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VALIDATION OF *SALMONELLA* THERMAL LETHALITY IN WHOLE MUSCLE MEAT PRODUCTS DURING PILOT-SCALE SLOW ROASTING PROCESSES

presented by

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has been accepted towards fulfillment
of the requirements for the

M.S. degree in Food Science

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7 Dec 2009

Date

VALIDATION OF *SALMONELLA* THERMAL LETHALITY IN WHOLE MUSCLE MEAT PRODUCTS DURING PILOT-SCALE SLOW ROASTING PROCESSES

By

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A THESIS

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

MASTER OF SCIENCE

Food Science

2009

ABSTRACT

VALIDATION OF *SALMONELLA* THERMAL LETHALITY IN WHOLE MUSCLE MEAT PRODUCTS DURING PILOT-SCALE SLOW ROASTING PROCESSES

By

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Previous research has shown that sub-lethal heating can increase subsequent thermal resistance of bacteria. If this phenomenon occurs during slow roasting of meat products, it might compromise the validity of thermal process validations. Therefore, this research evaluated the accuracy of a traditional log-linear inactivation model, developed via prior laboratory-scale, isothermal tests, applied to pilot-scale, slow cooking of whole muscle roasts. Irradiated turkey breast, beef rounds, and pork loin were inoculated with an 8-servovar *Salmonella* cocktail via vacuum tumble marination, in a salt/phosphate marinade. The resulting initial *Salmonella* population in the geometric center (core) was 7.0, 6.3, 6.3 log CFU/g for turkey, beef, and pork respectively. The experimental design consisted of seven different cooking combinations representing industry practices, in a pilot-scale, moist-air convection oven. Core temperature was recorded during cooking, and was used to calculate lethality real-time via the log-linear model. Calculated lethality, using the log-linear model, was greater ($P < 0.05$) than the actual lethality for turkey and beef. A path-dependent model accounting for sub-lethal history of *Salmonella* reduced the lethality error by 2.6 and 1.4 log CFU/g in turkey and beef, respectively, but did not reduce the error in pork. Results demonstrate that slow-cooked roasts, processed to a lethality (as calculated by a state-dependent model) at or near that required by the regulatory performance standards may be under-processed.

ACKNOWLEDGEMENTS

Heart felt and sincere appreciation to my major advisor, Dr. Bradley Marks, and my committee members, Dr. Alden Booren, and Dr. Elliot Ryser for your guidance, and expertise. My thanks are also expressed to Nicole Hall, who was always willing to take the time to guide me, and kindly taught me how to be an effective problem solver. Sanghyup Jeong and Michael James, were always a reliable resource, and took the time to help me resolve equipment issues. Undergraduate students in Dr. Marks' laboratory, and fellow graduate students; we all worked together to accomplish our goals. Finally and most importantly, I thank my entire family and all of my friends for always being there, and helping me to become the person that I am today.

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1. INTRODUCTION

With today's fast paced society, Americans demand convenience. The ever evolving on-the-go lifestyle is a delicate balance of long work days and full social schedules. In order to make these busy schedules work, Americans must optimize their time in many ways. One way Americans create more time in their days is by consuming a "prepackaged" diet. With the lack of time to prepare meals, consumers rely on processors to execute some or all food preparation steps in order save them time. These ready-to-eat (RTE) products have many obvious advantages and many drawbacks. The intricate supply chain that exists in the food industry adds to the food safety concern. Once food leaves the processing facility, the processor loses control of the product, yet ultimately is held accountable for its safety through consumption. Possibilities of risk factors are broad, spanning from inadequate processing, temperature abuse in distribution, and cross-contamination or non-compliance with cooking recommendations by consumers. The average consumer has minimal knowledge about food safety principles. Therefore, when processors rely on the consumer to perform the final kill step at home, there is an inherent risk of inadequate pathogen inactivation (Moss 2009).

Salmonella is a bacterial pathogen that originates in the intestinal tracts of poultry and other livestock, and as a result is introduced to the environment through fecal contamination. *Salmonella* poses a health risk to humans when contaminated food or water is ingested. The pathogen causes the disease salmonellosis; symptoms include diarrhea, fever, and abdominal pain. Populations most at risk for experiencing potentially life threatening salmonellosis infections are the elderly, newborns, and immunosuppressed. Based on the FoodNET surveillance data from the CDC (2005),

42% of all foodborne bacterial infections (confirmed in the laboratory) were due to *Salmonella*. *Salmonella* was second to norovirus, as the most common source of outbreaks in 2005 (CDC 2008a).

Due to the danger of *Salmonella,* the United States Department of Agriculture (USDA) and Food and Drug Administration (FDA) have introduced processing standards for products that are most vulnerable to contamination. Meat products have a particularly high risk of fecal contamination, because of the proximity of muscle tissue to the intestinal tract during processing. Lethality requirements have been developed for RTE meat products. The USDA Food Safety Inspection Service requires a 7- or 6.5- $\log_{10}$ reduction of *Salmonella* in fully cooked poultry and beef products, respectively (FSIS-USDA 1999). Setting standards based on log reduction of pathogens, as opposed to minimum cook time or temperature, ensures that meat products receive adequate heating to inactivate *Salmonella*, while allowing processors the flexibility to determine their own specific time and temperature parameters for a variety of products.

The flexibility of processing to a calculated lethality is possible because the USDA and FDA have established lethality standards using mathematical models that predict the destruction of pathogens based on time-temperature calculations. Many of the models utilized by the industry today are first-order kinetic models, which presume log-linear inactivation of bacteria under isothermal conditions. However, these model and lethality requirements typically have been developed using controlled, laboratory-scale studies, and not validated in commercial processing environments.

However, the inactivation of bacteria in full scale processing environments is much more complicated than a simple laboratory experiment; the processing environment,

conditions, and sample size, composition, and structure all impact the process outcomes. Slow cooking processes, in particular, challenge the modeling approach, because traditional models fail to account for sub-lethal injury of bacteria, which can occur approximately between 38 and 52°C (see section 2.2.2.1). Sub-lethal injury occurs when a bacterial cell is exposed to a temperature range that injures but fails to kill it, thereby allowing the cell to adapt and become more heat resistant. Subsequently, these injured cells require a higher temperature and/or a longer cook time to ensure complete inactivation. The application of current models to whole muscle foods that are cooked slowly to a calculated log reduction does not account for sub-lethal heating; therefore, the total kill might be overestimated. Stasiewicz and others (2008) demonstrated the error that occurs when applying a state-dependent inactivation model during slow cooking of 1 g samples of ground turkey thigh, subjected to varying sub-lethal heat treatments. However, such a modeling approach has not been tested in or applied to pilot or commercial – type products or processes.

The implications of overestimating the efficacy of cooking processes is potentially life threatening; therefore, the overall goal of this research was to evaluate the effect of slow cooking on the thermal resistance of *Salmonella* in whole muscle meat and poultry products. The hypothesis was that slow cooking of whole muscle meat products increases the thermal resistance of *Salmonella*. Evaluation of the overall goal and hypothesis included two main objectives: (1) Determine the D and z values for *Salmonella* in whole and ground beef via laboratory isothermal lethality tests, and (2) Evaluate the accuracy of traditional and alternative lethality models via pilot-scale, inoculated challenge studies with whole muscle products in a moist-air convection oven.

2. LITERATURE REVIEW

2.1. Foodborne Pathogens

Foodborne pathogens contaminate food products, and cause those whom consume a contaminated food product to become ill. It is "estimated that foodborne disease causes approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year" (Mead and others 1999). Foodborne bacterial pathogens responsible for the highest number of deaths each year include *Salmonella, Listeria*, and *Toxoplasma* (Mead and others 1999). The FDA (Food and Drug Administration) and USDA (United States Department of Agriculture) have developed regulations, and standards in the processing of food products to help reduce illness caused by foodborne pathogens. Food related illness results in billions of dollars in lost product, medical bills, and law suits (Bubzy and Frenzen 2001).

2.1.1 *Salmonella*

Salmonella was the foodborne bacterial pathogen with the highest incidence of infection in 2005 (CDC 2008a). *Salmonella* causes salmonellosis, which can result in diarrhea, fever, and abdominal cramping for most healthy individuals (CDC 2008b). Health complications can occur, such as reactive arthritis, Reiter's syndrome, and ankylosing spondylitis for susceptible populations, such as young children, the elderly, and immunocompormised individuals (Doyle 2001).

Salmonella spp. are members of the *Enterobacteriaceae* family, and are characterized as facultative anaerobic, Gram negative rods. *Salmonella* is motile with a peritrichous flagella, and optimal growth occurs at 37°C (Doyle 2001). Although several

thousand *Salmonella* serotypes exist, *Salmonella* Typhimurium and *Salmonella* Enteritidis are the two prevalent serotypes in the U.S. (FDA 2009). *Salmonella* Enteritidis is predominantly associated with poultry, its primary reservoir. *Salmonella* Typhimurium is a more widespread serotype, and is of great importance because of its multiple antibiotic resistance (FDA 2009).

Salmonella outbreaks are widespread, having occurred in numerous food products, including meat, poultry, peanut butter, vegetables, and nuts (FDA 2001, FDA 2009a, FDA 2009b). Now that the government has implemented new surveillance techniques, such as FoodNET, PulseNET, and NARMS, detection of foodborne illness is becoming more efficient (Richard and Arkin 2007). However, there still remains a need for more reliable control methods that will reduce the transmission of *Salmonella* (Juneja and Eblen 2000).

2.1.2 Pathogens in Meat

The predominant meat safety concerns of the 21st century will likely revolve around microbial pathogens. This is due to alterations in the production and distribution of our intricate food system (Sofos 2008). Consumers want convenient, high quality, inexpensive foods, and these demands will come with a price (Sloan 2009). The dangers associated with pathogens in meat products exist because fresh muscle tissue has a high nutrient value, and a high water activity of about 0.99 and a near neutral pH, all of which provide an environment ideal for bacterial proliferation (Doyle 2001). Temperature abuse of the product will also encourage pathogen growth. Pathogens readily exist within the environment of a slaughterhouse; therefore, monitoring and control of theses pathogens is crucial. HACCP (Hazard Analysis and Critical Control Points) is a program originally

developed by NASA, and now implemented in the food industry by the FDA and USDA, to assist in reducing product contamination during processing (van der Fels-Klerx and others 2008). The Food Safety and Inspection Service (FSIS), a division of the USDA, has labeled *Salmonella* as a target organism in major meat species (Duffy and others 2001).

2.1.2.1 Salmonella

Salmonella is a pathogen that occurs naturally within the intestinal tract of animals and is often present on animal hide due to fecal contamination. *Salmonella* is transmitted via the fecal-oral route (CDC 2008b). Raw meat products are a confirmed vehicle of *Salmonella* contamination and transmission (Stopforth and others 2006; Kegode and others 2008; Sofos 2008). *Salmonella* can attach to the surface of meat tissue from cross contamination, and is capable of migrating into the interior of a whole muscle product (Warsow and others 2008). Currently the limit of detection for microbial contamination is about one cell per 25 g of meat, therefore making it possible for a pathogen or other microorganism to go undetected, and multiply during periods of temperature abuse (Shimoni and Labuza 2000). *Salmonella* is capable of growing throughout a wide temperature range (4 to 54°C) and pH range (4.5 to 9.5), because of its ability to adapt to environmental conditions (Doyle 2001).

The possibility of meat product contamination exists throughout processing, with many opportunities for cross contamination. This danger, linked with the potential for insufficient processing, is an important concern for manufacturing ready-to-eat meat products. *Salmonella* is an adaptive pathogen, capable of surviving in many

environmental conditions. While *Salmonella* does not proliferate at freezing temperatures, it is not destroyed when subjected to freezing temperatures on beef trimmings (Dykes and Moorhead 2001). Dykes and Moorhead (2001) attributed the lack of reduction in *Salmonella* during freezing to the protective effect of the fat layer. *Salmonella* is generally salt susceptible, but increased salt tolerance has been observed at 10 to 30°C (Doyle 2001). At low water activity, *Salmonella* exhibits increased thermal resistance (Pearson and Dutson 1986; Carlson and others 2005). The ability of *Salmonella* to adapt to meat processing stressors, such as heat, cooled, acid, and salt causes it to be a major safety issue in regards to foodborne illness.

2.1.2.2 Mechanisms and modes of transfer

Mishandling of meat products is almost inevitable, given the complexity of the food distribution system. Cross-contamination of meat products can occur within the processing facility via equipment or handlers. For this reason, it is crucial to be sure that all RTE meat is properly cooked before distribution, therefore inhibiting pathogen growth during temperature abuse.

Currently, marination is used by processors as a method to increase meat tenderness, enhance water content, and amplify flavor and color. Most processors utilize marinades consisting of a combination of salt and phosphate. Salt functions as a flavor enhancer, while phosphate enhances marinade uptake and retention (Xiong and Kupski 1999).

The ability for bacteria to penetrate into the interior of whole muscle products that have been surface contaminated or marinated has been demonstrated, and poses a

safety issue during processing (Gill and Penney 1982; Gupta and others 1983). Vacuum tumbling aids in the penetration of marinade into intact muscle. If the marinade is contaminated with *Salmonella*, vacuum tumbling increases the migration of *Salmonella* into the interior of whole muscle turkey by forcing bacteria toward the center of the product (Warsow and others 2008). Vacuum tumbling facilitates the uptake of liquid marinade into whole muscle, but *Salmonella* can penetrate into whole muscle turkey without external intervention (Breslin and others 2007). The migration of *Salmonella* without intervention or a liquid marinade was demonstrated by inoculating whole muscle turkey using only the pellet from an 8 - serovar *Salmonella* cocktail following centrifugation (Breslin and others 2007).

2.2. Thermal Resistance

2.2.1 Product Factors Affecting Thermal Resistance

Regulatory standards for the thermal processing of muscle foods, based on a calculated lethality, have been established to allow processors flexibility in processing conditions (FSIS-USDA 1999). Measured lethality as a determination of product safety can prevent over processing, therefore increasing product yield, profits, and consumer acceptance. Contrary to the positive implications of this standard, safety concerns are associated with heating products to a lethality requirement, when determined by a model that fails to account for the multiple factors affecting thermal resistance of muscle foods.

Product composition and structure have been shown to affect the thermal resistance of *Salmonella* (Murphy and others 2000; Smith and others 2001; Orta-Ramirez and others 2005). Juneja (2001b) attributed the different D-values in ground beef, pork, turkey, and chicken to differences in product composition that affect bacterial

inactivation. In addition to the product variability, bacterial strains can vary widely in their heat resistance (Juneja and Eblen 2000). Thermal resistance of foodborne pathogens is typically reported as D and z values, (Smith and others 2001). D and z values are used in the log-linear model to calculate the lethality of pathogens within food products. Microorganisms and food matrixes similar to the product of interest must be used in order to accurately evaluate the safety of a final product (Murphy and others 1999; Smith and others 2001).

Fat is a major factor in the disparity of thermal resistance (Juneja and others 2001). Fat globules within the food matrix act as protective barriers for *Salmonella* cells, thereby increasing thermal resistance (Juneja and Eblen 2000).

Product structure, ground versus whole muscle, also has an impact on the thermal inactivation of *Salmonella* (Orta-Ramirez and others 2005; Tuntivanich and others 2008). Orta-Ramirez and others (2005) and Tuntivanich and others (2008) found that *Salmonella* was more resistant in whole muscle beef and turkey than in ground products of equivalent species, composition, and history. However, the fundamental mechanisms for those differences are not yet known.

The moisture composition of a food product has also been shown to have a major impact on *Salmonella* survival. If *Salmonella* exists in a food product with a low moisture content, it has an increased resistance to heat, as compared to the same product with a higher moisture content (Murphy and others 2001b; Carlson and others 2005; Naphaporn and others 2007). Water activity also profoundly affects the effectiveness of processing conditions on bacterial lethality. It needs to be evaluated when validating a process, because there is a high risk for *Salmonella* survival following the heating step.

Many researchers have found that pH of the product affects *Salmonella* survival. Despite these findings, pH is not a factor included in lethality calculations (Leguerinel and others 2007). Failing to account for the pH of the raw and processed product is likely to contribute to inaccurate lethality estimations. Currently pH is not incorporated into lethality models; however, this product factor is minimal when compared to other product factors (Leguerinel and others 2007).

Product composition and structure have a large influence on the thermal resistance of bacteria; therefore, in order to develop accurate models for evaluating the safety of RTE meat products, all of these intrinsic factors need to be considered.

2.2.2 Process Factors

In addition to product composition, processing factors also affect thermal inactivation of *Salmonella*. Dry cooking conditions increase the heat resistance of *Salmonella* on the surface during processing (Blankenship 1978; Goodfellow and Brown 1978; Murphy and others 2001b). However, in practice, even moderate added humidity (e.g. ~30% rh) can enable sufficient lethality during cooking to effectively eliminate *Salmonella* (Mann and Brashears 2007). Humidity during processing has been found to significantly affect the survival of *Salmonella* in meat (Murphy and others 2001b). However, Carlson (2002) concluded that the water activity of the meat product has a larger effect on the *Salmonella* thermal resistance than does the process humidity. Since process humidity significantly affects water activity, process humidity must be considered, not only for yield and heat transfer purposes, but more importantly for its impact on product safety.

2.2.2.1 Sub-lethal Injury, Non thermal

Processing intervention steps also are capable of causing injury to pathogens. These can include a broad range of factors including temperature, drying, irradiation, pressure, acid, sanitizers, preservatives, and antimicrobials (Wu 2008). Cells that have experienced stress injury, and repair under favorable conditions, are defined as sub-lethally injured (Wu 2008).

There are three broad categories of microbial reduction steps: thermal, chemical, and physical. Causative agents of non-thermal sub-lethal injury include acid washes, hydrostatic pressure, sanitizers, preservatives, and antimicrobials (Wu 2008). Bacteria are capable of adapting to their environment, and sub-lethally injured cells adapt to the causative agent that injured them, as well as cross protection to different stressors based on stress proteins. For example, *E. coli* has exhibited the capability to adapt to sanitizer stress on produce, thereby potentially decreasing the effectiveness of the cleaning process of fresh produce (Zook and others 2001). Sub-lethal injury of bacterial cells creates a safety risk in the food industry, but hurdle effects, when applicable, aid in eliminating the danger of sub-lethal injury.

2.2.2.2 Sub-lethal Injury, Thermal

Thermal sub-lethal injury has been investigated thoroughly because of the popularity of value-added food products and outbreaks that have occurred after a perceived lethality treatment. Thermal inactivation of *Salmonella* is significantly affected by prior thermal treatments (Wesche and others 2005). The range of values reported as the heat shock region are between 42 and 48 °C (Mackey and Derrick 1986; Xavier and

Ingham 1997), indicating that bacterial cells held at a temperature between 42 and 48 °C will not be destroyed, but rather injured, and capable of repairing. Following thermal sub-lethal injury, bacterial cells will require a longer exposure, or higher temperature for inactivation (Wesche and others 2005). Only mild temperature abuse is necessary to cause sub-lethal injury (Xavier and Ingham 1997), and this is quite possible, given the potential for improper temperature control during distribution (O'Bryan and others 2006). Heat injured cells are likely to display a lag period that has the potential to be days long before growth on nutrient rich media occurs (Juneja 2007). For this reason, knowledge of pathogen growth is crucial for analyzing the safety of RTE products following processing (Murphy and others 2001b); otherwise, a product may be deemed safe when in fact it is contains injured cells, which could result in ill consumers.

Storage conditions, temperature, and length of storage following processing greatly impact the growth of heat injured *Salmonella* (Murphy and others 2001b). Processing conditions also impact the survival of bacterial pathogens. Packaging conditions have been shown to affect *Listeria monocytogenes* thermal resistance in raw meat, with *L. monocytogenes* to be more heat resistant in vacuum packaged as compared to air-packaged products (Kim and others 1994).

