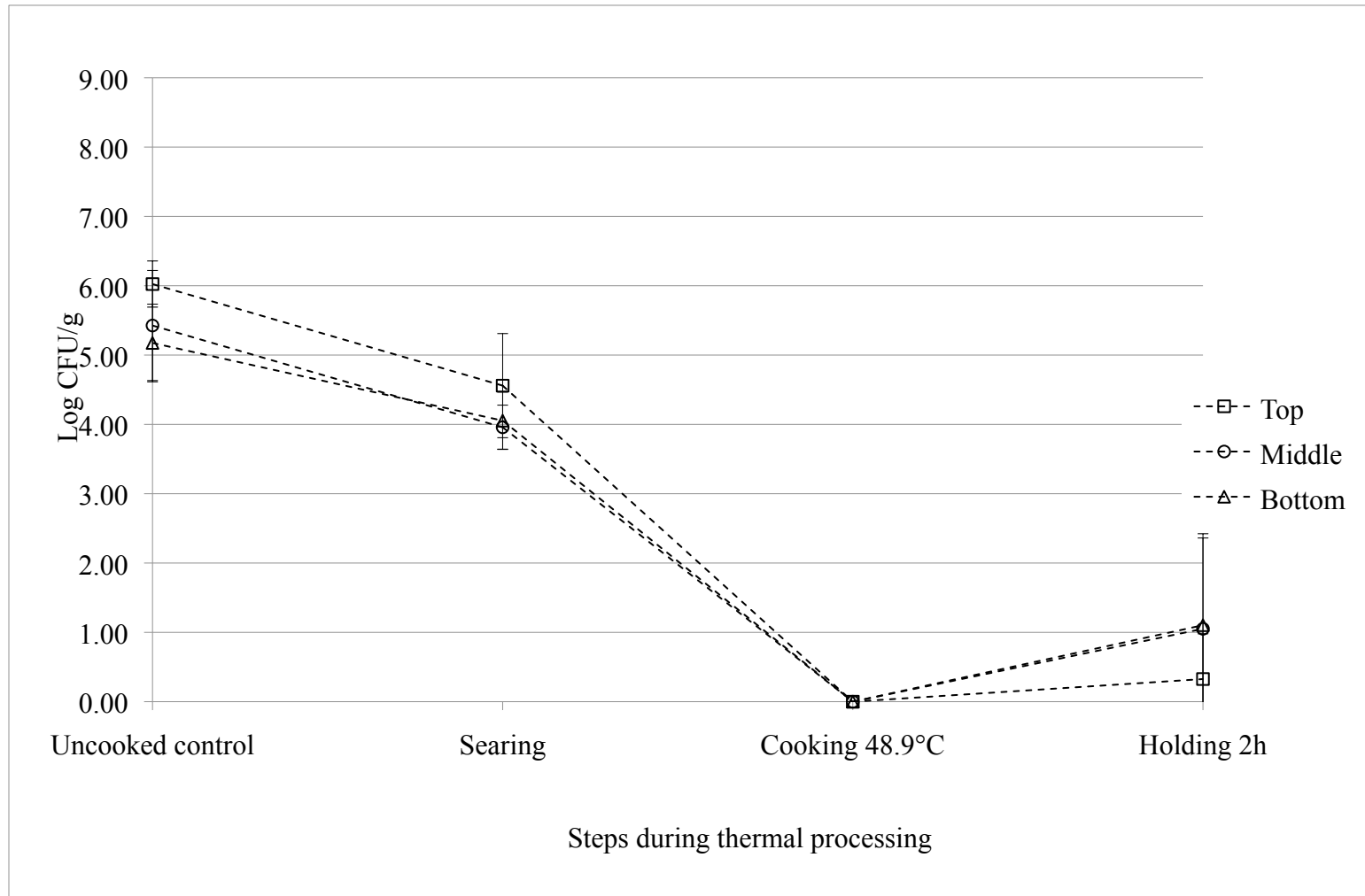


Figure 21. Survival of *Salmonella* spp. during the steps used for thermal processing of intact rib eye when samples were cooked to 48.9°C.

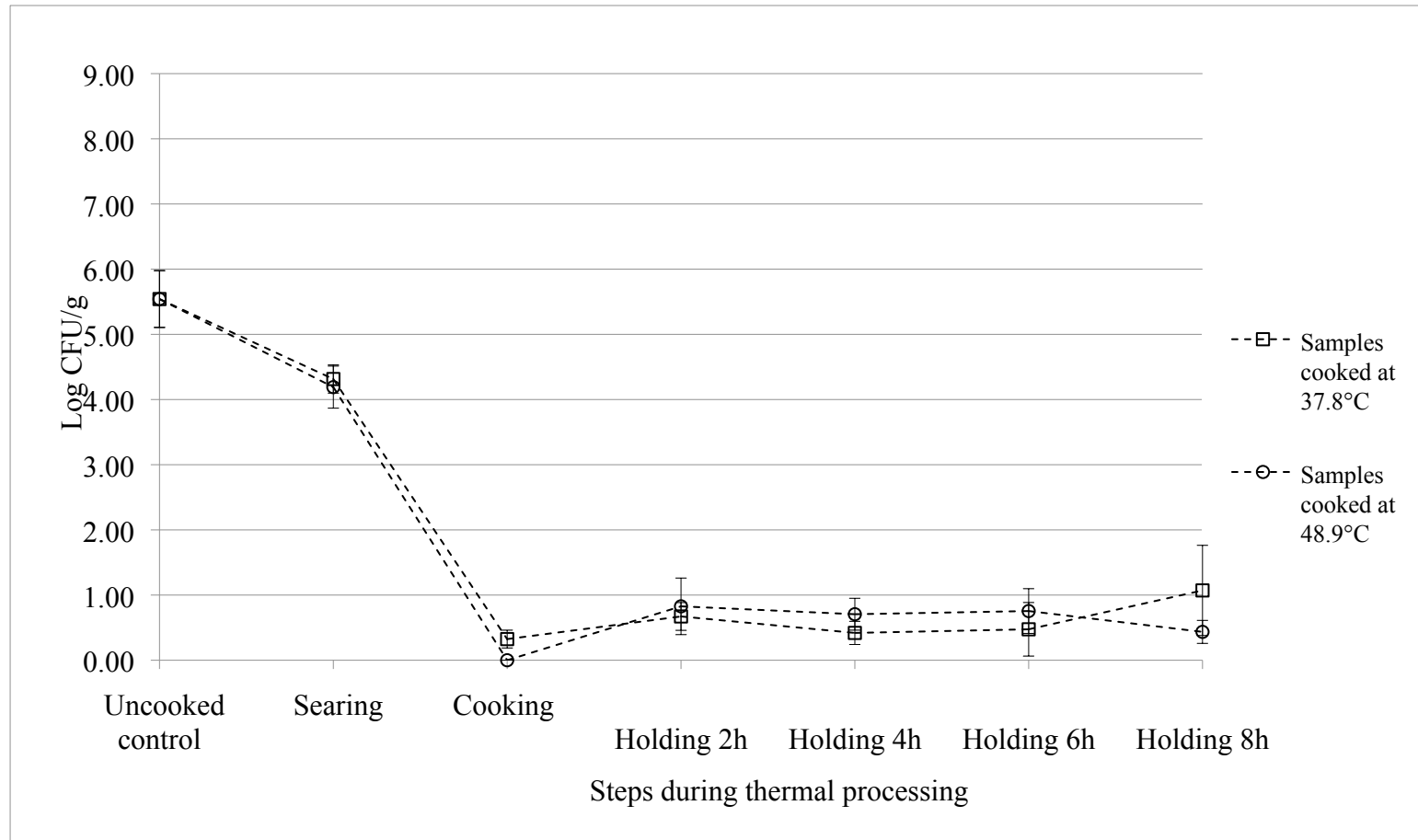


Figure 22. Survival of *Salmonella* spp. in intact meat during the steps used for thermal processing. Comparison between both set of samples, cooked to 37.8 °C and 48.9 °C.

Recovery of *Salmonella* spp. was different between the segments ($p \leq 0.05$).

