



Processed Meat Thermal Processing Food Safety - Generating D-Values for *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli*.

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Abstract: USDA, FSIS thermal processing guidelines (e.g. Appendix A for cooked beef, roast beef, and cooked corned beef, and Time-Temperature Tables for Cooking Ready-to-Eat Poultry Products) are widely used as validation support for cooking processes, but these procedures were developed and validated only for *Salmonella* in a limited number of products. To determine the extent to which Appendix A can safely be applied to other pathogens and products, a study was conducted to compare the thermal-death times of *Salmonella*, *Listeria monocytogenes*, and shiga toxin-producing *E. coli* (STEC) in model products representing roast beef, turkey breast, and boneless ham. Raw batter for each of the 3 products was inoculated with 8 log CFU/g of a multi-strain mixture of *L. monocytogenes*, *Salmonella*, or STEC. One-gram portions of inoculated roast beef, turkey breast, or ham batter were flattened into a thin film in moisture-impermeable pouches, vacuum-packaged, and heated at 54.4, 60.0, 65.6, or 71.1°C in a water bath. Triplicate samples were removed at predetermined time points and enumerated for surviving pathogens. The time needed to yield a 6.5-log reduction of *Salmonella* and STEC at 60.0, 65.6, or 71.1°C for the three product types was comparable to the times prescribed by USDA, FSIS Appendix A for *Salmonella* inactivation; however, at 54.4°C similar inactivation levels were not observed. *L. monocytogenes* showed greater thermostolerance than *Salmonella* and STEC for all 3 product types. These data suggest that current USDA, FSIS thermal processing guidelines are acceptable tools for ensuring the safety of cooking processes at 60.0°C or higher to inactivate *Salmonella* and STEC in the product types, but longer dwell times may be necessary to yield comparable log reduction of *L. monocytogenes*.

Keywords: D-values, *Escherichia coli*, FSIS Appendix A, *Listeria monocytogenes*, shiga toxin-producing *Salmonella*, thermal inactivation

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Introduction

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Thermal treatments are critical in controlling food-borne pathogens in ready-to-eat (RTE) meat and poultry products (U.S. Dept of Agriculture - Food Safety and Inspection Service, 2005a). Currently, the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) requires a ≥ 6.5 -log reduction of *Salmonella* spp. in RTE cooked beef, roast beef, and cooked corned beef and a ≥ 7 -log reduction in cooked

poultry products (USDA - FSIS, 1999). The USDA, FSIS Appendix A “Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products,” (Appendix A; USDA – FSIS, 2017) is used extensively by the meat and poultry industries to establish thermal processes that will meet these prescribed pathogen reduction requirements (USDA - FSIS, 1999). Appendix A guidelines are based on research conducted by Goodfellow and Brown (1978) on the fate of *Salmonella* in beef during cooking; however, they are applied by industry to a wide array of products and target pathogens. Of particular concern are *Listeria monocytogenes* and shiga toxin-producing *Escherichia coli* (STEC), 2 bacterial pathogens capable of causing serious illness that were not yet food safety concerns at the time Appendix A was developed. Additionally, the thermal processing procedures and formulations employed by the work of Goodfellow and Brown were not representative of the wide range of thermal processing procedures currently utilized by meat processors nor the wide array of products available to consumers in the U.S. Due to the limited scope of the research supporting Appendix A, it is important to examine the appropriateness of these guidelines for controlling *Salmonella*, *Listeria monocytogenes*, and STEC when applied to a wider range of conditions (i.e. product type, species, inclusion of sodium nitrite, etc.) by generating new and more encompassing data.

The heat resistance for any pathogen is not a static value. It is the result of interactions between the organism and its environment. As a result, the thermal tolerance of a pathogen at any given temperature can vary widely depending on numerous conditions. Factors such as fat content, water activity, pH, and solute levels (e.g. salt, sodium phosphates) have all been shown to impact the survivability of *Salmonella*, *Listeria*, and STEC, with Gram-positive pathogens being more resistant to thermal inactivation than Gram-negative pathogens (Aljarallah and Adams, 2007; Jay et al., 2005; Fernández et al., 2007; Schultze et al., 2007). Multiple studies have reported a range of D-values (the amount of time required to reduce the population of an organism by 90%, i.e. 1-log, at a specific temperature) for *L. monocytogenes*, *Salmonella*, and STEC when heated in different substrates such as pork, turkey, or beef products (Juneja, 2003; Juneja et al. 2001b; Murphy and Berrang, 2002; Murphy et al., 2000, 2002, 2003, 2004a, 2004b, 2004c, 2004d; O’Bryan et al., 2006). Physical structure can also affect thermotolerance, with *Salmonella* demonstrating greater survivability in whole-muscle products compared to comminuted products (Mogollón et al., 2009; Orta-Ramirez et al., 2005; Tuntivanich et al., 2008). Further complicating this issue is the relatively unknown impact of a multitude of

bacterial properties such as cell concentration, phase of growth, strain, and prior exposure to stressors on thermal tolerance (O’Bryan et al., 2006; Sherry et al., 2004).

