Executive Summary

Title: Developing Validated Time-Temperature Thermal Processing Guidelines for Ready-To-Eat Deli Meat and Poultry Products.

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Objectives:

- To validate the effect of thermal processing interventions on the survival of *Listeria monocytogenes*, *Salmonella*, and shiga-toxin producing *E. coli* (STEC) in roast beef, turkey deli-breast, and boneless hams;
- To use the thermal destruction data to develop scientifically-validated, easy-to-use time-temperature tables as tools for assuring regulatory compliance and pathogen destruction for ready-to-eat roast beef, turkey deli-breast, and boneless ham and;
- To develop the basis for a series of time-temperature tables organized in product categories that will cover the vast array of ready-to-eat meat products and thermal processes in the U.S. meat industry.

Conclusions: Thermal treatments are critical for controlling foodborne pathogens in ready-to-eat (RTE) meat and poultry products. Microbial resistance to thermal processes can be affected by several factors including the level and length of heat exposure and various intrinsic factors such as fat, salt or water concentration. To ensure that cooking protocols are effective in reducing pathogenic bacteria to safe levels, it is important that scientific support for the validation of thermal processes spans products in which it is used for; addresses the pathogens most commonly associated with certain products or animal species; and includes recently identified pathogenic bacteria of concern. This study investigated the validity of thermal processes for three different high moisture ready-to-eat processed deli-style products (ham, roast beef, and turkey breast). Results from this study confirmed that cooking temperatures and times that are currently being widely used in the meat and poultry industry following USDA, FSIS guidance supporting documentation for thermal lethality are sufficient to kill *Listeria monocytogenes*, *Salmonella*, and shiga-toxin producing *E. coli* (STEC) in roast beef, turkey deli-breast, and boneless hams in all the products tested when cooking temperatures met or exceeded 62.8°C. The pathogen reduction levels all met or exceeded regulatory requirements or recommendations for the products tested. However, when cooking roast beef to 54.4°C, current USDA, FSIS thermal processing guidance for *Salmonella* was not supported—suggesting that additional cooking time or higher cooking temperatures are needed to achieve a target pathogen reduction. Further, the integrated lethality of the products investigated was successfully determined by incorporating pathogen reduction results with thermal process profiles to create the integrated thermal lethality profiles.

Deliverable: Because of the wide variety of processed meat products, thermal process validations that encompass this wide range of products are important to confirm appropriate and expected pathogen destruction during cooking. The results of this study have enabled the development and generation of new easy-to-use time-temperature tables for boneless ham, roast beef, and deli-style turkey breast for validated reduction of the pathogens investigated.

Developing Validated Time-Temperature Thermal Processing Guidelines for Ready-To-Eat Deli Meat and Poultry Products.

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Technical Abstract: The objectives of this project were 1) to validate the effect of thermal processing interventions on the survival of *Listeria monocytogenes*, *Salmonella*, and shiga-toxin producing *E. coli* (STEC) in roast beef, turkey deli-breast, and boneless ham and 2) generate data that could be used for the development of scientifically-validated, easy-to-use time-temperature tools for assuring regulatory compliance and pathogen destruction for the products investigated having different compositional properties. Currently, U.S. meat industry establishments manufacturing RTE meat and poultry products have limited science-based supporting documentation to ensure and validate the thermal destruction of pathogenic microorganisms during cooking. As such, the limited thermal process supporting documentation currently available is routinely applied to a wide array of products -- including some for which the scientific support was never intended. This wide application of limited research may be of concern since the heat resistance of microorganisms can be affected by several factors including the bacterial properties such as the pathogen of interest to control, cell concentration, phase of growth, amount of strain, and exposure to stressors such as acid or salt. Further, the intrinsic properties of meat and poultry products such as fat content, water activity, or meat species also can influence the heat resistance of pathogens. As a result, a comprehensive investigation of pathogen destruction considering several of these factors is important to ensure that current thermal processing approaches are capable of reducing pathogenic bacteria to safe levels.

The scientific approach to reach the project objectives were to measure D- and z-values for *Salmonella*, *L. monocytogenes*, and STEC in roast beef, turkey deli-breast, and boneless ham following cooking temperatures and times commonly used in the meat industry. These generated D- and z- values could then be utilized in a number of ways such as in pathogen modeling programs or to generate simple thermal processing tools such as time/temperature thermal processing tables valid for specific product/pathogen combinations. However, validation of the measured D-values is also important and is accomplished by confirming the actual pathogenic log reduction in against the expected reduction according to the measured D-value. Further, temperature and microbial data collection provides opportunity for investigation of integrated lethality concepts and thermal processing tool development.

D- and z-values were determined using a ground meat mixture system. Ground turkey breast (containing 1.5% salt, 1.5% dextrose, 20% water), ground roast beef (containing 1.0% salt, 0.35% sodium phosphates, 0.75% sugar, 20% water), and ground ham (containing 2.5% salt, 1.65% sugar, 0.35% sodium phosphates, 547 ppm sodium erythorbate, 200 ppm sodium nitrite, 20% water) were inoculated with 8 log CFU/g *L. monocytogenes* or *Salmonella* (5-strain mix) or STEC (7-strain mix). All non-meat ingredient additions were based on the meat block weight. One-g portions (flattened to 0.5-1.0 mm in thick film in moisture-impermeable vacuum pouches) were heated at one of four temperatures (54.4, 60, 65.6, or 71.1°C) in a water bath. Triplicate samples were removed and immediately chilled to ≤4°C when meat reached target temperature and at seven additional times. Surviving *L. monocytogenes, Salmonella,* or STEC were enumerated using Modified Oxford, XLD, or Sorbitol MacConkey agar base, respectively,

with thin layer overlay of nonselective media to enhance recovery of injured cells. Linear regressions of the data were used to calculate D- and z-values for each treatment combination (3 product types x 3 pathogens x 4 temperatures). From this data, treatment combinations were selected for validation using commercial production processes.

Validation of D- and z- values took place by manufacturing commercial products using the same formulations as above. Treatment combinations included Turkey+*Salmonella* cooked to 71.1°C, Roast Beef+*Salmonella* cooked to 54.4, 62.8 and 71.1°C, Roast Beef+STEC cooked to 54.4, 62.8 and 71.1°C, and Ham+*L. monocytogenes* cooked to 62.8 and 71.1°C. Each treatment combination was inoculated with 8 log CFU/g of the designated pathogen cocktail, and stuffed into 4" (10.61cm) diameter casings. Treatments were cooked using either a step-up steam (turkey breast, roast beef) or wet bulb/dry bulb (ham) thermal process. Triplicate 25-g samples were removed from the core, midpoint, and surface of each chub for enumeration of surviving pathogens at 3 pre-determined time-points during each thermal process (54.4°C - sampled at 54.4, 54.4 +1 h, and 54.4°C +2 h; 62.8°C - sampled at 54.4, 62.8, and 62.8°C +5 min; 71.1°C - sampled at 54.4, 62.8, and 71.1°C). Additional samples were processed after chilling to <4°C to account for integrated lethality during cooling. Surviving *L. monocytogenes*, *Salmonella* or STEC were enumerated as previously discussed.

In all product types for D- and z- value studies, inactivation rates for STEC were similar to *Salmonella* at 60, 65.6, or 71.1°C, and were comparable to or less than times reported in Appendix A. In contrast, *L. monocytogenes* showed greater thermotolerance than *Salmonella* and STEC under all conditions. For example, a >5 log reduction of *Salmonella* and STEC in turkey was achieved instantaneously at 71.1°C, whereas *L. monocytogenes* was inactivated within 10 seconds. At 60°C, >5 log reduction of *L. monocytogenes* required 30 and 50 minutes in turkey and ham, respectively, as compared to <12 minutes for *Salmonella* and STEC. At the lowest temperature tested (54.4°C), >5-log reduction of *Salmonella,* STEC, and *L. monocytogenes* in all product types was achieved in <2, 2.8, and 4.6 hours, respectively. Since D- and z- values were generated using model systems with one gram meat samples, only immediate lethality was measured, while integrated lethality was not accounted for to determine expected total lethality in a commercial process.

Validation of D- and z-values using a commercial product system confirmed that cooking to 71.1°C was sufficient to kill >7 log of the 3 pathogens in all the products tested. STEC and *Salmonella* were similarly inactivated in roast beef when cooked to 62.8°C providing a 7 log reduction. For the ham and *L. monocytogenes* treatment combination cooked to a final temperature of 62.8°C, a 5 log cfu/g reduction observed but the additional lethality contributed during cooling to achieve an overall inactivation of >6 log. However, less than 4 log of *Salmonella* (3.21 log cfu/g) or STEC (3.44 log cfu/g) were inactivated in the core samples taken from the geometric center of beef heated to 54.4°C and held for 2 hours. Additional investigation is needed to identify hold times or other modifications necessary to achieve >5 or 6 log reductions of *Salmonella* and STEC when utilizing 54.4°C as the final cook temperature for roast beef.

This data supports the adequacy of current thermal processing practices for the products investigated with exception of the low temperature/roast beef thermal process treatment combination. Further, the results of this study have allowed the development and generation of new easy-to-use time-temperature tables for boneless ham, roast beef, and deli-style turkey breast for valid reduction of the pathogens investigated.

Objectives:

- To validate the effect of thermal processing interventions on the survival of *Listeria monocytogenes*, *Salmonella*, and shiga-toxin producing *E. coli* (STEC) in roast beef, turkey deli-breast, and boneless hams;

- To use the thermal destruction data to develop scientifically-validated, easy-to-use time-temperature tables as tools for assuring regulatory compliance and pathogen destruction for ready-to-eat roast beef, turkey deli-breast, and boneless hams and;
- To develop the basis for a series of time-temperature tables organized in product categories that will cover the vast array of ready-to-eat meat products and thermal processes in the U.S. meat industry.

Materials and Methods:

This study was divided into two phases. Phase 1 was conducted to determine D- and z-values for *L. monocytogenes*, *Salmonella*, and STEC using ground meat mixtures. The purpose of Phase 2 was to validate the D-values identified in Phase 1 using commercial products representing different product categories. Three low-fat products were selected for testing and chosen because of differences in species, moisture level and inclusion of sodium nitrite inclusion. The formulas for each product were selected to represent the worst-case for thermal destruction in its category, and would allow the thermal destruction data to be used as the basis for new Appendix A style time-temperature tables for those product categories.

To accomplish these goals, the experiment plan included the following approaches:

1. Measure D- and z-values for *Salmonella*, *L. monocytogenes*, and STEC in roast beef, turkey deli-breast, and boneless ham.
2. Validate the measured D-values in simulated commercial products and processes.
3. Generate data to develop new Appendix A style time-temperature tables for roast beef, turkey deli-breast and boneless ham product types/categories.

Phase 1: Determination of D- and z-values in model (ground) meat. Three low-fat products selected for testing were categorized by moisture level and inclusion of sodium nitrite and included 1) roast beef (lower moisture, uncured), 2) deli-style turkey breast (higher moisture, uncured) and 3) boneless ham (higher moisture, cured). The formulas for each product were selected to represent the worst-case for thermal destruction in its category, and this thermal destruction data will be used as the basis for new Appendix A style time-temperature tables for those product categories.

Ground roast beef (containing 2.0% salt, 0.35% sodium phosphates, 0.75% sugar, 20% water), ground ham (containing 2.5% salt, 1.65% sugar, 0.35% sodium phosphates, 547 ppm Na erythorbate, 200 ppm Na nitrite, 20% water), or ground turkey breast (containing 1.5% salt, 1.5% dextrose, 20% water) were inoculated with 8 log CFU/g *L. monocytogenes* or *Salmonella* (5-strain mix) or STEC (7-strain mix). One-g portions (0.5-1.0 mm in moisture-impermeable vacuum pouches) were heated at one of four temperatures (54.4, 60, 65.6, or 71.1°C; 130, 140, 150 and 160°F, respectively) in a water bath. Triplicate samples were removed and immediately chilled to ≤4°C/40°F when meat reached target temperature and at seven additional times. Surviving *L. monocytogenes, Salmonella,* or STEC were enumerated using Modified Oxford, XLD, or Sorbitol MacConkey agar base, respectively, with thin layer overlay of nonselective media to enhance recovery of injured cells. Each study was replicated twice.

Data was collected for log reduction of pathogens for all treatment combinations (3 product types x 3 pathogens; 4 temperatures) over time to attain time-temperature-log reduction relationships. Further, Data was analyzed using standard linear regression statistical methodology to generate linear regressions for each product type, pathogen and temperature combination incorporating at least 4 time-points for each combination to allow for generation of D- and z- values.

Phase 2: Validation of D- and z-values in commercial-type products. To validate the results found in Phase 1 and explore the impact of integrated lethality on pathogen reduction, a validation study was designed and conducted for all three products (roast beef, deli-style turkey breast, and boneless ham)

investigated in Phase 1. Products in this phase were manufactured following commercial production and thermal processing practices to mimic, as closely as possible, actual commercial manufacturing conditions. In the original experimental design, the pathogen with the highest D-value for each of the three products was to be chosen for validation in this phase. However, to benefit the overall product applicability and industry impact of the study, the experimental design was modified with the following treatment combinations listed below. For each treatment combination, initial (time 0) and 3 additional sampling points were chosen to validate the D-values generated in Phase I and document integrated lethality for the target internal temperature. Each treatment combination listed in the following table was manufactured/tested in duplicate for a total of 18 separate experimental runs.