Salmonella spp. populations of 2.77 and 3.33 log CFU/g were observed in the segments three and four respectively, the location of the geometric center of the steak, equivalent to 0.29%. Blade tenderization resulted in *Salmonella* spp. translocation of 4.24 log CFU/g vertically to the bottom of the prime rib. In segments five and six, an increase (0.59 and 1.90%, respectively) in *Salmonella* spp. population was observed as compared to the segments one through four (Fig. 23).

Disruption of meat tissues represents a major food safety concern, especially when the meat is undercooked (Laury et al. 2009). The effect of bacterial translocation has been reported (Sporing 1999, Phebus et al. 2000, Marsden et al. 2004, Huang et al. 2007, Luchansky et al. 2008 and 2009). Phebus et al. (2000) reported that blade tenderization carries 3 - 4% of surface contamination to the center of the subprimals, regardless of the initial level of surface contamination. The translocation of microorganisms throughout the muscle after mechanical tenderization is due to physical forces (blade insertion), and the rate of microbial displacement can be affected by factors such as blade shape, muscle structure, product temperature, and fat content (Thippareddi et al. 2000).

Gradual decrease in *Salmonella* spp. was observed from the surface of the rib eye to a point beyond the midpoint of the meat's thickness (segments one through four), after which an increase was observed (segments five and six). Possible explanations for this are: (i) the tips of the blades may be carrying bacteria that, due to being pressed between the blade surface and the meat tissue as the blade cuts through,

are not being deposited on the meat tissue, but are rather being transported. Once the blade reverses direction and begins to pull away from the meat, these cells become dislodged from the surface of the blade and therefore stay near the bottom of the blade's traveling path; (ii) juices from the inoculated surface (inoculated fat side) might have migrated to the bottom of the subprimal, carrying bacteria with them.

This trend of internalization of the microorganism has been reported by others using similar sampling methodology (Luchansky et al. 2008, 2009). In both studies, Luchansky et al. used blade tenderized subprimals inoculated on the fat side with a single pass of the blades. Luchansky et al. (2008) reported a translocation of 0.03 and 1.72% respectively to segments 5 and 6. Similar rates of translocation (0.10 and 0.82%) were observed in the same segments in the subsequent study (Luchansky et al. 2009). Findings in the present study agree with results from previous research and indicate that preventive measures and intervention strategies must be used when mechanical tenderization is applied to subprimals that are destined for the production of non-intact beef products.

Table 4. Level of *Salmonella* spp. recovered at different segments of the core samples.

Segment	log CFU/g	%	Std Dev
1	5.96	100	0.26
2	3.49	0.34	0.65
3	3.33	0.23	0.45
4	2.77	0.06	0.57
5	3.73	0.59	1.13
6	4.24	1.90	0.65

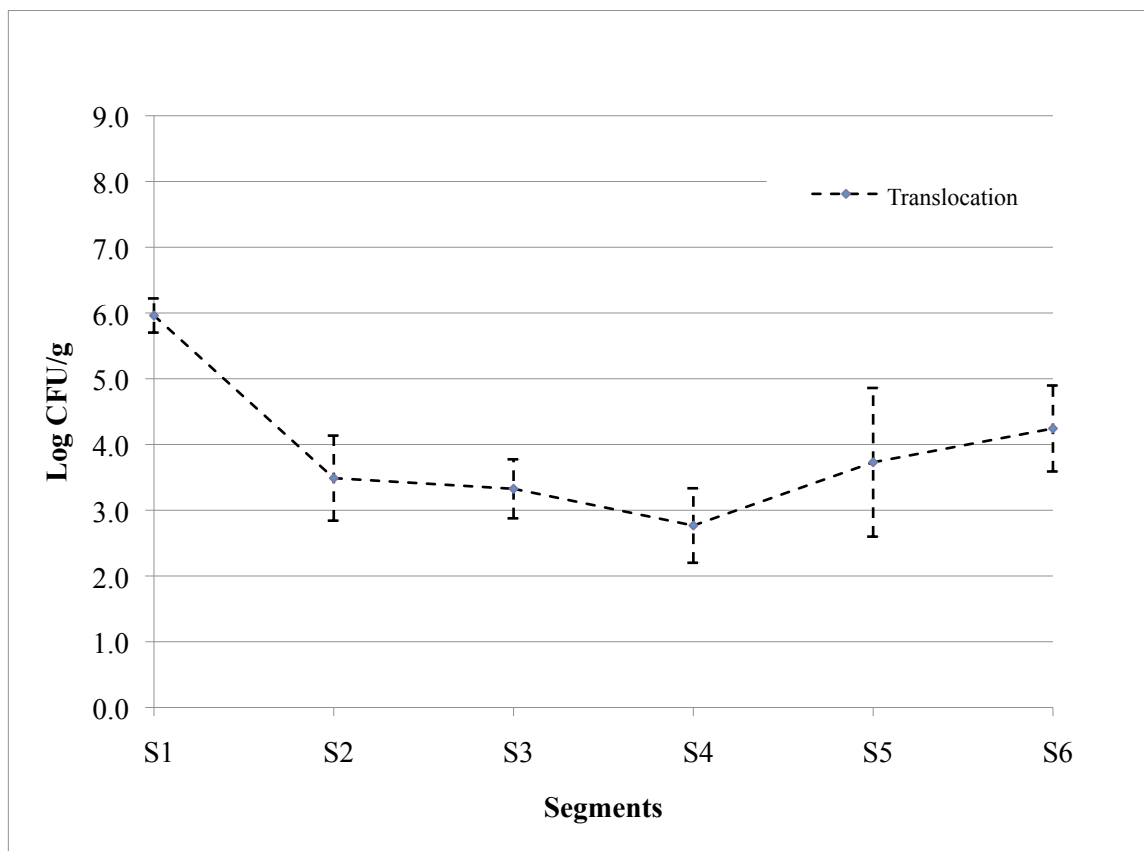


Figure 23. Degree of translocation of *Salmonella* spp. through the interior of the meat in non-intact blade tenderized rib eye.

Thermal processing of Non-Intact Prime Rib

Salmonella spp. populations of 5.73, 4.8, and 4.73 log CFU/g were achieved on the surface, middle and bottom surfaces, respectively, of the rib eye subsequent to inoculation. Searing the blade tenderized prime rib for 15 min at 260°C, resulted in *Salmonella* spp. population reductions ($p > 0.05$) by ca. 0.63 log CFU/g.

Subprimals cooked to a target temperature of 37.8°C showed a significant difference in bacterial growth between treatments (searing, cooking, and holding for 2h) ($p \leq 0.05$) (Table 5) and the vertical location of the sample as well (top, middle, and bottom) (Fig. 24).

Cooking of the rib eye to an internal temperature of 37.8°C resulted in a 2.86 log CFU/g ($p \leq 0.05$) reduction of *Salmonella* spp. in the three vertical strips, and subsequent growth of 1.72 log CFU/g was observed during the 2 h holding period ($p \leq 0.05$). Survival of *Salmonella* spp. was observed ($p > 0.05$) during the subsequent holding of the prime rib for 2, 4, 6 and 8 h.

Analyses corresponding to samples cooked to a target temperature of 48.9°C indicate that there is a significant difference in bacterial growth between treatments (searing, cooking, and holding for 2h) (Table 6), and no significant difference in the location of the samples (top, middle, and bottom) nor in the interaction between the treatments and the location (Fig. 25). Cooking of rib eye to an internal temperature of 48.9°C, resulted in reductions of 3.58 log CFU/g of *Salmonella* spp. ($p \leq 0.05$). This higher reduction lead to a lower *Salmonella* spp. population prior to holding, and holding for 2 h did not result in an increase (0.74 log CFU/g).

The two sets of samples were compared to evaluate differences between the subprimals that were seared, cooked to 37.8°C, and subsequently held for up to 2 h, and those that were seared, cooked to 48.9°C, and held for up to 2 h (Fig. 26). Differences ($p \leq 0.05$) in *Salmonella* spp. survival were observed between the cooking temperatures, with averages of 1.86 and 0.49 log CFU/g after cooking to 37.8 and

48.9°C, respectively. Similarly, holding the subprimals cooked to the two different target temperatures for up to 8 h showed a significant difference ($p \leq 0.05$) in *Salmonella* spp. populations. After 8 h of holding, prime rib cooked to 37.8°C had a bacterial load of 3.62 log CFU/g, compared to of 0.90 log CFU/g in prime rib cooked to an internal temperature of 48.9°C.