Though many conclusions have been made about the thermal resistance of bacterial cells in food products, a gap exists because the majority of studies have been conducted in controlled laboratory and small-scale environments. Small samples are much easier to control, because they eliminate the variability associated with larger sample sizes, such as non-uniform heating and sampling variability. However, in order for the industry to apply laboratory data to its processes, they must be validated in larger

commercial studies. The majority of thermal inactivation research has been conducted using isothermal tests to determine D values for various microorganisms in liquid media, or samples weighting less than 10 g, which are cooked for various times. These data then are applied directly by industry to their models, which can pose problems due to the length of time it takes to process a larger product. Hence, there is a necessity to develop lethality information that is directly applicable to industry processing conditions. Currently, no process validation method exists that quantifies the sub-lethal injury of bacterial cells during heat treatment. A processing model that accounts for sub-lethal injury of bacterial cells would assist the industry in ensuring the safety of ready-to-eat products.

2.2.3 Inactivation Models

In predictive microbiology, mathematical models are used to estimate changes in microbial populations during storage or processing (Doyle 2001). However, due to the complexity of model applications, and the difficultly in obtaining precise data, no gold standard model exists for all applications (Doyle 2001).

2.2.3.1 Primary Models

A primary model describes the correlation of microbial response as a function of time variation and cooking conditions (Metris and others 2008). Due to the various factors affecting thermal resistance, use of predetermined thermal inactivation data from other unrelated studies has been proven to be unreliable (Murphy and others 2000). Computer-based microbial pathogen growth/inactivation models can not be solely depended on to determine food safety (FSIS-USDA 2005). More sophisticated models

that account for a range of product and processing parameters need to be developed. This can be done by creating models that account for specific processing variables that most closely relate to the product in question. There is a need for inactivation models to be validated using actual processing conditions. Commercial processors desire models that incorporate the factors affecting thermal resistance, with a user friendly interface (Peleg and others 2005), in order to simply validate the safety of their final products.

Log-linear, first-order kinetic models are primarily utilized during or prior to most thermal treatments (Mattick and others 2001). Such models do not necessarily account for the vast difference between fast, high temperature cooking, and slow cooking at low temperatures.

The log-linear model does not account for exposure extent (Peleg and others 2005). The log-linear model utilizes D-values, defined as the time required at a given temperature to kill the initial population of bacteria by 90%. D-values are typically obtained from laboratory studies using broth cultures or small model food systems that do not represent true processing conditions. Using these data can result in inaccuracy in the lethality calculations during processing. Shoulders or lag periods can exist during processing (Peleg 2000). Hence, estimations made by a log-linear model using only data that fit the most linear trend can result in over or under processing (Peleg 2000; Peleg and Penchina 2000; Juneja and Eblen 2000; Juneja and others 2001). Despite the potential inaccuracy of the log-linear model, it is still highly utilized in the food industry via the USDA Pathogen Modeling Program (PMP) (USDA 2006) and other modeling tools, such as the American Meat Institute (AMI) lethality spreadsheet (AMI Foundation 2009). Modifications to the log-linear model that would account for changing heat resistance of

vegetative cells during thermal processing would provide more reliable lethality data (Mackey and Derrick 1986). Validation of modifications and new models should be conducted using industry relevant conditions, variability, and sample size.

An alternative primary model, the Weibull model, is described by the power law model $LogS(t) = -b(T)t^{n(T)}$ (Corradini and Peleg 2004). For situations where $n(T) > 1$ the curve has an upward concavity, which indicates that the microbial kill increases with time; for $n(T) < 1$, the survival curve is concave downward, indicating that resistant survivors remain persistent following death of weaker cells; if $n(T) = 1$, the survival curve is that of first-order log-linear kinetics (Corradini and Peleg 2004). The Weibull model is more flexible than the traditional log-linear model; however, it alone does not account for the effects of prior sub-lethal history (Stasiewicz and others 2008).

2.2.3.2 Secondary Models

Secondary models describe how primary model parameters depend on changing environmental factors (Doyle 2001; Metris and others 2008). Examples include the Arrhenius model, the square-root (Bèlerádek) model (primarily utilized for bacterial growth), and the Bigelow / z-value model, which relates the D-value to the temperature via the z-value, such that $D(T) = D_{ref}\, 10^{(Tref - T)/z}$.

Additional secondary models include polynomial models, which rely on the best fit assumption when comparing the independent and modeled variables (Doyle 2001). When recently applied to microbial thermal inactivation data, a 2-term fractional differential equation (FDE) model could not account for the shoulders and tails in the inactivation data (Kaur and others 2008). However, the model did account for concavity,

and remained accurate during extended processing (Kaur and others 2008). This alternative model seems appealing, but further research is needed, because these findings are based only on the application of the model to lab-scale pathogen inactivation data. There is a need to evaluate the 2-term FDE in large-scale, diverse processing conditions.

2.2.4 Modeling Effects of Sub-lethal History

Several researchers, including Corradini and Peleg (2009) and Valdramidis and others (2007), proposed lethality models that incorporated the effect of sub-lethal history on an organism during thermal processes. However, both of these studies modeled the inactivation parameters as functions of temperature and heating rate ($\frac{\partial T}{\partial t}$), even through the physiological response of heat shock has not been shown to be related to $\frac{\partial T}{\partial t}$. Also, neither of these models was validated using an actual food product. Valdramidis and others (2007) heated tubes containing suspended bacterial cells in a circulating waters bath, whereas Corradini and Peleg (2009) estimated their model parameters using previous thermal inactivation data generated from other laboratories.

In contrast, the thermal inactivation model proposed by Stasiewicz (2008) accounts for sub-lethal injury as an integral of the sub-lethal history during thermal processing. This model is referred to as a path-dependent model, and quantifies the sub-lethal history as the integral of the temperature profile within the defined heat shock region (Stasiewicz and others 2008). The model was applied to slow cooking conditions, and the error between the actual and predicted log reductions was evaluated. The error for a traditional Weibull / Arrhenius model was large during slow cooking, because it failed

to account for sub-lethal injury (Stasiewicz and others 2008). However, Stasiewicz's path-dependent, Weibull / modified-Arrhenius model accounts for sub-lethal history, and eliminated the systemmatic error that occurred using the state-dependent model. Additional research validating the path-dependant model needs to be conducted. The rationale for this model was evaluated using one product (turkey thigh meat). Another drawback of the research was that it was in a controlled laboratory environment, with very small samples sizes (1 g), which does not directly correlate to industrial processing situations.

2.3. Commercial Meat Cooking

2.3.1 Validation of Process

The United States Department of Agriculture (USDA) has implemented safety regulations for the processing of meat and poultry products, which concentrate on an endpoint internal temperature. Hence, a meat roast cooked to a specified core temperature, would be deemed fully cooked and safe. The USDA specifies an internal temperature of 71.1°C for the product to be labeled fully cooked (FSIS-USDA 1999). These standards have been developed for customers at home and as a reference temperature in cooking trials (Pittia and others 2008).

Endpoint lethality requirements are enforced by the USDA to assist processors in determining the safety of their product, without over-processing (FSIS-USDA 1999). End point temperature requirements can lead to over processing, which causes an increase in energy cost, and a decrease in product yield, both of which directly impact processors profits. Profits drive the industry; therefore, lethality requirements that maximize product

yield and minimize energy expenditure are desirable as long as safety of the product can be ensured.

2.3.2 Validation of Oven Conditions

Larger meat roasts are often cooked at lower temperatures for extended periods in order to achieve uniform heating, a more uniform color throughout, and a more tender and juicy end product. Several common cooking "schedules" can be used, depending on the quality goals.

Step-up oven conditions consist of a gradual increase in the oven temperature throughout the cooking process. Step-up cooking increases the surface and internal temperature at a similar rate, because a smaller temperature gradient exists, which helps to eliminate crust formation. This processing condition can provide a final product that has a rare center appearance, and is primarily utilized in roast beef processing.

Constant oven temperature processing is utilized to consistently increase the internal temperature over a short period. During this type of cooking, the oven remains at a steady temperature throughout cooking (e.g., ~93.3°C), which is higher than the desired product end temperature (~71.1°C), to encourage pathogen destruction (Murphy and others 2001a). The larger initial temperature gradient causes the internal temperature to increase more quickly during cooking. The result in turn yields a more time-efficient cook, as compared to the step-up process. Beef roasts cooked at a constant temperature will have a less pink center as compared with the step-up process. This is more appealing to some consumers, because it results in a product that "looks" cooked, but is less appealing to consumers who prefer rare or medium beef.

2.3.3 Humidity

Oven conditions influence the sensory characteristics of a processed product (James and Calkins 2008); therefore, depending on the desired end product, commercial processors may choose to control humidity in different manners. Introducing moisture into a hot air convection oven increases the heat transfer rate, resulting in a decreased cook time (Murphy and others 2001a). The increase in heat transfer rate is partly responsible for the more efficient killing of microorganisms when compared with dry heat (Naphaporn and others 2007). Also, *Salmonella* thermal resistance is affected by product moisture within different cooking environments (Carlson 2002). In addition to decreasing the processing time, and increasing lethality, increased humidity also minimizes water loss during cooking, which increases product yield, and thereby profits (Vittadini and others 2005).

2.3.3.1 FSIS Regulations of Humidity Use

Humidity requirements "apply only to those processes in which the surface moisture of the product can evaporate, and surface drying can occur, prior to destruction of the microorganisms"; exceptions include processes where humidity is inherently sustained around the product (FSIS-USDA 2005). Humidity requirements were developed to ensure complete inactivation of foodborne pathogens in RTE meat. Surface bacterial pathogens are more thermally resistant when the moisture level is low (Blankenship 1978; FSIS-USDA 1999b).

2.3.3.2 Cook-in-bag

In-bag cooking is used to trap humidity in the bagged product during cooking, and thereby enhance product yield, making this process more economical as compared to no-bag oven cooking. According to the FSIS humidity regulations (2005), "cooking the product in a sealed, moisture impermeable bag" avoids surface drying. Specifically engineered bags that withstand the cooking environment and are impermeable to moisture are used. Advances in cook-in-bag technology have provided processors with numerous bag options, depending on the desired end product. If a cooked roast with golden color is desired (e.g., turkey), then the bag remains closed throughout cooking, then just before ejection from the oven the bag splits open, to allow for top-surface color development. Other advantages of using cook-in-bags during processing include their ability to act as a barrier to the environment, preventing cross-contamination, decreasing purge loss, increasing yields, and retaining protein, mineral, and pigments components (Qiaofen and Da-Wen 2007).

Commercial processors choose cooking schedules that fulfill the end goal of yield, color, taste, and texture. Now, consumers and the government are putting the responsibility of providing safe ready to eat products on the processors; therefore, the industry needs to take appropriate steps to ensure the safety of their products.

3. MATERIALS AND METHODS

3.1 Isothermal Research

Isothermal laboratory studies were conducted with ground and whole beef and pork. The meat was acquired as stated below in section 3.2, and the *Salmonella* inoculum was the same as in section 3.3. Methods were the same as those performed by (Orta-Ramirez and others 2005), except experiments were performed at five different temperatures: 55, 58, 60, 62, and 63°C.

3.2 Acquisition of Meat

Fresh, skin-off turkey breast (*Pectoralis* major and minor muscles, acquired from a federally inspected commercial processor), top beef round (*Semitendinosus, Adductor, and Pectineus* muscles, commercial supplier from a federally inspected source), and center cut pork loin (*Longisimus Dorsi* muscle, commercial supplier from a federally inspected source) were used. All products were acquired directly from the processor/supplier, and shipped to Michigan State University's meat processing facility. Product temperature at time of receipt was verified to be < 4.4°C and documented. Meat was sectioned into approximately 0.68 kg roasts, vacuum packaged in double plastic bags, and frozen (-20°C).

Frozen roasts were then packaged in 57 L plastic storage boxes and shipped eight days after arrival to Tampa, FL for irradiation (FTSI, FDA registration 1054811). Products were shipped in a refrigerated truck at -20°C. Gamma rays were used to irradiate boxes (~10 kGy) to eliminate indigenous microflora, then transported frozen

back to Michigan State University's meat laboratory. Frozen irradiated samples remained frozen (-20°C) until use.

Sterility of the samples was confirmed by randomly testing 3 roasts of each species. A 25 g core was removed aseptically from the roast using a sterile scalpel blade (Rib-Back Carbon Steel Surgical Blades Bard-Parker, Becton Dickinson AcuteCare, Franklin Lakes NJ), placed in 8 oz Whirl-Pak® bags, diluted 1:10 in sterile triptic soy broth (Difco Laboratories, Sparks, MD) containing yeast extract (Difco Laboratories, Sparks, MD) (TSBYE), homogenized in a masticator (Neu-Tec Group Inc, Barcelona, Spain) for 180 s, and incubated for 24 h at 37°C. Following incubation, duplicate samples were plated on aerobic Pertrifilm™ (3M Microbiology Products, St. Paul, MN).

The moisture and fat composition of all species was determined using Association of Official Analytical Chemists (1990) methods 950.46B and 991.36, respectively.

3.3 Preparation of Salmonella

Triptic soy broth with yeast extract (TSBYE) was prepared by combining 30 g triptic soy broth, 6 g Yeast extract, and 1 L of deionized distilled water. The solution was dispensed into 250 (turkey), and 500 ml (beef, pork) capped, autoclavable bottles. The solution was autoclaved (Castle M/C 3522 Sterilizer, Getinge, Rochester, NY) for 20 min at 121°C.

The inoculum consisted of eight *Salmonella* serovars: *S.* Thompson FSIS 120 (chicken isolate), *S.* Enteritidis H3527 and H3502 (clinical isolates phage types 13A and 4 respectively), *S.* Typhimurium DT 104 H3380 (human isolate), *S.* Hadar MF60404 (turkey isolate), *S.* Copenhagen 8457 (pork isolate), *S.* Montevideo FSIS 051 (beef

isolate), and *S.* Heidelberg F5038BGI (human isolate), previously obtained from V.K. Juneja (Agricultural Research Service, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA). Each serovar was separately maintained at -80°C in vials containing tryptic soy broth and 10% glycerol. Cultures were started by transferring one loop of frozen culture into 9 mL of TSBYE, incubating at 37°C for 18-24 h. Cultures were maintained using consecutive daily transfers for up to one week, with a minimum of two consecutive transfers prior to use. One day prior to use, the strains were transferred separately into 250 mL (turkey) or 500 mL (beef, pork) bottles of TSBYE and incubated at 37°C for 24 h.

3.4 Preparation of Marinade

A typical commercial marinade containing 11.5% (w/v) salt and 3.7% (w/v) phosphate was prepared by adding 169 g of liquid phosphate (50% food grade liquid potassium phosphates, Butcher and Packer Supply Company, Detroit, MI) to 1,778 g of deionized distilled water, then stirring on a stir plate (Corning PC-420, Corning, NY) until the phosphate dissolved. Once the phosphate and water were completely combined, 253 g of NaCl was added to the solution, while stirring continued. Once the NaCl was dissolved into the mixture, aliquots of 520 mL and a small stir bar were added into 500 mL twist capped autoclavable bottles and autoclaved for 20 m on the liquid cycle at 121°C.

On the day of experimentation, the 8-serovar *Salmonella* cocktail was prepared by pipeting 16 mL of each strain into four individual 250 mL centrifuge bottles (turkey) or 20 mL of each strain into 18 (beef) or 22 (pork) 250 mL centrifuge bottles. The bottles were centrifuged at 6000 *x g* for 15 min, the supernant was poured off from each

centrifuge bottle, and the remaining pellets were transferred into 500 mL of sterile marinade, using a sterile spatula. The 500 mL bottle of marinade contained a sterile stirrer bar, which was added before autoclaving, in order to dissolve the pellet into the marinade. The pellet and marinade were stirred about 10 min, until the pellet had dissolved in the marinade to ensure a homogeneous suspension. Thereafter, the inoculum was plated onto duplicate aerobic Pertrifilm™ plates (3M Microbiology Products, St. Paul, MN) and incubated for 24 h at 37°C before enumeration. The 8-serovar *Salmonella* inoculum consistently had a *Salmonella* population ~10^9 CFU/ml.

3.5 Inoculation

Irradiated meat products were inoculated with the 8-serovar S*almonella* cocktail. For inoculation, each roast was pre-weighed and placed in a lab-scale, sterile vacuum tumbler (T-15 Vacuum Meat Tumbler, Kent Butcher Supply, Grandville, MI) that was modified with a stainless steel baffle insert. The targeted uptake was 15% (w/w) (turkey), or 10% (w/w) (beef, pork). Marinade uptake was calculated based on the following equation:

Where:

X = amount of marinade

Y = weight of meat

For 15% uptake:

$X / (X+Y) = 0.15$

$X = 0.15X + 0.15Y$

$X = 0.18Y$

For 10% uptake:

$X / X+Y = 0.10$

$X = 0.10X + 0.10Y$

$X = 0.11Y$

Following the addition of the proper amount of marinade and the roast(s) to the sterile tumbler, a vacuum pump (Welch Vacuum model 2534B-01, Thomas Compressors and Vacuum Pumps, Skokie, IL) was used to pull a vacuum of ~84.65 kPa on the tumbler. Roast(s) were then vacuum tumbled at 8 rpm for 20 min, rested for 5 min, and then tumbled at 8 rpm for an additional 20 min.

3.6 Inoculation Verification

The initial *Salmonella* inoculation level for turkey and beef was validated by removing the center core (16.4 cm^3) from the inoculated roast(s) with an electrosurgical unit (Valleylab SurgiStat II, Boulder CO). Tuntivanich and others (2008) previously confirmed that this electrosurgical unit allowed for the interior of the muscle to be sampled without exterior contamination for similar work with turkey breast.

To verify the inoculation process, the top 1.27 cm of the roast was sterilely removed using the electrosurgical knife, to avoid contamination from the exterior to the interior. Following removal of the contaminated surface, five 2.54 x 2.54 x 2.54 cm^3 samples (Figure 1) from near the center of each roast were removed with a sterile scalpel (Becton Dickinson AcuteCare, Franklin Lakes, NJ). The t-shaped figuration was used to confirm that the core sample had the lowest population of *Salmonella*, as compared to the surrounding sample locations.

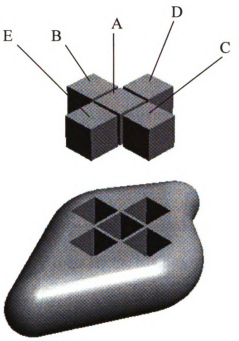

Figure 1. Whole muscle roast cutting diagram. Segment A was considered the center cut of the roast.

Once extracted from the roast, the sample cubes were diluted 1:5 with 0.1% buffered peptone water (Difco Laboratories, Sparks, MD) and homogenized in a masticator (NEUTEC Group Inc., Barcelona, Spain) for 180 s. Samples were then serially diluted with 0.1% buffered peptone water, plated on duplicate Petrifilm™ aerobic plates (3M Microbiology Products, St. Paul, MN), and enumerated following 24 h of incubation (Cenco, Central Sciences Co. Chicago, IL) at 37°C. Six roasts from each species were used to verify the inoculation procedure. The mean value from the six center cores (segment A) was used as the initial *Salmonella* population for the remainder of the study.

3.7 Sample Preparation

Before cooking, roasts were thawed and inoculated as described previously. For in-bag cook schedules, two septums (SSP 134, Spa, NY) were glued (Silicone II, General Electric Company, Huntersville, NC) to the exterior of the bag at least 24 h before use, to ensure adequate adhesion. Inoculated samples that were cooked in-bag were inserted aseptically into boil-in-bags, which were vacuum sealed (VacMaster, Kansas City, MO). The septums allowed for thermocouples to be inserted into the roast through the bag, while maintaining the vacuum. Except for the bagging step, the in-bag and out-of-bag samples were prepared identically.

3.8 Oven

A commercial, moist air roasting oven (CO151FWUA12B2083, Cres Cor®, Mentor, OH) was modified to control the cooking temperature profile and to log the time-temperature data. The control and recording system consisted of a LabVIEW® (National Instruments, Austin, TX) data acquisition unit (CompactDAQ, National Instruments, Austin, TX), thermocouple signal conditioning modules (NI 9211), a digital output module (NI 9401), and a universal relay module (URM-800, Omega Engineering, Inc., Stamford, CT). Thermocouples consisted of 12 short needle probes (type K, PA1454B, length 1.5 m, diameter 1.6 mm, barb end, accuracy ± 1.1°C, max temperature 265°C, Datapaq, Inc., Wilmington, MA), which were used to measure core temperature of the samples. The control system was used to generate the step-up temperature profiles. Oven humidity ranging from 20-100 % RH was controlled by a built-in dial on the oven, but the humidity was measured by using a humidity sensor (Hydro Clip, serial number 36737

009, Rotronic, Huntington, NY) and a data logger (Datapaq, Inc., Wilmington, MA). However, the humidity control was not a feedback system, based on the continuous humidity measurement, as would be typical in an industrial system.