The vast number of variables contributing to heat resistance of a pathogen in a meat product can make the selection of appropriate supporting documentation for a thermal process difficult. Numerous studies employing a range of methodologies have determined D- and Z-values (the number of degrees the temperature must be reduced in order to obtain a 1-log reduction of the D-value) for *Salmonella*, *L. monocytogenes*, and STEC. Small differences in the methodologies of these studies, such as varying heating substrates (broth vs. ground meat), sample sizes, or pathogen strain(s) (single strain vs. multi-strain cocktail) can impact the resulting D- and Z-values, making comparisons across this range of studies difficult and complicating their application to real thermal processes. Additionally, most of the D-values reported in the literature are commonly considered to have limited use when utilized for assessing actual thermal processes because the isothermal experiments used to develop most of these D-values do not account for the integrated lethality of a process. Integrated lethality incorporates, amongst other factors, atmospheric relative humidity, rate of temperature change, product geometry, and fluctuations in product moisture content. All of these factors have been shown to impact the total lethality of commercial product cooking processes (Murphy et al., 2001a, 2001b; Pradhan et al., 2007). As such, D-values for a given pathogen become more useful when they are experimentally validated under conditions that, as closely as possible, mimic a commercial processing environment in order to account for integrated lethality. Unfortunately, many published D-values are not accompanied by such validation studies. This difficulty in applying published D-values to an array of different meat product thermal processes (for many different products) combined with a lack of validation studies identifies scientific gaps while also suggesting the current body of literature may not sufficiently assess the true robustness of Appendix A guidelines with all of the products for which it is currently used. No comprehensive study has examined these 3 pathogens in multiple products following a single methodology that incorporates validation using commercial thermal processes. The use of a single methodology for assessing thermal tolerance could allow more thorough and useful comparisons to be made between pathogens and product types.

For a single methodology approach (commercial product validation of D- and Z-values), it is first necessary to establish thermal tolerance in a model system with a variety of product types (e.g. beef, pork, poultry, etc.) and intrinsic factors (e.g. proximate composition,

concentration of salt and other non-meat ingredients, inclusion of sodium nitrite, etc.). Any differences in heat resistance found using this model system could then identify specific areas where Appendix A guidelines may not be adequate or where thermotolerance exists. Resulting D-values that suggest any pathogen investigated may not be adequately controlled by following Appendix A could then be validated in a pilot plant-scale system using a thermal process appropriate to the product type and representative of standard industry practices.

As such, the objective of this study was to determine the temperature-death times of *Salmonella*, *L. monocytogenes*, and shiga toxin-producing *E. coli* (STEC) in model, ready-to-eat (RTE) roast beef, turkey breast, and boneless ham systems.

Materials and Methods

Strain selection

Five strains of *L. monocytogenes* (FSL-C1-109, clinical isolate, processed turkey outbreak, serotype 4b; LM 101, hard salami isolate, serotype 4b; LM 108, hard salami isolate, serotype 1/2a; LM 310 goat milk cheese isolate, serotype 4b, and V7 raw milk isolate, serotype 1/2b); 5 strains of *Salmonella enterica* (Enteritidis 6424, phage type 4, baked cheesecake isolate; Enteritidis E40, chicken ovary isolate; Heidelberg S13, clinical isolate; Typhimurium S9, clinical isolate, and Typhimurium M-09-0001-A1, peanut butter isolate); and 7 strains of shiga toxin-producing *E. coli* [O111:H8-strain 00-3142, clinical isolate; O103:H2-strain 01-3002, clinical isolate; O121:H9-strain 01-3434, clinical isolate; O45:H2-strain 01-3510, clinical isolate; O145:NM-strain 99-3311, clinical isolate; O26:H11-strain H30, clinical isolate, and O157:H7 strain FRIK47 (ATCC 43895), clinical isolate] were used in this study (Riley et al., 1983; Wells et al., 1983). All strains were obtained from the Food Research Institute stock culture collection or from the Wisconsin State Hygiene Laboratory (Madison, WI).