1) Deli-style turkey breast – *Salmonella* – 160°F final internal temperature
2) Roast beef – *Salmonella* – 130°F (54.4°C) final internal temperature
3) Roast beef – *Salmonella* – 145°F (62.8°C) final internal temperature
4) Roast beef – *Salmonella* – 160°F (71.1°C) final internal temperature
5) Roast beef – STEC – 130°F final internal temperature
6) Roast beef – STEC – 145°F final internal temperature
7) Roast beef – STEC – 160°F final internal temperature
8) Boneless ham – *L. monocytogenes* – 145°F final internal temperature
9) Boneless ham – *L. monocytogenes* – 160°F final internal temperature

Manufacturing procedures for all products included grinding muscles through a ¾" plate, addition of ingredients (dissolved in water) and tumbling on medium speed under vacuum (1.0 hour for ham/turkey and 1.5 hour for roast beef). For ham, an overnight holding period was also included after vacuum tumbling. After product manufacture at the UW Meat Science and Muscle Biology Laboratory, meat mixtures were transported to the Applied Research Laboratory at the Food Research Institute (FRI). At FRI, meat mixtures were placed in a paddle mixer and inoculated with 8 log CFU/g of the designated pathogen cocktail. Inoculated meat mixtures were then stuffed into 4" diameter impermeable plastic (turkey and beef) or permeable fibrous (ham) casings to a length of approximately 14 inches. Chubs of beef and turkey product were thermally processed in a combi-oven (Alto-Shaam Combitherm, model 6.10 ESI SK, Alto-Shaam Inc., Menomonee Falls, WI) using ramped steam cook cycles (Tables 1-3) similar to those commonly used in the meat industry for these types of products. Chubs of ham product were thermally processed in a smokehouse (Alkar Model 1000, Alkar Engineering Corp., Lodi, WI) at the Alkar-RapidPak Research and Technology Center. For ham treatment combinations, smokehouse schedules (Tables 4-5) were designed with the intent of 1) being similar to that commonly used in the meat industry containing appropriate wet bulb, dry bulb and relative humidity configured temperature steps and 2) including a wet bulb spike for preliminary investigation of surface humidity/surface log reduction concepts that will be investigated in a subsequent AMIF study. Internal temperature probes (iButtons; DS1922T-F5) were placed at three locations inside the chubs to continuously monitor the surface, midpoint, and geometric center (core) temperature. Product temperature data was logged at one-minute intervals for use in integrated lethality calculations; oven environment conditions (i.e. dry bulb, wet bulb, air velocity) was also recorded as appropriate. Thermal process profiles were generated for each treatment combination by measuring product core, midpoint, and surface temperatures as well as environmental (steam or wet-bulb/dry-bulb) conditions.

Microbial sampling/pull time points for each product were established based upon the final cook temperature target goal for the treatment, comparative time/temperature combinations from USDA, FSIS Appendix A, and to achieve uniform experimental design and data distribution for analysis. Table 6 outlines the sampling/pull points that were followed for the 9 different product and pathogen treatment combinations. For each sampling time point, a single inoculated chub was removed from the oven when target internal temperatures or time sampling/pull time point target was reached. After removal at each time/temperature point, a 2 inch piece from the each end of the chub was removed and discarded. The

remaining portion of the 12 inch long chub was cut into three, 4-inch long pieces, and 25 g core samples (4" long x 0.75" diameter) were removed from the geometric center (core) and midpoint of each section using a metal bore inserted parallel to the length of the chub (Figure 1). Surface samples were extracted by first removing the casing and shaving 2-3 mm thick pieces from the outside surface. For each chub, three surface, three midpoint, and three center samples were collected for microbial enumeration of surviving pathogens. Samples were immediately chilled on ice after removal from the chub and enumerated to determine cfu/g *L. monocytogenes*, *Salmonella* or STEC. Additional samples were processed after chilling to < 40°F to account for integrated lethality during cooling. Surviving *L. monocytogenes*, *Salmonella* or STEC were enumerated using Modified Oxford, XLD or Sorbitol MacConkey agar, respectively, with thin layer overlay of nonselective media to enhance recovery of injured cells.

Proximate and chemical analysis: Moisture (5 h, 100°C, vacuum oven method AOAC 950.46), pH (10 g homogenized portion diluted in 90 ml distilled water, pH of slurry measured with Accumet Basic pH meter and Orion 8104 combination electrode, Thermo Fisher Scientific, Waltham, MA), NaCl (measured as % Cl^-, $AgNO_3$ potentiometric titration, Mettler DL22 food and beverage analyzer), and water activity (Decagon AquaLab 4TE water activity meter, Pullman, WA) were assayed by the Food Research Institute for triplicate samples of each lot.

Data analysis: The microbiological data reported are average values and standard deviations (log CFU/g) for triplicate samples and two separate trials for each test formulation (n=6).

Results and Discussion:

Phase 1: Determination of D- and z-values in model (ground) meat. For D-value calculations, 36 graphs were developed (Figure 2a-2c) plotting pathogen log reduction over time using linear regression analysis. The "Ham-*Salmonella*-60°C"figure found in Figure 2b provides an example of a standard linear regression calculated for a ham/*Salmonella* treatment combination at 60°C (140°F) where populations of *Salmonella* were determined at 0, 120, 240, 360, and 480 seconds. To account for the initial come-up time to the first time-point (0 seconds), time adjustments were made to all subsequent time-points. For example, in the "Ham-*Salmonella*-60°C" figure, "0" time was adjusted 8 seconds for replication 1 and 11 seconds for replication 2 to signify the actual time the internal sample temperature reached the pre-determined experiment temperature. From this linear regression, a calculated D- value of 90.1 seconds was less than the D- value of 102.6 seconds calculated from the USDA, FSIS Appendix A for the same temperature. This would suggest Appendix A is a conservative predictor of *Salmonella* reduction, even when raw materials sources were not the same. Similarly, the D-values calculated for *Salmonella* in turkey were less than those identified in the USDA-FSIS Time-Temperature Tables for Cooking Ready-To-Eat Poultry Products. Table 7 displays calculated D- and z- values for all roasts beef, deli-style turkey breast, boneless ham, *L. monocytogenes*, *Salmonella*, and STEC combinations. Z-values were calculated (Figures 3-5) for *Salmonella, Listeria monocytogenes,* and STEC in roast beef, turkey deli-breast, and boneless ham by potting the D- values for each treatment combination against temperature.

In all product types (roast beef, ham and turkey), inactivation rates for STEC were similar to *Salmonella* at 140, 150 and 160°F (60, 65.6, or 71.1°C, respectively) and were comparable to or less than times reported in USDA, FSIS Appendix A. In contrast, *L. monocytogenes* showed greater thermotolerance than *Salmonella* and STEC under all conditions. For example, >5-log reduction of *Salmonella* and STEC in turkey was achieved instantaneously at 71.1°C, whereas *L. monocytogenes* required a 10 second hold time. At 60°C (140°F), >5-log reduction of *L. monocytogenes* required 30 and 50 minutes in turkey and ham, respectively, as compared to <12 minutes for *Salmonella* and STEC. At the lowest temperature tested (54.4°C/130°F), >5-log reduction of *Salmonella,* STEC and *L. monocytogenes* in all product types was achieved in <2, 2.8 and 4.6 hours, respectively.

Results from Phase I support the USDA Appendix A as an acceptable tool for *Salmonella* and STEC lethality; as expected *L. monocytogenes* was more thermo-tolerant than *Salmonella* or STEC. Since Phase I utilized a model type approach using one gram meat samples for all lethality investigations, only immediate lethality was measured while integrated lethality was not accounted for nor considered to determine an actual and/or expected total lethality. It is important to consider integrated lethality because significantly different thermal processing conditions exist in a 1 g sample (Phase I sample size) versus a larger mass, thickness, or diameter. Due to these physical differences, longer temperature increases and slower come-up times naturally exist. Generally, the longer come-up times result in higher lethality processes because the longer times at lethal temperatures enhance pathogen destruction. However, extended cook times at sub-lethal temperatures can also potentially allow bacterial survival due to 1) the lack of achieving required lethality temperatures or 2) allowing the opportunity for temperature adaptation by bacteria resulting in greater survival during normal lethal processes. Therefore, care and consideration must be taken when interpreting Phase I data and results to recognize this important thermal processing condition. As such, it is important to validate pathogenic log reductions estimated using D-values in commercial meat products.

Phase 2: ***Validation of D- and z-values in commercial-type products.*** Table 8 provides a summary of the total log reductions observed for each product and time/temperature combination (internal). Cooking to 160°F resulted in >6.5 log reduction for all pathogens in all products tested--confirming that sufficient log reduction expectations/requirements of 6.5 log *Salmonella* and 5.0 log STEC were easily met in these products. Further, cooking ham to 145°F and holding at this temperature for 5 minutes also resulted in >5 log reductions for *L. monocytogenes* while >7.0 log reduction was noted after chilling the chub to < 40°F -- suggesting that integrated lethality was responsible for the additional log reduction. For roast beef cooked to 130°F, a 3.2 log reduction of *Salmonella* and a 3.4 log reduction of STEC were observed when held at this temperature for 2 hours. These results undershot the target goal reductions of 5.0 and 6.5 for STEC and *Salmonella*, respectively. This lack of lethality requires further study, but may have been caused by a sub-lethal heating phenomenon (heat adaptation when bacteria are exposed to sub lethal temperatures for long periods of time) that has been found to provide partial protection against overall lethality (Tenorio-Bernal et al., 2013).

In addition to heat-adaptation as a potential explanation for the lower than expected log reductions, the actual temperature being utilized to provide lethality for this research should also be considered. Generally, as exposure temperatures decrease for pathogenic bacteria, the true lethality potential at those temperatures also decreases. This phenomenon has been shown to be more evident at temperatures that may offer marginal lethality. Research reviewed by O'Bryan et al. (2006) illustrates this trend. For example, their review of D-values for various meat and poultry products reported D-values for *Escherichia coli* O157:H7 in ground to be 42.3 minutes at 125°F (51.6°C), 12.5 minutes at 131°F (55°C), and 2.8 minutes at 135°F (57.2°C). These reported D-values show that at low temperatures, where a lesser lethality would be expected, small changes in temperature at these lower temperatures provide significant changes in pathogen reduction.

To better understand the relationship between thermal processing and microbial reductions, thermal process data that was collected by continuously monitoring the surface, midpoint, and geometric center (core) product temperatures was combined with microbial survival data to create integrated thermal process/pathogen reduction profiles. These profiles were developed for all treatment combinations utilizing microbial and temperature data collected (Figure 6-14). By closely analyzing these figures, it can be quickly determined what affect different temperatures during a thermal process had on pathogen reduction at the surface of a product, the midpoint of the product and at the core of that product. As expected, pathogen lethality differences existed between the three sampling locations and that the temperature and length of a given temperature exposure were both important for pathogen reduction.

Generally, as the surface temperatures increased from 120°F to 140°F (Figure 9 vs. 10) due to the different thermal processes used (Table 1 vs. 2 showing 120°F vs. 130°F starting temperatures), achieving >5 log CFU/g reduction for STEC occurred at a faster rate at the product surface for the 145°F final internal treatment than the 130°F final internal treatment. However, additional pathogen reductions at a similar rate did not continue for the 160°F final internal treatment (Figure 11). These results suggest that surface temperature (and dwell time) where important for providing lethality at the surface. This phenomenon, although not always as evident, was also observed for midpoint and core temperatures between all the final cook temperature treatments, and suggests that integrated lethality plays an important role in overall or total lethality. It appears that when pathogens were exposed to lethal temperatures for sufficient time, adequate reductions were noted. However, when sub-lethal temperatures existed during long holding times (at any of the sampling locations), overall pathogen reduction was significantly slowed or failed to achieve target reduction goals.

For the ham/*L. monocytogenes* treatment combinations following a varying relative humidity cook process, a wet-bulb spike was incorporated to begin the eventual investigations of the surface humidity/temperature/lethality relationship. The 145 and 160°F final temperature treatments showed similar patterns for increased lethality with increasing temperatures as was observed with the roast beef. However, a closer examination reveals a longer thermal process time was needed to achieve similar pathogen log reductions. This result can be partly explained by the differences in the thermal process schedule itself (Table 2 vs. 4) or by the thermal resistance differences that exist between pathogenic bacteria strains (Juneja, 2003; Murphy et al., 2002; Murphy and Berrang, 2002; Murphy et al., 2003; Murphy et al., 2004). But, if comparing surface log reductions, approximately 225 minutes was needed to achieve a 5 log CFU/g reduction of *L. monocytogenes* for ham heated to 145°F compared to approximately 125 minutes need to achieve a similar reduction for STEC in roast beef. This additional time needed could be partly due to the drying of the product surface during the cooking. Because, impermeable casings were used for the roast beef, the relative humidity was essentially 100% compared to lower humidities of less than 20% throughout all but 60 minutes of the ham thermal process. Since humidity is important for lethality, surface dryness is another factor important for consideration of total lethality.

D- and z- values were also utilized to construct Appendix A style time-temperature tables. Tables 9-11 outline time and temperature tables for achieving lethality for different pathogen/product combinations and can be easily followed and implemented in current manufacturing processes to provide validation of thermal processes. Other thermal processing tools are also being investigated for feasibility and usefulness.

Treatment combinations were also analyzed for physiochemical properties including proximate moisture, pH, salt, and water activity (Table 12). All analytical results for proximate moisture, salt, and water activity were as expected. The pH of ham was slightly higher than expected and may be attributed to the randomly received raw materials having a higher than normal pH.

Conclusions:

This study investigated the validity of thermal processes for three different high moisture ready-to-eat processed deli-style products (ham, roast beef, turkey breast). Results from this study confirmed that cooking temperatures and times that are currently being widely used in the meat and poultry industry following USDA, FSIS guidance supporting documentation for thermal lethality are sufficient to kill *Listeria monocytogenes*, *Salmonella*, and shiga-toxin producing *E. coli* (STEC) in roast beef, turkey deli-breast, and boneless hams in all the products tested when cooking temperatures met or exceeded 62.8°C. The pathogen reduction levels all met or exceeded regulatory requirements or recommendations for the products tested. However, when cooking roast beef to 54.4°C, current USDA, FSIS thermal processing guidance for *Salmonella* was not supported, suggesting that additional cooking time or higher cooking

temperatures are needed to achieve a target pathogen reduction. Further, the integrated lethality of the products investigated was determined by incorporating pathogen reduction results with thermal process profiles to create integrated thermal lethality profiles for the products investigated. The integrated process lethality profiles will be useful tools for establishments to better evaluate overall pathogen lethality of their products and processes. Finally, the results of this study have also enabled the development of new easy-to-use time-temperature tables for boneless ham, roast beef, and deli-style turkey breast for validated reductions of the 3 pathogens investigated.

Results from this study will be incorporated into a subsequent AMIF study "Investigating the Development of Thermal Processing Tools to Improve the Safety of Ready-To-Eat Meat and Poultry Products" to further develop thermal processed food safety technologies and tools. The information collected in this study will be used to investigate compositional, physical, and intrinsic properties in higher fat, small and large diameter, thick and thin products, slow and fast cook processes and in different species. The continuing project will also attempt to determine if a more (or most) effective and pathogen lethal thermal process step can be identified based on a minimum relative humidity, wet-bulb temperature, and time requirements for assuring the pasteurization of product surfaces.

Recommendations for Future Research:

This study investigated the validity of Appendix A thermal processes for 3 different products and 3 different pathogenic bacteria. The variation included in this experimental design included species (beef, pork, and poultry), inclusion of sodium nitrite, and the thermal process itself. However, other variables that warrant further research and can impact thermal lethality are intrinsic factors (fat, water activity, pH, etc), physical differences (diameter, size, shape, etc), or extrinsic factors (type of heat source, presence/level of humidity, speed of cooking, etc). Thus, it would be valuable to investigate these factors to better understand their effects on thermal lethality. Further, because our research was not able to validate low temperature cooking temperatures (54.4°C), it would be useful to further investigate this lack of lethality to determine if longer cook times at a given temperature or if slightly higher cook temperatures would result in a valid process. Finally, the topic of thermal processing in food safety is vast with many unknowns and significant opportunity for discovery and understanding to ensure the safety of processed meat products -- given the diversity of product and thermal process technologies that exist. Thus, previous comments are not inclusive to the many research opportunities that could surface as future research on this topic.