3.9 Cooking

Prior to cooking, stainless steal corers (2.54 cm diameter) were wrapped in aluminum foil, and autoclaved for 20 min at 121°C to ensure sterility. Thereafter, the wires for each of the 12 thermocouples were sterilized using sanitizing wipes (Sani-Cloth Plus, PDI, Orangeburg, NY). The sterile corers were then strung onto the sterile thermocouple wires, which recorded product core temperature in real time throughout the cook (Figure 2).

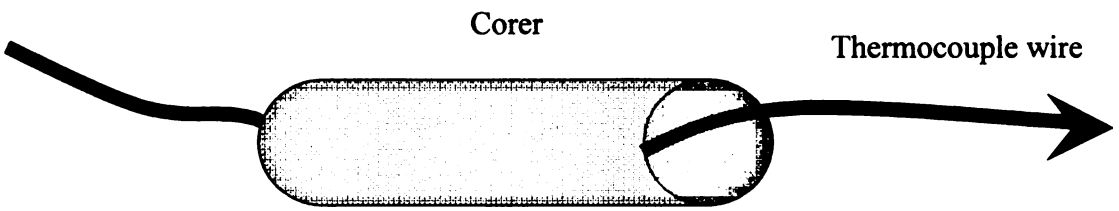

Figure 2. Thermocouple wire threaded through a sterile stainless steel corer.

After the thermocouple wire had been strung through the corers, two thermocouple probes were inserted near the center of each roast. For in-bag samples, these probes were inserted through the septum, to ensure integrity of the vacuum during cooking. The temperature data from the colder of the two probes for each roast were used to evaluate the roast's endpoint temperature or predicted lethality, which was calculated

in real-time, based on the log-linear model with parameters obtained from isothermal research (section 3.11.1).

When the software program indicated that the cold spot of the roast neared the targeted end point, 71.1°C, or calculated 7.0 / 5.5 / 3.0 log reductions, the roast was immediately removed from the oven and placed into a sterile stainless steel pan. A core was quickly and aseptically taken of the recorded cold spot using the 2.54 cm sterile corer that was strung onto the thermocouple wire. After the entire core was removed from the roast, about 2.54 cm were removed from the top and bottom of the sample, using a sterile scalpel. The remaining center of the core, ~8 g sample ("cold spot"), was then quickly inserted into a Whirl Pak bag containing 18 g of sterile refrigerated (4°C) 0.1% buffered peptone water to cool the sample below 15°C in less than 10 s after coring. The computed lethality used in the subsequent analysis included all of the temperature history of the core through cooling to < 15°C.

Following cooling, the ~8 g samples removed from the treated roasts were diluted 1:5 with 0.1% buffered peptone water, homogenized for 180 s in a masticator, serially diluted, plated on duplicate Petrifilm™ aerobic plates, and enumerated for *Salmonella* survivors following 48 h of incubation at 37°C. The limit of detection for *Salmonella* on Petrifilm™ aerobic plates following dilution was 0.4 log CFU/g.

3.10 Cooking Schedules

Following inoculation and preparation for cooking, roasts were processed using a variety of pre-determined, industry relevant cooking schedules. Cook schedules varied in containment, time, temperature, and humidity (Figure 3). Each schedule included two

roasts cooked to a target end point temperature of 71.1°C, and three roasts cooked to a target endpoint for *Salmonella* lethality.

For turkey, the targeted end point lethality was 7 $\log_{10}$, based on the USDA *Salmonella* lethality standards (FSIS - USDA 1999). For beef roasts, the targeted end point lethality was 5.5 $\log_{10}$ rather than the USDA standard of 6.5 $\log_{10}$. This was because our inoculation process achieved a core *Salmonella* population of only 6.27 $\log_{10}$ CFU/g. It was necessary to choose a lethality endpoint lower than our initial concentration in order to quantify the actual lethality of *Salmonella* during the treatments. Had the sample been cooked to the USDA *Salmonella* lethality standard of 6.5 $\log_{10}$ for beef, survivors would have been recovered only from cooks that over-predicted lethality, which would have biased the study results.

For pork, the targeted end point lethality was 3.0 $\log_{10}$. The reason for the low targeted lethality for pork was again due to the low initial *Salmonella* population within the core (6.27 ± 1.04 log CFU/g), and a high level of variability between roast cores. It was concluded that if the targeted lethality was lowered to (3.0 $\log_{10}$), survivors could be consistently quantified, because the initial population was always going to be larger than 3.0 $\log_{10}$. The experimental design (Figure 3) consisted of seven different cooking combinations representing typical industry conditions.

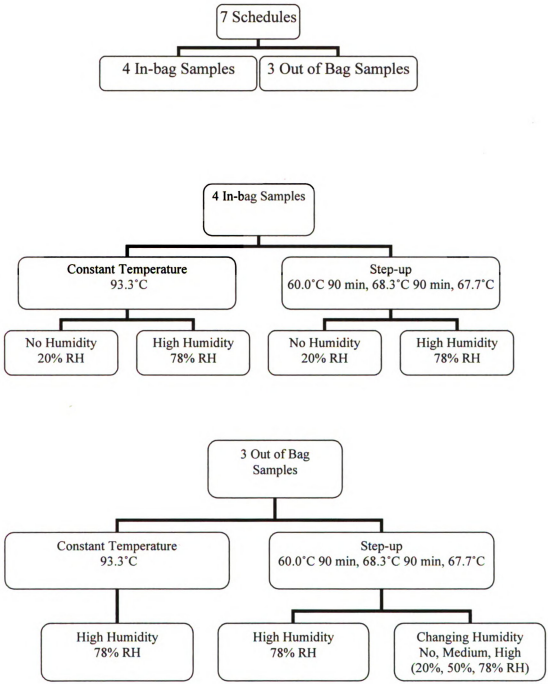

Figure 3. Predetermined cook schedules utilized to process whole muscle roasts in a modified Cres Cor® moist air convection oven.

Of the seven cook schedules, four consisted of roasts that were contained within vacuum packaged boil-in-bags (Smurfit-MBI, Butcher and Pack Supply, Detroit, MI). Two of the in-bag schedules were cooked at a constant temperature of 93.3°C, one with high humidity, and the other with no humidity. The two other in-bag schedules used a step-up temperature condition, 60.0°C for 90 min, 68.3°C for 90 min, and 76.7°C until the product reached its targeted end point. This condition included one treatment with high humidity and one with added humidity.

Humidity was also a variable. For each of the temperature profiles, roasts were processed with the humidity control set to no humidity, medium humidity, high humidity, or a combination of the three. Relative humidity was measured by using a humidity sensor and a data logger, following completion of the cook, the relative humidity data was copied into a spreadsheet for analysis. The terms no, medium, and high refer to settings on the oven control panel. The high humidity setting on the oven resulted in a relative humidity >98%, but the average relative humidity throughout the course of an entire cook was 78%, because the door was opened to remove samples. The average relative humidity for the medium setting was 50%. The no humidity setting only inhibited the oven from producing steam, but the environmental humidity had an impact, making the relative humidity average 25%.

In the three remaining cook schedules, product was processed out-of-bag, exposing the roast to the oven environment. For out-of-bag cooking, one schedule was at a constant temperature with high humidity. Two others were step-up cook schedules, one with high humidity, and the other with changing humidity. The changing humidity

condition increased the humidity every hour, from none to medium to high, based on the settings of the oven control panel.

Figure 4 shows the oven air temperature and product core temperature for a constant temperature cook schedule, whereas Figure 5 shows the oven air temperature and product core temperature for a step-up temperature cooking profile.

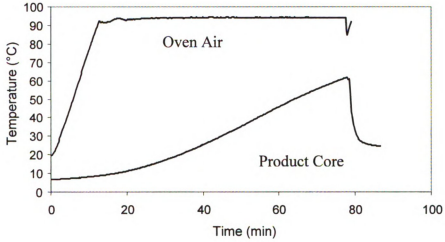

Figure 4. Sample cooking profile for constant temperature cook schedule.

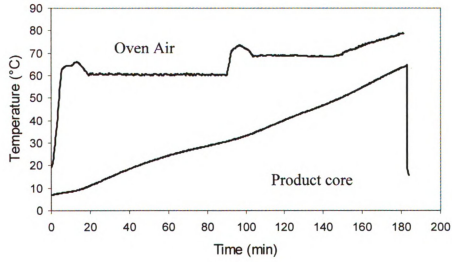

Figure 5. Sample cooking profile for step-up temperature cook schedule.

3.11 Model Computed Lethality

3.11.1 State-Dependent Model

The state-dependent model calculated the predicted lethality of the samples in real-time, based on the log-linear model, using D and z values obtained from isothermal research. Data obtained from the data acquisition system at the end of each cook were used to verify the real-time lethality calculations using the log-linear (Bigelow) model:

$$\log S = \log \frac{N}{N_0} = \frac{-t}{D(T(t))} \tag{1}$$

where S is the survivor ratio, N is the population of microorganisms at time t, and N_0 is the initial microbial population. The D-value, D(T) is the time required at a certain temperature to achieve a one log reduction. The z-value is the temperature change required to achieve a one log reduction in the D-value.

The state-dependent model parameters, D and z, for turkey were obtained from Tuntivanich and others (2008) (Table 1). For beef, the parameters were obtained from unpublished work (Appendix 7.A) based on the methods of Orta-Ramirez and others (2005) (Table 1). The pork parameters were obtained from Velasquez and others (2009) (Table 1).

Table 1. D and z values calculated from isothermal testing of the 8-serovar *Salmonella* cocktail in turkey, beef, and pork (Tuntivanich and others 2008; Velasquez and others 2009).

	Turkey	**Beef**	**Pork**
z (°C)	5.38	5.52	5.29
D_{ref}(s)	114.7	111.5	120.8
T_{ref} (°C)	60	60	60

For a direct comparison of the state-dependent and path-dependent model (section 3.11.2), a log-linear equation was used, but with a different formulation, such that:

$$\log S = \log \frac{N}{N_0} = -b(T(t))\, t \tag{2}$$

where S is the survivor ratio, N is the population of microorganisms at time t, and N_0 is the initial microorganism population. Parameter b is the temperature-dependent rate of inactivation.

To be consistent with Stasiewicz and others (2008), the effect of temperature on b was modeled as a modified Arrhenius dependency:

$$b(T) = b_{ref} \cdot \exp\left\{ -\beta_1 \left[\frac{1}{T(t)} - \frac{1}{T_{ref}} \right] \right\} \tag{3}$$

where β_1 determines the effect of temperature on b, using the same data as described below (Table 2).

Table 2. State-dependent model parameters for whole turkey, beef, and pork (Marks and others 2009).

Product	b_{ref}	β_1 *(K)*
Whole-Muscle Turkey	0.5350	46,395
Whole-Muscle Beef	0.5632	44,854
Whole-Muscle Pork	0.5705	34,859

3.11.2 Path-Dependent Model

The path-dependent model accounts for the effect of sub-lethal history (Stasiewicz and others 2008):

$$b(T) = b_{ref} \cdot \exp\left\{-\beta_1\left[\frac{1}{T(t)} - \frac{1}{T_{ref}}\right] - \beta_2\tau\right\} \quad (4)$$

where $\beta_2\tau$ accounts for sub-lethal thermal injury of the microorganism within the organism's region of sub-lethal injury. Sub-lethal history was quantified as an integral of the temperature vs. time curve when the temperature was within the heat shock region (38 and 52°C) (Stasiewicz and others 2008):

$$\tau = \int_{t_{T=HS_{lower}}}^{t_{T=HS_{upper}}} \left(T(t) - HS_{lower}\right)dt \quad (5)$$

The path-dependent model was calibrated with 30 cook schedules using 1 g of ground product (Tenorio-Bernal and others 200X), to obtain the model parameters (b_{ref}, β_1, and β_2, table 3), by minimizing the sum of squared errors, where error refers to the difference between the observed and predicted lethality (Marks and others 2009). These previously determined parameters were then applied to the cook schedules of the whole muscle roasts in this study, in order to determine whether the path-dependent model reduced the error between the observed and predicted lethality as compared to the state-dependent model, at the pilot-scale.

Table 3. Path-dependent model parameters and goodness of fit obtained from nonisothermal calibration data sets for turkey, beef, and pork (Tenorio-Bernal and others 200X).

Product	B_1	β_1 *(K)*	$\beta_2(K^{-1}\ min^{-1})$	*RMSE (log CFU/g)*	*Bias (log CFU/g)*
Turkey	0.91	50,752	0.0018	0.67	0.09
Beef	0.96	44,243	0.0019	0.87	0.11
Pork	0.82	53,952	0.0032	1.27	0.55

3.12 Data Analysis

T-tests using R-2.7.2 software were performed to determine whether a difference existed between the mean predicted and observed log reductions. The null hypothesis was that no difference existed between the predicted and observed log reductions, and the alternative hypothesis was that a difference did exist.

An Analysis of Variance (ANOVA) using Excel was also performed to evaluate the relationship between the error (observed – predicted log reductions) vs. the sub-lethal history. The potential for an error bias existed for the turkey and beef tests where no cells were recovered following cooking. In this case, it is not possible to conclude whether the state-dependent model under-predicted the lethality; therefore, the result of the ANOVA could be skewed negatively.

The root mean square error (RMSE) and mean residual for each species and the lethality prediction model were calculated to compare the accuracy and average error, respectively, of the state-dependent and path-dependent model.

4. RESULTS AND DISCUSSION

4.1 Isothermal Results

The D and z values used for whole turkey and pork were reported by Tuntivanich and others (2008), and Velasquez and others (2009). The beef isothermal D and z values were new for this study and were reported in Table 1 in section 3.11.1. The individual experimental replication averages (log CFU/g), and log N vs. time (min) graphs for these results are reported in Appendix 7.A.

4.2 Product Composition

The moisture content of the turkey, beef, and pork was 74.3 ± 0.8%, 73.6 ± 0.2%, and 68.5 ± 0.8%, moisture respectively. The fat content of the turkey, beef, and pork was 1.09 ± 0.1%, 8.08 ± 2.8%, and 9.99 ± 2.0%, respectively.

4.3 Initial *Salmonella* Concentration in Whole Muscle Roasts

Verification of the inoculum level (Table 4) was performed as described in section 3.6.

Unfortunately, there is a large standard deviation associated with research using microorganisms, and the large standard deviation associated with the initial *Salmonella* population introduced an aspect of variability into these experiments. This was because the exact initial population for every sample (roast) could not be quantified before cooking (as that would have destroyed the sample before cooking), which thereby affected the accuracy of the measured log reductions for each individual sample.

Table 4. Initial core *Salmonella* population following inoculation, the USDA established regulatory lethality target (FSIS-USDA 1999), and the experimental lethality target (calculated) for turkey, beef, and pork.

	Initial *Salmonella* concentration	Regulatory Target Lethality	Experimental Target Lethality
Turkey log CFU/g	6.96 ± 0.53	7	7.0
Beef log CFU/g	6.27 ± 0.89	6.5	5.5
Pork log CFU/g	6.27 ± 1.06	6.5	3.0

The marinade uptake and *Salmonella* population varied between meat species, due to differences in product structure, protein, fat content, location of fat, connective tissue, and moisture content. In addition to product composition, other factors can also contribute to the high variability between samples, such as sampling location, sampling technique, plating, and enumeration methods. Larger sample sizes inherently introduce variability into this process, and it is more difficult to achieve uniform bacterial concentrations in whole muscle as compared to ground (See section 4.9).

4.4 End Point Temperature

For all cook schedules, duplicate roasts of each species were cooked to 71.1°C. FSIS states that 71.1°C "...is the minimum (temperature) that must be achieved..." in order to eliminate all existing *Salmonella* for all of the species used in this research (FSIS-USDA 1999).

Cooking the roasts to an end point temperature of 71.1°C resulted in the near elimination of all countable cells (Table 5). For turkey and beef, three of the total 14 roasts cooked to 71.1°C had quantifiable numbers of *Salmonella*. For both turkey and

beef, the maximum surviving *Salmonella* population was 0.7 log CFU/g which corresponds to 6.3 and 5.6 log reductions for the turkey and beef, respectively. Complete elimination of all *Salmonella* was not confirmed, because enrichment was not performed on samples following cooking. None of the 14 pork roasts cooked to 71.1°C yielded any surviving salmonellae after plating.

Table 5. The average *Salmonella* population (CFU/g) recovered after duplicate plating on aerobic Petrifilm™ plates for turkey, beef and pork roasts cooked to 71.1°C. Limit of detection 0.4 log CFU/g.

Cook Schedule	Turkey Average Plate Count (CFU)	Beef Average Plate Count (CFU)	Pork Average Plate Count (CFU)
93.3C In Bag, High humidity			
Replication 1	0.0	0.0	0.0
Replication 2	0.0	0.5	0.0
93.3C In Bag, No Humidity			
Replication 1	0.0	0.0	0.0
Replication 2	0.0	0.5	0.0
93.3C No Bag, High humidity			
Replication 1	0.0	0.0	0.0
Replication 2	0.0	0.0	0.0
Step Up, In Bag, High Humidity			
Replication 1	1.0	0.0	0.0
Replication 2	1.0	0.0	0.0
Step Up, In Bag, No Humidity			
Replication 1	0.0	0.0	0.0
Replication 2	0.5	0.0	0.0
Step Up, No Bag, High Humidity			
Replication 1	0.0	0.0	0.0
Replication 2	0.0	0.0	0.0
Step-up, No Bag, Changing Humidity (No, Med, High)			
Replication 1	0.0	1.0	0.0
Replication 2	0.0	0.0	0.0

The turkey, beef, and pork roasts were inoculated to contain an initial *Salmonella* population, in the center core of 6.96 ± 0.53, 6.27 ± 0.89, and 6.27 ± 1.06 log CFU/g. Of course, an initial *Salmonella* population this high within the core of whole-muscle meat/poultry products is very unlikely in normal commercial processing. A product that contained an initial bacterial load greater than 6.0 log CFU/g is likely to have a tainted aroma, and be labeled rotten. Following processing to 71.1°C, the products had achieved at least a 5.6 log reduction. Given the high initial population of *Salmonella* and the negligible number of total recovered cells supports the safety of processing to the USDA suggested internal temperature of 71.1°C.

Cooking roasts to an end point temperature of 71.1°C is very likely to eliminate *Salmonella*. This has positive implications for meat processors, because it reiterates that a product cooked to an internal temperature of 71.1°C is likely to yield a safe product, therefore providing processors with a reliable slow cooking technique.

4.5 Lethality Error

The difference between the calculated and experimentally observed *Salmonella* log reductions are referred to here as the lethality error, or error.

The mean predicted lethality for *Salmonella* in turkey and beef roasts was significantly greater than the observed lethality ($P<0.05$), reported in section 4.8. This result indicated that the state-dependent model resulted in a large negative (dangerous) error (-3.0 and -1.2 log CFU/g for turkey and beef, respectively), and inaccurately over-predicted the lethality of *Salmonella* within whole muscle turkey and beef roasts during pilot-scale, slow cooking processes.

The mean predicted lethality for *Salmonella* in whole muscle pork roasts was not significantly different from the mean observed lethality (P=0.36), reported in section 4.8. Because no significant difference was seen between the predicted and observed lethality, the state-dependent model had a small lethality error, and was appropriate for predicting the lethality of *Salmonella* within whole muscle pork roasts, cooked to a targeted 3 $\log_{10}$ reduction, during pilot-scale slow cooking.

The lethality error for whole turkey roasts was significantly greater for samples that were cooked in-bag compared to those out-of-bag (P=0.026). In addition, the samples processed in-bag without humidity had a significantly greater negative error (P<0.05) than those processed in-bag with humidity for both the constant and step-up temperature schedules.