Culture preparation

Strains were grown individually in 9 ml of Trypticase Soy Broth (TSB, Difco, BD Biosciences, Sparks, MD) with shaking for 18 h at 37°C. For each strain, 0.2 ml aliquots of overnight culture were spread onto 5 Trypticase Soy Agar (TSA, BD Biosciences) plates and incubated at 37°C for 18 to 22 h. Cells were harvested by scraping the surface of

the TSA plates with a sterile inoculating loop and suspending them in 4.5 ml of 0.1% peptone water (PW, pH 7.2) to achieve approximately 10 log CFU/ml. Equivalent populations of each strain were combined and diluted to 2.0 ml with PW to provide a mixture with a concentration of approximately 10 log CFU/ml. Populations of each strain and the mixture were verified by plating on TSA and on modified Oxford agar (MOX, *Listeria* Selective Agar base, BD Biosciences), Xylose-Lysine-Desoxycholate agar (BD Biosciences), or MacConkey-Sorbitol agar (BD Biosciences) for *L. monocytogenes*, *Salmonella*, and *E. coli*, respectively. Plates were incubated at 37°C for 18 to 22 h.

Product manufacture

Three categories of low-fat (< 3% fat), RTE products (roast beef, deli-style turkey breast, and boneless ham) were selected to represent a range of meat species, moisture levels, and inclusion of sodium nitrite. Formulations for all 3 products, listed in Table 1, were standardized for other critical factors including salt, phosphate, pH, and fat content, which have been shown to affect thermal lethality of pathogens.

Products were manufactured using Good Manufacturing Practices in the Meat Science and Muscle Biology Laboratory at the University of Wisconsin-Madison. Uncured roast beef was manufactured using fresh, closely trimmed beef top round muscles (*Semimembranosus*) obtained from a commercial supplier stored at ≤ 4.0°C until used (within 3 d of receipt). Deli-style turkey breast was manufactured using frozen boneless, skinless turkey breasts obtained from a commercial supplier. Frozen turkey breasts were thawed and stored at ≤ 4.0°C until used (within 24 h). Boneless, deli-style ham was made using fresh, boneless, closely trimmed ham inside muscles (*Semimembranosus*) ob-

Table 1. Product formulations for manufacture of uncured roast beef, deli-style turkey breast, and boneless cured ham¹

Ingredient	Product		
	Roast beef	Turkey breast	Ham
Water	20.0	20.0	25.0
Salt	1.20	1.50	2.50
Sugar	0.90	-	1.65
Sodium tripolyphosphate	0.42	-	0.35
Dextrose	-	1.50	-
Sodium nitrite	-	-	0.020
Sodium erythorbate	-	-	0.055

¹Formulated ingredients reported as ingoing percentage on a meat/poultry weight basis.

tained from a commercial supplier stored at $\leq 4.0^{\circ}\text{C}$ for ≤ 3 d prior to use. For all products the muscles were ground through a 19.05-mm plate attached to a grinder (Model 4732, Hobart Corp., Troy, OH).

For each product, all non-meat ingredients (Table 1) were first dissolved in water to create a brine. To ensure complete dissolution in the brine, ingredients were added in the following order: sodium tripolyphosphates, salt, and sugar (or dextrose). For the ham formulation, sodium nitrite and sodium erythorbate were added after the sugar had fully dissolved. Brines were then placed in a vacuum tumbler (Lance Model LT-5, Koch Industries, Kansas City, KS) with the appropriate coarsely ground meat and tumbled under vacuum continuously at 6 rpm at $\leq 4.0^{\circ}\text{C}$ for either 90 min (roast beef and turkey breast) or 120 min (boneless ham) to ensure uptake of the brine and facilitate protein extraction.

After tumbling, all product mixtures were then ground twice through a 4.5-mm plate, vacuum-packaged (3-mil high-barrier pouches; Part number 120218-200; Ultra Source LLC, Kansas City, MO), and transported to the Food Research Institute at the University of Wisconsin-Madison for subsequent thermal inactivation testing. Products were stored at 4°C prior to inoculation and were used within 48 h of manufacture.

Sample inoculation and preparation

For inoculation, 200 g of the appropriate meat formula was placed into a food processor (KitchenAid, KFC3511, Benton Harbor, MI) and mixed for 30 s. A 1% inoculum was then added to the meat and mixed for 30 s to yield a concentration of approximately 8.0 CFU/g of meat. Portions of 1 g of inoculated meat were then placed into water- and oxygen-impermeable vacuum pouches (16.51 cm \times 20.32 cm, 3 mil high barrier EVOH pouches, Deli 1 material; oxygen transmission rate, 2.3 cm^3/cm^2 ; 24 h at 23°C ; water transmission rate, 7.8 g/m^2 ; 24 h at 37.8°C ; and 90% relative humidity; WinPak, Winnipeg, Manitoba, Canada). Meat was flattened to a uniform target thickness of 1.0 mm. Pouches were then vacuum sealed (Multivac AGW, Sepp Hagemuller KG, Wolfertschewenden, Germany) and held at 4.0°C for no longer than 1 h prior to thermal processing.