Table 5. Statistical comparisons based on P-values for steaks cooked at 37.8°C. Means (log CFU/g of *Salmonella* spp.) at each processing step.

Control	Searing	Cooking	Holding (2h)
5.09	4.72	1.86	3.58
a	a	b	c

Table 6. Statistical comparisons based on P-values for steaks cooked at 48.9°C. Means (log CFU/g of *Salmonella* spp.) at each processing step.

Control	Searing	Cooking	Holding (2h)
5.09	4.07	0.5	1.24
a	a	b	b

The processing parameters (time-temperatures) used in this study were based on a survey of local restaurants. Searing, regardless of the oven temperature used, resulted in minimal reductions in *Salmonella* spp. population in intact or non-intact rib eye. Prior to thermal processing, subprimals were stored under in refrigeration, and during searing at 260°C for 15 min, the mean internal temperature at the geometric center of the rib eye was ca. 13.3°C, a temperature at which reductions are not likely to be achieved in non-intact beef.

Cooking of non-intact rib eye to an end point temperature of 48.9°C resulted in a greater reduction of *Salmonella* spp. (3.58 log CFU/g) as compared to cooking to an end point temperature of 37.8°C (2.86 log CFU/g). Johnston et al. (1978) reported *Salmonella* survival in mechanically tenderized roasts cooked in an oven to an internal temperature of 54.4°C. *Salmonella* spp. survival was observed in both the surface and core samples, indicating that such a temperature represents a public health problem.

The relationship between internal cooking temperature and the resulting microbial reduction affected survival and further growth of *Salmonella* spp., which was evident after 2 h of holding. The average of the internal temperatures monitored during the 8 h of holding, indicated that the subprimals cooked to 37.8°C remained at ca. 42.8°C, and those cooked to 48.9°F were at ca. 46.1°C. Survival of *Salmonella* spp. during preparation of roast beef was related to the location of the microorganism, and the heating process applied (Doyle and Mazzota 1999).

Marsden et al. (2001) reported that at an internal temperature of 43.3 and 48.9°C, followed by 1 h holding at 48.9°C, resulted in a considerable reduction in numbers of *Salmonella* spp. (4.54 and 4.80 log CFU/g) population. In a later report on blade tenderized beef rounds, Marsden et al. (2004) stated that reductions in *Salmonella* of 2.73 log CFU/g were achieved in mechanically tenderized steaks that were cooked at a target internal temperature of 48.9°C.

The thermal process of mechanically tenderized prime rib subprimals that were seared, cooked to 48.9°C, and held for 8 h, resulted in a reduction of 5.08 log CFU/g of *Salmonella* spp. population, whereas those that were seared, cooked to 37.8°F, and

held for 8 h, resulted in a 2.26 log CFU/g reduction. Thus, the prime rib cooking process is not adequate to assure the safety of non-intact prime rib.

4.3 STUDY TWO. Germination and outgrowth of *C. perfringens* spores during hot holding and cooling of prime rib

4.3.1 Evaluation of hot holding

C. perfringens spore population of ca. 1.97 log CFU/g was achieved subsequent to inoculation. Holding of inoculated ground rib eye at 43°C, resulted in an increase of *C. perfringens* to 1.9, 5.3, 6.1, 6.5, and 6.5 log CFU/g of after 2, 4, 6, 8 and 10 h of holding, respectively (Table 7). Similarly, at a holding temperature of 49°C, the *C. perfringens* increase was 6.05 log CFU/g after 10 h with a consistent gradual increase during holding (Table 7; Fig. 27).

The optimal temperature for *C. perfringens* reported in literature is between 43 to 45°C; however, it may be able to multiply at temperatures up to 50°C (R. Labbe, *Clostridium perfringens* Gastroenteritis 1992). Roy et al. (1981) described the active growth of this pathogen at 37, 41, 45, and 49°C, along with the thermal inactivation of the bacteria. In their study they concluded that in a roast cooked to 55°C, at a rate of 6°C/h an increase of 3.5 log of *C. perfringens* is expected.

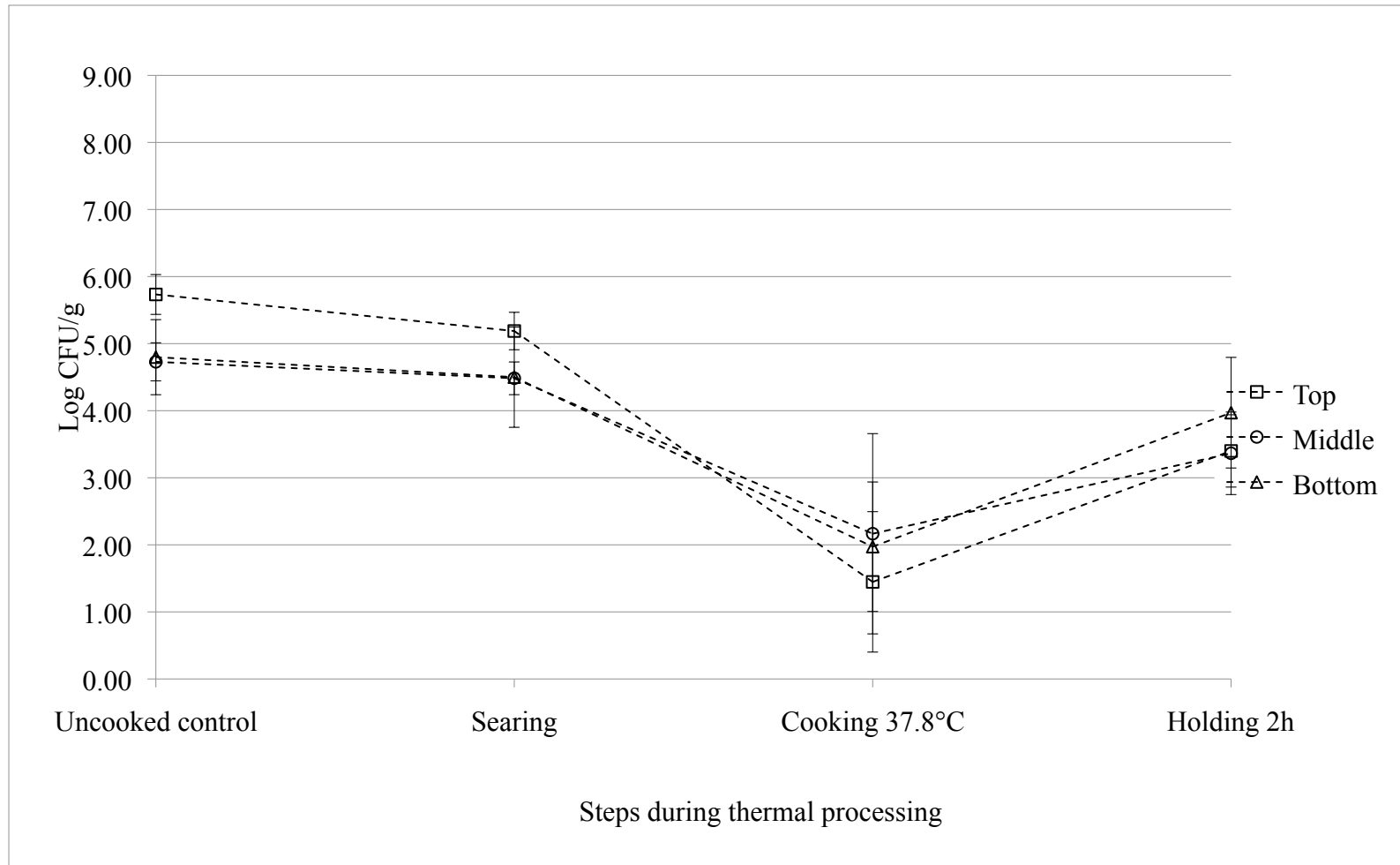


Figure 24. Survival of *Salmonella* spp. during the steps used for thermal processing of non-intact rib eye when samples were cooked to 37.9°C.