The lethality errors for whole beef roasts were not significantly different for the in-bag vs. out-of-bag cook schedules (P=0.29). The lethality error for the cook schedules processed at a constant temperature, in-bag, with humidity was significantly greater than the lethality error for the constant temperature samples processed in-bag without humidity (P=0.04). No statistically significant difference in errors was seen for the step-up temperature profiles in-bag with and without humidity (P=0.8). In addition, no significant differences were evident between the lethality errors among any of the pork roast cook schedules.

The lethality error was inconsistent across the three species. Statistically significant interactions based on the cook schedules could not be tested, due to the small number of samples available for analysis (8 for turkey and 11 beef), because only

samples with recoverable *Salmonella* cells were used in the analysis. In contrast, all 20 pork samples yielded quantifiable *Salmonella* for analysis.

Turkey and beef were processed to a targeted lethality near the initial *Salmonella* concentration, therefore making it more difficult to quantify positive lethality errors (section 4.8). Positive lethality errors are when the experimental log reductions exceed the values predicted by the state-dependent model. The inability to quantify positive lethality errors negatively skews the data, and may attribute to the inconsistency in statistical significance between the three species.

Inconsistencies in the inoculation process could have also led to irregularities in the results between species. The standard deviation for the initial *Salmonella* population was as large as 1 log CFU/g, with this high variability perhaps impacting the observed lethality, thereby affecting the lethality error.

There are no fundamental explanations for why humidity would have impacted turkey, but not beef or pork. The statistically significant results could be attributed to the small number of samples with recoverable cells in turkey and beef samples that yielded surviving salmonellae.

4.6 Replication Error

The experimental replication errors for the recovered *Salmonella* in turkey, beef, and pork were 1.42, 0.93, and 1.03 log CFU/g, respectively. The issue of high variability was consistent across all three species, suggesting that there was substantial variability inherent in the method of scaling-up to pilot-scale processing. A study considered to have low variability would have a replication error less than 0.2 log CFU/g.

Many factors within the experimental methods had the potential to contribute to the overall experimental error. These factors included, but were not limited to, inaccurate determination of the initial *Salmonella* population within the core, ability to insert the thermocouple probe directly into the geometric center of the product, uncertainty in the state-dependent model parameters, sampling of the roast core, enumeration of survivors, and the recovery of sub-lethally injured cells.

Replication error (i.e., the standard deviation among replicates) increased with increasing sample size. The increase previously occurred when the experiment was scaled-up from 1 g ground samples cooked in a thermocycler (Tenorio-Bernal and others 200X), to 25 g ground and whole muscle samples cooked in a bench-top convection oven (Jones and others 200X), to 500-1000 g whole muscle samples of turkey, beef, and pork cooked in a pilot scale moist air convection oven (this study). The small, controlled, laboratory samples had a very low replication error, while the larger bench-top and pilot-scale samples both had substantially larger replication errors (Table 6), even though all three studies were conducted with the same basic laboratory procedures in the same research group. This suggests that considerable variability in the processing of larger products is unavoidable, due to the various causes mentioned above.

The laboratory scale experiments included a larger number of treatments, 30 for every species, as compared with five treatments for bench-top experiments and seven treatments for pilot-scale experiments. All experiments were performed with three replicates per treatment per species.

Table 6. Replication error for measured process lethality at three experimental scales log CFU/g (Tenorio-Bernal and others 200X, Jones and others 200X).

	1 g ground	25 g ground	25 g whole	500 - 1000 g whole
Turkey	0.15	0.56	0.99	1.37
Beef	0.15	0.93	1.16	0.93
Pork	0.08	NA	1.51	1.02

In order to confirm that the low replication error for the 1 g samples was not due to the larger sample set, survivor (log CFU/g) data from 21 of the 1 g samples were randomly selected to match the sample set size with the present study. The replication error for these samples was computed and compared to the replication error for all of the samples. This process was performed three times for every species (Table 7). The average replication error including all 90 samples and the average replication error with only 21 samples did not differ more than 0.02 $\log_{10}$ CFU/g (Table 7 vs. Table 6), indicating that the increase in replication error scaling up the experiments was not due to differences in the number of samples, but rather differences inherent in the experimental system.

Table 7. Replication error (log CFU/g) for 21 randomly selected samples from the 1 g laboratory experiments, with three replication for each species.

	Turkey	**Beef**	**Pork**
Replication 1	0.13	0.14	0.10
Replication 2	0.10	0.16	0.07
Replication 3	0.17	0.12	0.07
Average	0.13	0.14	0.08

4.7 Sub-lethal History vs. Model Error

The sub-lethal history of *Salmonella* was quantified during every cook according to equation 5. As a simple test of any systematic failure of the traditional, state-dependent model for slow cooking, a linear regression was conducted for error vs. sub-lethal history. The sub-lethal history was compared with the model error (observed – predicted log reductions) to determine whether the error of the state-dependent model changed linearly with sub-lethal history (Figures 6, 7, and 8). Based on the variability in the data collected for the pilot-scale research, another method of analysis may have been more suitable for data with this amount of dispersion. Clearly, the data points do not follow a linear trend, or any obvious alternative trend. However, when a linear regression was used, error did not increase (P = 0.25) with sub-lethal history, which was inconsistent with the hypothesis of this study and the prior results of Stasiewicz and others (2008).

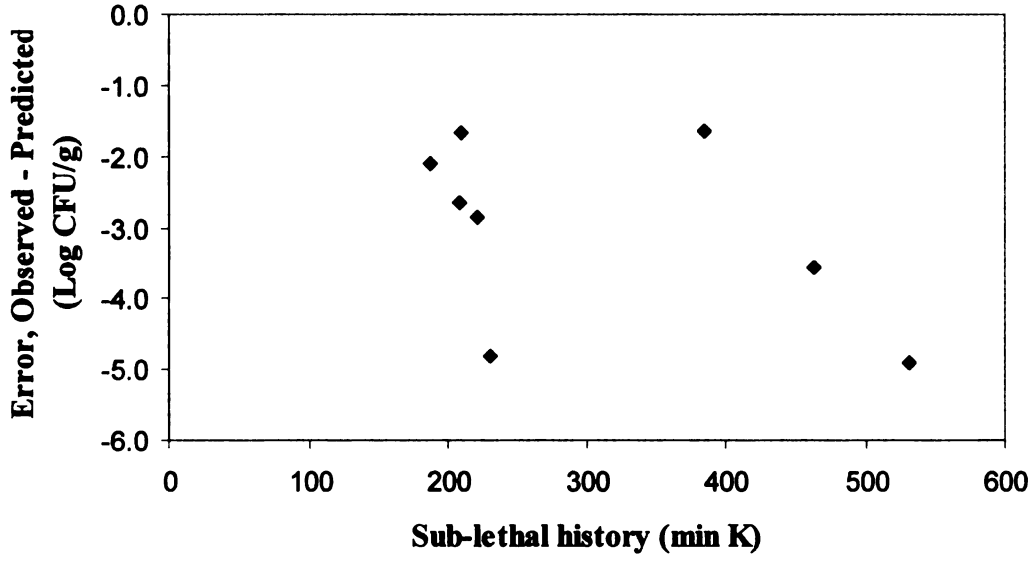

Figure 6. *Salmonella* lethality error (observed - predicted) vs. sub-lethal history for turkey breast. Lethality calculations based on laboratory derived isothermal D and z values (Tuntivanich and others 2008).

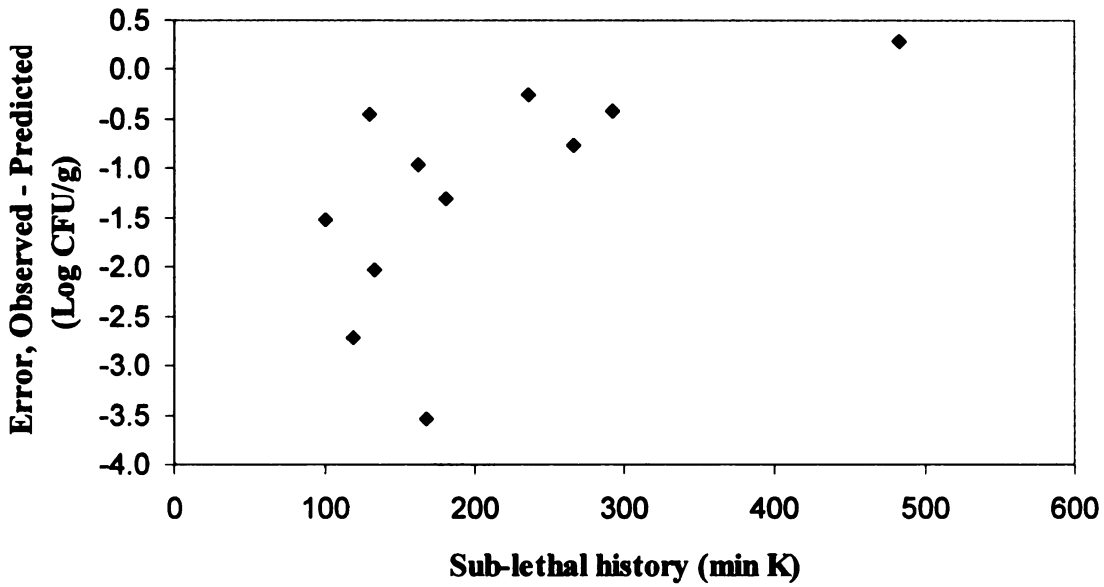

Figure 7. *Salmonella* lethality error (observed - predicted) vs. sub-lethal history for beef roasts. Lethality calculations based on laboratory derived isothermal D and z values, Appendix 7.A

A linear regression evaluating the relationship between the error and sub-lethal history for beef roasts was statistically significant (P=0.04). This indicated that the lethality error was significantly changed with sub-lethal history; however, the linear relationship was negative, giving a result that again was inconsistent with our hypothesis. According to the statistical analysis for this experiment, the absolute error decreased with an increase in sub-lethal history for beef roasts.

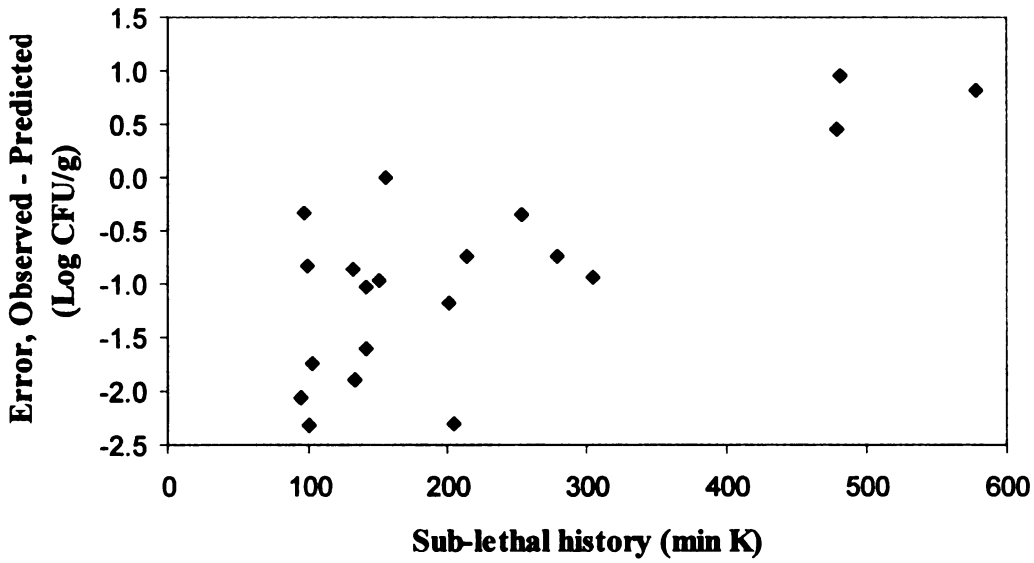

Figure 8. *Salmonella* lethality error (observed - predicted) vs. sub-lethal history for pork roasts. Lethality calculations based on laboratory derived isothermal D and z values (Velasquez and others 2009).

For pork roasts, no statistically significant linear relationship was seen between the lethality error estimated by the state-dependent model and the sub-lethal history (P=0.80).

Slow cooked roasts processed to a targeted lethality in a pilot-scale convection oven demonstrated an error between the observed and predicted log reductions when the state-dependent model was used to estimate *Salmonella* lethality, but this error did not linearly increase with an increase in the sub-lethal history. These particular results lack a common trend between the error and sub-lethal history, perhaps because of the large amount of variability associated with the pilot-scale data as referred to in section 4.6 and/or the inconsistencies associated with recovering usable data following lethality treatments.

4.8 Comparison of State-Dependent and Path-Dependent Models

A comparison of the lethality error (observed – predicted log reductions) for the state-dependent and path-dependent models was performed only on cook schedules that had quantifiable *Salmonella* (n=8 turkey, n=11 beef, n=20 pork). The path-dependent model parameters were obtained by minimizing the sum of squares from the laboratory 1 g, ground data calibration set. The root mean square error (RMSE) was used to validate the accuracy of each model, by comparing the RMSE of the state-dependent and path-dependent models to determine which model provided a smaller RMSE. Reported below, the RMSE was reduced by applying the path-dependent model for whole muscle turkey and beef, but not for whole muscle pork (Table 8).

Table 8. Prediction error and mean residual of the state-dependent and path-dependent models for whole muscle turkey, beef, and pork.

	n	Model parameters used	RMSE (log CFU/g)	Mean Residual (log CFU/g)
Whole Turkey	8	State-dependent	3.3	-3.0
		Path-dependent	1.1	0.4
Whole Beef	11	State-dependent	1.7	-1.2
		Path-dependent	0.8	0.2
Whole Pork	20	State-dependent	0.8	-0.07
		Path-dependent	2.3	1.8

A definitive explanation for why the state-dependent model had a lower RMSE as compared with the path-dependent model for whole pork is unknown. A possible explanation for the increased RMSE for only pork could be attributed to the experimental methods, because the pork was processed to a lower targeted lethality (3.0 $\log_{10}$ CFU/g)

than turkey (7.0 $\log_{10}$ CFU/g) and beef (5.5 $\log_{10}$ CFU/g) roasts, which allowed only the pork cooks to quantify positive errors. A positive error occurs when the predicted log reductions were smaller than the observed log reductions.

The mean residual (bias) for each species and lethality prediction model was calculated in order to compare average errors. The state-dependent model for turkey and beef over predicted lethality for the final product. The large negative error could be due to an experimental bias, because these products were cooked to targeted lethalities near the initial *Salmonella* inoculum level, making it difficult to quantify positive errors.

If the hypothesis that slow cooking increases the thermal resistance of *Salmonella* was true for every observation, then every individual treatments would have resulted in quantifiable *Salmonella* following cooking. But if the hypothesis was not true for an individual observation, then that treatment would result in un-useable data. Unfortunately this appears to have been the case; therefore, quantification of cells when the predicted log reductions were less than the observed log reductions was inconsistent for turkey and beef, because the targeted lethality was too near the initial *Salmonella* concentration. Ideally, if the initial *Salmonella* population was 10 log CFU/g, then survivors could have been consistently recovered following every lethality treatment.

In the situation with pork, unlike turkey and beef, roasts were cooked to a targeted lethality 3.0 log below the initial *Salmonella* population, which allowed for quantification of recoverable *Salmonella* when the observed log reductions exceeded the predicted log reductions. Turkey roasts did not yield any positive errors, with the maximum positive errors being 0.62, and 2.33 log CFU/g for beef and pork, respectively.

The mean residual for turkey and beef showed that the state-dependent model over-predicted *Salmonella* lethality in the final product, which is dangerous, because it falsely indicates that the product is safe. The average errors of the state-dependent model were -3.0 and -1.2 log CFU/g for turkey and beef, respectively. A negative error means that the model over-predicted the actual lethality. Over-predicting the final product safety can be dangerous in regard to food safety.

The largest over-predictions of lethality for individual roasts, using the state-dependent model were -5.07, -3.31, and -0.96 log CFU/g for turkey, beef, and pork, respectively (Figures 9, 10, and 11). A negative value indicates that the lethality model over-predicted product safety, and a positive value indicates that the lethality model under-predicted the lethality of the product, making it more safe.

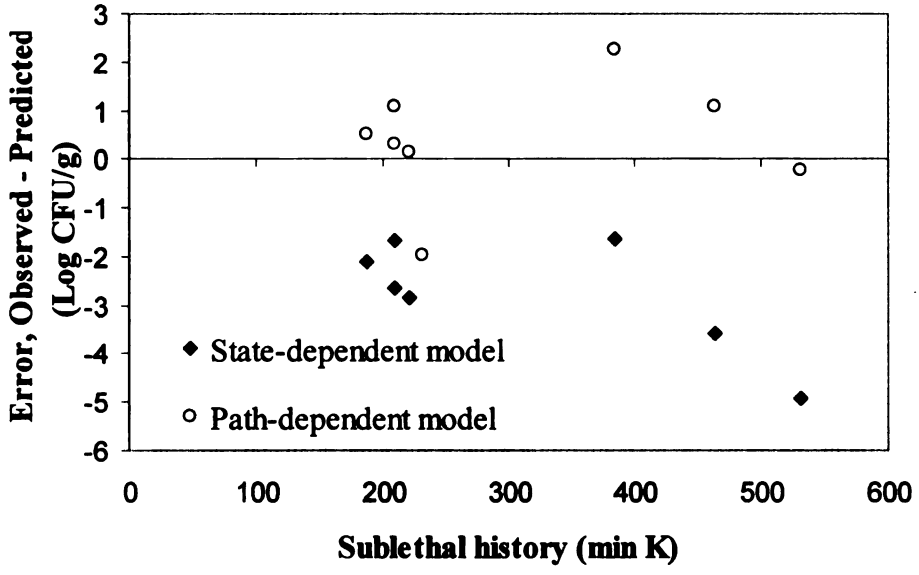

Figure 9. Lethality errors for the state-dependent and path-dependent model applied to the whole muscle turkey data set.

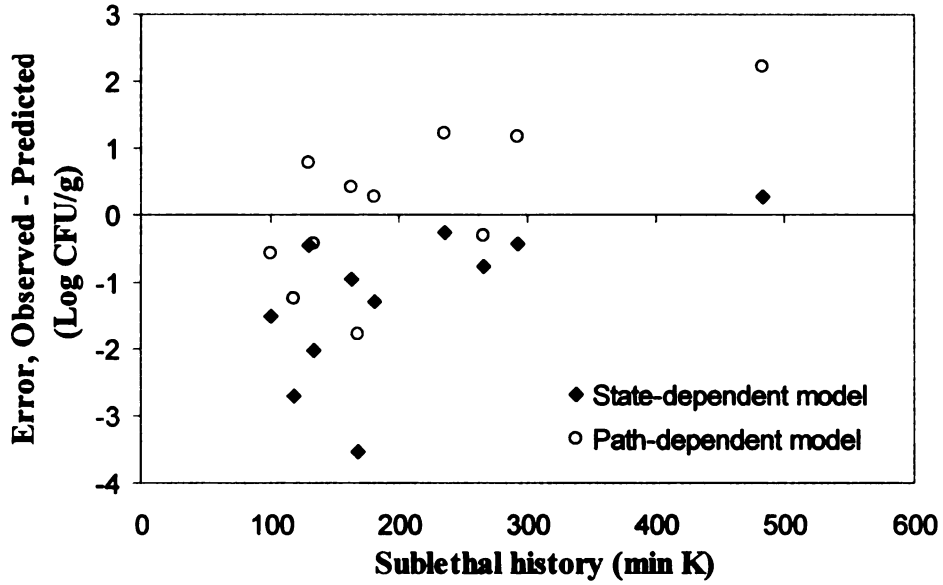

Figure 10. Lethality errors for the state-dependent and path-dependent model applied to the whole muscle beef data set.