Presentations and Publications:

- **Poster:** King, A.M., R.P. McMinn, K.A. Glass, A.L. Milkowski, and J.J. Sindelar. 2013. Developing Time-Temperature Thermal Processing Guidelines for Ready-to-Eat Meat and Poultry Products. International Congress of Meat Science and Technology; Izmir, Turkey (August 2013).
- **Poster:** McMinn, R.P., A.M. King, J.J. Sindelar, R. Hanson, and K. A., Glass. 2013. Validation of D- and Z- Values for *Listeria Monocytogenes, Salmonella*, and Shiga-toxin Producing Escherichia coli in Ready-to-Eat Meat and Poultry Products. Food Research Institute. 2013 Spring Meeting, Madison, WI (May 2013).
- **Poster:** King, A.M., R.P. McMinn, A.L. Milkowski, and R. Hanson K.A. Glass, J.J. Sindelar. 2013. Validation of D- and Z- Values for *Listeria Monocytogenes, Salmonella*, and Shiga-toxin Producing Escherichia coli in Ready-to-Eat Meat and Poultry Products. Reciprocal Meat Conference; Auburn, AL (June 2013).
- **Poster:** McMinn. R. P., J.J. Sindelar, and K.A. Glass. 2012. Thermal Inactivation of *Listeria monocytogenes, Salmonella* and Shiga-Toxin Producing *E. coli* in Ready-to-Eat Roast Beef. Food Research Institute 2012 Spring Meeting; Madison, WI (May 2012).

- **Poster:** King, A.M., R.P. McMinn, J.J. Sindelar, K.A. Glass, and R. Hanson. 2012. Thermal Inactivation of *Listeria monocytogenes, Salmonella* and Shiga-Toxin Producing *E. coli* in Ready-to-Eat Ham and Turkey Breast. Reciprocal Meat Conference; Fargo, ND (June 2012).
- **Poster:** McMinn. R. P., J.J. Sindelar, and K.A. Glass. 2012. Thermal Inactivation of *Listeria monocytogenes, Salmonella* and Shiga-Toxin Producing *E. coli* in Ready-to-Eat Roast Beef. Food Research Institute International Association for Food Protection; Providence, RI (July 2012).
- **Manuscript in preparation:** Thermal Inactivation of *Listeria monocytogenes, Salmonella* and Shiga-Toxin Producing *E. coli* in Ready-to-Eat Meat and Poultry Products. R.P. McMinn, A.M. King, A.L. Milkowski, R. Hanson, K.A. Glass, and J.J. Sindelar.
- **Manuscript in preparation:** Validation of D- and Z- Values for *Listeria Monocytogenes, Salmonella*, and Shiga-toxin Producing Escherichia coli in Ready-to-Eat Meat and Poultry Products. R.P. McMinn, A.M. King, A.L. Milkowski, R. Hanson, K.A. Glass, and J.J. Sindelar.

Acknowledgements

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Figure 1. Cross-sectional view of a 3" section of ham displaying locations (surface, center (core) and midpoint) sample removal location in Phase II.

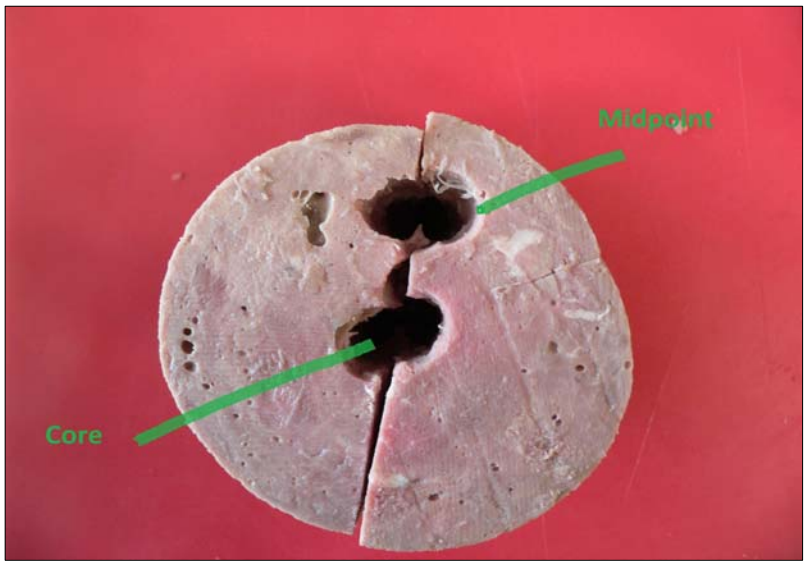
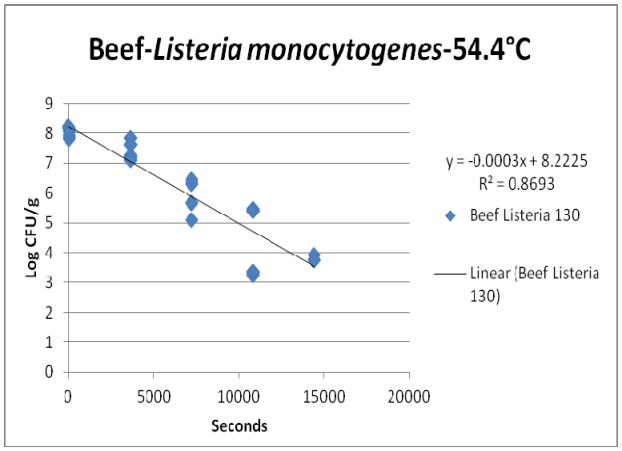
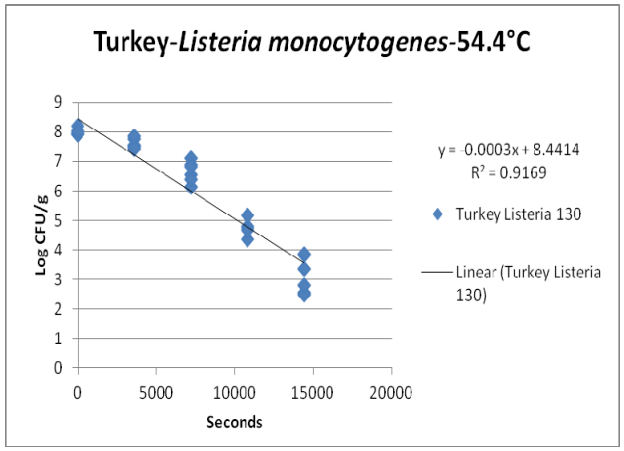
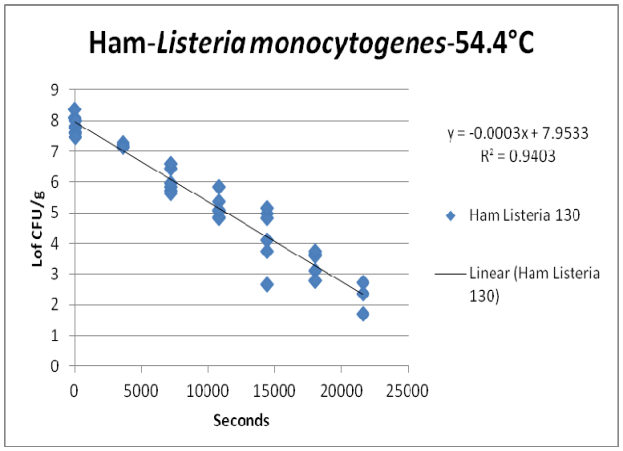
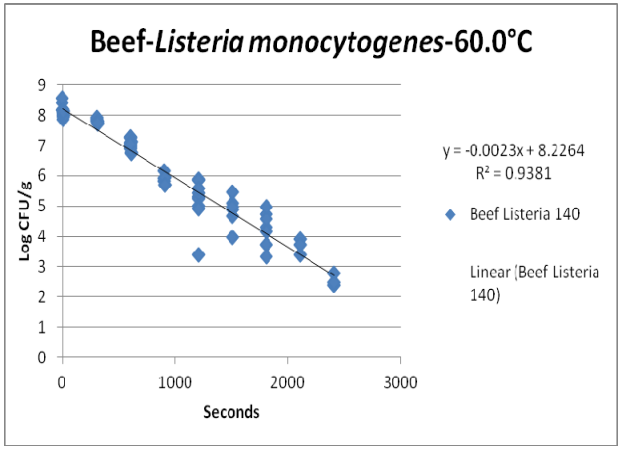


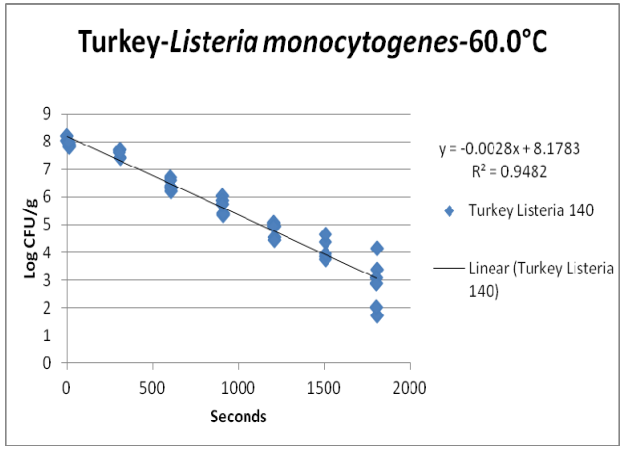
Figure 2a: Determination of D-values for *Listeria monocytogenes* in roast beef, turkey deli-breast, and boneless ham by plotting Log CFU/g pathogen levels against temperature.

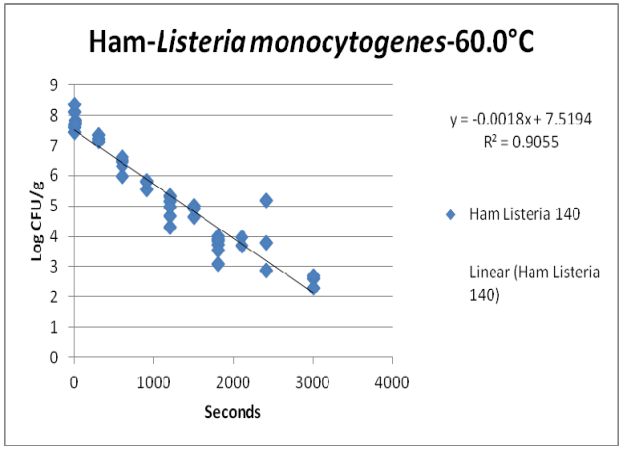


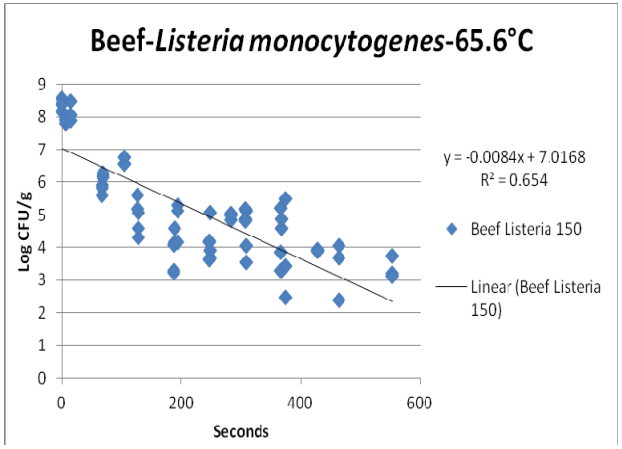


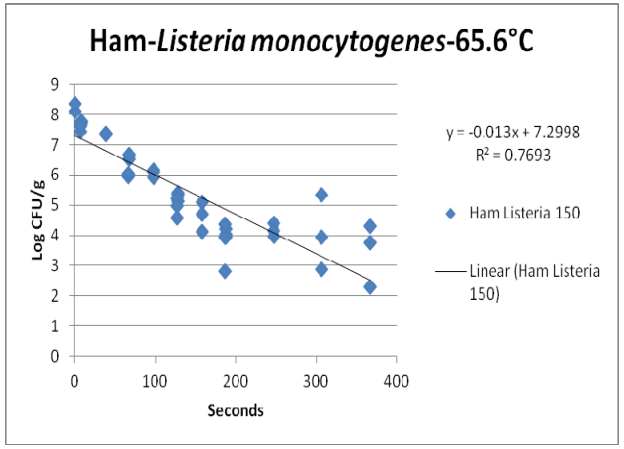


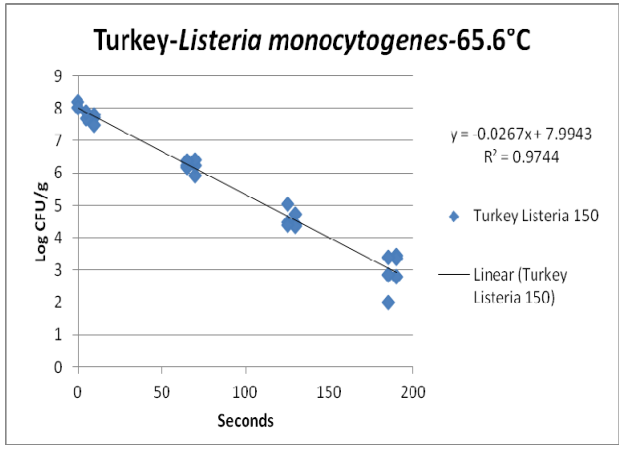


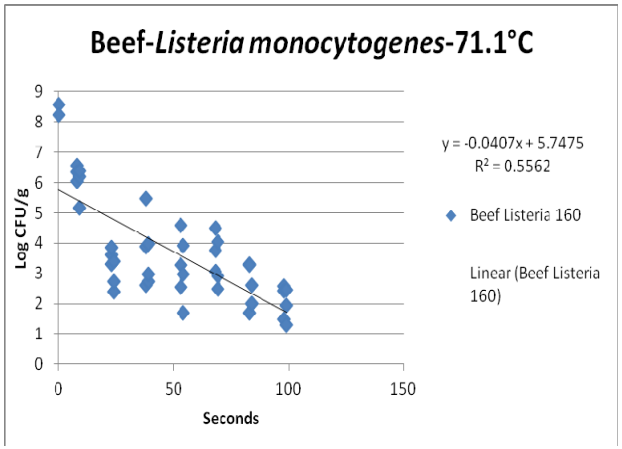


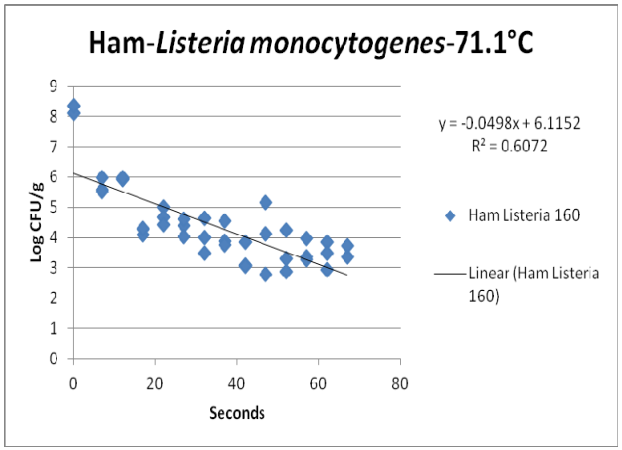



Beef-*Listeria monocytogenes*-65.6°C
Log CFU/g
Seconds
y = -0.0084x + 7.0168
R² = 0.654
Beef Listeria 150
Linear (Beef Listeria 150)