Testing and enumeration

Treatment pouches were attached to a fabricated sampling rack to provide even distribution of sample bags within the water bath as well as to allow for simultaneous and efficient immersion into the water. The sampling rack was then submerged in a circulating water

bath (Magniwhirl Constant Temp Bath, Blue M Electric Company, Blue Island, IL) heated to 1 of 4 target temperatures (54.4, 60, 65.5, or 71.1°C) with samples being removed at pre-determined time intervals. Each trial consisted of one pathogen-product combination tested at all 4 temperatures, and trials were conducted in duplicate.

For all trials, sample temperature was monitored with a digital thermocouple (Fisher Scientific Traceable Thermometer and type K probe, Thermo Fisher Scientific, Waltham, MA) inserted through a rubber septum into a vacuum-sealed pouch containing 1 g of uninoculated meat. The time needed for the sample to reach the target cook temperature (come-up time) was recorded for each temperature within a trial. Come-up times ranged from 4 to 12 s across all trials and time-0 samples were not removed from the water bath until samples reached the specified target temperature.

At each sampling time interval, triplicate inoculated samples were removed and immediately submerged in an ice water bath for a minimum of 2 min to reach $\leq 4.0^{\circ}\text{C}$. Chilled sample pouches were then removed from the ice bath, dried, and the outside was sanitized with 70% ethanol before opening. Contents of the pouch were mixed with 9.0 ml of sterile Butterfield phosphate buffer (1:10 dilution) by massaging externally by hand for 2 min, then serially diluted (1:10 dilutions in 0.1% peptone water buffer, pH 7.2), and surface plated on duplicate plates of appropriate selective media. Selective media for each pathogen were prepared with a thin layer overlay of TSA to enhance recovery of injured cells (Kang and Fung, 2000). Plates were incubated for either 24 h (STEC and *Salmonella*) or 48 h (*L. monocytogenes*) at 35°C after which colonies were counted. Typical colonies were considered confirmatory.

Proximate analysis

Triplicate uninoculated, raw samples from each trial were assayed for moisture (5 h, 100°C , vacuum oven method, AOAC, 2000), NaCl (measured as % Cl^- , AgNO_3 potentiometric titration, Mettler DL22 food and beverage analyzer, Columbus, OH), and water activity (Decagon AquaLab 4TE water activity meter, Pullman, WA). In addition, the pH (Accumet Basic pH meter and Orion 8104 combination electrode, Thermo Fisher Scientific) was measured on a slurry obtained by removing a representative 10 g of the uninoculated sample and homogenizing it with 90 ml deionized water using a lab blender (Stomacher 400, A.J. Steward, London, England). Boneless ham samples were also analyzed for sodium nitrite content (Colorimetric Method, AOAC, 2000b).

Data and statistical analysis

Colony counts were transformed to log CFU/g and plotted versus heating time. To account for any lethality that may have occurred during the time it took for samples to reach the target temperature, the come-up time for each experiment was included in the respective survival curve. Linear regressions were fitted to the linear portion of the survival curve for each trial. The linear portion of the curve excluded tailing effects (defined as a decrease < 0.3 log CFU/g in the 2 consecutive time points prior to achieving a 5.0 log decrease; Vasan et al., 2013). For each pathogen-product combination, D-values were estimated as the average of the absolute inverse of the slopes of the regression lines for 2 replicate trials. Survival curves were used to determine D-values only if there were at least 4 time points in the linear portion of the curve. Therefore, if survival counts were below the limit-of-detection (2.0 log CFU/g) prior to a third or fourth time point, no accurate D-value could be calculated. The Z-values for each product-pathogen combination were determined by plotting log D-values versus cooking temperature and fitting a linear regression. The Z-value was estimated as the absolute inverse of the slope of the regression line. Linear regressions were fitted using JMP Pro software (version 11.0, SAS Inst. Inc., Cary, NC). Survival curves with prominent tailing were also assessed using the Log-Linear + Tail and Weibull + Tail models in the GlnaFiT add-on for Excel (Microsoft Excel 2016, Microsoft, Redmond, WA). However, D-values calculated from linear regressions were deemed to be more appropriately conservative and reported for this paper (Geeraerd et al., 2005).

Analysis of variance was used to test the effects of pathogen and meat type on log D-values within each temperature. Mean values were compared using Fisher's least significant difference test ($\alpha = 0.05$). All statistical analyses were performed using SAS (Version 9.4, SAS Inst. Inc.).