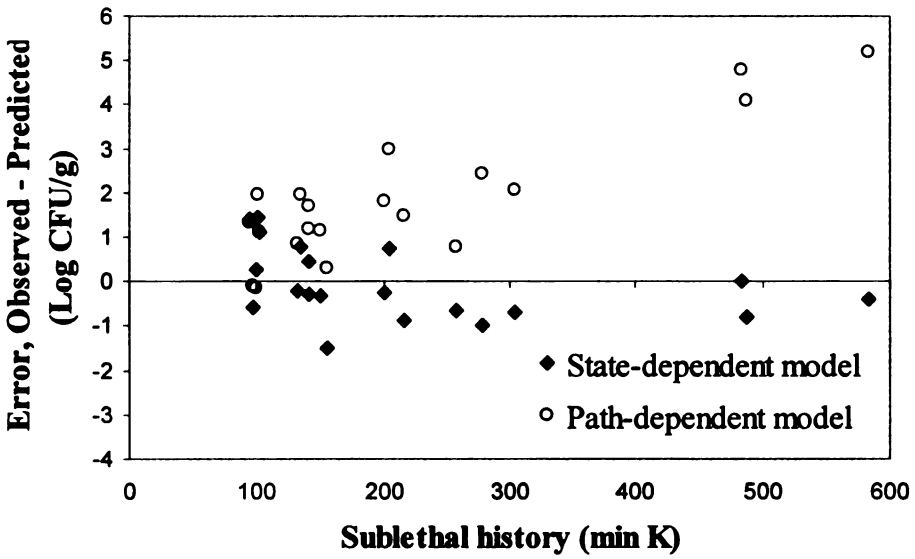

Figure 11. Lethality errors for the state-dependent and path-dependent model applied to the whole muscle pork data set.

Using the state-dependent model to predict product safety of slow cooked whole muscle turkey and beef roasts can be dangerous, because it over estimates the safety of the final product.

When the path-dependent model was applied to the experimental data from turkey and beef, the average error decreased from -3.0 to 0.4 and -1.2 to 0.2 log CFU/g, respectively. The path-dependent model resulted in a decreased error for turkey and beef, making the model a more accurate prediction of *Salmonella* lethality during slow cooking of whole muscle. Furthermore, the average error of the path-dependent model was positive, indicating that the model under-predicted the safety of the final product, therefore resulting in a final product that has achieved, or slightly exceeded, its predicted log reductions.

Application of the path-dependent model to the experimental data for pork species yielded different results than for turkey and beef. For pork, the state-dependent model had a small average error of -0.07 log CFU/g. The path-dependent model had an average error that was greater than that of the state-dependent model, 1.8 log CFU/g. Application of the path-dependent model to pork increased the average lethality error, but the increase resulted in a larger under-prediction of lethality. For pork roasts cooked to targeted lethality of 3.0 $\log_{10}$, the state-dependent model was more accurate than the path-dependent model, but the path-dependent model produced a safer product.

While for pork the state-dependent model had a lower error (-0.07 log CFU/g) as compared with the error of the path-dependent model (1.8 log CFU/g), the state-dependent model still had a negative error. The state-dependent model over-predicted the average safety of the final product for all turkey, beef, and pork. In contrast, the path-

dependent model always resulted in an average positive error, showing that it under-predicted the safety of the final product.

The state-dependent model more accurately estimated *Salmonella* lethality for only slow-cooked whole muscle pork as compared with the path-dependent model. As mentioned previously, a possible explanation for the disparity between the species could have been because pork was cooked to a lower targeted lethality.

4.9 Safe Harbors

Safe harbors are a regulatory paradigm that specifies the amount of time that cooked meat need to be held at or above a given temperature in order to be acceptable (FSIS-USDA 1999 and 2005). These time-temperature conditions are specified by FSIS in tables that help processors determine the conditions of their process. The safe harbors published by FSIS were based on data generated in laboratory-scale studies, with small sample sizes (Juneja and others 2001; FSIS-USDA 2005). Examples of FSIS specified safe harbor temperatures and times for beef to achieve a 6.5 $\log_{10}$ reduction include 60°C for 12 min, or 65.6°C for 67 sec, or 71.1°C for 0 sec (FSIS-USDA 1999).

As another evaluation of the cooking treatments in this study, the present data were compared to the FSIS-specified safe harbors. The amount of time that the roasts were held at a certain temperature was evaluated based on the time and temperature data recorded during each cook. Only the roasts cooked to a targeted lethality were evaluated (Table 9).

From these evaluations, none of the cook conditions (Table 9) complied with the safe harbors as defined by FSIS (1995 and 2005). None of the products were held at a

single temperature for the amount of time specified by the safe harbors; all samples failed to satisfy the time requirement.

Although all of the turkey cooking treatments achieved a calculated 7.0 $\log_{10}$ reduction based on the state-dependent model parameters, a 7.0 $\log_{10}$ reduction was not actually achieved in all cases. Therefore, given that the processes did not comply with the safe harbors, but not all samples achieved a 7.0 $\log_{10}$ reduction, this analysis does not allow any general conclusions about the validity of the safe harbors for these processes.

Table 9. Documented times (s) that roasts cooked to a targeted lethality were held at a specific temperature, for turkey and beef.

	Turkey			Beef		
	Temp (°C)	Time (s) at Temp		Temp (°C)	Time (s) at Temp	
Cook		Required	Actual		Required	Actual
Constant Temp, in bag, high humidity	60	1512	171	60	720	181
	60	1512	181	61	480	188
	60	1512	182	61	480	116
Constant Temp, in bag, no humidity	60	1512	64	60	720	170
	60	1512	182	59	900	212
	60	1512	191	60	720	85
Constant Temp, no bag, high humidity	60	1512	169	60	720	96
	60	1512	235	61	480	107
	60	1512	117	61	480	149
Step-up, in bag, high humidity	60	1512	215	60	720	117
	60	1512	203	60	720	202
	60	1512	256	60	720	106
Step-up, in bag, no humidity	59	1896	404	58	1380	342
	59	1896	364	58	1380	372
	59	1896	364	58	1380	254
Step-up, no bag, changing humidity	60	1512	171	59	900	192
	60	1512	171	60	720	128
	60	1512	223	59	900	160
Step-up, no bag, high humidity	60	1512	213	60	720	116
	60	1512	244	60	720	138
	60	1512	202	59	900	170

4.10 Challenges of Whole Muscle Pilot-Scale Research

In scientific studies, the method of experimentation has an influence on the results. During this research, many challenges centered around developing an experimental method that would be relevant to industry processing techniques. Very little prior thermal inactivation research has been done using inoculated whole muscle

products (particular at the pilot or commercial scale); therefore, a standard method of experimentation has not been developed.

The critical challenge associated with whole muscle, pilot-scale, thermal inactivation studies is getting the pathogen into and out of the product, while still replicating industry practices as closely as possible. While other studies have reported on the migration of pathogens into whole muscle (Warsow and others 2008; Luchansky and others 2009), a reliable inoculation method for achieving a high number of the target pathogen in the core of whole muscle products does not yet exist. Warsow and others (2008) did show penetration of *Salmonella* into whole muscle, but at core concentrations less than 3.0 log CFU/g.

In this study, an inoculation marinade was used to achieve a *Salmonella* population greater than nearly 7.0 log CFU/g within a large (500-1000 g) sample. The vacuum tumble marination method used in this experiment worked relatively well, but it was difficult to consistently achieve the targeted core population for our experimental protocol. Using needle injection and a 30 min rest period, Pradhan and others (2007) were able to achieve an initial internal *Listeria innocua* population of 10^6 to 10^7 log CFU/g in chicken breast. Though a standard deviation for the initial population was not reported, based on their reported inoculation level of 10^6 to 10^7 log CFU/g, one might assume that similar results would have been obtained using a method similar to vacuum tumble marination. However, chicken breasts are substantially smaller than the roasts used in this cooking study.

Luchansky and others (2009) showed *Escherichia coli O*157:H7 has the capability to move from the surface to the interior of beef roasts during blade tenderization.

Contamination levels were $\geq$ 3.4 $\log_{10}$ CFU/g within the interior of the meat. High numbers of internalized *E. coli* O157:H7 were not achieved because blade tenderizing does not inoculate uniformly within a roast. Following blade tenderization, *E. coli* O157:H7 reductions were quantified for steaks cooked over a commercial open-flame grill (Luchansky and others 2009). Though steaks cooked over an open flame differ in multiple ways from roasts cooked in a pilot-scale convection oven, both studies experienced challenges. A limitation to the study conducted by Luchansky and others (2009) was that post-cook samples were not rapidly cooled in order to inhibit carry-over cooking lethality. Similar to the whole muscle pilot-scale cooking research, quantifying the post-cooking pathogen reduction proved to be a challenge for Luchansky and others (2009), as evident from their large and varying standard deviations, ranging from 0.16 to 2.13 log CFU/g.

Most researchers do not perform pathogen inactivation research using intact whole muscle products. This is likely due to the challenges associated with inoculation and sample collection after processing. When intact whole muscle products are used, the sample sizes are typically small, and not applicable to industry relevant processing. In a bench-top study, small (5 g) samples were used to evaluate the effect of grinding on the thermal resistance of *Salmonella* in beef (Mogollon and others 2009). The marination process for smaller whole muscle samples was also quite different to that used for larger sample sizes. After Mogollon and others (2009) immersed their whole beef muscle sample in an inoculated marinade (10^8 CFU/mL); the reported initial *Salmonella* population was ~ 7.8 $\log_{10}$ CFU/g. For whole muscle beef roasts used in pilot-scale

Salmonella inactivation research, the inoculated marinade had a concentration of 9.54 ± 0.56 $\log_{10}$ CFU/mL, but the average *Salmonella* population at the center of the roast following vacuum tumble marination was 6.27 $\log_{10}$ CFU/g, lower than that in the smaller samples used by Mogollon and others (2009).

In order for this research to be both applicable to industry standards and investigate our objectives, it was necessary to overcome several experimental challenges, such as achieving a high initial *Salmonella* population within the interior of a large, whole muscle roast, quantifying the initial *Salmonella* population, quickly cooling the roast post-cooking, and aseptically removing a sample from the interior of the roast. These challenges impacted the experimental methods used, and the variability of the results. There are few scientific studies that have cooked large, whole muscle roasts in pilot-scale ovens, because this type of research introduces a great deal of variability into the process and results, as compared with laboratory studies. While the variability of results can be discouraging, such research is critically important in validating industry processes. This study evaluated the accuracy of two microbial process lethality models, and demonstrated that prior results from laboratory-scale research with slow-cooked product were not necessarily well replicated at the pilot scale. However, a modified, path-dependent model generally yielded improved predictions of process lethality for slow cooked roasts.

5. CONCLUSIONS

5.1 Implications of Process Scale-Up Variability

Currently, the industry is validating the safety of their final product using predictive lethalities calculated with state-dependent models developed from isothermal laboratory studies. Most isothermal thermal inactivation tests have been conducted using liquid media, slurries, or ground meat, rather than whole muscle products. Depending on laboratory-scale data to validate the safety of industrial processes can be risky, because large commercial processes are far more variable compared to controlled laboratory studies. The increase in replication error associated with scaling up from a small, controlled laboratory study to bench-top and pilot-scale slow cooking experiments was demonstrated in the results (section 4.6). This suggests that commercial applications of state-dependent models validated with laboratory studies may need to be re-evaluated at the processing level. Clearly, pathogen inactivation studies can not be conducted in industrial processing facilities; therefore, pilot plants are the next best option in terms of applicability to validating models and accounting for the variability associated with industrial processing conditions.

5.2 Lethality Error vs. Sub-lethal History

There was no obvious trend in the relationship between sub-lethal history and calculated lethality error, even though prior laboratory-scale research had shown this to be true. However, application of the path-dependent model did reduce the prediction error (RMSE) and the risk of over predicting the *Salmonella* lethality in the cooked product.

For whole muscle roasts cooked in a pilot-scale convection oven, the path-dependent model under-predicted lethality, thereby increasing the safety of the final product.

5.3 Implications of Using a State-Dependent vs. Path-Dependent Model

The path-dependent model did not decrease the RMSE for every species of meat processed, but it did always result in a positive mean error, or under-prediction of lethality. The state-dependent model had a negative mean error, which over-predicts lethality, making the product less safe.

The path-dependent model was applied to whole muscle data sets using parameters previously developed from 1 g samples of ground muscle. These parameters were previously estimated using 30 randomly selected treatments, and verified with 15 randomly selected treatments from the experimental protocol of each species. When applying parameters that have been developed in a controlled laboratory experiment, it would be ideal to apply ground parameters to ground product, and whole parameters to whole product. Unfortunately it is not feasible to conduct an experiment using 1 g whole muscle samples, because samples of this size lose much of their whole muscle structure.

In order to understand the usefulness of the path-dependent model in pilot-scale processes, ground muscle parameters were applied to the whole muscle data in order to demonstrate the path-dependent models potential to account for the sub-lethal history of *Salmonella* during slow cooking. To evaluate this, the RMSE for the state-dependent and path-dependent models were compared. The path-dependent model had a lower RMSE for turkey and beef roasts, suggesting that the path-dependent model may be an improved model for predicting the lethality of *Salmonella* in slow cooked, whole muscle roasts, as compared with the state-dependent model.

The actual product used for the three different experiments – 1 g laboratory (Tenorio-Bernal and others 200X), 25 g bench-top convection oven (Jones and others 200X), and 500-1000 g whole muscle pilot-scale – were all from the same original lot of meat for each species. Only the structure (whole vs. ground) and size of the samples varied between the three experiments.

Based on previous research, *Salmonella* is 2-3 times less heat resistant in ground than in whole muscle products (Orta-Ramirez and others 2005; Tuntivanich and others 2008; Velasquez and others 2009). In this study, the path-dependent model (based on data from ground product) under-predicted the lethality of slow-cooked, whole muscle roasts. Based on the effect that meat structure has on heat resistance, it could be suggested that if the path-dependent model were applied to ground product and the same thermal conditions as for the samples in this study, then the path-dependent model (using the existing parameters) might have under-predict the *Salmonella* lethality as compared with the whole muscle roasts. This needs further investigation.

6. FURTHER WORK

Additional research and analysis of the path-dependent model need to be conducted in order to make these findings more applicable for industry use. Currently, the path-dependent model is being applied to whole muscle data, based on ground meat parameters. Additional whole muscle data from the methods performed by Tenorio-Bernal and others (200X), with larger whole muscle samples (~ 10 g) needs to be collected in order to estimate the whole muscle parameters of the path-dependent model. These parameters can then be applied to a randomly selected data set for validation. This will provide an apples-to-apples comparison of the state-dependent and path-dependent models for whole muscle products in pilot-scale slow cooking applications.

The various sources of error contributing to the large experimental variability associated with the scale-up of cooking processes needs to be evaluated. All possible sources of error should be evaluated individually and as a group to determine whether any have a synergistic effect. For example, if it is determined that the inoculation method and sampling location do not have a significant impact on the overall error when evaluated individually, but together these two sources of error provide a significant increase in experimental variability, then it could be concluded that the two sources have a synergistic effect. Individually they do not have an impact, but together they interact with each other to have a significant impact.

The average lethality error should be evaluated with alternative end point lethality values. The pre-determined, industry relevant cook schedule used in this research can be re-run with turkey and beef cooked to obtain 3.0 $\log_{10}$ reductions, in order to determine

whether the end point lethality has an impact on the accuracy of the state-dependent model. Cooking these products to a 3.0 $\log_{10}$ reduction will allow the quantification of any positive errors, thereby eliminating possible experimental bias. Also, after the present results for cooking pork roasts to a targeted 3.0 $\log_{10}$ reduction, it would also be beneficial to evaluate the results from cooking pork roasts to a targeted 6.5 $\log_{10}$ reduction, to determine whether cooking to increased end point lethality has an impact on the average lethality error. Tenorio-Bernal and others (200X) demonstrated that for 1 g laboratory experiments, there was an increase in the lethality error when the targeted lethality was increased.

Alternative inoculation methods should also be investigated. Various methods should be compared to the vacuum marination technique to determine whether alternative marination methods facilitate a higher initial *Salmonella* population, lower variability of the initial pathogen level, and/or lower variability of the final population. Various inoculation methods need to be tested; a few examples include injection, injection followed by vacuum tumbling, injection followed by a rest period, a "dry" surface inoculation, or a "dry" surface inoculation on an exposed interior surface that is then closed into the core prior to cooking (Table 10).

Table 10. Possible pros and cons of alternative whole muscle pathogen inoculation methods.

Inoculation Method	Pros	Cons
Needle injection	High pathogen population in interior	Uncertain uniformity of distribution
Needle injection followed by vacuum tumbling	High pathogen population in interior, and potentially improved uniformity of distribution	Potential for bacterial migration out of product
Needle injection followed by a rest period	High pathogen population in interior, and potentially improved uniformity of distribution	Uncertain uniformity of distribution and potential for bacterial migration out of product
"Dry" inoculation on interior of muscle surface	High pathogen population in specific interior location	Integrity of the whole muscle is sacrificed, and unknown potential for migration away from inoculated interior surface

All alternative inoculation techniques will need to be verified for uniform distribution of *Salmonella* throughout the roast. An alternative inoculation process should be evaluated, because if it does decrease the large variability in initial numbers of *Salmonella*, then it could more accurately account for the log reductions obtained after cooking.

Additionally, bacterial transport during whole muscle cooking should be assessed. During this research, it was assumed that the initial bacterial population within the center of the roast was altered only by heating; bacterial transport during cooking was not assessed. An experiment evaluating bacteria transport during cooking, relative to liquid (purge) transport, would be useful. A proposed method to evaluate bacteria movement

would be to remove a core from a roast, then burry a small metal tube containing inoculated whole muscle product into the middle, cored location of the roast prior to cooking, and cook the roast according to the methods in section 3.9. Following cooking, the contents of the product within the tube cooked inside the roast could be evaluated and compared with an inoculated roast cooked without a metal insert.

7. APPENDICES

Appendix 7.A: Isothermal tests on whole and ground beef

Initial cooking trials revealed that the previously published inactivation parameters for *Salmonella* in beef (Orta-Ramirez and others 2005) were insufficient and yielded extremely large errors. Therefore, the methods of Orta-Ramirez and others (2005) were repeated, using the same beef as in this study, and with more temperatures (55, 58, 60, 62, and 63°C) in an attempt to generate more robust model parameters. The results from the isothermal tests are reported below (Table 11). Raw, average data from the isothermal tests are reported in Tables and Figures 12 -21.

Table 11. D and z values calculated from beef isothermal inactivation tests of 8-serovar *Salmonella* cocktail.

Ground		Whole	
z	5.63 °C	z	5.52 °C
D_{ref}	64.22 sec	D_{ref}	111.52 sec
T_{ref}	60 °C	T_{ref}	60 °C

Table 12. *Salmonella* survivors (CFU/g) in ground beef during 55°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average
0	17,250,000	18,250,000	11,000,000
308	8,575,000	10,250,000	3,100,000
616	5,600,000	2,850,000	702,500
924	1,550,000	130,000	74,750
1232	235,000	14,750	10,250
1540	14,000	10,875	3,775
1848	15,750	2,425	3,375
2156	6,150	2,897.5	500

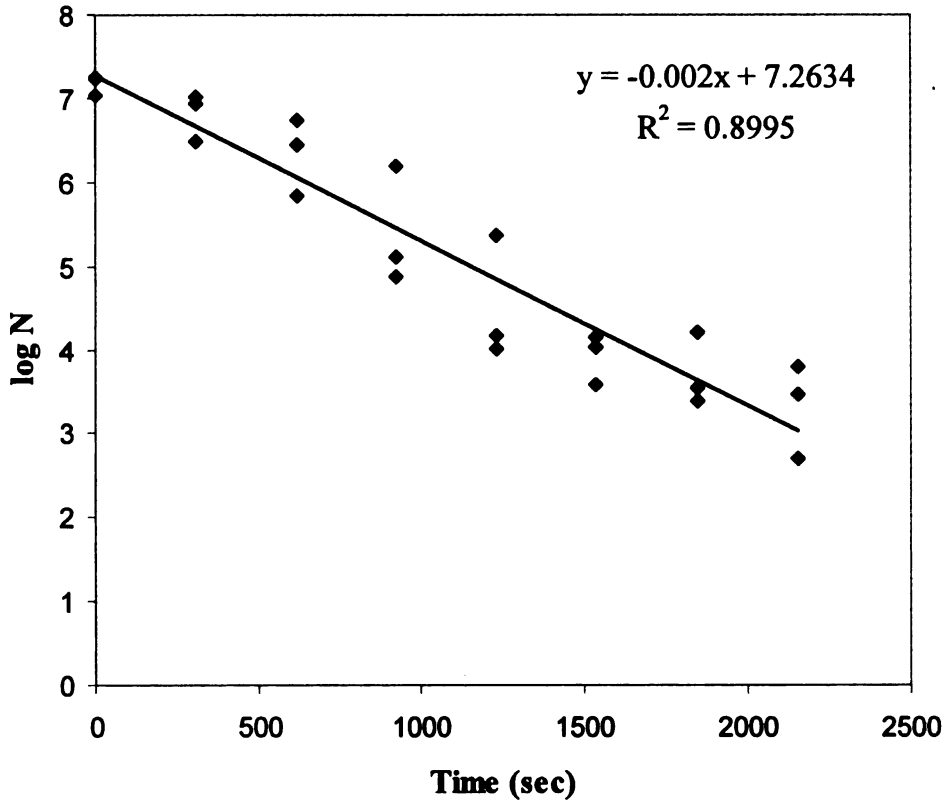

Figure 12. *Salmonella* survivors (CFU/g) in ground beef during 55°C isothermal inactivation.