Ham-*Listeria monocytogenes*-65.6°C
Log CFU/g
Seconds
y = -0.013x + 7.2998
R² = 0.7693
Ham Listeria 150
Linear (Ham Listeria 150)


Turkey-*Listeria monocytogenes*-65.6°C
Log CFU/g
Seconds
y = 0.0267x + 7.9943
R² = 0.9744
Turkey Listeria 150
Linear (Turkey Listeria 150)


Beef-*Listeria monocytogenes*-71.1°C
Log CFU/g
Seconds
y = -0.0407x + 5.7475
R² = 0.5562
Beef Listeria 160
Linear (Beef Listeria 160)


Ham-*Listeria monocytogenes*-71.1°C
Log CFU/g
Seconds
y = -0.0498x + 6.1152
R² = 0.6072
Ham Listeria 160
Linear (Ham Listeria 160)

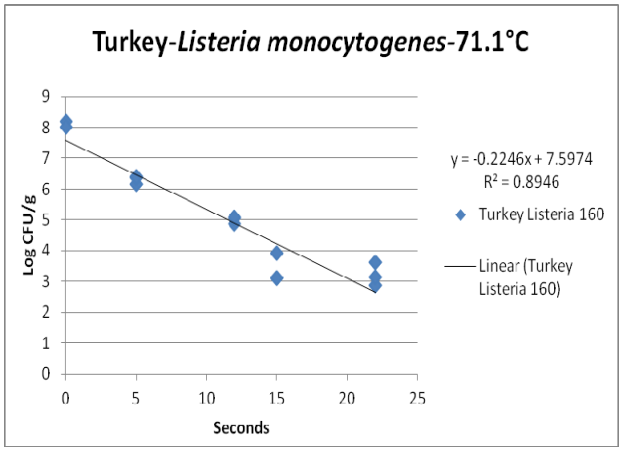
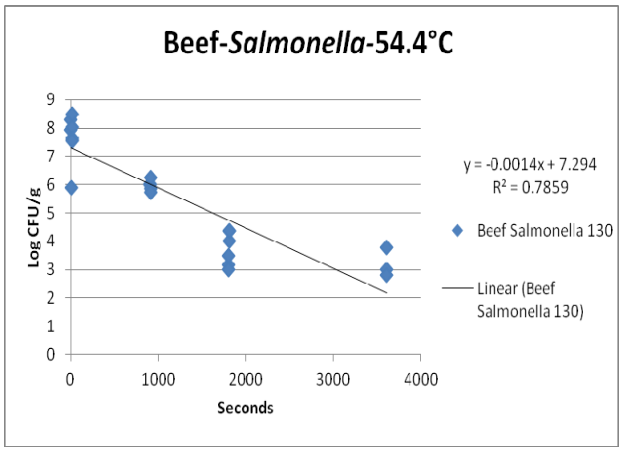
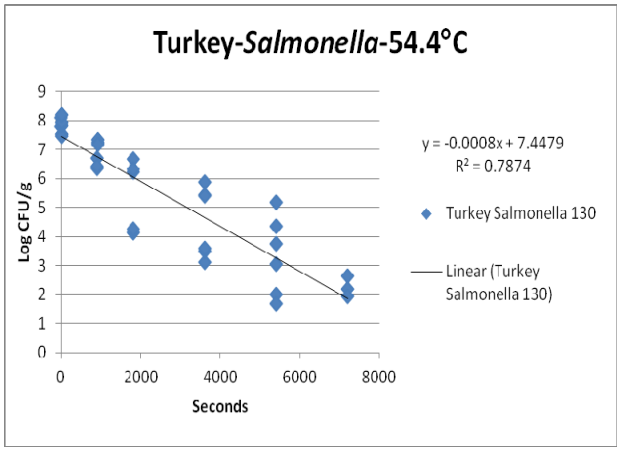
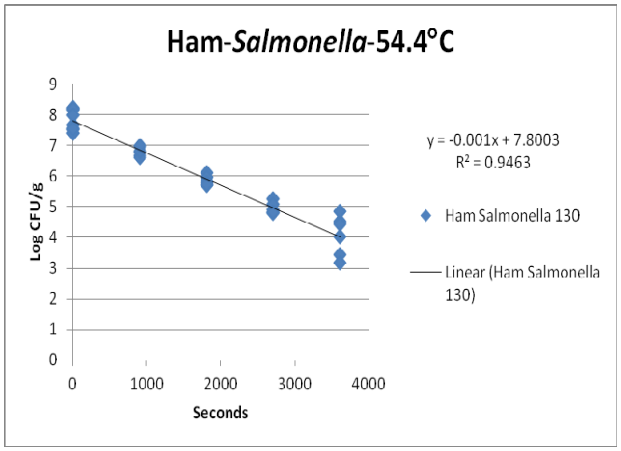
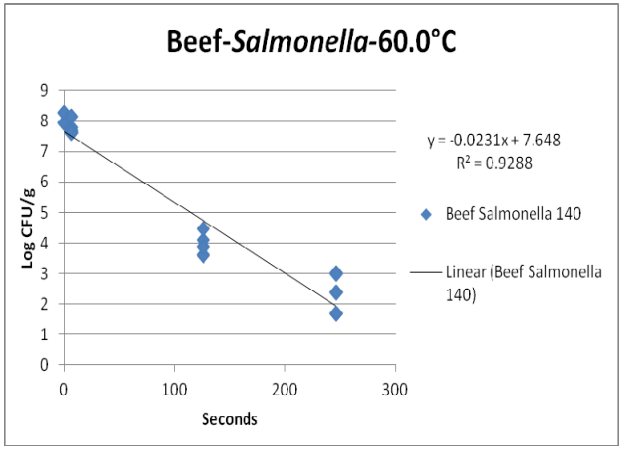

Turkey-*Listeria monocytogenes*-71.1°C
Log CFU/g
Seconds
y = -0.2246x + 7.5974
R² = 0.8946
Turkey Listeria 160
Linear (Turkey Listeria 160)

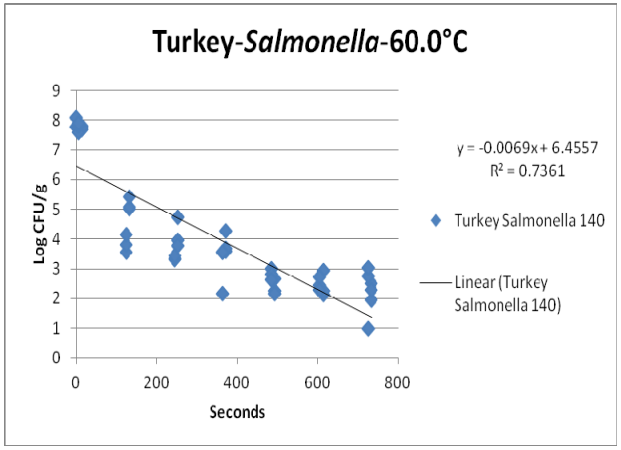
Figure 2b: Determination of D-values for *Salmonella* in roast beef, turkey deli-breast, and boneless ham by plotting Log CFU/g pathogen levels against temperature.

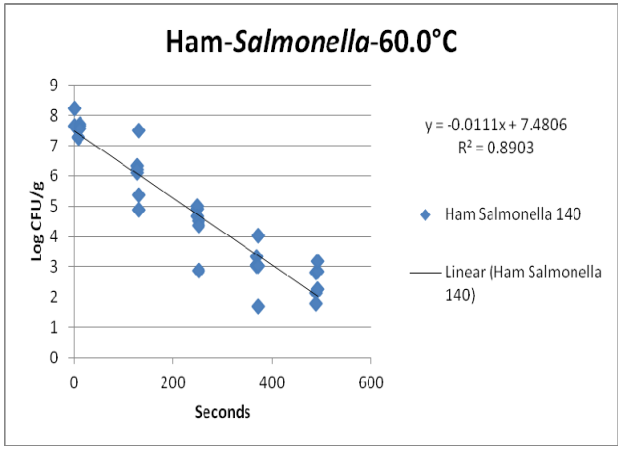


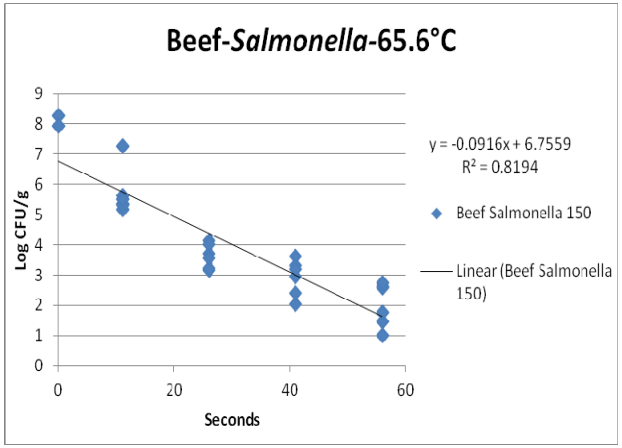


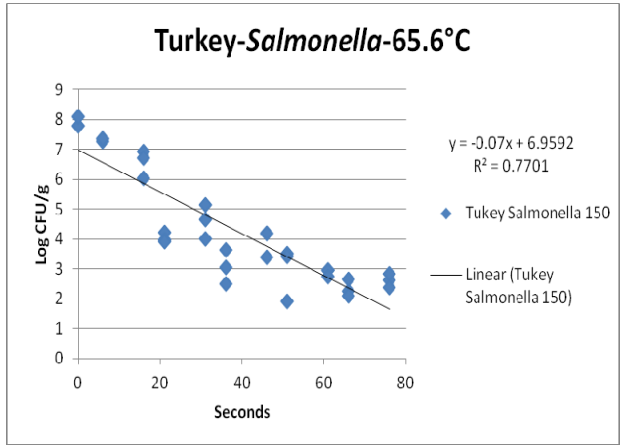


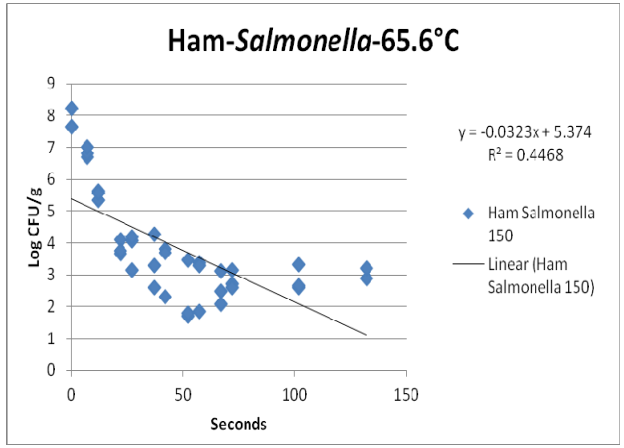


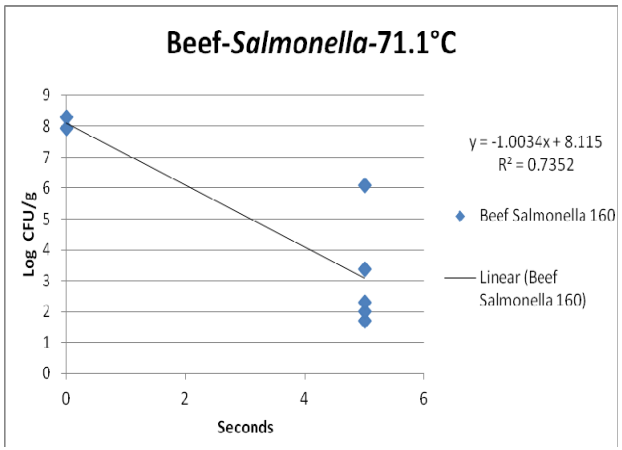


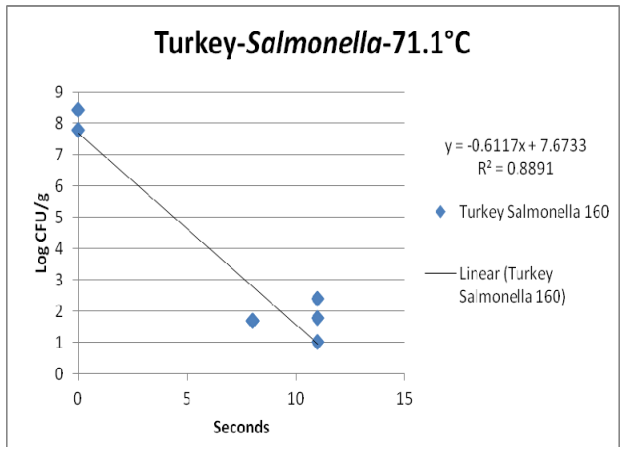



Beef-Salmonella-65.6°C
Log CFU/g
Seconds
y = -0.0916x + 6.7559
R² = 0.8194
Beef Salmonella 150
Linear (Beef Salmonella 150)


Turkey-Salmonella-65.6°C
Log CFU/g
Seconds
y = -0.07x + 6.9592
R² = 0.7701
Tukey Salmonella 150
Linear (Tukey Salmonella 150)


Ham-Salmonella-65.6°C
Log CFU/g
Seconds
y = -0.0323x + 5.374
R² = 0.4468
Ham Salmonella 150
Linear (Ham Salmonella 150)


Beef-Salmonella-71.1°C
Log CFU/g
Seconds
y = -1.0034x + 8.115
R² = 0.7352
Beef Salmonella 160
Linear (Beef Salmonella 160)