Results and Discussion

Proximate analysis

Proximate analysis results are presented in Table 2. Moisture content among the formulations ranged from 74.48 to 75.65%. Roast beef and turkey breast had comparable salt concentrations (1.13 and 1.38%, respectively) and pH levels (5.84) while the boneless ham formulation had a higher salt content (1.99%) and pH (5.97). Fat content was standardized to $< 3\%$ based on calculated values for incoming ingredients (fat not analyzed). Proximate

Table 2. Physiochemical properties¹ of raw, uncured roast beef, deli-style turkey breast, and boneless ham

Product	% Moisture ²	% NaCl ³	pH ⁴	a _w ⁵
Roast Beef	75.65 ± 1.85	1.13 ± 0.07	5.84 ± 0.24	0.980 ± 0.000
Turkey Breast	74.61 ± 0.46	1.38 ± 0.07	5.84 ± 0.09	0.979 ± 0.001
Ham	74.48 ± 1.57	1.99 ± 0.12	5.97 ± 0.11	0.974 ± 0.002

¹Values expressed as mean ± standard deviation from all replications (n = 6 for each product type). For each replication, triplicate samples were analyzed for physiochemical properties.

²Vacuum oven method, 5 h, 100°C; Association of Official Analytical Chemists, method 950.46.

³Measured as % Cl-, AgNO₃ potentiometric titration, Mettler DL22 food and beverage analyzer.

⁴Indirect pH by using an Accumet Basic pH meter with an Orion 8104 combination electrode, 10 g of meat to 90 ml of distilled water.

⁵Measured using a Decagon Aqua lab 4TE water activity meter.

analysis results were as expected to confirm that treatment formulations mimicked commercial products.

Evaluation of D- and Z-values

Survival curves for all 3 pathogens revealed a linear decrease in populations at all test temperature-product combinations, with tailing observed as the populations approached the minimum detection limit of 2.0 log CFU/g by direct plating (> 5 log decrease, data not shown). Figure 1 illustrates an example plot of log survival versus time at target temperature showing the linear decrease and standard deviations that were typically observed. Samples used in the generation of survival curves in this study were small (1 g), which allowed for rapid sample heating (come-up time ranged between 4 and 12 s). To generate conservative D-values, the linear portion of these curves included lethality that occurred between the initial application of heat and the first sampling point (come-up time) where temperature was below target. The relatively high standard deviations at the lower cook temperatures appear larger due to the greater lengths of time between sampling intervals timing (e.g. every 15 min at 54.4°C and every 1.5 min at 60°C).

The D- and Z-values determined for the 36 pathogen-substrate-temperature combinations are reported in Table 3. Figures 2 through 4 show the log D-value vs. temperature plots that were used to calculate Z-values for all 3 pathogens in roast beef, turkey breast and boneless ham, respectively. Survival curves were plotted for both *Salmonella* and STEC at 71.1°C; however, populations were reduced to below detectable limits at the first sampling point in all 3 products (during come up time of ~4 to 12 s). Linear regression of these plots could not provide an accurate estimate of the rates of thermal inactiva-

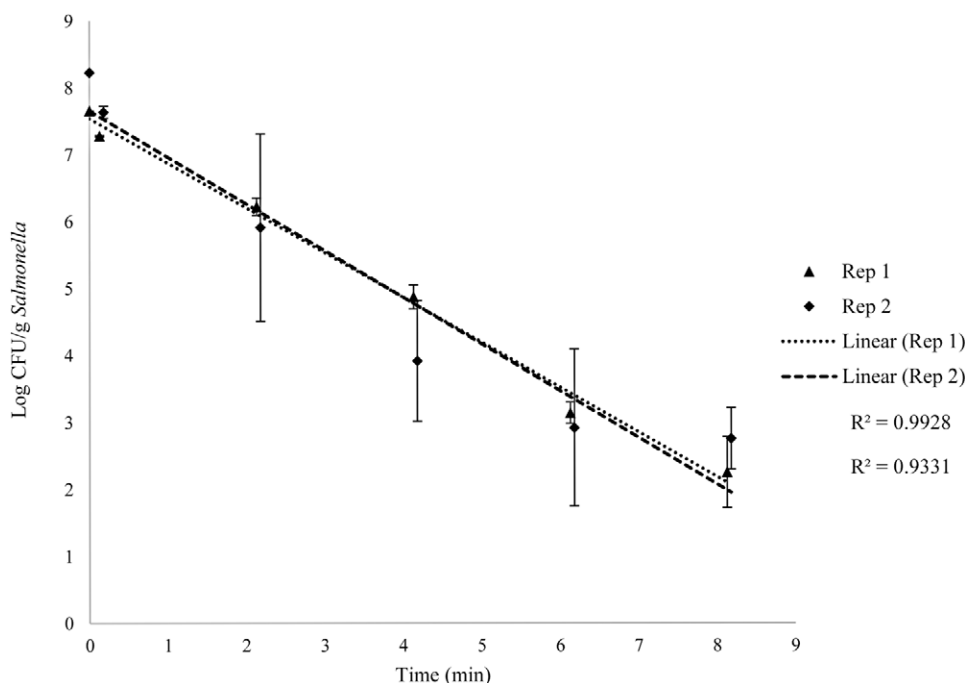


Figure 1. Determination of D-value for *Salmonella* in deli-style ham at 60.0°C. Log CFU/g *Salmonella* (y-axis) against time (min; x-axis). Data points represent the mean population of 3 samples and error bars represent the standard deviation for each time-point. Replicate thermal inactivation trials are labeled as “Rep”. The linear regression line for the survival curve from each “Rep” is labelled as “Linear”.