Table 13. *Salmonella* survivors (CFU/g) in ground beef during 58°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average
0	41,000,00	11,500,000	3,975,000
65	1,075,000	7,400,000	772,500
130	242,500	2,825,000	110,000
195	17,500	860,000	30,000
260	10,500	83,500	7,250
325	7,225	43,750	13,150
390	3,700	23,750	5,125
455	6,050	10,750	32,750

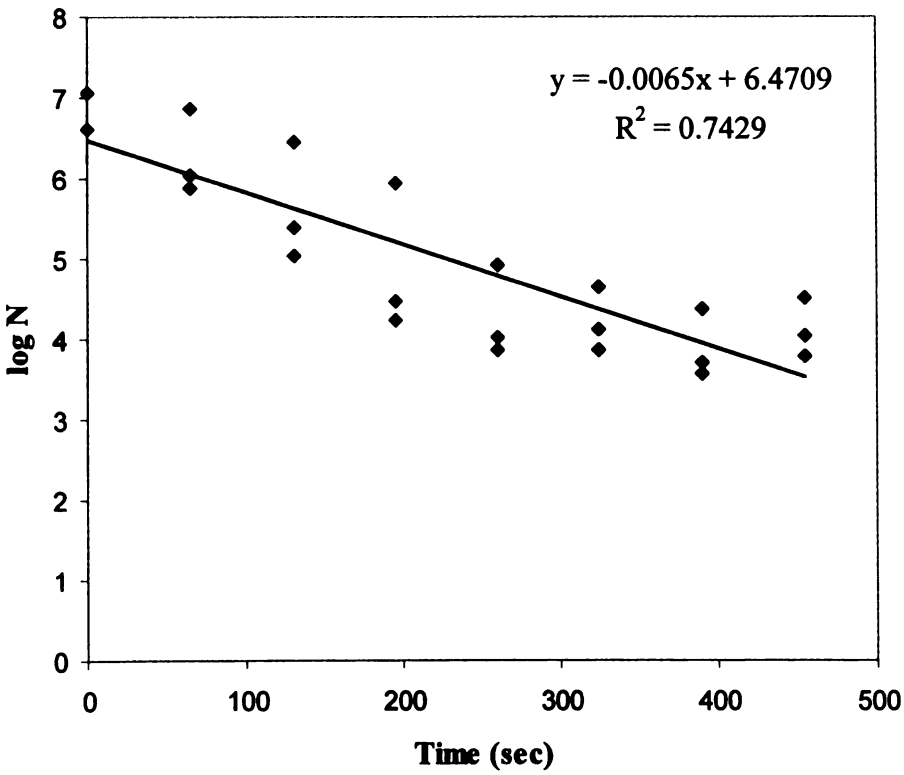

Figure 13. *Salmonella* survivors (CFU/g) in ground beef during 58°C isothermal inactivation.

Table 14. *Salmonella* survivors (CFU/g) in ground beef during 60°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average	Rep4 average	Rep5 average
0	495,000	1,725,000	495,000	775,000	172,500
14	2,325,000	6,250	2,550	587,500	285,000
28	NA	24,000	97,250	1,375,000	1,525
42	75	11,025	4,650	307,500	97.5
56	38,300	175,000	7,175	3,250	20
70	15	292.5	161,000	310,000	40
84	0	1,100	1,565	37.5	7.5
98	0	1,050	1,225	780	90

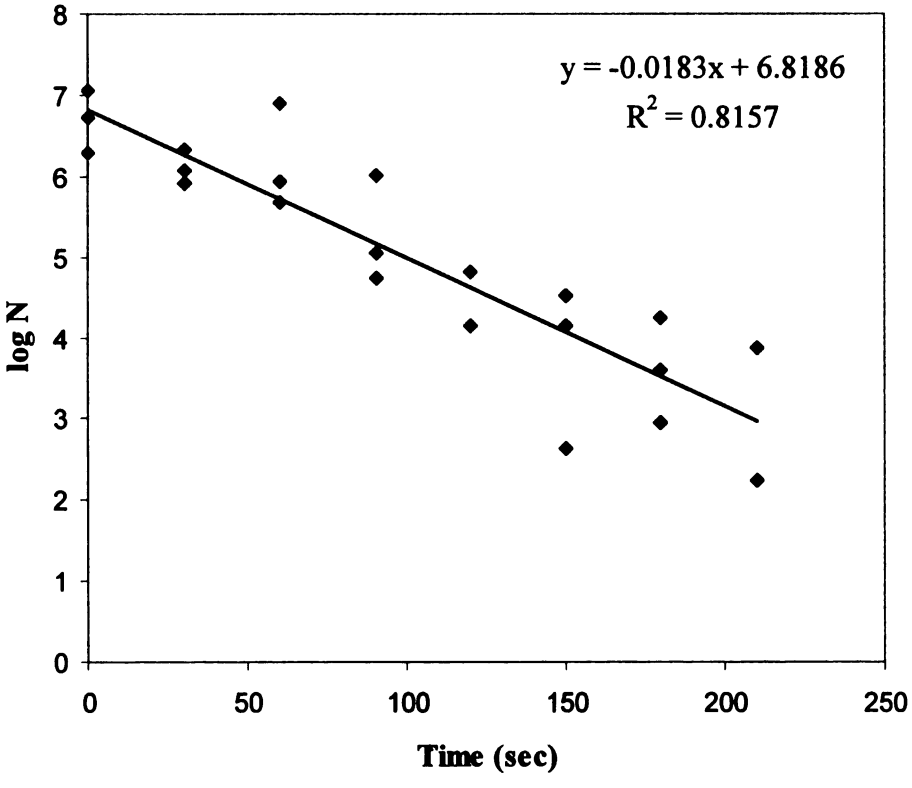

Figure 14. *Salmonella* survivors (CFU/g) in ground beef during 60°C isothermal inactivation.

Table 15. *Salmonella* survivors (CFU/g) in ground beef during 62°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average	Rep4 average	Rep5 average
0	495,000	1,725,000	495,000	775,000	172,500
14	2,325,000	6,250	2,550	587,500	285,000
28	NA	24,000	97,250	1,375,000	1,525
42	75	11,025	4,650	307,500	97.5
56	38,300	175,000	7,175	3,250	20
70	15	292.5	161,000	310,000	40
84	0	1,100	1,565	37.5	7.5
98	0	1,050	1,225	780	90

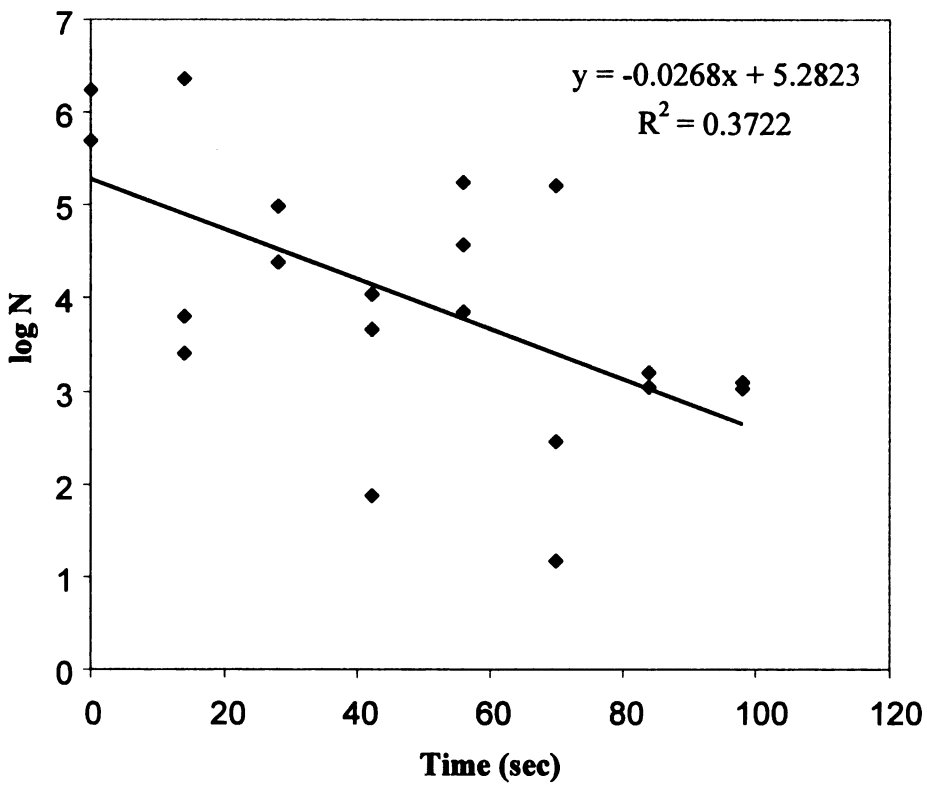

Figure 15. *Salmonella* survivors (CFU/g) in ground beef during 62°C isothermal inactivation.

Table 16. *Salmonella* survivors (CFU/g) in ground beef during 63°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average	Rep4 average	Rep5 average
0	165,000	2,525,000	577,500	2,250,000	1,700,000
10	685,000	950,000	10,250	975,000	21,250
20	NA	975,000	6,450	100,000	950
30	17,250	582,500	310	270,000	9,700
40	NA	625	152.5	11,250	9,800
50	525	46,000	155	308,750	NA
60	375	19,950	210	665	4850
70	60	680	47.5	797.5	2.5

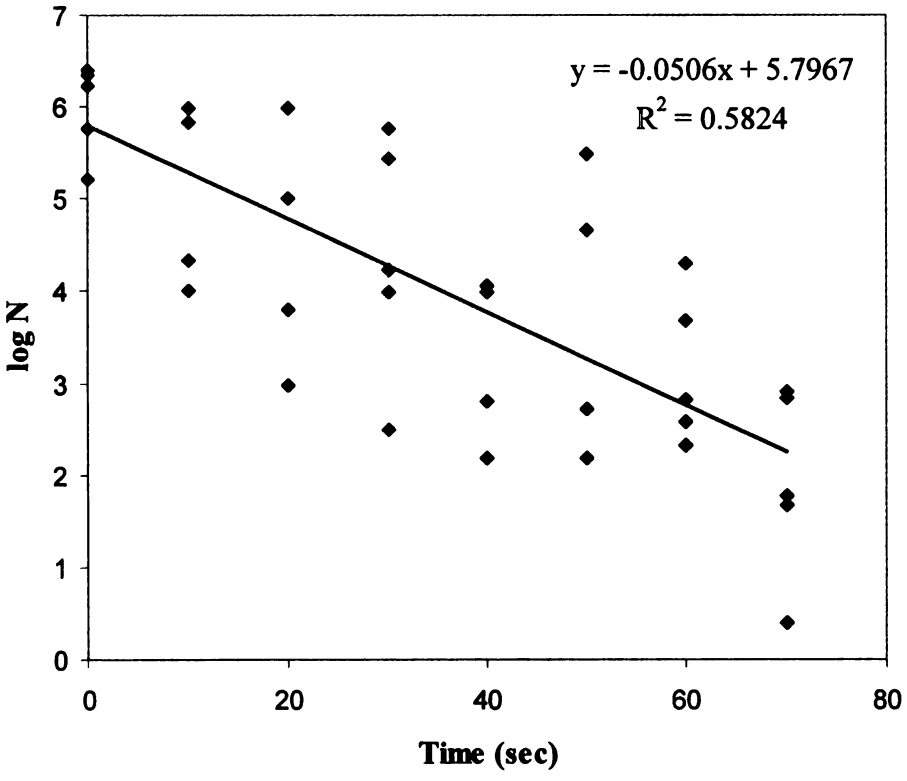

Figure 16. *Salmonella* survivors (CFU/g) in ground beef during 63°C isothermal inactivation.

Table 17. *Salmonella* survivors (CFU/g) in ground beef during 55°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average
0	19,750,000	1.25E+08	9,625,000
760	4,950,000	2,300,000	1,225,000
1520	812,500	402,500	305,000
2280	107,500	52,500	12,500
3040	25,250	2,375	14,000
3800	6,975	1,025	600
4560	700	142.5	165
5320	225	622.5	940

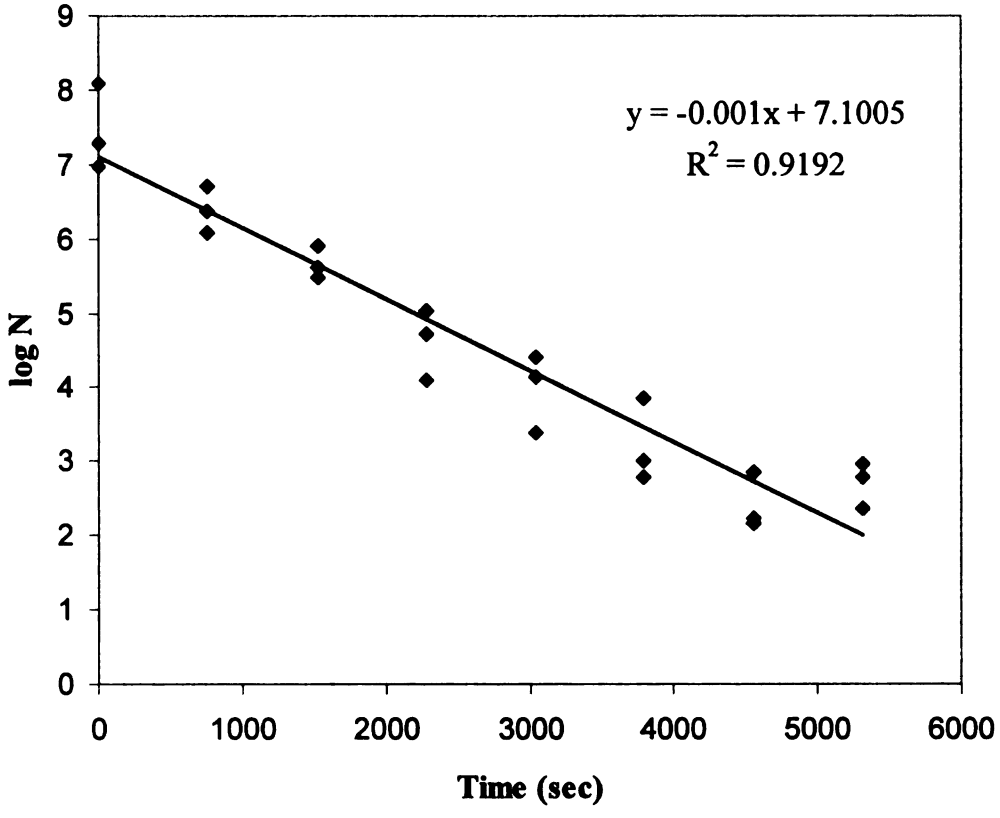

Figure 17. *Salmonella* survivors (CFU/g) in ground beef during 55°C isothermal inactivation.

Table 18. *Salmonella* survivors (CFU/g) in ground beef during 58°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average
0	2,600,000	12,175,000	4,150,000
140	507,500	2,275,000	720,000
280	255,000	760,000	537,500
420	4,575	43,000	55,500
560	6,175	6,000	16,375
700	877.5	4,775	2,625
840	292.5	1,375	2,537.5
980	160	1,100	160

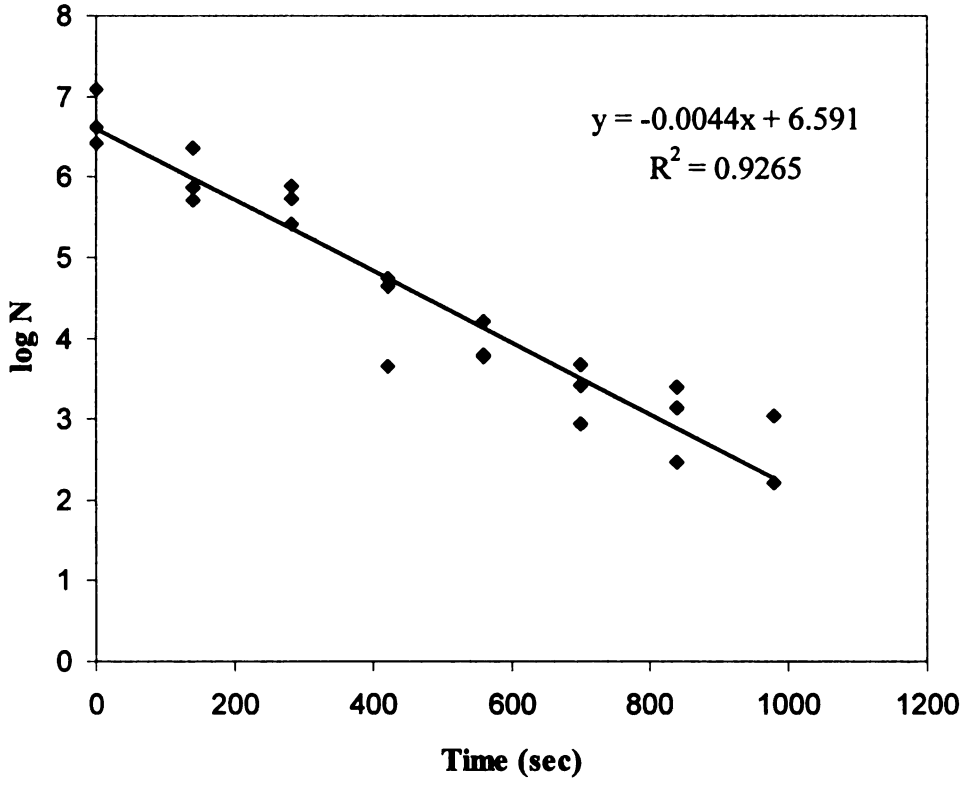

Figure 18. *Salmonella* survivors (CFU/g) in ground beef during 58°C isothermal inactivation.