Turkey-Salmonella-71.1°C
Log CFU/g
Seconds
y = -0.6117x + 7.6733
R² = 0.8891
Turkey Salmonella 160
Linear (Turkey Salmonella 160)

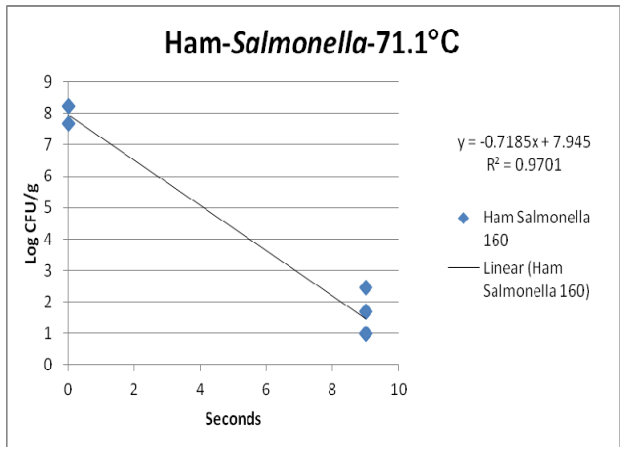
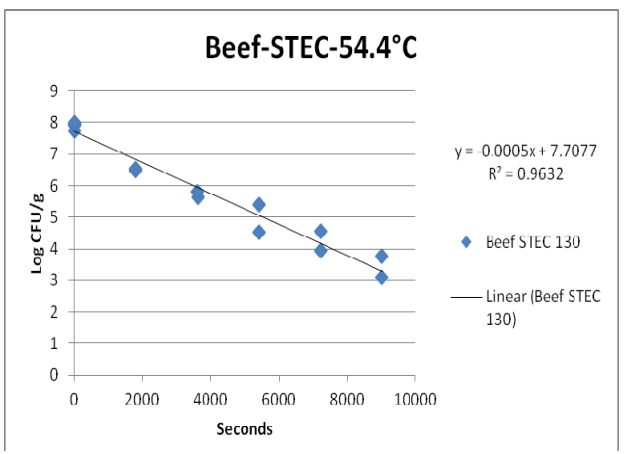
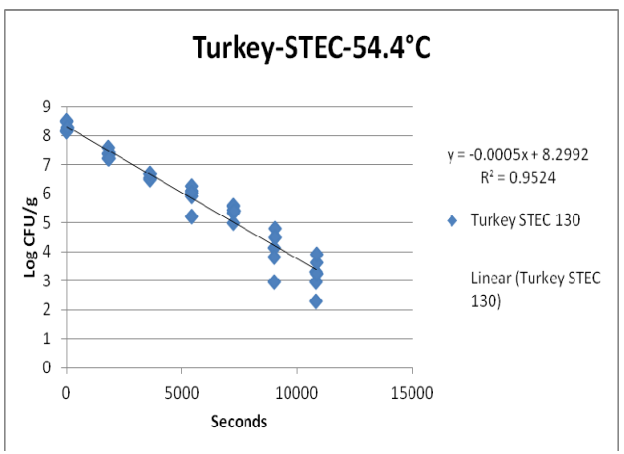
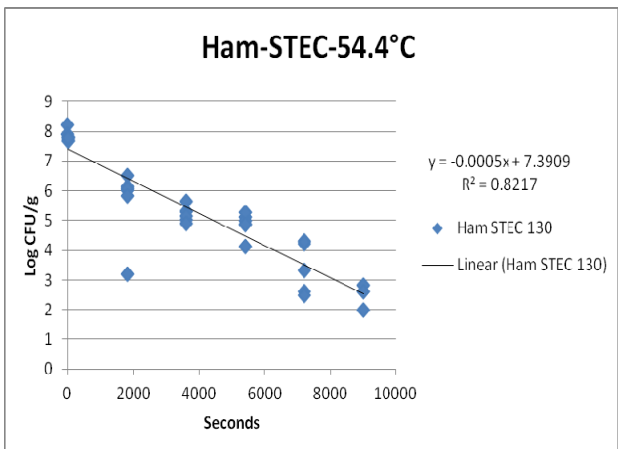
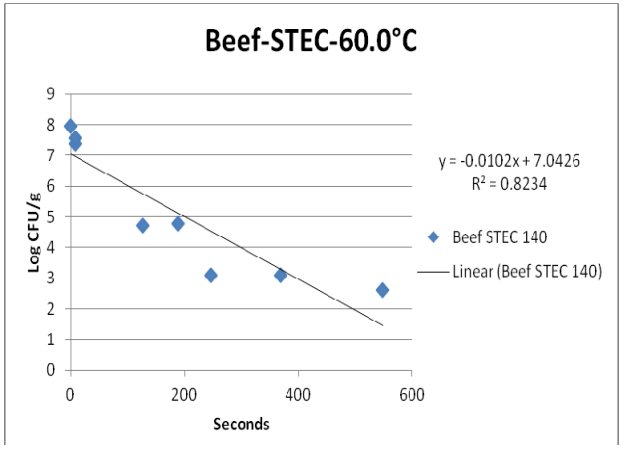

Ham-Salmonella-71.1°C
Log CFU/g
Seconds
y = -0.7185x + 7.945
R² = 0.9701
Ham Salmonella 160
Linear (Ham Salmonella 160)

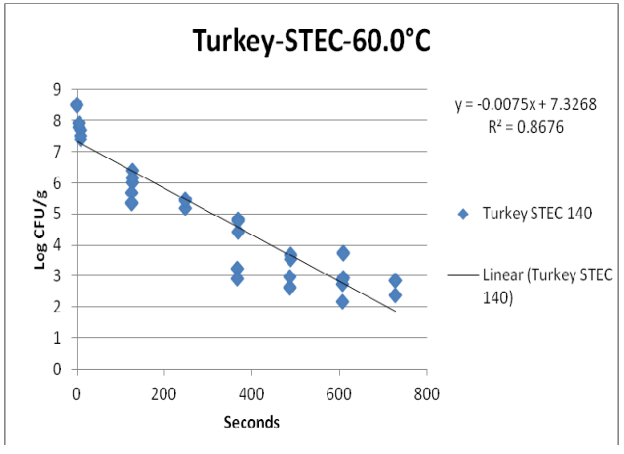
Figure 2c: Determination of D-values for STEC in roast beef, turkey deli-breast, and boneless ham by plotting Log CFU/g pathogen levels against temperature.

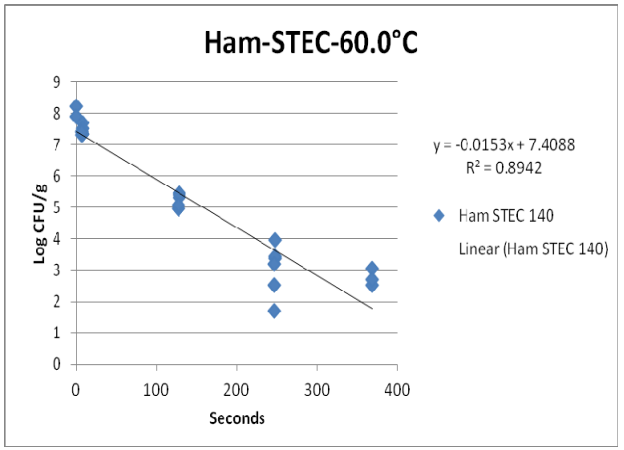


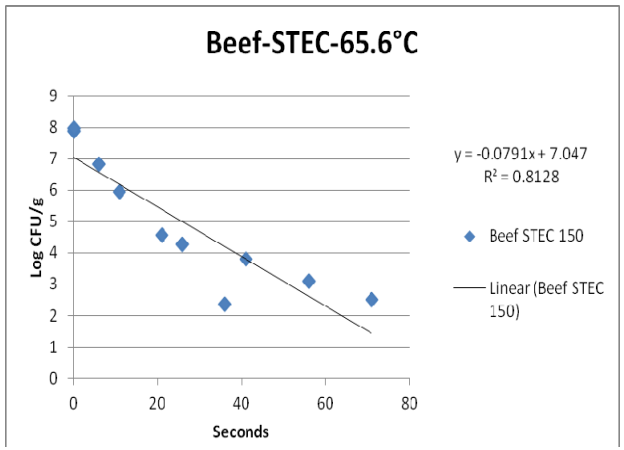


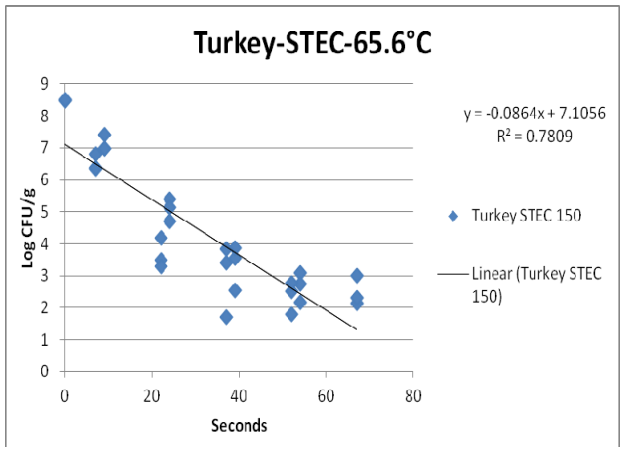


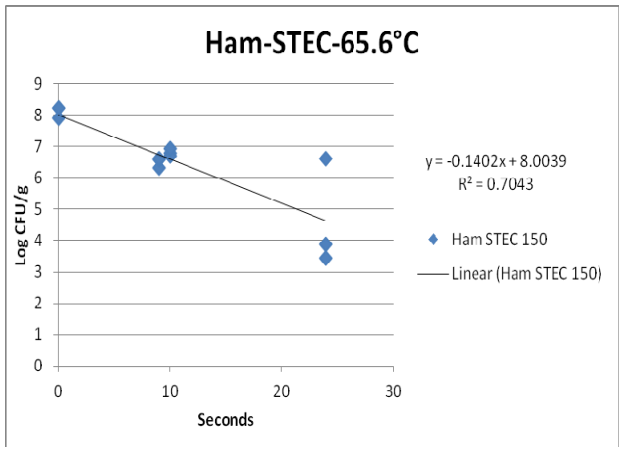


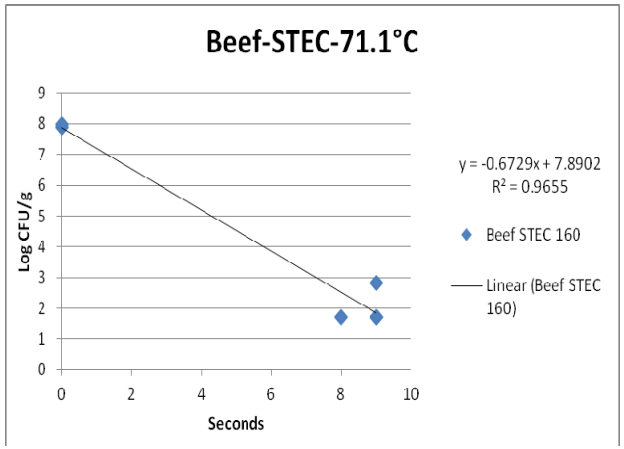


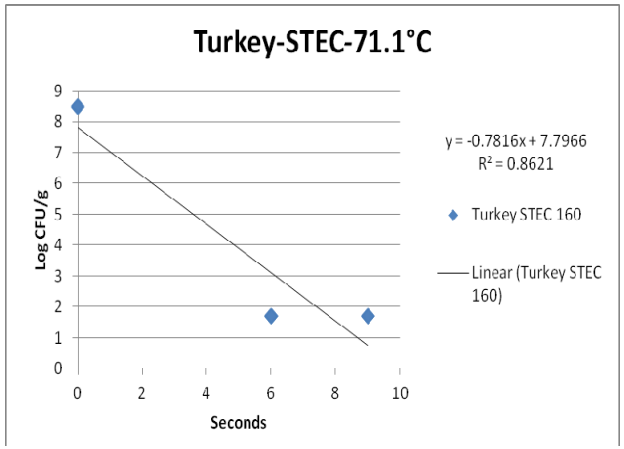



Beef-STEC-65.6°C
Log CFU/g
Seconds
y = -0.0791x + 7.047
R² = 0.8128
Beef STEC 150
Linear (Beef STEC 150)


Turkey-STEC-65.6°C
Log CFU/g
Seconds
y = -0.0864x + 7.1056
R² = 0.7809
Turkey STEC 150
Linear (Turkey STEC 150)


Ham-STEC-65.6°C
Log CFU/g
Seconds
y = -0.1402x + 8.0039
R² = 0.7043
Ham STEC 150
Linear (Ham STEC 150)


Beef-STEC-71.1°C
Log CFU/g
Seconds
y = -0.6729x + 7.8902
R² = 0.9655
Beef STEC 160
Linear (Beef STEC 160)


Turkey-STEC-71.1°C
Log CFU/g
Seconds
y = -0.7816x + 7.7966
R² = 0.8621
Turkey STEC 160
Linear (Turkey STEC 160)

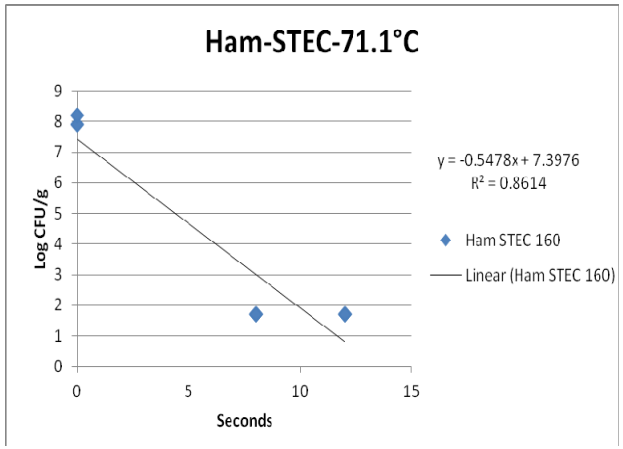

Ham-STEC-71.1°C
Log CFU/g
Seconds
y = -0.5478x + 7.3976
R² = 0.8614
Ham STEC 160
Linear (Ham STEC 160)

Figure 3. Determination of z-values for *Salmonella* in roast beef, turkey deli-breast, and boneless ham by plotting D-values (y-axis) against temperature (x-axis).