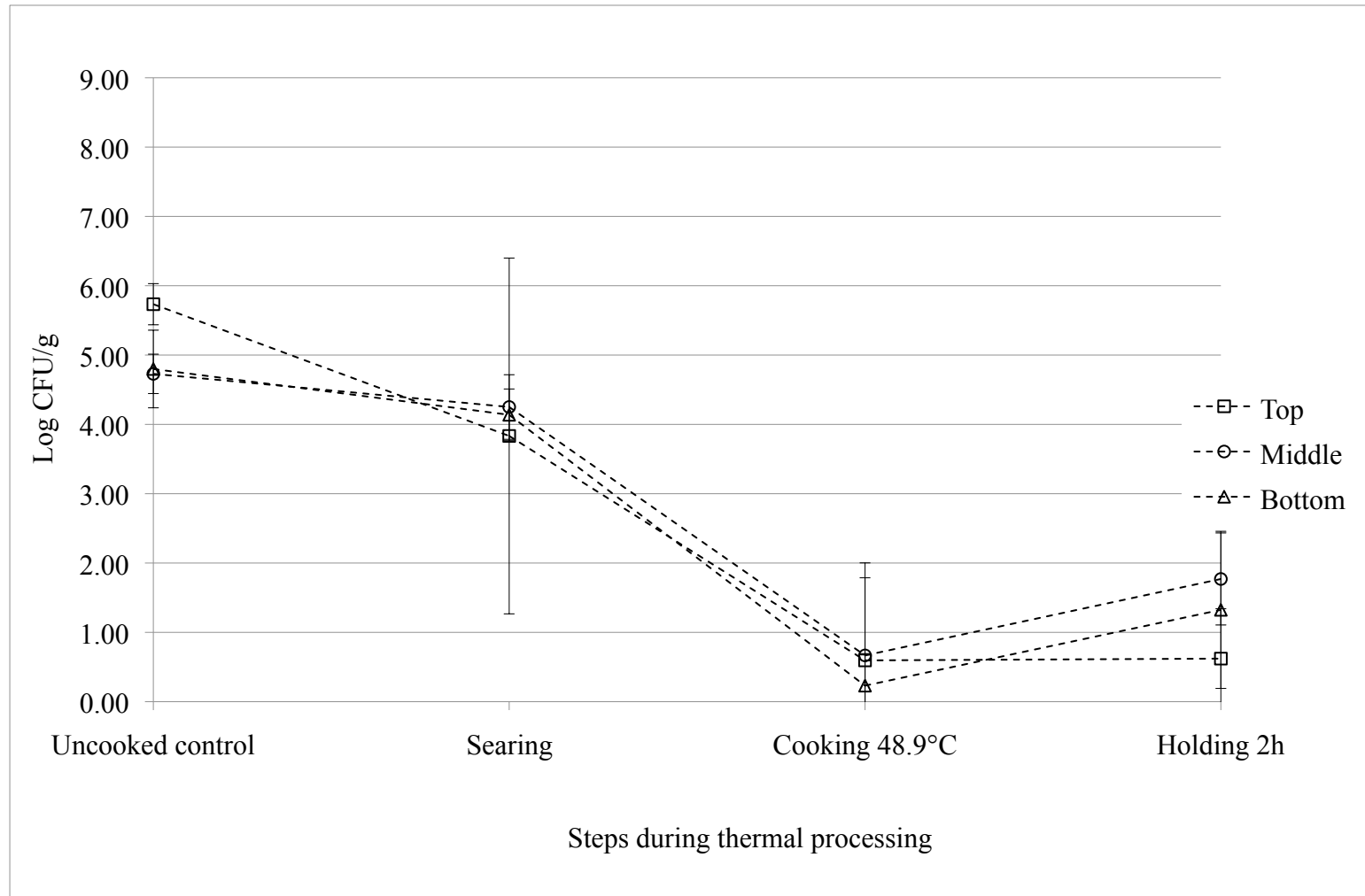


Figure 25. Survival of *Salmonella* spp. during the steps used for thermal processing of non-intact rib eye when samples were cooked to 48.9°C.

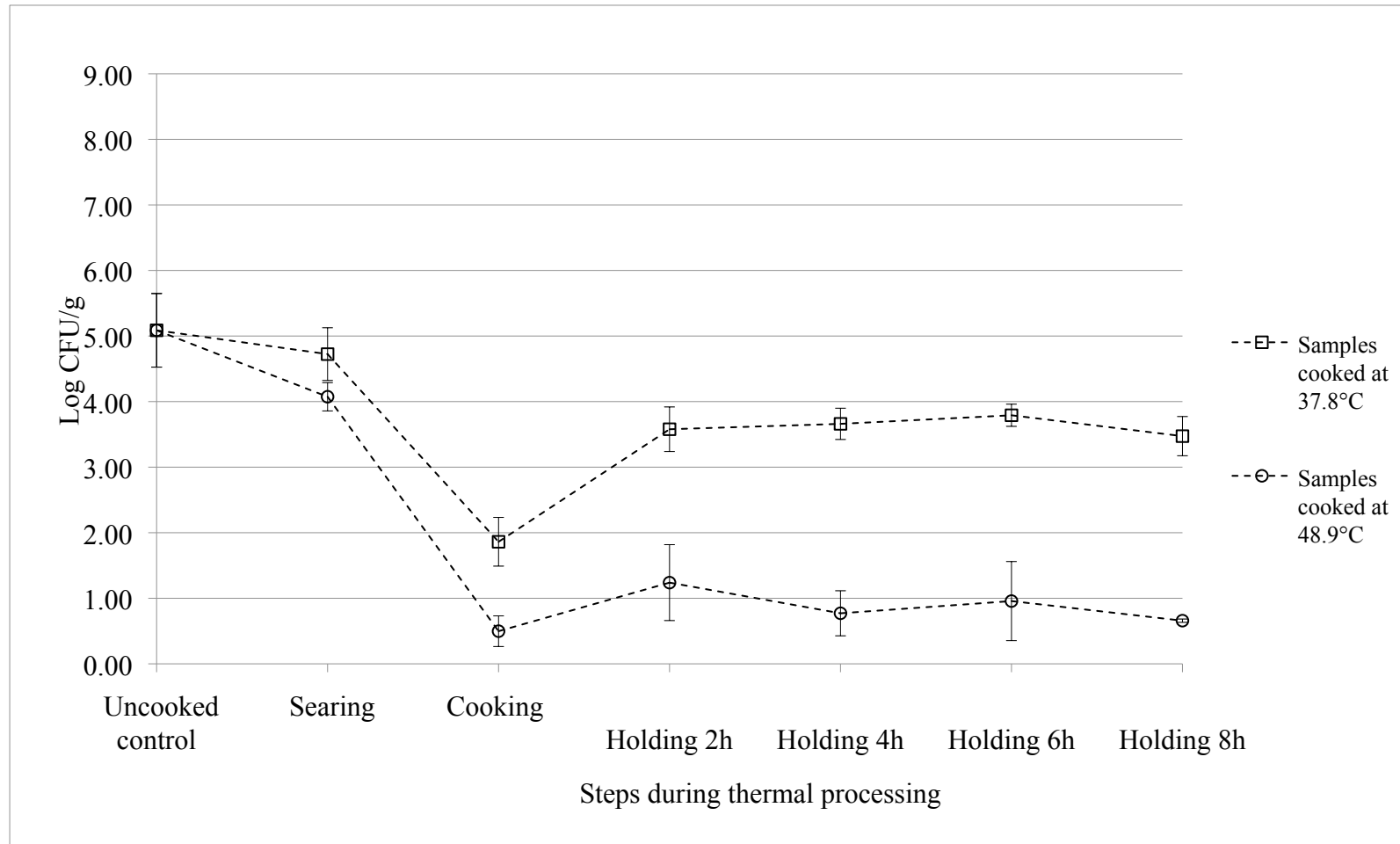


Figure 26. Survival of *Salmonella* spp. in non-intact meat during the steps used for thermal processing. Comparison between both set of samples, cooked to 37.8 °C and 48.9 °C.

Table 7. Increase of *C. perfringens* during 10 h of holding at 43 and 49°C. Log CFU/g at different sampling time.