Table 3. Means for D-values¹ at 4 temperatures (54.4, 60.0, 65.6, and 71.1°C) and Z-values² for *Salmonella*, *Listeria monocytogenes*, and shiga toxin-producing *Escherichia coli* in uncured roast beef, uncured deli-style turkey breast, and cured boneless ham

Pathogen	Product	D-value, min, for Temperature, °C				z-value (°C)
		54.4	60.0	65.6	71.1	
<i>Salmonella</i>	Roast beef	9.34 ± 4.71 ^{e,3}	0.70 ± 0.07 ^{cd}	0.14 ± 0.03 ^{cd}	≤ 0.02 ⁴	7.87
	Turkey	19.45 ± 5.84 ^{cd}	2.21 ± 0.10 ^{abc}	0.21 ± 0.03 ^c	≤ 0.02 ⁴	5.70
	Ham	16.40 ± 4.52 ^d	1.46 ± 0.04 ^{bc}	0.20 ± 0.02 ^{cd}	≤ 0.02 ⁴	6.90
STEC	Roast beef	34.11 ± 3.68 ^{bc}	1.26 ± 0.60 ^{bc}	0.17 ± 0.09 ^{cd}	≤ 0.02 ⁴	4.86
	Turkey	36.54 ± 4.44 ^c	2.00 ± 0.41 ^{abc}	0.17 ± 0.03 ^{cd}	≤ 0.02 ⁴	4.80
	Ham	28.81 ± 3.56 ^{bcd}	0.95 ± 0.23 ^d	0.10 ± 0.00 ^d	≤ 0.02 ⁴	4.38
<i>Listeria</i>	Roast beef	48.14 ± 12.1 ^{ab}	7.25 ± 0.47 ^a	1.71 ± 0.10 ^a	0.34 ± 0.01	6.14
	Turkey	50.59 ± 6.25 ^{ab}	5.99 ± 0.36 ^{ab}	0.62 ± 0.03 ^b	0.06 ± 0.01	5.69
	Ham	66.21 ± 9.75 ^a	8.56 ± 1.06 ^a	1.08 ± 0.42 ^{ab}	0.27 ± 0.07	5.85

^{a-d}Means within a column bearing a common uppercase letter are not significantly different (P > 0.05).

¹D-values shown are the mean of 2 replicate experiments and expressed as mean ± standard deviation.

²Z-values for *Salmonella* and STEC calculated using D-values from 54.4-65.6°C. Z-values for *L. monocytogenes* calculated using D-values from 54.4-71.1°C.

³This D-value was not supported by subsequent validation experiments that may be attributed to lack of thermal adaptation during short come-up times for experiments described in this paper.

⁴D-value could not be accurately determined due to rapid cell death at this temperature. This value is an estimate that assumes a 6.0 log reduction in 10 s (the average come-up time for samples at this temperature).

tion with only 2 data points as it was unclear how quickly the limit of detection was reached within that nearly instantaneous timeframe. Therefore, we conservatively estimated D-values at 71.1°C for *Salmonella* and STEC based on a 6-log reduction in an average 10 s for come up (D-value <0.02 min). Our inability to accurately es-

tablish a D-value at 71.1°C was not unexpected. Very few studies have reported D-values for *Salmonella* or STEC at temperatures greater than 70°C due to the rapid lethality and practical limitations in being able to pull samples quickly enough to establish a survival curve. In one study, Murphy et al. (2003) reported the D-value for *Salmonella*