Table 19. *Salmonella* survivors (CFU/g) in ground beef during 60°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average	Rep4 average
0	13,000,000	21,250,000	1,925,000	17,125,000
65	875,000	2,152,500	165,000	672,500
130	217,500	220,000	12,275	50,750
195	230,000	15,750	11,250	18,000
260	575	2,450	1,000	5,500
325	3,725	232.5	115	900
390	110	30,500	130	1,175
455	375	12.5	2.5	367.5

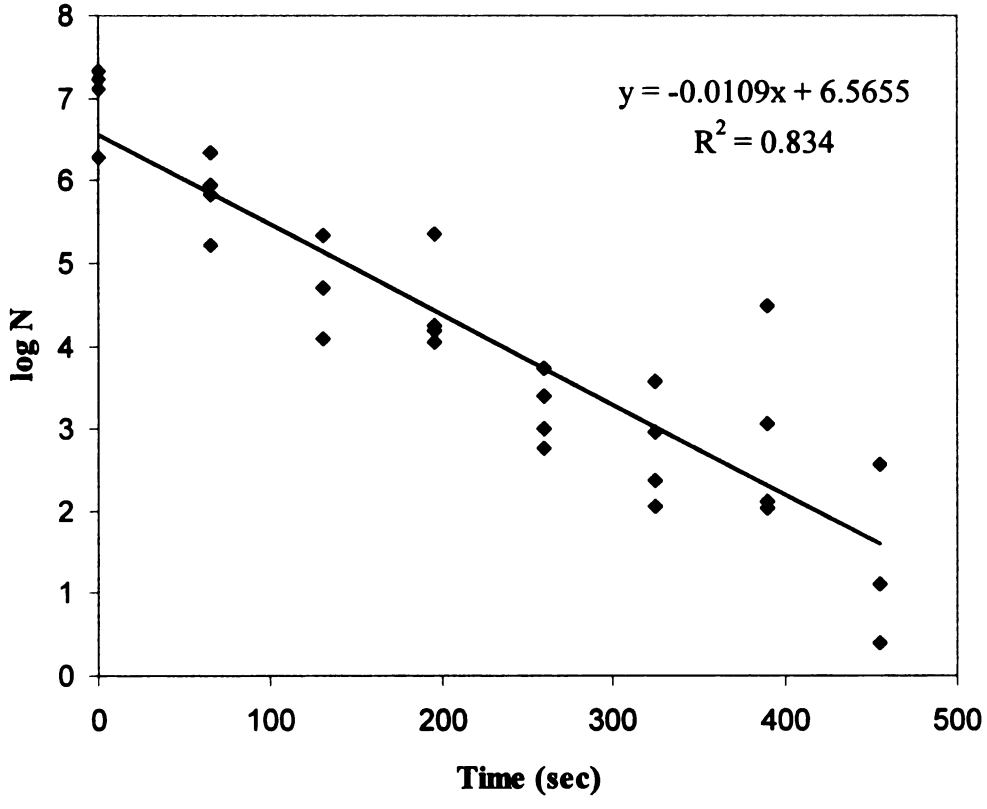

Figure 19. *Salmonella* survivors (CFU/g) in ground beef during 60°C isothermal inactivation.

Table 20. *Salmonella* survivors (CFU/g) in ground beef during 62°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average	Rep4 average	Rep5 average
0	232,500	6,750	3,075,000	3,475,000	2,550,000
30	840,000	59,750	895,000	4,750,000	52,750
60	830,000	6,900	217,500	1,107,500	13,750
90	11,300,000	520	24,250	92,500	6,525
120	1,450	615	2,450	142,500	2,925
150	350	25	822.5	1,250	1,900
180	NA	50	1,075	525	5,450
210	850	7.5	2.5	12.5	320

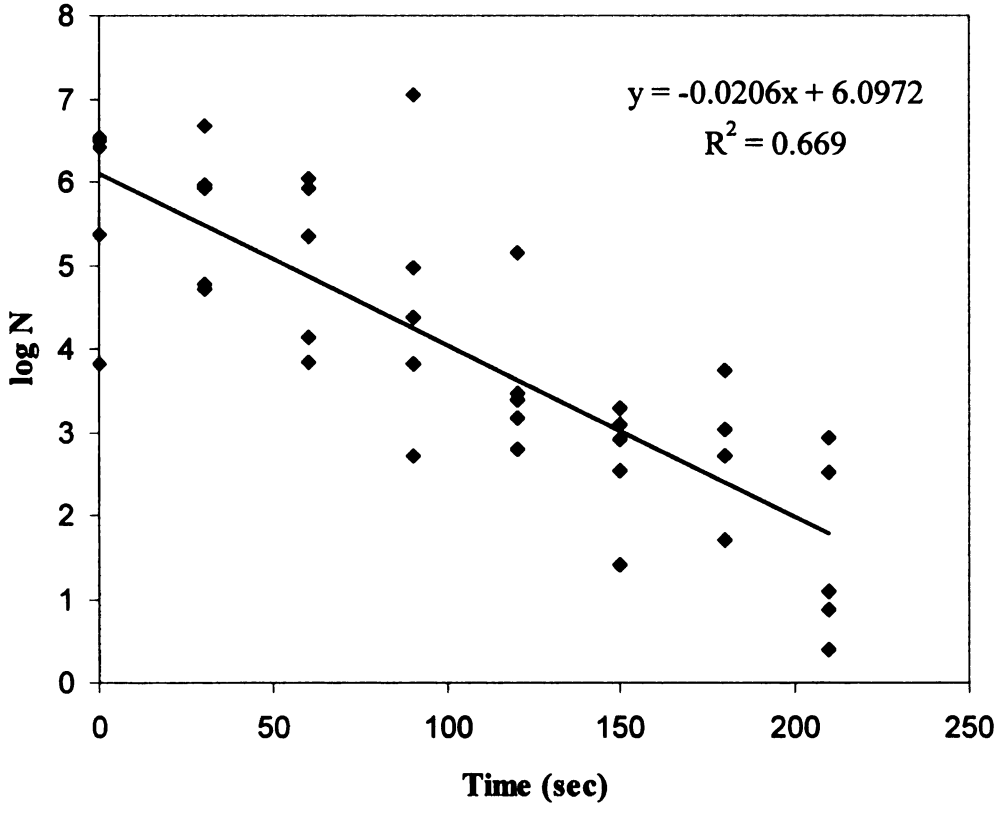

Figure 20. *Salmonella* survivors (CFU/g) in ground beef during 62°C isothermal inactivation.

Table 21. *Salmonella* survivors (CFU/g) in ground beef during 63°C isothermal inactivation.

Time (sec)	Rep1 average	Rep2 average	Rep3 average	Rep4 average	Rep5 average	Rep6 average
0	6,150,000	10,750,000	3,450,000	747,500	3,350,000	762,500
20	63,750	5,875,000	657,500	3,500	2,510,000	1,825,000
40	1,100,000	2,875,000	141,000	2,250	NA	54,000
60	200,000	667,500	632,500	11,500	11,600	20,500
80	15,250	395,000	875	525	2,875	1,302.5
100	532.5	765,000	2,200	15	8,750	832.5
120	70	405,000	8250	2.5	252.5	287.5
140	NA	6,050	625	NA	395,000	10

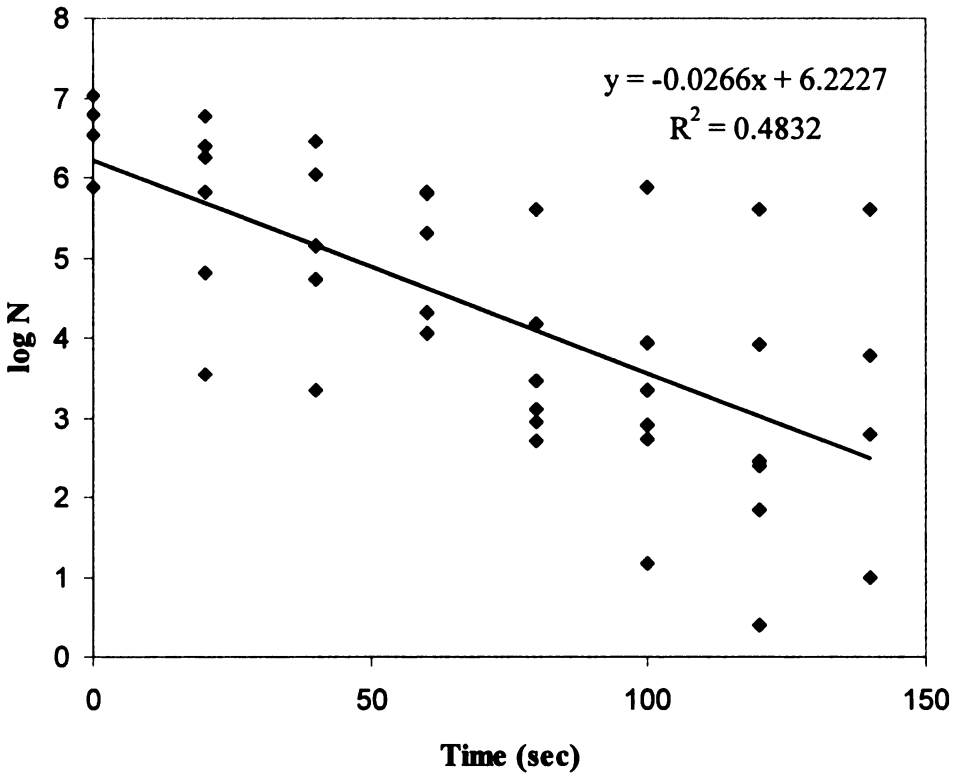

Figure 21. *Salmonella* survivors (CFU/g) in ground beef during 63°C isothermal inactivation.

Appendix 7.B: Inoculation verification summary data

Inoculation levels were verified as described in section 3.6 (Tables 22, 23, and 24). Only the aerobic Petrifilm™ data from the core labeled "A" were used. The average of the entire A data set on aerobic Petrifilm™ was deemed the initial *Salmonella* population. *TNTC indicates plates that where too numerous to count.

Table 22. Summary of inoculation verification data for turkey roasts.

	Sample Replicates											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Average**	**St. Dev**
Marinade	8.95	8.65	8.95	NA	9.45	9.28	9.53	9.53	9.61	9.61	9.28	0.35
A	6.71	6.90	7.83	6.38	7.56	6.24	7.57	6.82	6.62	6.97	**6.96**	**0.53**
B	7.59	7.52	7.63	6.43	7.54	6.02	7.77	6.66	7.16	7.61	7.19	0.61
C	6.79	6.87	7.58	6.89	7.24	6.56	7.14	7.07	7.04	7.15	7.03	0.28
D	6.96	7.12	7.69	6.89	7.67	6.96	8.00	5.89	6.33	6.94	7.04	0.63
E	7.14	7.85	7.82	6.18	7.24	6.15	6.99	7.80	6.95	NA	7.13	0.65

Table 23. Summary of inoculation verification data for beef roasts.

	Sample Replicates									
	1	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**Average**	**St. Dev**
Marinade	9.31	9.31	9.17	9.17	9.17	9.31	9.04	9.04	9.19	0.11
A	6.11	6.69	6.75	6.99	7.14	6.62	4.70	5.18	**6.27**	**0.89**
B	6.45	6.59	6.76	6.99	7.61	6.63	4.70	5.54	6.41	0.90
C	6.25	6.95	8.09	7.10	7.11	6.86	5.54	4.88	6.60	1.01
D	6.44	7.11	6.93	6.33	7.25	6.21	4.70	4.70	6.21	1.00
E	6.05	6.74	6.73	7.19	6.74	7.38	4.88	6.42	6.52	0.78

Table 24. Summary of inoculation verification data for pork roasts.

	Sample Replicates							
	1	**2**	**3**	**4**	**5**	**6**	**Average**	**St. Dev**
Marinade	TNTC*	TNTC*	9.68	9.68	9.68	9.68	9.68	0.00
A	6.63	6.55	4.46	5.71	6.74	7.56	**6.27**	**1.06**
B	TNTC*	6.15	4.39	5.87	6.60	7.04	6.01	1.01
C	6.35	6.47	4.63	5.53	6.19	7.26	6.07	0.90
D	6.20	6.25	4.49	6.00	6.83	7.99	6.29	1.14
E	7.12	7.16	5.05	7.33	7.52	7.16	6.89	0.91

Appendix 7.C: Effect of the roast mass on the initial inoculums concentration

A linear regression comparing the turkey roast mass to the core *Salmonella* population was performed (Figure 22). The mass span of 500 – 1200 g is representative of the variation in roast mass throughout all experimental trials. No significant relationship between the roast mass, and the inoculation level at the core of the roast was found (P=0.76). This conclusion indicates that using a mean value as the initial core inoculation level is appropriate to determine the log reductions occurred during cooking.

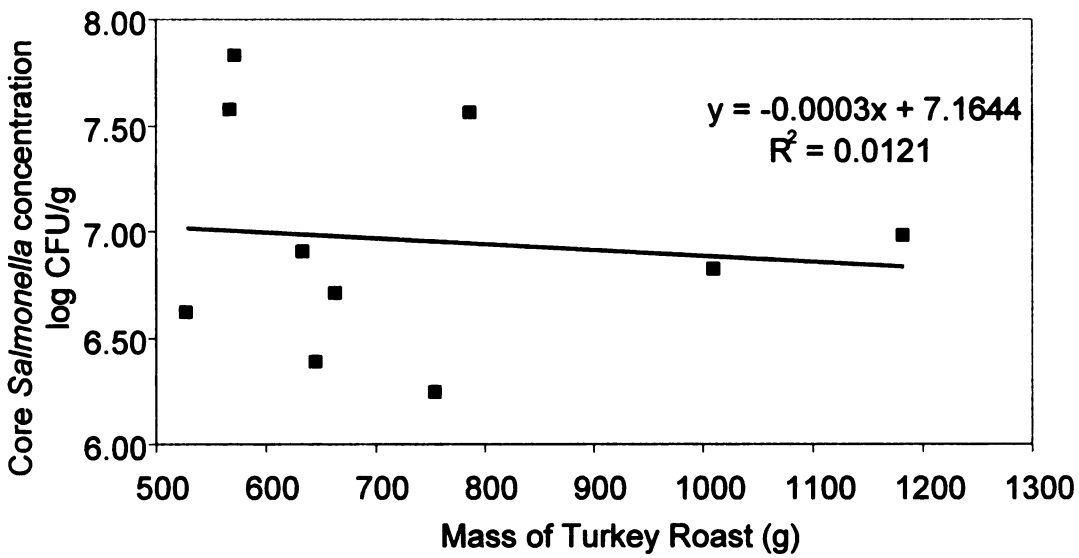

Figure 22. Whole muscle turkey roast weight (g) vs. core *Salmonella* concentration (log CFU/g).

Appendix 7.D: Verification of coring method following cooking

Large roasts (500-1000 g) were cooked to a calculated log reduction in a pilot-scale oven. In order to achieve the targeted log reduction, and stop carry-over cooking after the roast is removed from the oven, it needs to be cooled quickly. It was impractical to cool an entire roast quickly enough with an ice bath to stop heating at the center.

A rapid cooling method was needed. The method proposed was to core the sample after it had been cooked to its targeted end point, aseptically cut off the top and bottom surfaces of the sample core, and then submerge the sample into chilled peptone water. Before utilizing the alternative method, it had to be validated that the mechanical pressure of coring a hot roast was not pushing *Salmonella* from the sample. The method was verified by sampling the turkey purge following cooking (when it was sterile), before and after coring the sample. The results in CFU/g are shown in Table 25.

Table 25. Validation of the post-cook sampling method, coring for whole muscle roasts. Purge from the cook-in bag was collected immediately after cooking *Salmonella* inoculated whole muscle roasts in a pilot-scale moist air convection oven and again after coring the whole muscle roasts with a sterile stainless steel corer.

	Sample	Media	Plate Count A (CFU/g)	Plate Count B (CFU/g)	Average Plate Count (CFU/g)	Amount Plated (ml)	Initial Dilution	Log CFU/g
Purge Before Coring	4	PF	0	0	0	1	1	<0.4
		Plate	0	0	0	10	1	<1.4
	5	PF	0	0	0	1	1	<0.4
		Plate	0	0	0	10	1	<1.4
	6	PF	0	0	0	1	1	<0.4
		Plate	0	0	0	10	1	<1.4
Purge After Coring	4	PF	0	0	0	1	1	<0.4
		Plate	0	0	0	10	1	<1.4
	5	PF	0	0	0	1	1	<0.4
		Plate	0	0	0	10	1	<1.4
	6	PF	0	0	0	1	1	<0.4
		Plate	0	0	0	10	1	<1.4

The recovered purge data verifies that the coring method did not change the amount of *Salmonella* in the purge. The result demonstrated that the coring method was not forcing *Salmonella* out of the sample; therefore, the coring method was used to rapidly chill the samples core.

Appendix 7.E: Alternative inoculation verification for pork

An alternative method for verifying the initial *Salmonella* inoculation level in pork prior to cooking was tested in order to attempt an improved direct measure of the initial level of *Salmonella* contamination for each roast, rather than using a mean value (as was done for turkey and beef). When the electrosurgical knife was used to determine a mean initial inoculation value for pork roasts (section 3.6), the standard deviation was very large (1.06 log CFU/g); therefore, a biopsy method was investigated.

The alternative, biopsy method consisted of determining the initial *Salmonella* population within the pork roasts by extracting five 0.02 g samples from the near center of each roast. The extraction was performed with an E-Z core single action biopsy needle, 2.7 mm x 9 cm (Products Group International, Lyons, CO). Before extracting the samples using the biopsy needle, the portion of the surface to be penetrated (4 cm diameter) was flamed using the electrosurgical knife, in order to prevent external contamination of the interior of the roast. After the roasts were inoculated via vacuum tumbling, the biopsy needle was sterilized by immersing it in 250 ml of ethyl alcohol (Cecon Labs, Inc, King of Prussia, PA) for 20 s. The sterilized needle then was placed into 250 ml of sterile 0.1% buffered peptone water, in order to remove any excess ethanol.

The biopsy was performed five consecutive times per roast, aseptically removing each sample independently from the needle, and collecting them into a single Whirl-Pak™ bag (Nasco, Fort Atkinson, WI). Samples were then diluted, stomached, plated on aerobic Petrifilms™, and incubated for 24 h at 37°C before enumeration. The biopsy needle was sterilized after each roast, as mentioned previously with ethyl alcohol.

After the samples were cooked, plated, and enumerated, the average and standard deviation of the error (observed - predicted) of the biopsy and electrosurgical knife (ESU) methods were compared (Table 26).

Table 26. Post-cook, comparison of the lethality error (observed - predicted) for the biopsy and electrosurgical unit methods following pork inoculation. The standard deviations of the lethality error post cook were compared to determine the most repeatable process.

Cook	Error of Biopsy Method	Standard Deviation	Error of ESU method	Standard Deviation
93.3C In-bag, High humidity	-0.71329		-0.32815	
	-0.0635		-2.05474	
	-1.47631	0.707162	-1.74199	0.919953
93.3C In-bag, No Humidity	-1.85408		-2.30453	
	-0.41037		-1.18206	
	0.611733	1.238897	-0.74445	0.804703
93.3C No Bag, High humidity	-0.26895		-0.82714	
	-0.47897	0.148505	-2.32572	1.059655
140F 90m, 155F 90m, 170 In-bag, High Humidity	-0.66655		0.002974	
	0.031757		-0.86167	
	0.259171	0.482406	-1.0263	0.552889
140F 90m, 155F 90m, 170 In-bag, No Humidity	-1.12742		0.451134	
	-1.49841		0.816101	
	-1.52813	0.223264	0.955023	0.260258
140F 90m, 155F 90m, 170F No Bag, High Humidity	0.848762		-0.97408	
	-0.07181		-1.89465	
	0.223681	0.470015	-1.59916	0.470015
140F 90m, 155F 90m, 170F No Bag, Changing Humidity (No, Med, High)	0.446033		-0.35562	
	-0.7282		-0.73813	
	-1.31046	0.894717	-0.94397	0.298564

Based on the comparison of the standard deviations for the biopsy and electrosurgical knife methods, it was determined that the methods resulted in similar variability. Due to the fact that the biopsy method did not reduce experimental variability by improving the prediction of the initial level of *Salmonella* contamination it was not

used for analysis. The method using the average core value from the electrosurgical unit sampling method was used in order to maintain inoculation validation uniformity between species.

Appendix 7.F: Summarized cook schedules and spreadsheets

Using Excel, master spreadsheets for each species were made in order to compile a summary of each experimental run (Tables 27-29). These sheets do not include all of the raw data, but summarize it with averages, making the data easier to interpret. Column labels were used help compare the cooks to each other. This allowed for the determination of similar trends. An explanation to the table labels is below.

Date: Indicates the date that they experiment was performed.

Cook: Identifies the cook schedule performed.

Target End Point: Identifies when the sample was removed from the oven - either at an end point temperature of 71.1°C or a specified log reduction. Species were removed at different log reductions; therefore, the targeted log reductions for each sample are labeled.

Weight (g): This column indicates the initial weight of the raw, irradiated, thawed roast before marination or cooking.

Media: Specifies the growth media used. The data from the aerobic Petrifilms™ (PF) were the only data used in analysis. The modified Triptic Soy Agar plates (plates) were only used to assure that *Salmonella* was being recovering on the Petrifilms™. In all cases, the plates only contained colonies with the characteristics of *Salmonella.* Samples plated on modified Triptic Soy Agar plates were not used in the analysis, because it was difficult to consistently recover cells, because the level of detection (25 CFU/g), was too high; therefore, the aerobic Petrifilms™ were used.