Holding time (h)	43°C	49°C
2	1.9	1.4
4	5.3	4.2
6	6.1	5.9
8	6.5	6.0
10	6.5	6.0

C. perfringens is an ubiquitous microorganism, and is prevalent in food products (Hall and Angelotti 1965). Spores present in the meat can be heat activated during cooking (Walker 1975), resulting in germination and outgrowth to a dangerous levels during hot holding of prime rib. Standard processing of prime rib in the restaurants during hot holding utilizes temperatures within the growth range optimal for *C. perfringens* representing an imminent public health concern.

4.3.2 Evaluation of cooling during storage of prime rib

An increase of *C. perfringens* population was observed in all exponential cooling rates of rib eye from 54.4°C to 5°C within 6, 9, 12, and 15 h (Fig. 27). However, statistical analysis revealed a significant ($p \leq 0.05$) *C. perfringens* increase of 2.2 log CFU/g during exponential cooling of rib eye within 15 h, whereas cooling within 6, 9, and 12 h resulted in a minimal increase of *C. perfringens* population (< 0.8 log CFU/g).

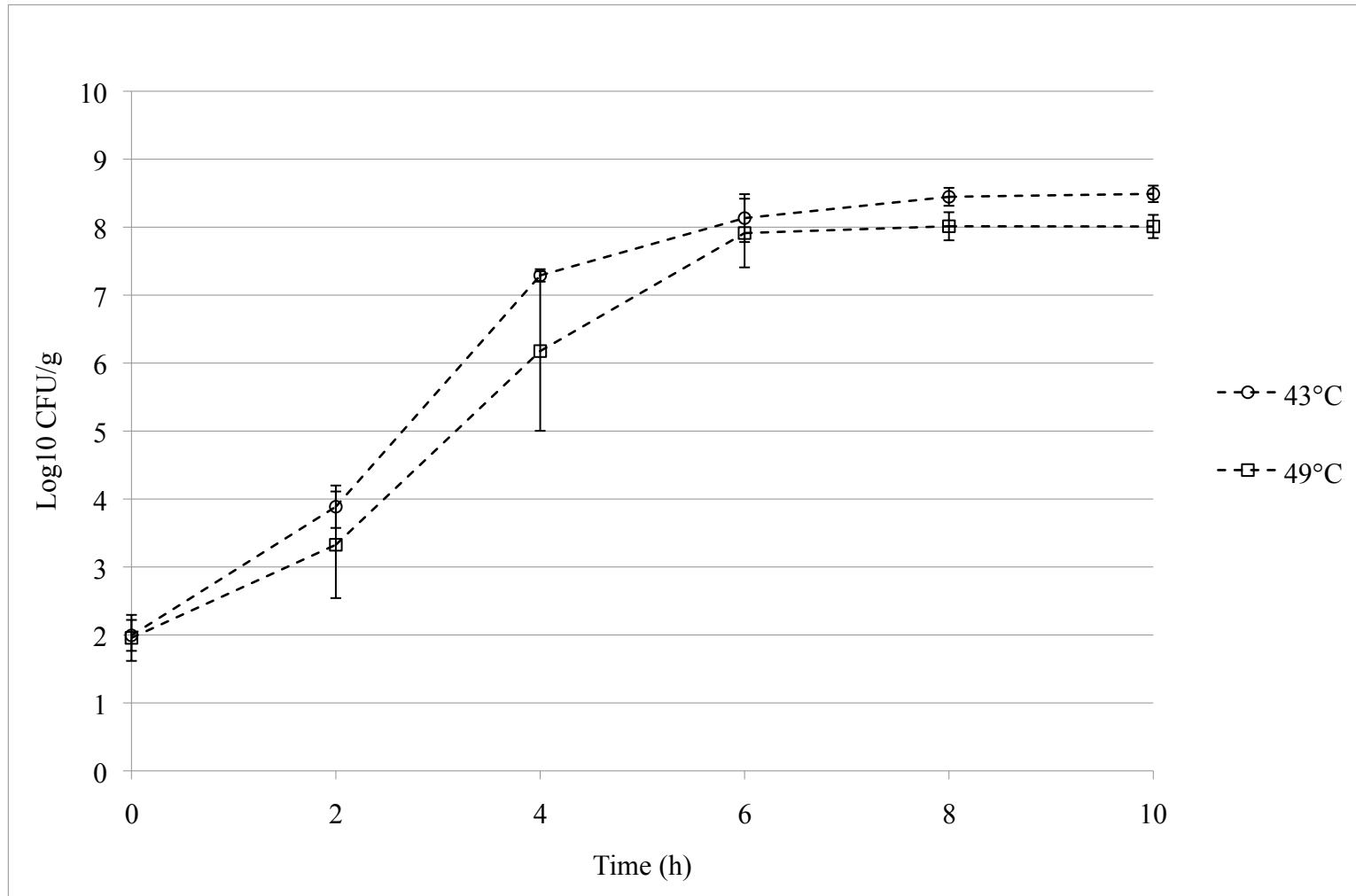


Figure 27. Growth of *C. perfringens* during hot holding of rib eye at 43°C and 49°C.

This finding indicates that the rate of cooling has an impact on the microbiological safety of the prime rib, and therefore restaurants need to be aware of monitoring the temperatures of their coolers as well as the internal temperature of the prime rib during cooling.

A greater increase in *C. perfringens* population of 1.1 log in 1.5 h, and 3.6 log in 3 h was reported during cooling of cooked uncured beef from 54.4°C to 27°C. Similarly, Doyle (2002) found that a rapid increase of *C. perfringens* population at a slow cooling rates (5 and 7.5°C per hour) occurs; however, an appreciable growth was not observed when cooling from 54.4 to 7.2°C takes 15 h.

This finding indicates that the rate of cooling has an impact on the food safety of the prime rib. Considering that refrigerated leftovers are to be used the following day for serving to consumers, there is an imminent risk of outbreaks that need to be prevented. Thus, restaurants need to be aware of monitoring temperatures of their cool rooms as well as the internal temperature of the prime rib during this process.