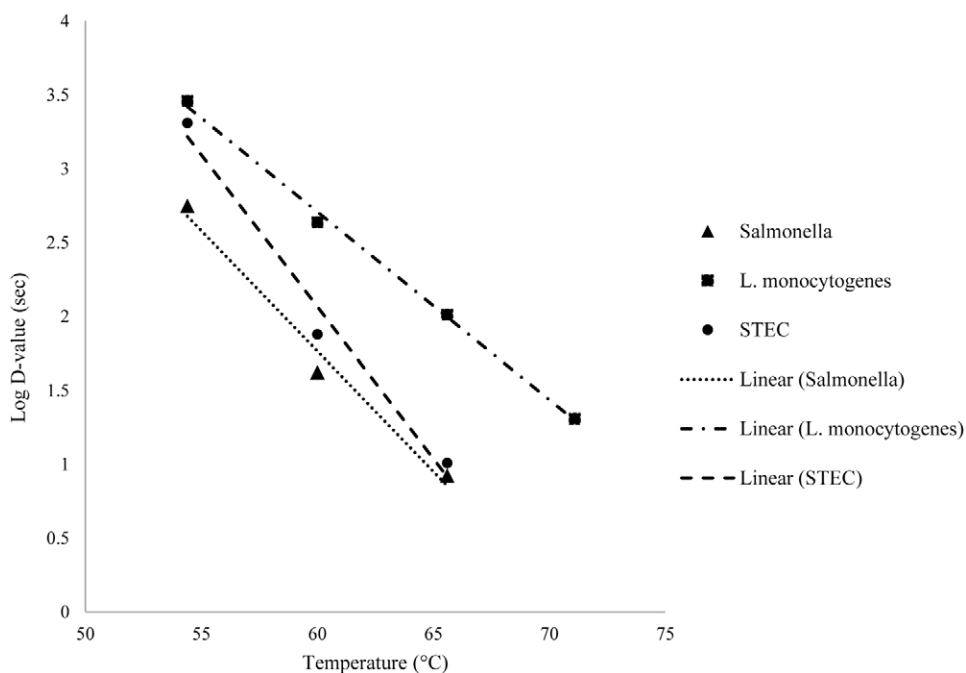


Figure 2. Determination of Z-values for *Salmonella*, *L. monocytogenes*, and STEC in uncured roast beef. Log D-values (y-axis) against temperature (°C; x-axis). The linear regression line for each curve is labelled as “Linear”.

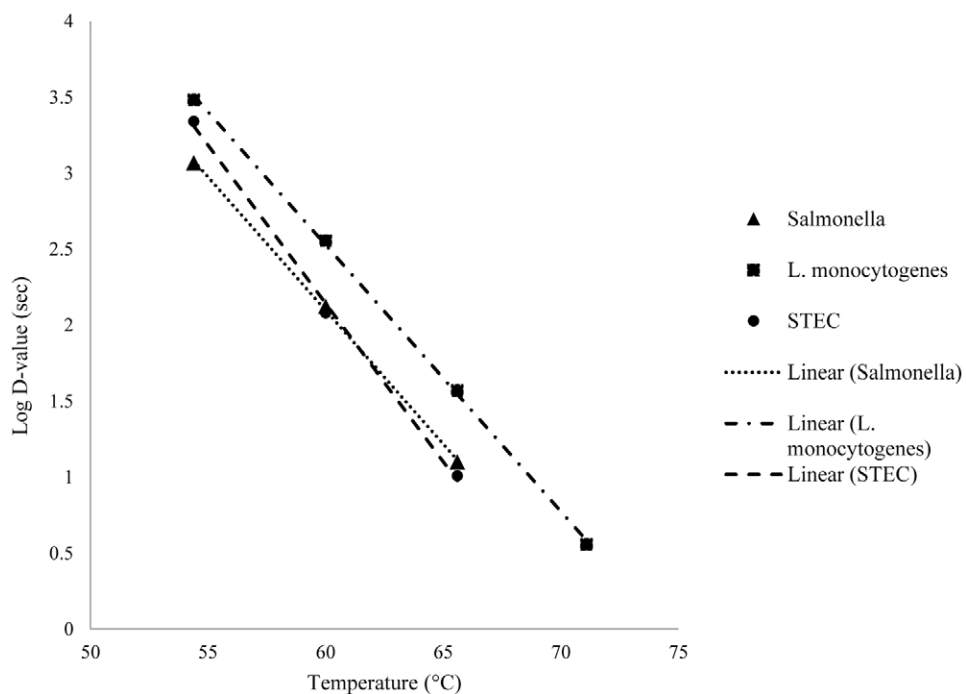


Figure 3. Determination of Z-values for *Salmonella*, *L. monocytogenes*, and STEC in deli-style turkey breast. Log D-values (y-axis) against temperature (°C; x-axis). The linear regression line for each curve is labelled as “Linear”.

at 70°C to be 0.12 min in fully-cooked turkey breast. In another study, Murphy et al. (2004c) determined the D-value for *Salmonella* at 70°C to be 0.07 and 0.08 min in chicken thigh/leg meat with and without sodium lactate, respectively. In addition to the lower target temperature in these 2 studies by Murphy et al. (2003, 2004c),

the investigators used a 6-strain cocktail that included *Salmonella* Senftenberg, an exceptionally heat-tolerant strain, which may explain their slightly higher D-values (Davidson et al., 1966). Our findings (Table 4) are in agreement with Appendix A covering beef, pork, or lamb, or with the draft compliance guidelines for poultry prod-