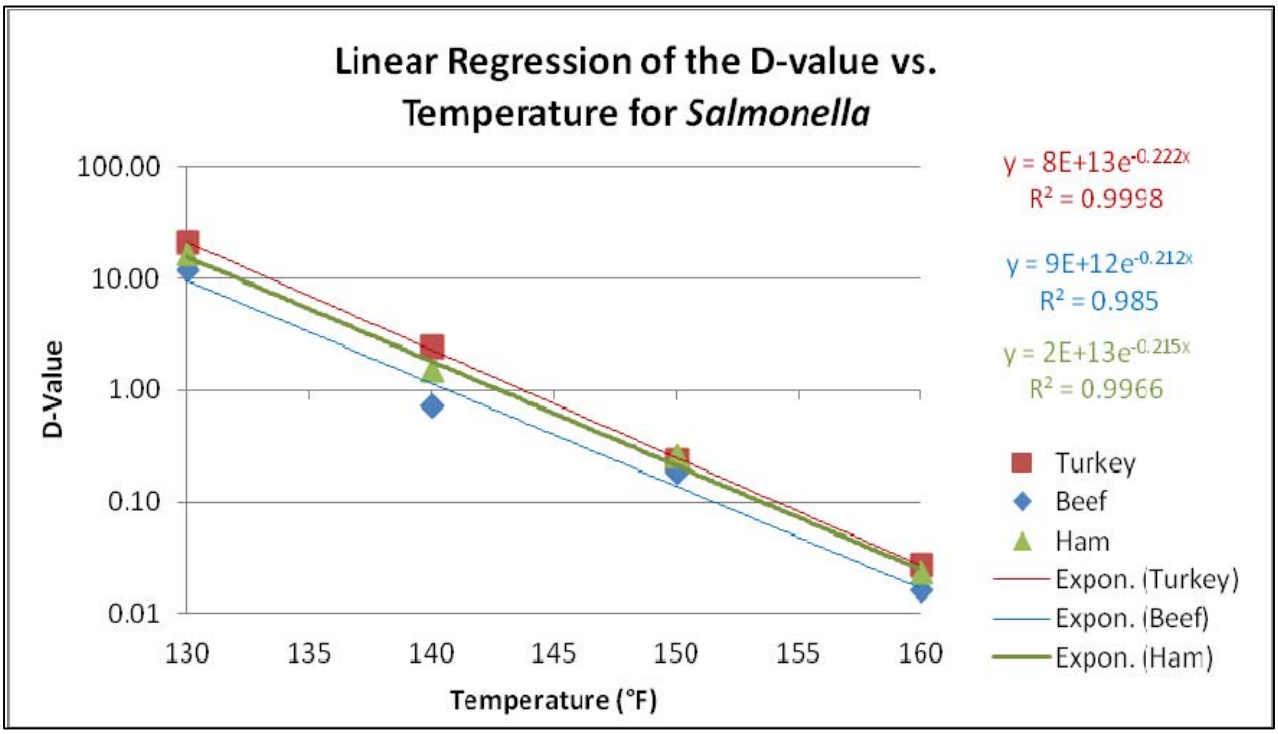


Figure 4. Determination of z-values for *Listeria monocytogenes* in roast beef, turkey deli-breast, and boneless ham by plotting D-values (y-axis) against temperature (x-axis).

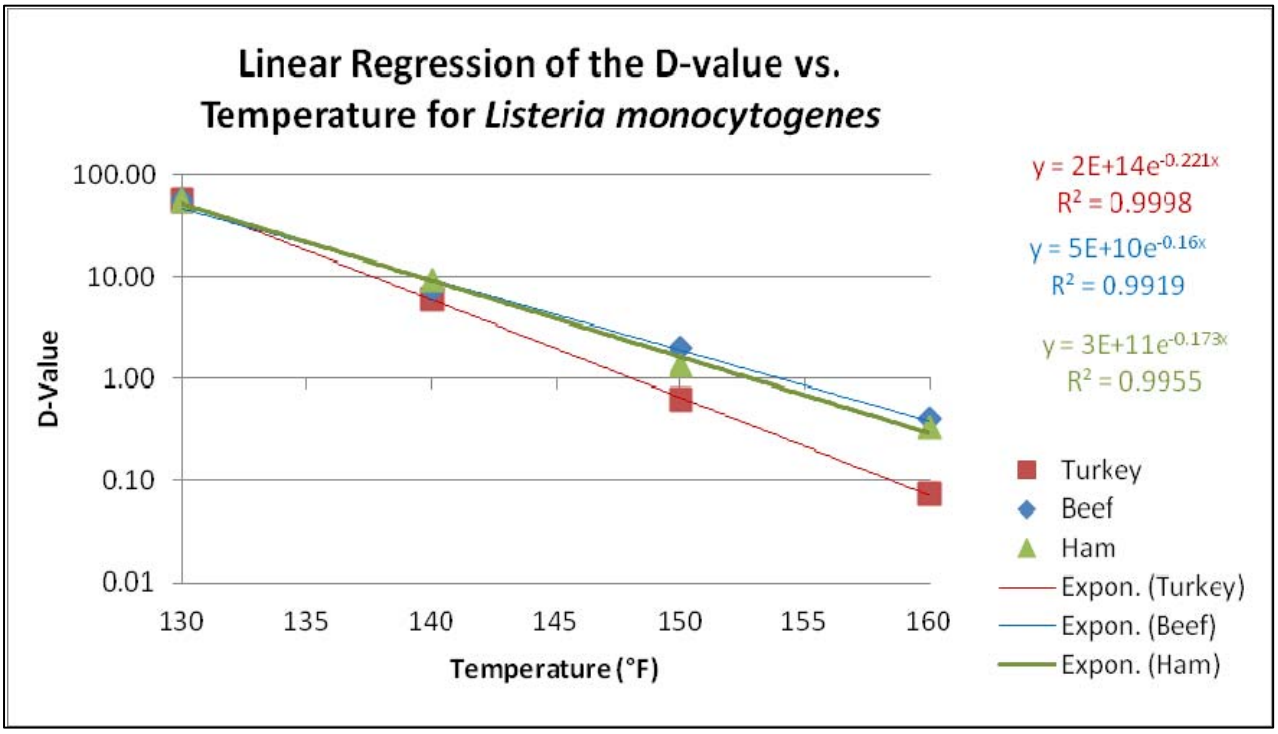


Figure 5. Determination of z-values for STEC in roast beef, turkey deli-breast, and boneless ham by plotting D-values (y-axis) against temperature (x-axis).

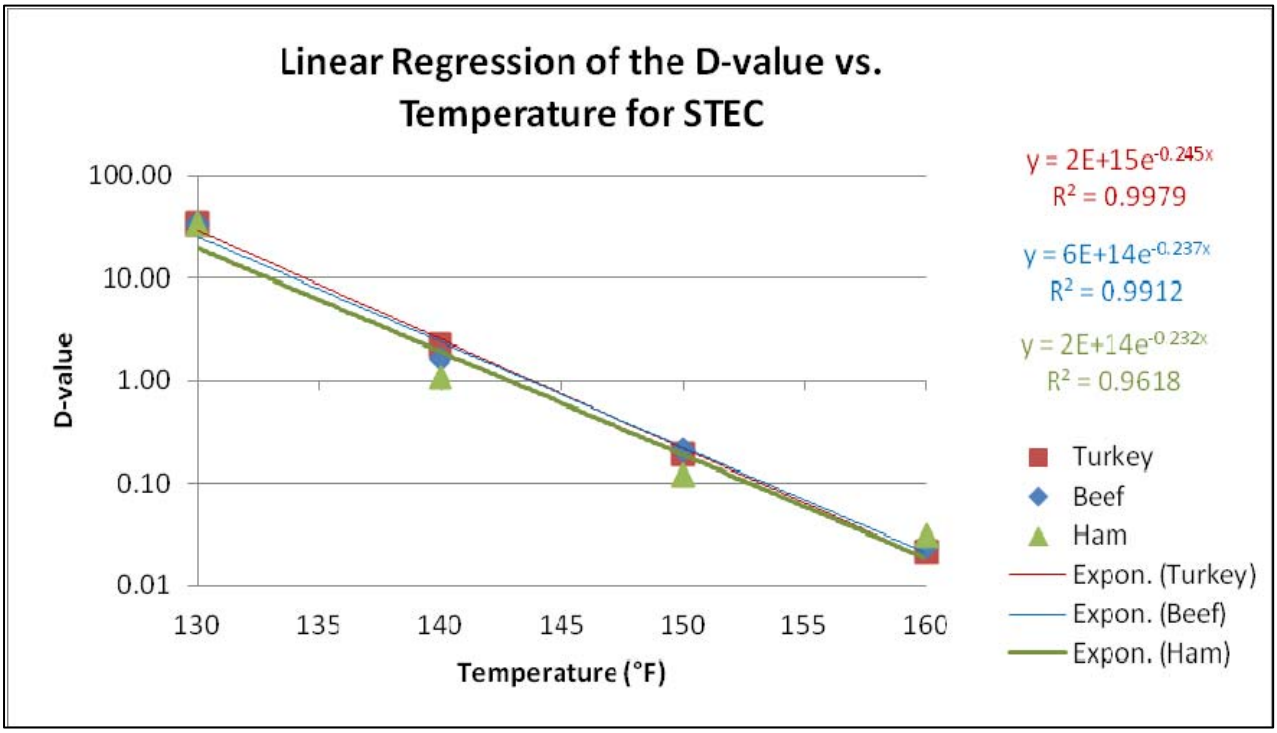


Figure 6. Integrated thermal process /pathogen lethality profile for roast beef inoculated with *Salmonella,* stuffed in 4" diameter moisture impermeble casings , and heated to a final internal temperature of 130°F

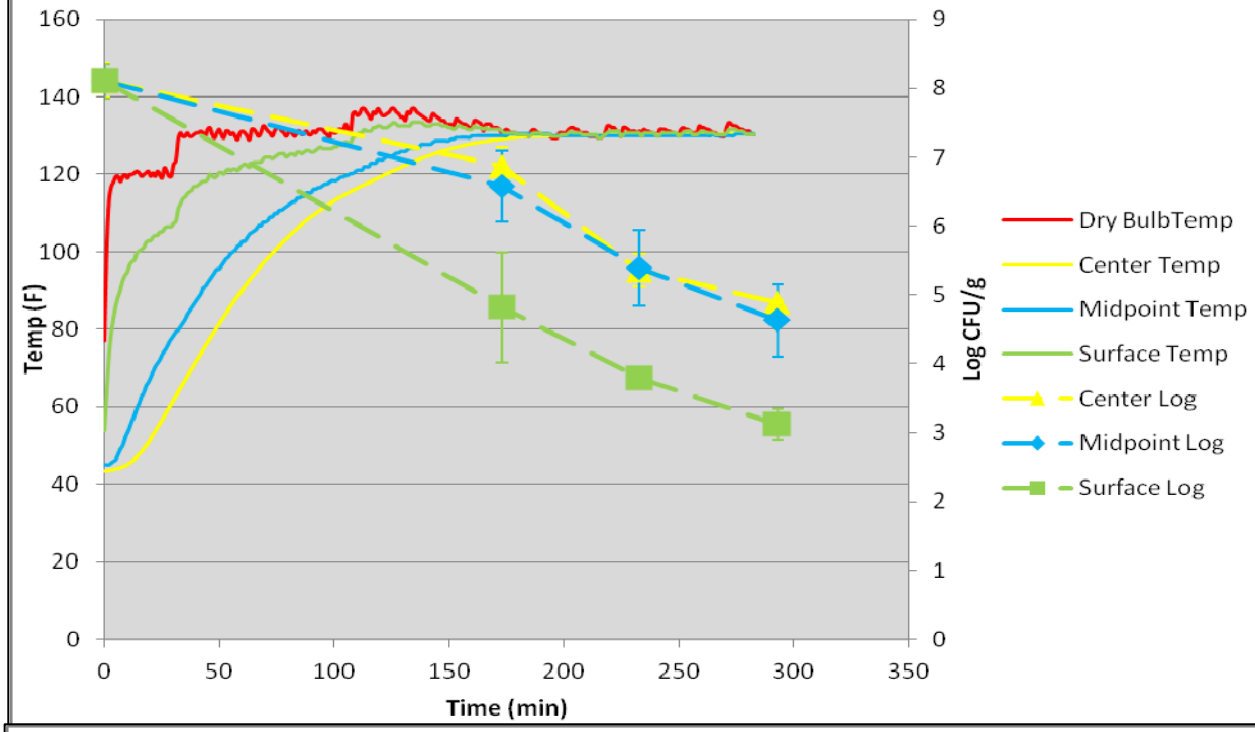


Figure 7. Integrated thermal process /pathogen lethality profile for roast beef inoculated with *Salmonella,* stuffed in 4" diameter moisture impermeble casings , and heated to a final internal temperature of 145°F

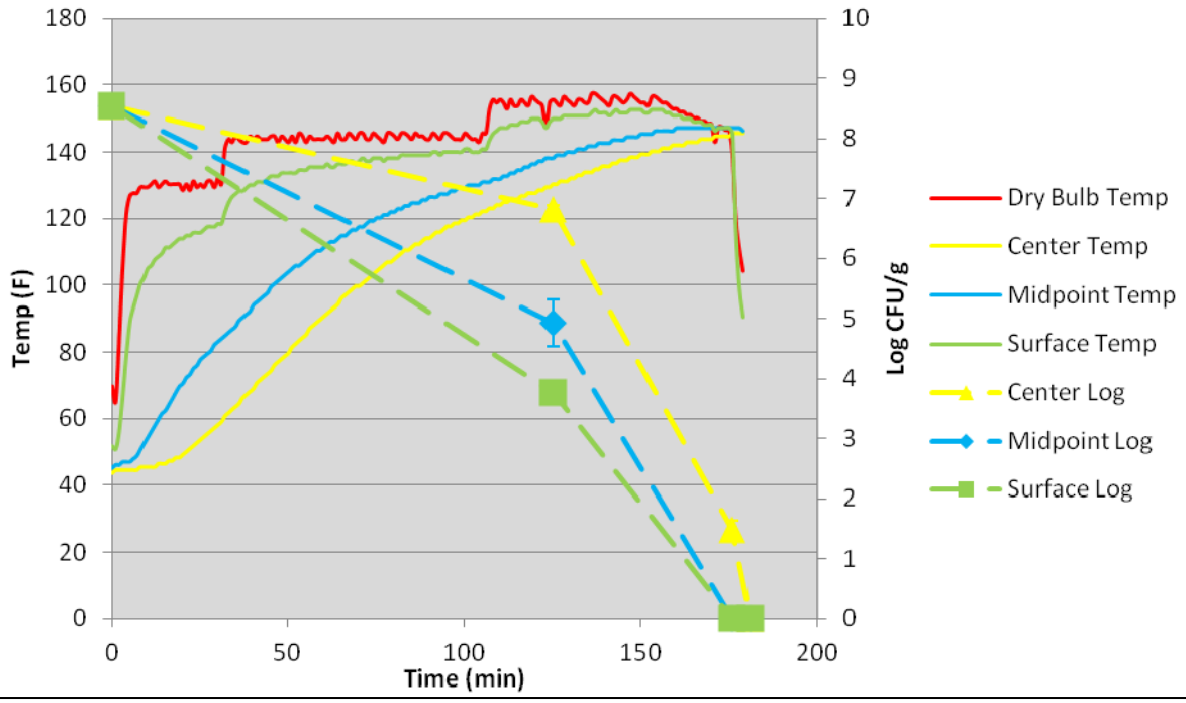


Figure 8. Integrated thermal process /pathogen lethality profile for roast beef inoculated with Salmonella, stuffed in 4" diameter moisture impermeble casings , and heated to a final internal temperature of 160°F.