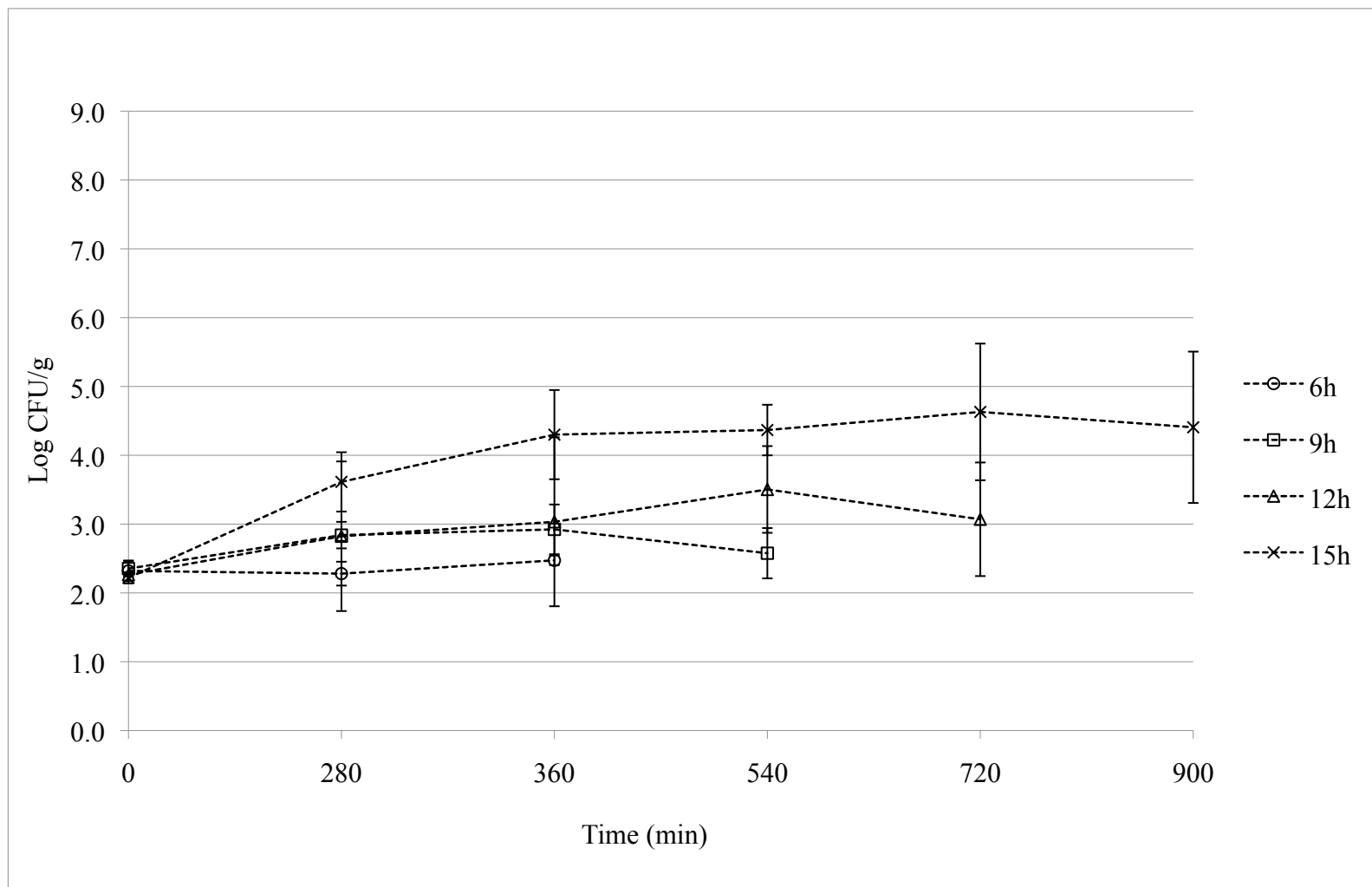


Figure 28. Growth of *C. perfringens* during cooling of rib eye at four different exponential profiles.

5. CONCLUSIONS

Based on the surveys conducted, restaurants are not following the local and state regulations for the preparation of prime rib. Those regulations include 2009 Food Code specifications and USDA compliance guidelines for lethality and stabilization (cooling heat-treated meat).

Searing of intact rib eye for 45 min in a kitchen oven at 260 or 204°C attained a surface meat temperature of ca. 63°C, and changes color of the meat as specified in the US FDA Food Code (2009). Searing of rib eye resulted in *Salmonella* spp. reduction on the surface of 4.61 and 3.25 log CFU/cm², respectively. Searing of rib eye does not meet the USDA-FSIS (1999) performance standards for the reduction of *Salmonella* spp. as 6.5 log CFU/g was not achieved

The thermal process for preparation of prime rib from either intact meat or non-intact (mechanically tenderized) rib eye is not sufficient to meet the lethality performance standards of the USDA-FSIS (1999). Searing of prime rib in a kitchen oven at 260°C followed by cooking to either 37.9 or 48.9°C achieved a *Salmonella* spp. reduction of ca. 5 log CFU/g in intact meat. The same processing variables applied to mechanically tenderized prime rib achieved lower *Salmonella* spp. reductions (3.23 and 4.59 log CFU/g, respectively) due to the translocation of the pathogen to the interior of the meat. Therefore, use of non-intact meat requires

additional antimicrobial interventions prior to mechanical tenderization to minimize the risk of *Salmonella* spp. from prime rib.

Regardless of the type of meat and the thermal processes applied, holding prime rib at 60°C allowed the growth of *Salmonella* spp. Although the increase in *Salmonella* spp. population is low, extended periods of holding make the finished product unsafe for consumption.

C. perfringens spore germination and outgrowth was observed during hot holding of prime rib at 43 and 49°C; holding at these temperatures is not adequate to assure food safety as $> 6 \log$ CFU/g increase of *C. perfringens* population was observed within 6 h. Cooling of prime rib resulted in *C. perfringens* spore germination and outgrowth by 1 log CFU/g from 57.2 to 5°C during 6, 9, or 12 h. However, extending the cooling time to 15 h resulted in *C. perfringens* spore germination and outgrowth by 2.2 log CFU/g.

To prevent repeated violations by food service establishments and reduce the risk of foodborne illness outbreaks from prime rib, regulatory agencies, through the state and local health departments, should encourage validation of the prime rib preparation process and similar products, and request a food safety variance along with a HACCP plan from the establishments.

REFERENCES

- Abdunaser, D., F. Almabrouk, W. Ashraf, M. Yves, C. Olivier, and S. Moez. 2009. Quantitative risk assessment of human salmonellosis linked to the consumption of ground beef. *Iraqi J. Veterin. Sciences* 23: 263-273.
- Alban, L., A. Olsen, B Nielsen, and R. and Jessen, B. Sorensen. 2002. Qualitative and quantitative risk assessment for human salmonellosis due to multiresistant *Salmonella* Typhimurium DT104 from consumption of Danish dry-cured pork sausages. *Prev Vet Med.* 52: 251-265.
- Amy, B. 2008. Understanding Food: Principles and Preparation. 3rd Edition. Thompson Learning Inc. Belmont, CA
- Anderson, R., and L. Ziprin. 2001. Bacteriology of *Salmonella*. Vol. I. In Y. Hui, M. Pierson and J. Gorham (ed.), Foodborne Disease Handbook. Vol. I. Marcel Dekker, Inc. New York, NY.
- Armstrong, G., J. Hollingsworth, and G. Morris. 1998. Bacterial Foodborne Disease. In: Bacterial Infections of Humans. Epidemiology and Control, edited by A. Evans and P. Brachman, 109-138. New York, NY: Plenum Publishing Corporation.
- Bailey, S., J. Richardson, N. Cox, and D. Cosby. 2010. *Salmonella*. In (J. Vijay and Sofos, J. ed.), Pathogens and Toxins in Foods: Challenges and Interventions. Washington, DC: ASM Press.

- Bhunja, A. 2008. Foodborne microbial pathogens: Mechanisms and Pathogenesis. Springer. West Lafayette, IN
- Blankenship, L. 1978. Survival of a *Salmonella* Typhimurium experimental contaminant during cooking of beef roast. *Appl. Environ. Microbiol.* 35: 1160-1165.
- Blankenship, L., C. Davis, and G. Magner. 1980. Cooking methods for elimination of *Salmonella typhimurium* experimental surface contaminant from rare dry-roasted beef roasts. *J. Food Sci.* 45:270-273.
- Boles, J. 2010. Thermal Processing. In (F. Toldra, ed.), Handbook of Meat Processing. Blackwell Publishing. Ames, IA
- Brynstad, S. and P. Granum. 2002. *Clostridium perfringens* and foodborne infections. *Int. J. Food Microbiol.* 73: 195-202.
- Buffo, R., and R. Holley. 2006. Effects of food processing on disease agents. In (H. Riemann and D. Cliver, ed.), Foodborne Infections and Intoxications. Academic Press. Burlington, MA.
- Centers for Disease Control and Prevention (CDC). 1995. Outbreak of *Salmonella* serotype Typhimurium infection associated with eating raw ground beef - Wisconsin, 1994. *MMWR*. Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00039761.htm>. Accessed October 21, 2010.

- CDC. 2002. Outbreak of Multidrug resistant *Salmonella* Newport. US, 2002. Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5125a1.htm>. Accessed December 11, 2010.
- CDC. 2006. Multistate outbreak of *Salmonella* Typhimurium associated with eating ground beef. Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5507a4.htm>. Accessed December 10, 2010.
- CDC. 2010. *Salmonella* homepage. CDC. Available at: <http://www.cdc.gov/Salmonella/general/technical.html>. Accessed December 19, 2010.
- CDC. 2010. FoodNet. Facts and Figures 2009. Available at: <http://www.cdc.gov/FoodNet/factsandfigures/2009/incidence.html>. Accessed October 28, 2010.
- Cooke, F., J. Threlfall, and J. Wain. 2007. Current trends in the Spread and Occurrence of Human salmonellosis: Molecular Typing and Emerging Antibiotic Resistance. In (Mikael Rhen, Duncan Maskell, Pietro Mastroeni and John Threlfall, ed.), *Salmonella: molecular biology and pathogenesis*. Horizon Bioscience. Norfolk.
- Chris, B., and A. Kyriakides. 2002. *Salmonella*. A practical approach to the organism and its control in foods. Blackwell Science. Malden, MA.