Sample: This indicates a number label for the sample. All samples with a label of 1 or 2 were cooked to an end point temperature of 71.1°C. Samples labeled 4, 5 and 6 were cooked to targeted end point lethality.

Total time (hr): This is the time in hours, of the cook, from placing the roasts in the oven to when they were completely cooled.

Marinade inoculum level log(CFU/g): The amount of *Salmonella* within the salt, phosphate marinade.

Initial inoculum level log(CFU/g): The initial concentration of *Salmonella* within the core of the roast before cooking. This was determined using the procedure explained in section 3.6.

Survivors log(CFU/g): The amount of recovered cells following enumeration and cooking.

Observed log reductions: The difference between the initial level of *Salmonella* concentration, and the enumerated cells following cooking.

Predicted log reductions: The log reductions estimated in real-time via the log-linear model, using isothermal D and z values.

Max sub-lethal history: The total sub-lethal history of the *Salmonella* cells during cooking. A detailed explanation of the quantification of this value is provided in section 3.11.2.

Table 27. Turkey roast spread sheet summary **of all cook schedules.**

Date	Cook Schedule	Target End Point	Weight (g)	Media	Sample	Total Time (Hr)	Marinade Inoculum Level Log(CFU/ml)	Initial Inoculum Level Log(CFU/g)	Survivors (Log CFU/g)	Observed Log Reductions	Predicted Log Reduction	Max Sublethal History (min K)
	93.3C In Bag, High humidity											
7/1/2008		71.1 C	695.8	PF	1	2.31	8.64	6.96	<.39794	>6.94	663.92	
		71.1 C	866.6	PF	2	2.31	8.64	6.96	<.39794	>6.94	666.82	
6/19/2008		7.0 log	851.4	PF	4	2.41	9.49	6.96	2.19	4.77	6.72	234.42
		7.0 log	1071.6	PF	5	2.41	9.49	6.96	<.39794	>6.94	6.54	258.82
		7.0 log	660.4	PF	6	2.41	9.49	6.96	1.65	5.31	6.83	206.18
	93.3C In Bag, No Humidity											
7/1/2008		71.1 C	762.7	PF	1	2.94	8.73	6.96	<.4	>6.94	534.98	
		71.1 C	762.7	Plate		2.94	8.73	6.82	1.40	5.42		
		71.1 C	608.5	PF	2	2.94	8.73	6.96	<.4	>6.94	574.73	
		71.1 C	608.5	Plate		2.94	8.73	6.82	<1.4	>6.82		
6/24/2008		7.0 log	553.1	PF	4	2.08	9.30	6.96	2.20	4.76	6.67	179.28
		7.0 log	511.1	PF	5	2.08	9.30	6.82	4.92	1.89	6.56	229.53
		7.0 log	730.5	PF	6	2.08	9.30	6.96	<.4	>6.94	7.39	229.48
	93.3C No Bag, High humidity											
7/8/008		71.1 C	573.6	PF	1	2.94	8.69	6.96	<.4	>6.94	1360.17	
		71.1 C	573.6	Plate		2.94	8.69	6.82	<1.40	>6.82	1360.17	
		71.1 C	1129.6	PF	2	3.16	8.69	6.96	<.4	>6.94	1322.05	
		71.1 C	1129.6	Plate		3.16	8.69	6.82	<1.40	>6.82	1322.05	
7/8/2008		7.0 log	726.1	PF	4	1.88	8.69	6.96	<.4	>6.94	6.96	136.58
		7.0 log	726.1	Plate		1.88	8.69	6.82	<1.40	>6.82	6.96	136.58
		7.0 log	922.5	PF	5	1.77	8.69	6.96	<.4	>6.94	6.77	176.82
		7.0 log	922.5	Plate		1.77	8.69	6.82	<1.40	>6.82	6.77	176.82
		7.0 log	444.1	PF	6	1.43	8.69	6.96	<.4	>6.94	7.52	107.52
		7.0 log	444.1	Plate		1.43	8.69	6.82	<1.40	>6.82	7.52	107.52

Table 27. Continued

	Step Up, In Bag, High Humidity												
6/11/2008		71.1 C	767.3	PF	1	2.43	9.46	6.96	0.70	6.26	1154.71		
		71.1 C	745.3	PF	2	3.32	9.46	6.82	0.70	6.26	2780.78		
7/3/2008		7.0 log	773.1	PF	4	2.12	8.42	6.96	<.4	>6.94	6.73	215.93	
		7.0 log	773.1	Plate	4	2.12	8.42	6.82	<1.4	>6.82	6.73	215.93	
		7.0 log	622.2	PF	5	2.12	8.42	6.96	2.22	4.74	7.21	207.64	
		7.0 log	622.2	Plate	5	2.12	8.42	6.82	<1.4	>6.82	7.21	207.64	
		7.0 log	949	PF	6	2.15	8.42	6.96	2.51	4.46	7.12	212.72	
		7.0 log	949	Plate	6	2.15	8.42	6.82	<1.4	>6.82	7.12	212.72	
	Step Up, In Bag, No Humidity												
7/2/2008		71.1 C	698.2	PF	1	5.51	8.56	6.96	<.4	>6.94	1890.38		
		71.1 C	698.2	Plate	1	5.51	8.56	6.82	<1.4	>6.82	1890.38		
		71.1 C	512.3	PF	2	5.51	8.56	6.96	0.40	6.56	1707.65		
		71.1 C	512.3	Plate	2	5.51	8.56	6.82	<1.4	>6.82	1707.65		
6/24/2008		7.0 log	553.1	PF	4	4.01	9.34	6.96	2.20	4.76	7.10	462.19	
		7.0 log	553.1	Plate	4	4.01	9.34	6.82	1.88	4.94	7.10	462.19	
		7.0 log	511.3	PF	5	4.01	9.34	6.96	4.92	2.04	7.10	532.43	
		7.0 log	511.3	Plate	5	4.01	9.34	6.82	3.75	3.06	7.10	532.43	
		7.0 log	730.5	PF	6	4.01	9.34	6.96	<.4	>6.94	6.83	471.73	
		7.0 log	730.5	Plate	6	4.01	9.34	6.82	<1.4	>6.82	6.83	471.73	

Table 27. Continued

	Step Up, No Bag, High Humidity											
		71.1 C	682	Plate	1	4.48	8.74	6.82	<1.4	>6.82	2186.15	
		71.1 C	774.4	Plate	2	4.48	8.74	6.82	<1.4	>6.82	2708.43	
7/9/2008		7.0 log	763	Plate	4	2.29	8.74	6.82	<1.4	>6.82	6.62	216.78
		7.0 log	947.7	Plate	5	2.29	8.74	6.82	<1.4	>6.82	6.82	231.19
		7.0 log	1056.1	Plate	6	2.29	8.74	6.82	<1.4	>6.82	6.59	237.74
	Setp Up, No Bag, Changing Humidity (No, Med, High)											
7/10/2008		71.1 C	495.6	PF	1	4.23	8.53	6.96	<.4	>6.94	1033.23	
		71.1 C	495.6	Plate		4.23	8.53	6.82	<1.4	>6.82	1033.23	
		71.1 C	608.7	PF	2	4.23	8.53	6.96	<.4	>6.94	1069.61	
		71.1 C	608.7	Plate		4.23	8.53	6.82	<1.4	>6.82	1069.61	
7/10/2008		7.0 log	594.7	PF	4	4.23	8.53	6.96	1.70	5.26	6.75	383.59
		7.0 log	594.7	Plate		4.23	8.53	6.82	<1.4	>6.82	6.75	383.59
		7.0 log	589.4	PF	5	4.23	8.53	6.96	<.4	>6.94	6.75	383.59
		7.0 log	589.4	Plate		4.23	8.53	6.82	<1.4	>6.82	6.75	383.59
		7.0 log	830	PF	6	4.23	8.53	6.96	<.4	>6.94	6.86	465.93
		7.0 log	830	Plate		4.23	8.53	6.82	<1.4	>6.82	6.86	465.93

Table 28. Beef roast spread sheet summary of all cook schedules.

	93.3C In Bag, High humidity											
8/8/2008		71.1 C	Plate	1	726.70	2.07	9.48	6.64	<1.4	>6.64	283.88	
		71.1 C	PF	1	726.70	2.07	9.48	6.27	<.4	>6.71	283.88	
		71.1 C	Plate	2	400.50	1.47	9.31	6.64	<1.4	>6.64	143.73	
		71.1 C	PF	2	400.50	1.47	9.48	6.27	0.40	5.87	143.73	
10/9/2008		5.5 log	Plate	4	622.80	1.31	10.49	6.64	4.48	2.16	4.02	96.90
		5.5 log	PF	4	622.80	1.31	10.49	6.27	3.60	2.67	4.02	96.90
		5.5 log	Plate	5	721.90	1.40	10.49	6.64	<1.4	>6.64	5.97	105.80
		5.5 log	PF	5	721.90	1.40	10.49	6.27	2.10	4.17	5.97	105.80
		5.5 log	Plate	6	642.50	1.43	10.49	6.64	4.11	2.53	5.51	117.32
		5.5 log	PF	6	642.50	1.43	10.49	6.27	3.26	3.01	5.51	117.32
	93.3C In Bag, No Humidity											
8/6/2008		71.1 C	Plate	1	606.30	2.52	9.34	6.64	<1.4	>6.64	288.14	
		71.1 C	PF	1	606.30	2.52	9.60	6.27	0.40	5.87	288.14	
		71.1 C	Plate	2	623.30	2.34	9.34	6.64	<1.4	>6.64	747.02	
		71.1 C	PF	2	623.30	2.34	9.60	6.27	<.4	>6.71	747.02	
10/7/2008		5.5 log	Plate	4	570.60	2.11	9.17	6.64	2.51	4.13	5.98	166.59
		5.5 log	PF	4	570.60	2.11	9.17	6.27	3.61	2.66	5.98	166.59
		5.5 log	Plate	5	677.30	2.11	9.17	6.64	<1.4	>6.64	4.88	234.30
		5.5 log	PF	5	677.30	2.11	9.17	6.27	1.35	4.92	4.88	234.30
		5.5 log	Plate	5	760.20	2.11	9.17	6.64	<1.4	>6.64	4.71	215.48
		5.5 log	PF	6	760.20	2.11	9.17	6.27	<.4	>6.71	4.71	215.48

Table 28. Continued

	93.3C No Bag, High humidity			6								
8/11/2008		71.1 C	Plate	1	703.10	1.92	uncountable	6.64	<1.4	>6.64	243.74	
		71.1 C	PF	1	703.10	1.92	uncountable	6.27	<.4	>6.71	243.74	
		71.1 C	Plate	2	708.60	1.92	uncountable	6.64	<1.4	>6.64	260.03	
		71.1 C	PF	2	708.60	1.92	uncountable	6.27	<.4	>6.71	260.03	
10/20/2008		5.5 log	Plate	4	847.20	1.51	9.39	6.64	<1.4	>6.64	5.95	153.61
		5.5 log	PF	4	847.20	1.51	9.39	6.27	<.4	>6.71	5.95	153.61
		5.5 log	Plate	5	840.40	1.41	9.39	6.64	<1.4	>6.64	4.75	124.51
		5.5 log	PF	5	840.40	1.41	9.39	6.27	1.78	4.49	4.75	124.51
		5.5 log	Plate	6	747.20	1.43	9.39	6.64	1.40	5.24	5.78	129.26
		5.5 log	PF	6	747.20	1.43	9.39	6.27	2.28	3.99	5.78	129.26

Table 28. Continued

	Step Up, In Bag, High Humidity											
8/5/2008		71.1 C	Plate	1	496.50	1.93	8.77	6.64	<1.4	>6.64	209.62	
		71.1 C	PF	1	496.50	1.93	9.70	6.27	<.4	>6.71	209.62	
		71.1 C	Plate	2	643.60	2.34	8.77	6.64	<1.4	>6.64	440.90	
		71.1 C	PF	2	643.60	2.34	9.70	6.27	<.4	>6.71	440.90	
10/10/2008		5.5 log	Plate	4	592.20	1.77	10.37	6.64	<1.4	>6.64	4.74	151.01
		5.5 log	PF	4	592.20	1.77	10.37	6.27	<.4	>6.71	4.74	151.01
		5.5 log	Plate	5	572.30	1.84	10.37	6.64	<1.4	>6.64	5.62	151.26
		5.5 log	PF	5	572.30	1.84	10.37	6.27	<.4	>6.71	5.62	151.26
		5.5 log	Plate	6	451.30	1.60	10.37	6.64	<1.4	>6.64	5.02	145.04
		5.5 log	PF	6	451.30	1.60	10.37	6.27	<.4	>6.71	5.02	145.04
	Step Up, In Bag, No Humidity											
7/29/2008		71.1 C	Plate	1	608.70	5.00	9.49	6.64	<1.4	>6.64	24.98	
		71.1 C	PF	1	608.70	5.00	9.53	6.27	<.4	>6.71	24.98	
		71.1 C	Plate	2	537.00	5.11	9.49	6.64	<1.4	>6.64	772.12	
		71.1 C	PF	2	537.00	5.11	9.53	6.27	<.4	>6.71	772.12	
10/14/2008		5.5 log	Plate	4	849.80	3.75	9.51	6.64	<1.4	>6.64	4.86	521.51
		5.5 log	PF	4	849.80	3.75	9.51	6.27	<.4	>6.71	4.86	521.51
		5.5 log	Plate	5	680.00	3.97	9.51	6.64	<1.4	>6.64	4.93	555.98
		5.5 log	PF	5	680.00	3.97	9.51	6.27	<.4	>6.71	4.93	555.98
		5.5 log	Plate	6	533.80	3.33	9.51	6.64	<1.4	>6.64	4.55	479.14
		5.5 log	PF	6	533.80	3.33	9.51	6.27	1.10	5.17	4.55	479.14

Table 28. Continued

	Step Up, No Bag, High Humidity											
8/7/2008		71.1 C	Plate	1	694.60	2.10	7.95	6.64	<1.4	>6.64	446.67	
		71.1 C	PF	1	694.60	2.10	8.23	6.27	<.4	>6.71	446.67	
		71.1 C	Plate	2	649.80	2.10	7.95	6.64	<1.4	>6.64	291.62	
		71.1 C	PF	2	649.80	2.10	8.23	6.27	<.4	>6.71	291.62	
10/17/2008		5.5 log	Plate	4	675.10	1.96	9.20	6.64	<1.4	>6.64	7.13	158.18
		5.5 log	PF	4	675.10	1.96	9.20	6.27	1.98	4.29	7.13	158.18
	-	5.5 log	Plate	5	646.90	1.86	9.20	6.64	<1.4	>6.64	5.99	176.18
	-	5.5 log	PF	5	646.90	1.86	9.20	6.27	1.97	4.30	5.99	176.18
		5.5 log	Plate	6	880.50	2.14	9.20	6.64	<1.4	>6.64	6.66	167.25
		5.5 log	PF	6	880.50	2.14	9.20	6.27	0.40	5.87	6.66	167.25
	Step Up, No Bag, Changing Humidity											
10/16/2008		71.1 C	Plate	1	680.50	3.67		6.64	NA	NA	650.11	
		71.1 C	PF	1	680.50	3.67	9.31	6.27	0.70	5.57	650.11	
		71.1 C	Plate	2	613.90	3.64		6.64	NA	NA	614.69	
		71.1 C	PF	2	613.90	3.64	9.31	6.27	<.4	>6.71	614.69	
		5.5 log	Plate	4	881.80	3.42		6.64	<1.4	>6.64	4.80	254.77
		5.5 log	PF	4	881.80	3.42	9.31	6.27	<.4	>6.71	4.80	254.77
		5.5 log	Plate	5	790.10	3.15		6.64	2.00	4.64	4.68	262.66
		5.5 log	PF	5	790.10	3.15	9.31	6.27	2.08	4.19	4.68	262.66
		5.5 log	Plate	6	753.10	3.03		6.64	<1.4	>6.64	4.63	290.81
		5.5 log	PF	6	753.10	3.03	9.31	6.27	1.78	4.49	4.63	290.81

Table 29. Pork roast spread sheet summary of all cook schedules.

8/14/2009	93.3C In Bag, High humidity	71.1 C	PF	1	412.50	1.37	9.45	0.40	5.87	437.31	98.59
		71.1 C	PF	2	394.80	1.37	9.45	<0.4	> 6.27	500.47	89.71
		3.0 log	PF	4	486.69	1.12	9.45	3.18	3.09	2.76	96.50
		3.0 log	PF	5	385.94	1.12	9.45	0.88	5.39	3.34	94.82
		3.0 log	PF	6	487.33	1.12	9.45	1.18	5.09	3.35	102.28
8/27/2009	93.3C In Bag, No Humidity	71.1 C	PF	1	443.62	2.01	9.27	<0.4	> 6.27	1726.04	168.75
		71.1 C	PF	2	443.67	2.06	9.27	<0.4	> 6.27	432.61	182.06
		3.0 log	PF	4	527.18	1.77	9.27	1.10	5.17	2.87	204.47
		3.0 log	PF	5	481.46	1.69	9.27	1.83	4.44	3.26	200.42
		3.0 log	PF	6	543.17	1.85	9.27	3.03	3.24	2.49	213.58
8/28/2009	93.3C No Bag, High humidity	71.1 C	PF	1	461.60	1.59	9.37	<0.4	> 6.27	829.22	106.83
		71.1 C	PF	2	468.97	1.59	9.37	<0.4	> 6.27	458.08	104.20
		3.0 log	PF	4	592.13	1.30	9.37	<0.4	> 6.27	2.52	112.73
		3.0 log	PF	5	473.67	1.13	9.37	1.18	5.09	4.27	98.66
		3.0 log	PF	6	492.88	1.20	9.37	1.24	5.03	2.70	100.89

Table 29. Continued

8/20/2009	Step Up In Bag, High Humidity	71.1 C	PF	1	430.04	2.24	9.40	<0.4	> 6.27	1449.42	131.65
		71.1 C	PF	2	456.10	2.24	9.40	<0.4	> 6.27	525.72	139.52
		3.0 log	PF	4	552.48	1.54	9.40	3.84	2.43	2.43	154.80
		3.0 log	PF	5	458.23	1.27	9.40	2.72	3.55	2.69	131.97
		3.0 log	PF	6	499.45	1.43	9.40	2.82	3.45	2.42	140.91
8/18/2009	Step Up, In Bag, No Humidity	71.1 C	PF	1	427.70	4.75	9.45	<0.4	> 6.27	3282.07	399.40
		71.1 C	PF	2	430.08	4.75	9.45	<0.4	> 6.27	1459.52	413.16
		3.0 log	PF	4	525.34	3.34	9.45		2.35	2.80	478.57
		3.0 log	PF	5	508.00	3.67	9.45	1.10	1.98	2.79	578.09
		3.0 log	PF	6	450.11	3.39	9.45	1.40	1.89	2.85	480.60
9/2/2009	Step Up, No Bag, High Humidity	71.1 C	PF	1	386.45	1.97	9.57	<0.4	> 6.27	1133.62	123.42
		71.1 C	PF	2	399.28	1.97	9.57	<0.4	> 6.27	672.09	128.90
		3.0 log	PF	4	479.29	1.38	9.57	2.40	3.87	2.89	150.49
		3.0 log	PF	5	461.82	1.31	9.57	1.98	4.29	2.40	132.99
		3.0 log	PF	6	434.60	1.37	9.57	2.04	4.23	2.63	140.93
9/1/2009	Step Up, No Bag, Changing Humidity	71.1 C	PF	1	461.26	3.33	8.93	<0.4	> 6.27	870.68	297.54
		71.1 C	PF	2	455.47	3.33	8.93	<0.4	> 6.27	505.71	324.50
		3.0 log	PF	4	511.41	2.75	8.93	4.91	1.36	1.01	253.57
		3.0 log	PF	5	465.30	2.79	8.93	0.88	5.39	4.66	278.61
		3.0 log	PF	6	547.00	2.68	8.93	2.95	3.32	2.38	304.51

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