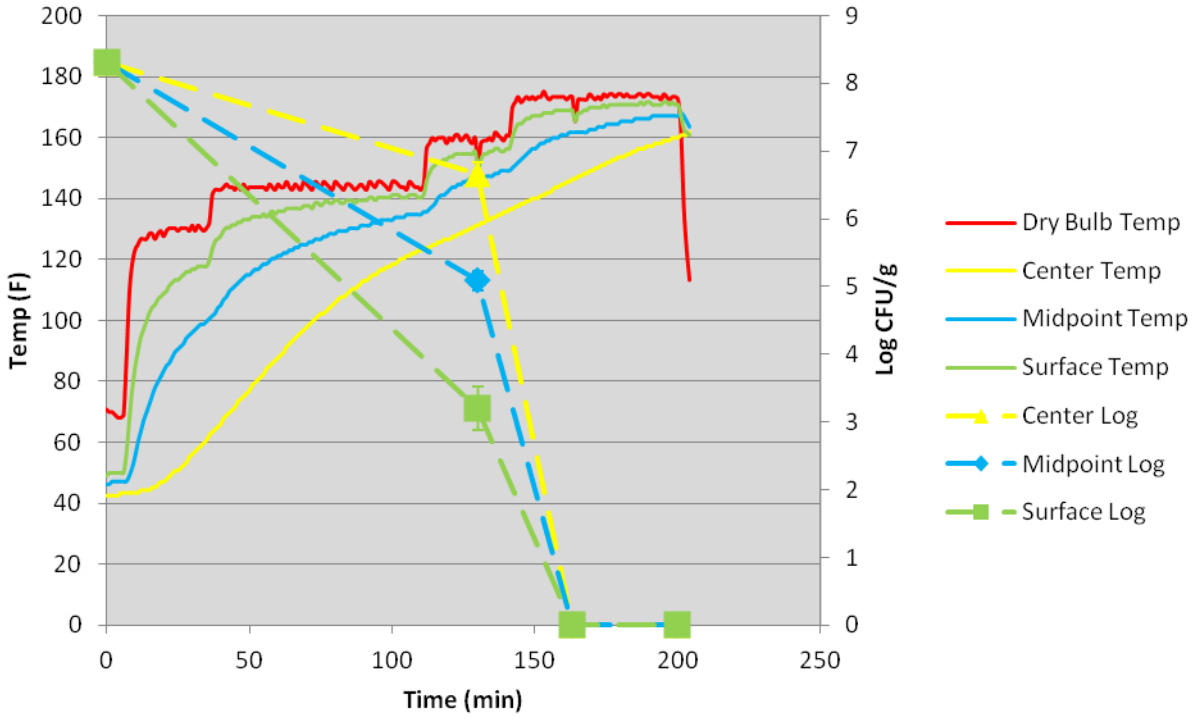


Figure 9. Integrated thermal process/pathogen lethality profile for roast beef inoculated with shiga-toxin producing *Escherichia coli,* stuffed in 4" diameter moisture impermeable casings and heated to a final internal temperature of 130°F.

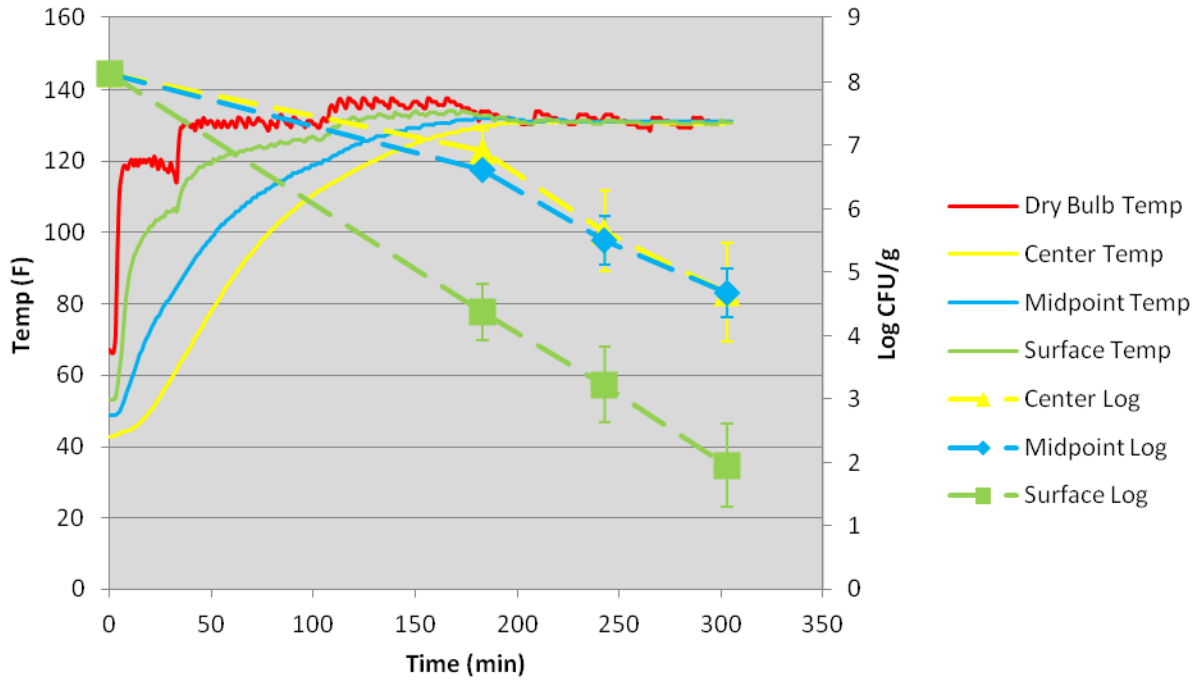


Figure 10. Integrated thermal process/pathogen lethality profile for roast beef inoculated with shiga-toxin producing *Escherichia coli,* stuffed in 4" diameter moisture impermeable casings and heated to a final internal temperature of 145°F.

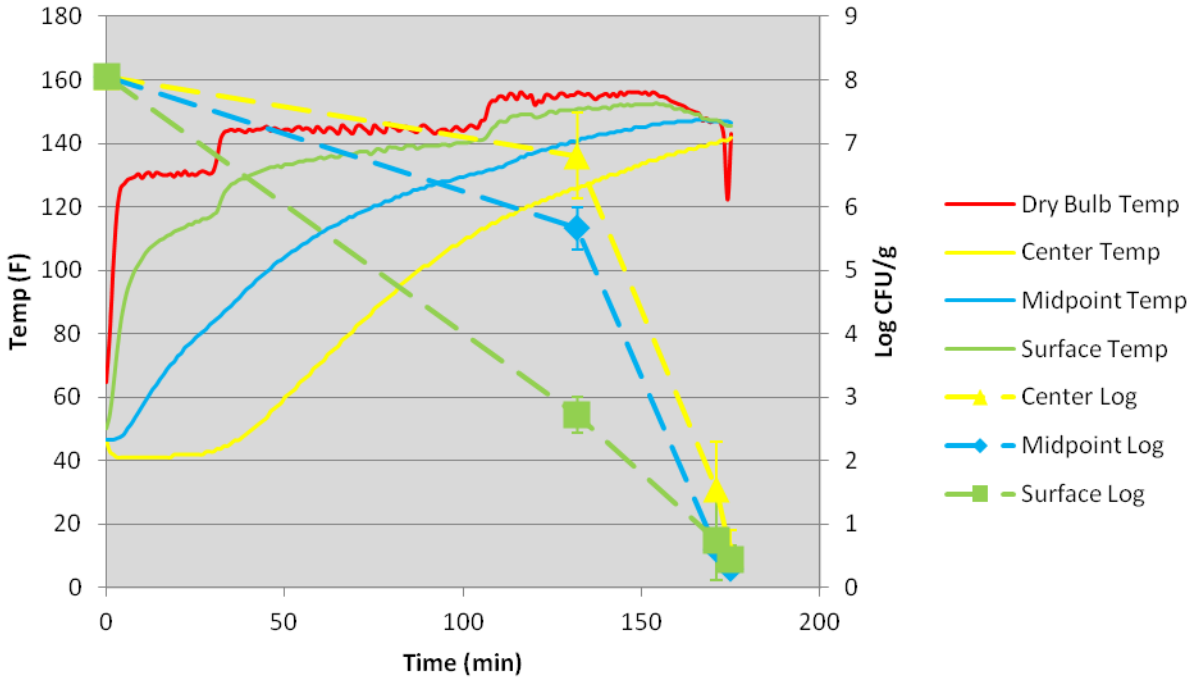


Figure 11. Integrated thermal process/pathogen lethality profile for roast beef inoculated with shiga-toxin producing *Escherichia coli,* stuffed in 4" diameter moisture impermeable casings and heated to a final internal temperature of 160°F.

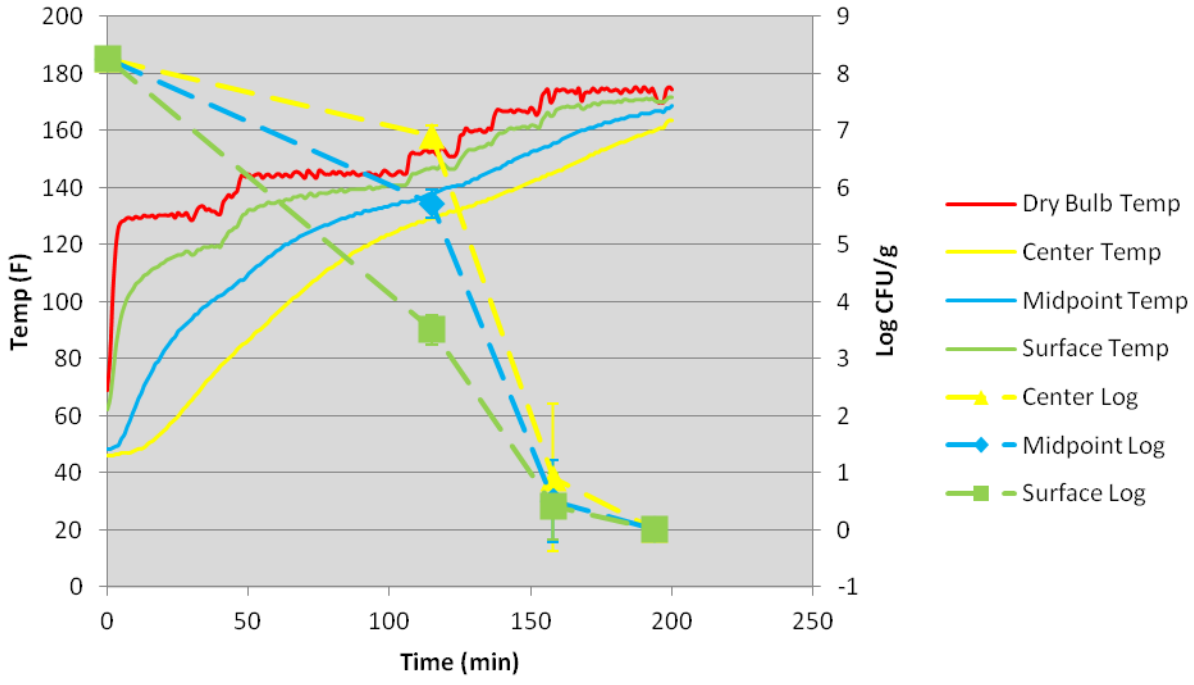


Figure 12. Integrated thermal process/pathogen lethality profile for deli-style turkey breast inoculated with *Salmonella,* stuffed in 4" moisture impermeable casings and heated to a final internal temperature of 160°F.

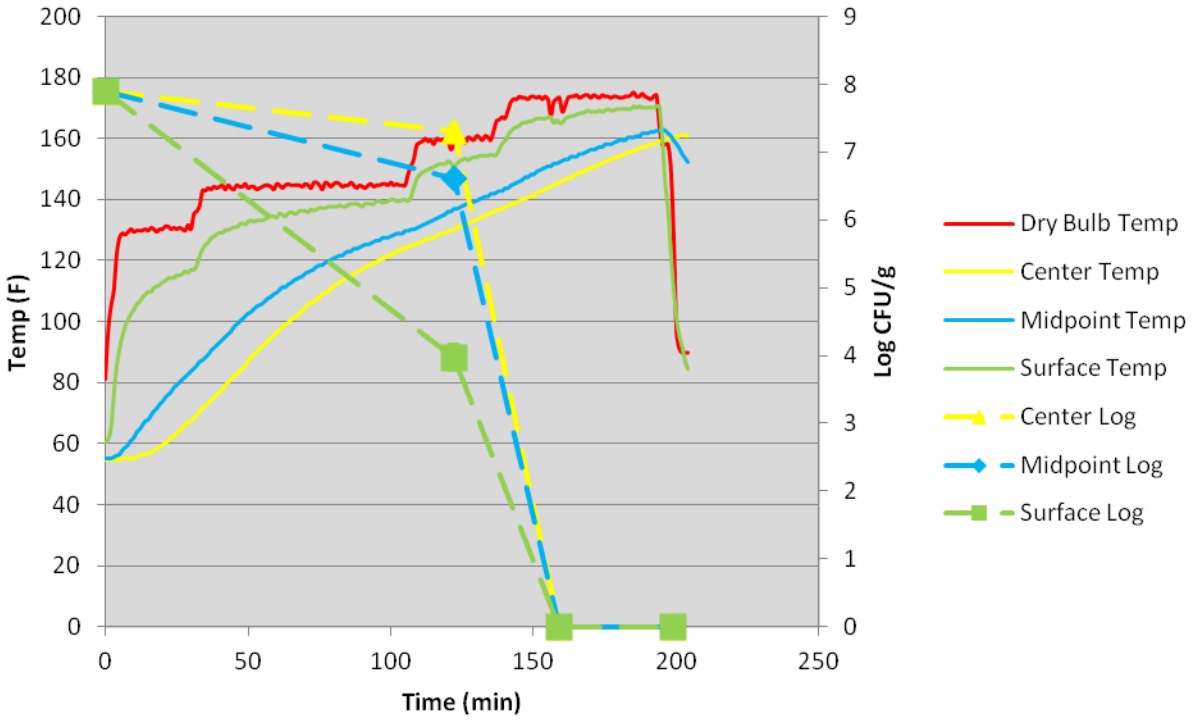


Figure 13. Integrated thermal process/pathogen lethality profile for boneless ham inoculated with *Listeria monocytogenes,* stuffed in 4" permeable casings and heated with controlled humidity to a final internal temperature of 145°F.