- Crouch, E., and N. Golden. 2005. A risk assessment for *Clostridium perfringens* in ready-to-eat and partially coked meat and poultry products. Draft for public review, Cambridge Environmental, Inc., and FSIS (Food Safety Inspection Service).
- D'Aoust, J. 1991. Psychrotrophy and foodborne *Salmonella*. *Int. J. Food Microbiol.* 13: 129-158.
- D'Aoust, J. 2001. *Salmonella*. In (Ronald Labbe and Santos Garcia, ed.), Guide to foodborne pathogens. John Wiley and Sons, Inc. New York, NY.
- D'Aoust, J., J. Maurer, and J. Stan Baile. 2001. *Salmonella* Species. In (M. Doyle, Beuchat, L. and Montville, ed.), Food Microbiology. Fundamentals and Frontiers, ASM Press. Washington, DC.
- Dahl, C., and J. Petska. 1985. Foodservice systems: Presence of injured bacteria in foods during food product flow. *Ann. Rev. Microbiol.* 39:51-67.
- Dominguez, S., and D. Schaffner. 2009. Microbial Quantitative Risk Assessment. In (F. Toldra, ed.), Food microbiology and food safety. Safety of meat and processed meat, Springer Science. New York, NY.
- Doyle, E. 2002. Survival and growth of *Clostridium perfringens* during cooling step of thermal processing of meat products. A review of the scientific literature. Food Research Institute, University of Wisconsin, Madison, WI.
- Doyle M., and A. Mazzota. 1999. Review of studies on the thermal resistance of *Salmonellae*. *J. Food Prot.* 63:779-795.

- Duncan, C. 1970. *Clostridium perfringens* food poisoning. *J. Milk Food Technol.* 33: 35-41.
- Food and Drug Administration (FDA). 1992. The Bad Bug Book. Center for Food Safety and Applied Nutrition. Available at: <http://www.cfsan.fda.gov/~mow/intro.html>. Accessed September 21, 2010.
- FDA. 2009. U.S. Public Health Service. Food Code. Department of Health and Human Services. College Park, MD.
- FDA. 2010. About FDA. Available at: <http://www.fda.gov/AboutFDA/Transparency/Basics/ucm194244.htm>. Accessed September 23, 2010.
- FSIS. 1999. Appendix A. Compliance Guidelines For Meeting Lethality Performance Standards For Certain Meat And Poultry. Washington, DC.
- FSIS. 1999. Appendix B. Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products (Stabilization)." Washington, DC.
- Gill, C. 1979. Intrinsic bacteria in meat. *J. Appl. Bacteriol.* 47: 367-378.
- Gill, C. 1998. Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. *In* (Board, A. and R. Davies, ed.), The microbiology of meat and poultry, Blackie Academic and Professional. London, UK.
- Hajmeer, M., E. Ceylan, R. Phebus, and J. Marsden. 2000. Translocation of microflora from muscle surface to interior by blade tenderization. Cattleman's Day. 2000. Conference paper, p. 125-126. Kansas State University, Manhattan.

- Hale, D., J. Goodson, and J. Savell. 2010. Beef Quality and Yield Grades. Texas A&M University. Available at: <http://meat.tamu.edu/beefgrading.html>. Accessed September 22, 2010.
- Hall, H., and R. Angelotti. 1965. *Clostridium perfringens* in meat and meat products. *Applied Microbiol.* 13: 352-357.
- Helms, M., P. Vastrup, P. Gerner-Smidt, and K. Mølbak. 2002. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg Infect Dis.* 8: 490-495.
- Heredia, N., and R. Labbe. 2001. *Clostridium perfringens*. In (R. Labbe and S. Garcia, ed.), Guide to Foodborne Pathogens, John Wiley & Sons, Inc. New York, NY.
- Hobbs, B., M. Smith, G. Oakley, H. Warrack, J. Cruickshank. 1953. *Clostridium welchii* food poisoning. *Epidemiol. Infect.* 51: 75-101.
- Huang, H., D. Raju, D. Paredes, and M. Sarker. 2007. *Clostridium perfringens*: Sporulation, Spore Resistance and Germination. *Bangladesh J Microbiol* 24: 18.
- Huang, H., and S. Sheen. 2011. Quantitative analysis of vertical translocation and lateral cross-contamination of *Escherichia coli* O157:H7 during mechanical tenderization. *J. Food Saf.* 31: 108-114.
- ICMSF. 1996. Microorganisms in Food 5: Characteristics of Microbial Pathogens, Blackie Academic and Professional. London, UK.
- ICMSF. 1998. Microorganisms in Foods 6. Blackie Academic and Professional. London, UK

- Ilungo, X., and I. Steven. 1997. Increased D-Values for *Salmonella* Enteritidis following heat shock. *J. Food Prot.* 60: 181-184.
- Ingham, S., J. Losinski, B. Dropp, L. Vivio, and D. and Buege. 2004. Evaluation of *Staphylococcus aureus* growth potential in ham during a Slow-Cooking Process: Use of predictions derived from the U.S. Department of Agriculture pathogen modeling program 6.1 predictive model and an inoculation study. *J. Food Prot.* 67: 1512-1516.
- Jay, J., M. Loessner, and D. Golden. 2005. Modern Food Microbiology. Springer. New York.
- Johnston, R., M. Harris, and A. Moran. 1978. The effect of mechanical tenderization of beef rounds inoculated with salmonellae. *J. Food Saf.* 1: 201-209.
- Jones, S., A. Guru, V. Singh, B. Carpenter, C. Calkins, and D. Johnson. 2004. Bovine Myology and Muscle Profiling. Available at: <http://bovine.unl.edu>. Accessed september 23, 2010.
- Juneja, V. 2002. Thermal inactivation. In (V. Juneja and J. Sofos, ed.), Control of Foodborne Microorganisms. Marcel Dekker, Inc. New York, NY:.
- Juneja, V. 2010. *Clostridium perfringens*. In (V. Juneja and J. Sofos, ed.), Pathogens and Toxins in Foods, ASM Press. Washington, DC.
- Juneja, V. 2010. *Clostridium perfringens*. In (V. Juneja and J. Sofos, ed.), Pathogens and Toxins in Foods, ASM Press. Washington, DC

- Juneja, V. and H. Thippareddi. 2004. Inhibitory effects of organic acid salts on growth of *Clostridium perfringens* from spore inocula during chilling of marinated ground turkey breast. *Int. J. Food Microbiol.* 93: 155-163.
- Juneja, V. , H. Marks, and H. Thippareddi. 2007. Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured beef. *Food Microb.* 25: 42-55.
- Juneja, V., H. Marks, and H. Thippareddi. 2008. Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured beef. *Food Microbiol.* 70: 218-222.
- Juneja, V., J. Call, and A. Miller. 1993. Evaluation of methylxanthines and related compounds to enhance *Clostridium perfringens* during cooling of cooked cured chicken. *Food Microbiol.* 2: 203-218.
- Kang, C., M. Woodburn, A. Pagenkopf, and R. Cheney. 1969. Growth sporulation and germination of *Clostridium perfringens* in media of controlled water activity. *Appl. Microbiol.* 18: 798-805.
- Labbe, R. 1989. *Clostridium perfringens*. In (Marcel Dekker, ed.) Foodborne Bacterial Pathogens, Marcel Dekker Inc. New York, NY.
- Labbe, R. 1992. *Clostridium perfringens* Gastroenteritis., In (A. Tu, ed.), Food Poisoning. Handbook of Natural Toxins. Vol. 7, Marcel Dekker, Inc. New York, NY

- Labbe, R. 1995. *Clostridium perfringens*. In (T. Lund, C. Baird-Parker and G. Gould, ed.), The microbial safety and quality for food. Vol. 2, Aspen Publishers Inc. Gaithersburg, MD.
- Labbe, R. J., and J. Juneja. 2002. *Clostridium perfringens*. In (D. O. Cliver and H. Riemann Foodborne Diseases, ed.), Academic Press, Inc. San Diego , CA.
- Laine, E., J. Scheftel, D. Boxrud, K., Danila, R. Vough, K. Elfering, and K. Smith. 2005. Outbreak of *Escherichia coli* O157:H7 infections associated with non-intact blade tenderized frozen steaks sold by door-to-door venders. *J. Food Prot.* 68: 1198-1202.
- Laury, A., A. Echeverry, and M. Brashears. 2009. Fate of *E. coli* O157:H7 in Meat. In (F. Toldra, ed.), Safety of Meat and Processed Meats, Springer. New York, NY.
- Luchansky, J., A. Porto-Fett, B. Shoyer, R. Phebus, H. Thippareddi, and J. Call. 2009. Thermal inactivation of *Escherichia coli* O157:H7 in blade-tenderized beef steaks cooked on a commercial open-flame gas grill. *J. Food Prot.* 72: 144-1411.
- Luchansky, J., R. Phebus, H. Thippareddi, and J. Call. 2008. Translocation of surface-inoculated *Escherichia coli* O157:H7 into beef subprimals following blade tenderization. *J. Food Prot.* 71:2190-2197.
- Mackey, B., and C. Derrick. 1986. Elevation of the heat resistance of *Salmonella* Typhimurium by sublethal heat shock. *J. Appl. Bacteriol.* 61: 389-393

- Marks, B. 2010. Food safety beyond guidelines and regulations. *In* (C. L., and Rust R. E. Knipe, ed.), Thermal processing of ready-to-eat meat products, Wiley-Blackwell. Ames, IA.
- Marks, B. 2010. Introduction to lethality equations. *In* (C. L., and Rust, R. E. Knipe, ed.), Thermal processing of ready-to-eat meat products, Wiley-Blackwell. Ames, IA.
- Marsden, J., R. Phebus, H. Thippareddi, C. Kastner, and J. Gosh. 2004. *Salmonella* spp. and *Listeria monocytogenes* risk assessment for production and cooking of blade tenderized beef steaks. Kansas State University, Manhattan.
- Marsden, J., R. Phebus, H. Thippareddi, C. Kastner, and J. Gosh. 2001. *Salmonella* spp. risk assessment for production and cooking of blade tenderized prime rib. Department of Animal Sciences and Industry, Kansas State University, Manhattan.
- McClane, B. A. 2001. *Clostridium perfringens*. *In* (M. Doyle, L. Beuchat and T. Montville, ed.), Food Microbiology: Fundamentals and frontiers, ASM Press. Washington, DC.
- McDonough, F., and R. Hargrove. 1968. Heat resistance of *Salmonella* in dried milk. *J. Dairy Sci.* 51.
- McLauchlin, J, and K. Grant. 2007. *Clostridium botulinum* and *Clostridium perfringens*. *In* (Sharbir Simjee, ed.) *Foodborne Diseases*, edited by Humana Press Inc. Totowa, NJ.

- Mead, P. S., et al. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5: 607-625
- Molbak, K., J. Olsen, and H. Wegener. 2006. *Salmonella* infections. In (Riemann, H. and D. Cliver, ed.) Foodborne infections and intoxications, Academic Press. San Diego, CA.
- Montville, T. 2008. Food Microbiology. 2nd edition. ASM Press. Washington, DC.
- Montville, T., and K. Matthews. 2005. Food microbiology, an introduction. 2nd. edition, ASM Press. Washington, DC.
- NAMP, North American Meat Processors Association. 2007. The Meat Buyer's Guide. John Wiley and Sons, Inc. Hoboken, NJ
- Norrung, B., J. Kirk, and S. Buncic. 2009. Main concerns of pathogenic microorganisms in meat . In (F. Toldra, ed.), Food microbiology and food safety. Safety of meat and processed meat, Springer Sciences, New York, NY.
- Olsen, S., J. MacKinon, J. Goulding, N. Bean, and L. Slutsker. 2000. CDC, Surveillance for foodborne-disease outbreaks - United States 1993-97. Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/ss4901a1.htm>. Accessed September 18, 2010.
- Pace, P., C. Duncan, V. Dowell, J. Antonmattei, and H. Wisniewski. 1976. Perfringens illness associated with beef prime rib served in a restaurant. *J. Milk Food Tech.* 39: 305.
- Parry, C. 2006. Epidemiological and clinical aspect of human thypoid fever. In (P. Mastroeni and D. Maskell, ed.), *Salmonella* infections. Clinical Immunological

and Molecular Aspects, edited by, Cambridge University Press. New York, NY

Pearson, A., and T. Gillet. 1996. Processed meats. 3rd Edition. Aspen Publishers Inc. New York, NY

Phebus, R., H. Thippareddi, S. Sporing, L. Marsden, and C. Kastner. 2000. *Escherichia coli* O157:H7 Risk Assessment for Blade Tenderized Beef Steaks. Report of Progress, Kansas State University, 117-118.

Podollak, R., E. Enache, W. Stone, D. Black, and P. Elliott. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *J. Food Prot.* 73: 1919-1936.

Romans, J., W. Costello, C. Carlson, M. Greaser, and K. Jones. 2001. The meat we eat. 14th Edition. Interstate Publishers, Inc., Danville, IL.

Roy, R., F. Busta, and D. Thompson. 1981. Thermal inactivation of *Clostridium perfringens* after growth at several constant and linearly rising temperatures. *J. Food Sci.* 46: 1586-1591.

Saito, M. 1990. Production of enterotoxin by *Clostridium perfringens* derived from human, animals, food, and the natural environment in Japan. *J. Food Prot.* 53: 115-118.

Savell, J. et al. 2006. *National Beef Tenderness Survey*. Survey, Beef Checkoff, National Cattlemen's Beef Association, National Cattlemen's Beef Association. Centennial, CO

- Scallan, E., R. Hoekstra, F. Angulo, R. Tauxe, M. Widdowson, and S. Roy. Foodborne illness acquired in the United States – major pathogens. *Emerg. Infect. Dis.* 2011. Jan; (Epub ahead of print).
- Shandera, W., C. Tacket, and P. Blake. 1983. Food poisoning due to *Clostridium perfringens*. *J. Infect. Diseases* 147: 167-170.
- Shapiro, R., et al. 1999. *Salmonella* Thompson associated with improper handling of roast beef at a restaurant in Sioux Falls, South Dakota. *J. Food Prot.* 62: 118-122.
- Smith, C., G. Hicks, B. Kritbarlow, L. Vegosen, and L. Alderton. 2006. Foods associated with food-borne illness outbreaks from 1990 through 2003. *Food Prot. Trends* 26: 466-473.
- Sporing, S. 1999. *Escherichia coli* O157:H7 Risk Assessment for production and cooking of blade tenderized beef steaks. Kansas State University, Manhattan, KS, 1999.
- Thippareddi, H., L. Dee Dee, R. Phebus, J. Mardsen, C. Kastner, E. Boyle, M. Hunt, and D. Fung. 2000. *Salmonella* risk assessment for blade tenderized, immersion marinated, needle injected and fibrinogen processed pork cuts during processing, storage and cooking. National Pork Producers Council, Des Moines, IA.
- USDA, FSIS. 1999. Federal Register, 9 CFR, Chapter III, Volume 64, Number 11. Beef products contaminated with *Escherichia coli* O157:H7. Available at:

<http://www.fsis.usda.gov/oppde/rdad/FRPubs/99-1123.htm>.

Accessed

September 2, 21010).

USDA, FSIS. 1999. Federal Register, 9 CFR Parts 301, 317, 318, 320 and 381.

Performance standards for the production of certain meat and poultry products.

Volume 64, Number 3. Available at:

<http://www.fsis.usda.gov/OPPDE/rdad/frpubs/95-033F.htm>.

Accessed August 30,

2010.

USDA- FSIS. 2008. Inspection & grading of meat and poultry: What are the differences?. Available at:

[http://www.fsis.usda.gov/PDF/Inspection_and_Grading_What_Are_the_Differences.p](http://www.fsis.usda.gov/PDF/Inspection_and_Grading_What_Are_the_Differences.pdf)

[df](http://www.fsis.usda.gov/PDF/Inspection_and_Grading_What_Are_the_Differences.pdf). Accessed September 20, 2010.

Walker, H. 1975. Foodborne illness from *Clostridium perfringens*. CRC Crit. Rev. *Food Sci. Nutr.* 7: 71-104.

Wrigley, D., H. Hanwella, and B. Thon. 1995. Acid exposure enhances sporulation of certain strains of *Clostridium perfringens*. *Anaerobe* 1: 295-269.

Ziprin, R., and E. Hume. 2001. Human salmonellosis: General medical aspects. *In* (Y. Hui, M. Pierson and J. Gorham, ed.), Foodborne disease handbook: Bacterial pathogens, Vol. 1. Marcel Dekker, Inc., New York, NY.