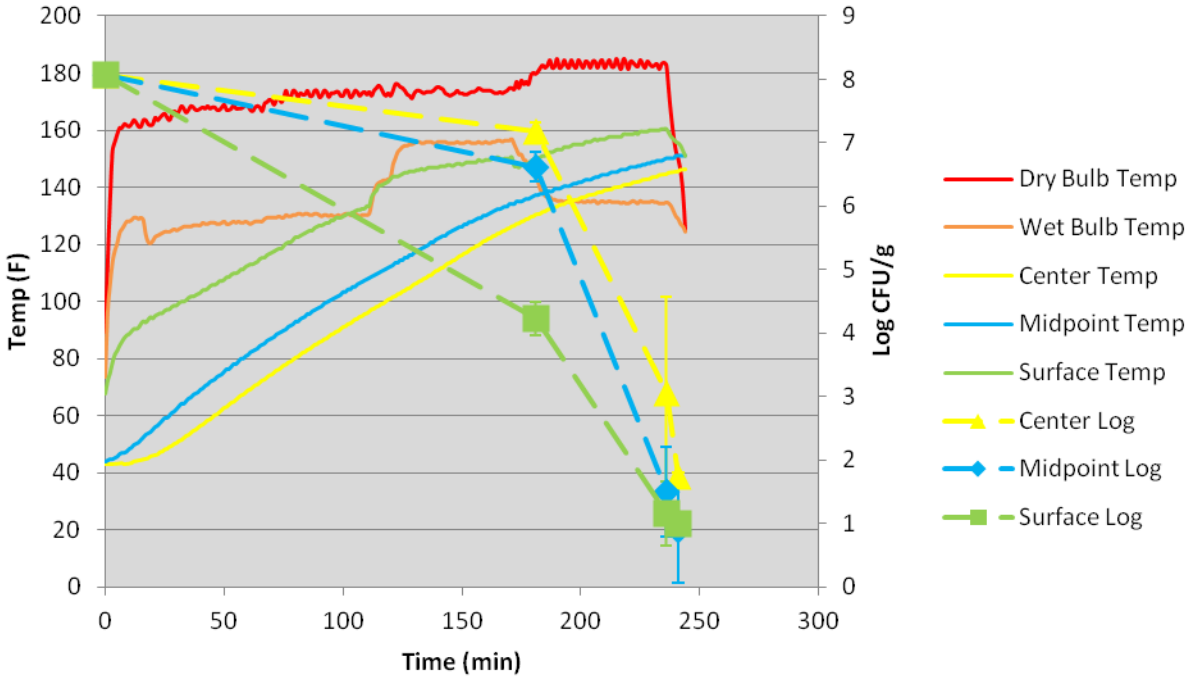


Figure 14. Integrated thermal process/pathogen lethality profile for boneless ham inoculated with *Listeria monocytogenes*, stuffed in 4" permeable casings and heated with controlled humidity to a final internal temperature of 160°F.

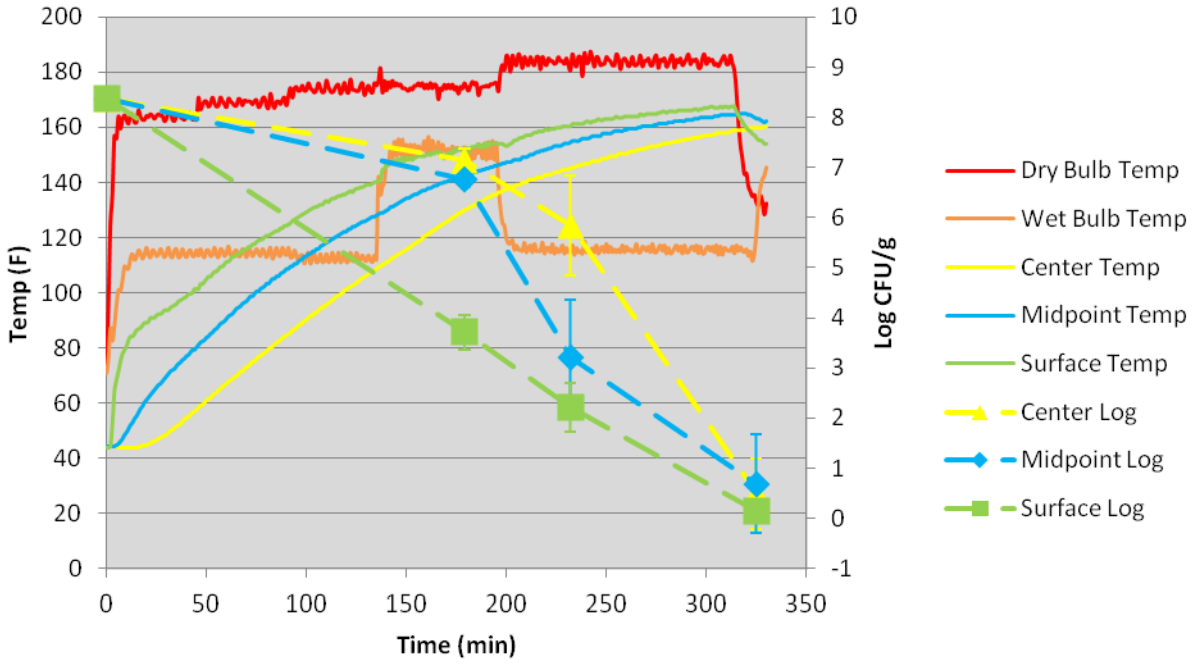


Table 1. Thermal processing schedule utilized for cooking roast beef and deli-style turkey breast (4.0" diameter) stuffed in impermeable plastic casings and heated to the 130°F internal final target temperature.

Step	Type	Time	DryBulb (°F)
1	Steam	30 min	120
2	Steam	75 min	130
3	Steam	to IT of 128°F	135
4	Steam	hold	130

Table 2. Thermal processing schedule utilized for cooking roast beef (4.0" diameter) stuffed in impermeable plastic casings and heated to the 145°F internal final target temperature.

Step	Type	Time	DryBulb (°F)
1	Steam	30 min	130
2	Steam	75 min	145
3	Steam	to IT of 142°F	155
4	Steam	hold for 5 min	145

Table 3. Thermal processing schedule utilized for cooking roast beef and deli-style turkey breast (4.0" diameter) stuffed in impermeable plastic casings and heated to the 160°F internal final target temperature.

Step	Type	Time	DryBulb (°F)
1	Steam	30 min	130
2	Steam	75 min	145
3	Steam	30 min	160
4	Steam	to IT=160^{o}F	175

Table 4. Thermal processing schedule utilized for cooking boneless ham (4.0" diameter) to 145°F stuffed into a fibrous casing.

Step	Type	Time	DryBulb (°F)	WetBulb (°F)
1	Dry	30 min	165	105
2	Dry	45 min	170	105
3	Dry	45 min	175	105
4	Cook	60 min	175	150
5	Cook	to IT=145°F	185	105

Table 5. Thermal processing schedule utilized for cooking boneless ham (4.0" diameter) to 160°F stuffed into a fibrous casing.

Step	Type	Time	DryBulb (°F)	WetBulb (°F)
1	Dry	30 min	165	105
2	Dry	45 min	170	105
3	Dry	45 min	175	105
4	Cook	60 min	175	150
5	Cook	to IT=160°F	185	105

Table 6. Product, pathogen, target final temperature, and experimental sampling/pull time points followed for roast beef, turkey breast, and boneless ham treatments inoculated with *Salmonella, L. monocytogenes,* and STEC.

			Sampling/Pull Time Points (°F)			
Product	**Pathogen**	**Target Final Temperature (°F)**	**#1**	**#2**	**#3**	**#4**
Turkey	*Salmonella*	160	130	145	160	<40
Roast beef	*Salmonella*	160	130	145	160	<40
Roast beef	*Salmonella*	145	130	145	145 / 5min	<40
Roast beef	*Salmonella*	130	130	130 / 1hr	130 / 2hr	<40
Roast beef	STEC	160	130	145	160	<40
Roast beef	STEC	145	130	145	145 / 5min	<40
Roast beef	STEC	130	130	130 / 1hr	130 / 2hr	<40
Ham	*Listeria*	160	130	145	160	<40
Ham	*Listeria*	145	130	145	145 / 5min	<40

Table 7. D- and z-values, in minutes, for *Salmonella*, *Listeria*, and STEC in roast beef, turkey breast, and ham.

	130°F	**140°F**	**150°F**	**160°F**	**Z-value**
Salmonella					
Beef	11.90	0.72	0.18	0.02	10.86
Turkey	20.83	2.42	0.24	0.03	10.37
Ham	16.67	1.50	0.25	0.02	10.71
Listeria					
Beef	55.56	7.25	1.98	0.41	14.39
Turkey	55.56	5.95	0.62	0.07	10.42
Ham	55.56	9.26	1.33	0.33	13.31
STEC					
Beef	33.33	1.63	0.21	0.02	9.72
Turkey	33.33	2.22	0.19	0.02	9.39
Ham	33.33	1.09	0.12	0.03	9.93

Table 8. Treatments (meat product, pathogen, final temperature/time) and total log reduction observed during validation of D- and Z-values.

Product	Pathogen	Final Temperature/ Hold Time	Total Reduction During Cooking (Log CFU/g)
Turkey	Salmonella	160°F/0 sec	>7.00
Roast beef	Salmonella	130°F/2 hr	3.21 ±0.21
Roast beef	Salmonella	145°F/5 min	>7.00
Roast beef	Salmonella	160°F/0 sec	>7.00
Roast beef	STEC	130°F/2 hr	3.44 ±0.64
Roast beef	STEC	145°F/5 min	>7.00
Roast beef	STEC	160°F/0 sec	6.95 ±0.08
Ham	Listeria	145°F/5 min	5.01 ±1.63
Ham	Listeria	160°F/0 sec	>7.00

Table 9: Time and Temperature Table for Achieving Lethality of *Salmonella* in Deli-Style Turkey.

Time and Temperature Table for Achieving Lethality of *Salmonella* in Deli-Style Turkey

		Minimum Time Needed in Minutes after Temperature is Reached	
Degree (F)	D-Value*	6.5 log lethality	7.0 log lethality
130	20.83	135.395	145.8
131		123.4	132.9
132		111.5	120.0
133		99.5	107.1
134		87.5	94.3
135		75.6	81.4
136		63.6	68.5
137		51.6	55.6
138		39.7	42.7
139		27.7	29.8
140	2.42	15.73	16.9
141		14.3	15.4
142		12.9	13.9
143		11.5	12.4
144		10.1	10.8
145		8.6	9.3
146		7.2	7.8
147		5.8	6.3
148		4.4	4.7
149		3.0	3.2
150	0.24	1.56	1.7
151		1.4	1.5
152		1.3	1.4
153		1.2	1.2
154		1.0	1.1
155		0.9	0.9
156		0.7	0.8
157		0.6	0.7
158		0.5	0.5
159		0.3	0.4
160	0.03	0.195	0.2

* D-values from AMIF conducted research product "Developing Validated Time-Temperature Thermal Processing Guidelines for Ready-To-Eat Deli Meat and Poultry Products"

Table 10: Time and Temperature Table for Achieving Lethality of *Listeria monocytogenes* in Boneless Ham.

Time and Temperature Table for Achieving Lethality of *Listeria monocytogenes* in Boneless Ham			
		Minimum Time Needed in Minutes after Temperature is Reached	
Degree (F)	D-Value	4.0 log lethality	7.0 log lethality
130	55.56	222.24	388.9
131		203.7	356.5
132		185.2	324.1
133		166.7	291.7
134		148.2	259.3
135		129.6	226.9
136		111.1	194.5
137		92.6	162.1
138		74.1	129.6
139		55.6	97.2
140	9.26	37.04	64.8
141		33.9	59.3
142		30.7	53.7
143		27.5	48.2
144		24.4	42.6
145		21.2	37.1
146		18.0	31.5
147		14.8	26.0
148		11.7	20.4
149		8.5	14.9
150	1.33	5.32	9.3
151		4.9	8.6
152		4.5	7.9
153		4.1	7.2
154		3.7	6.5
155		3.3	5.8
156		2.9	5.1
157		2.5	4.4
158		2.1	3.7
159		1.7	3.0
160	0.33	1.32	2.3
* D-values from AMIF conducted research product "Developing Validated Time-Temperature Thermal Processing Guidelines for Ready-To-Eat Deli Meat and Poultry Products"			

Table 11: Time and Temperature Table for Achieving Lethality of *Listeria monocytogenes* in Boneless Ham.

Time and Temperature Table for Achieving Lethality of Shiga-toxin producing *E. coli* (STEC) in Roast Beef			
		Minimum Time Needed in Minutes after Temperature is Reached	
Degree (F)	D-Value	5 log lethality	7.0 log lethality
130	33.33	166.65	233.3
131		150.8	211.1
132		135.0	188.9
133		119.1	166.7
134		103.3	144.6
135		87.4	122.4
136		71.6	100.2
137		55.7	78.0
138		39.9	55.8
139		24.0	33.6
140	1.63	8.15	11.4
141		7.4	10.4
142		6.7	9.4
143		6.0	8.4
144		5.3	7.4
145		4.6	6.4
146		3.9	5.4
147		3.2	4.5
148		2.5	3.5
149		1.8	2.5
150	0.21	1.05	1.5
151		1.0	1.3
152		0.9	1.2
153		0.8	1.1
154		0.7	0.9
155		0.6	0.8
156		0.5	0.7
157		0.4	0.5
158		0.3	0.4
159		0.2	0.3
160	0.02	0.1	0.1
* D-values from AMIF conducted research product "Developing Validated Time-Temperature Thermal Processing Guidelines for Ready-To-Eat Deli Meat and Poultry Products"			

Table 12. Proximate composition of turkey, roast beef, and ham.

Product	**Moisture (%)**	**a_w**	**pH**	**NaCl (%)**
Turkey	73.6 ±2.00	0.983 ±0.002	6.25 ±0.05	1.40 ±0.05
Roast beef	73.6 ±0.77	0.974 ±0.004	6.01 ±0.03	1.67 ±0.10
Ham	73.0 ±1.93	0.974 ±0.004	6.40 ±0.08	2.15 ±0.11