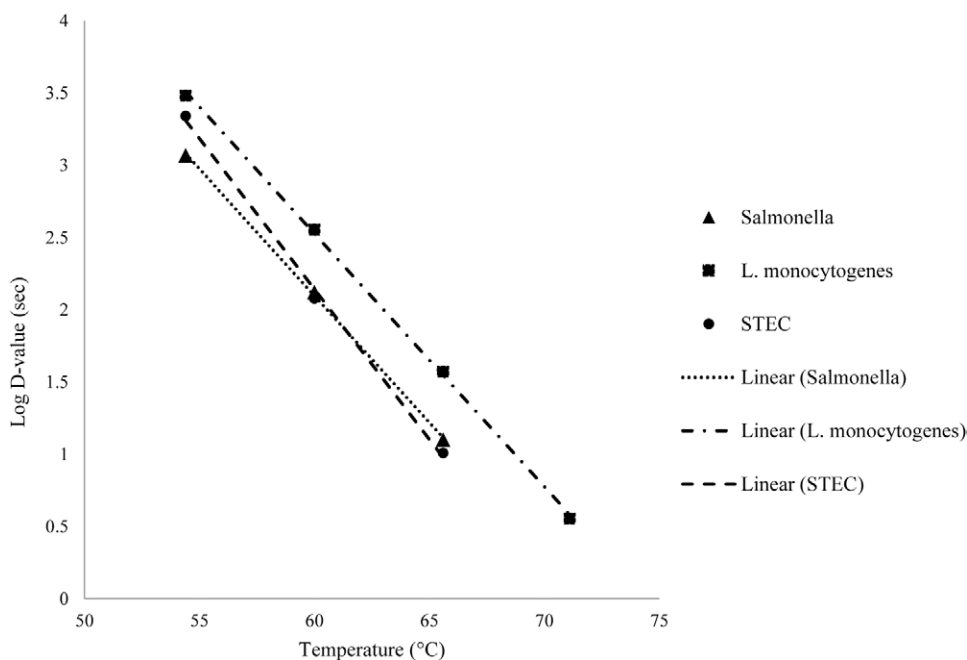


Figure 4. Determination of Z-values for *Salmonella*, *L. monocytogenes*, and STEC in boneless ham. Log D-values (y-axis) against temperature (°C; x-axis). The linear regression line for each curve is labelled as “Linear”.

Table 4. Time-temperature tables generated utilizing D- and Z-values for *Salmonella*, shiga toxin-producing *Escherichia coli*, and *Listeria monocytogenes* for uncured roast beef, deli-style turkey breast, and boneless ham

Category ¹	Temperature ²	<i>Salmonella</i>			STEC			<i>L. monocytogenes</i>		
		5.0 ³	6.5 ⁴	7.0 ⁵	5.0 ³	6.5 ⁴	7.0 ⁵	5.0 ³	6.5 ⁴	7.0 ⁵
Roast Beef	54.4 (130)	46.7	60.7	65.4	170.6	221.7	238.8	240.7	312.9	337.0
	60.0 (140)	3.5	4.6	4.9	6.3	8.2	8.8	36.3	47.1	50.8
	65.6 (150)	0.7	0.9	1.0	0.9	1.1	1.2	8.6	11.0	12.0
	71.1 (160)	0.0	0.0	0.0	0.0	0.0	0.0	1.7	2.2	2.4
Turkey Breast	54.4 (130)	NI ⁶	NI	136.2	182.7	237.5	255.8	253.0	328.8	354.1
	60.0 (140)	NI	NI	15.9	10.0	13.0	14.0	30.0	38.9	41.9
	65.6 (150)	NI	NI	1.5	0.9	1.1	1.2	3.1	4.0	4.3
	71.1 (160)	NI	NI	0.0	0.0	0.0	0.0	0.3	0.4	0.4
Boneless Ham	54.4 (130)	82.0	106.6	114.8	144.1	187.3	201.7	331.1	430.4	463.5
	60.0 (140)	7.3	9.5	10.2	4.8	6.2	6.7	42.8	55.6	59.9
	65.6 (150)	1.0	1.3	1.4	0.5	0.7	0.4	5.4	7.0	7.6
	71.1 (160)	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.8	1.9

¹Roast beef, turkey breast, boneless ham investigated in this study compared with USDA, FSIS Appendix A “Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products” (USDA – FSIS, 2017).

²Target meat temperature prescribed by experimental treatments reported as °C (°F).

³Time in min to achieve target 5.0 log reduction of specified pathogen.

⁴Time in min to achieve target 6.5 log reduction of specified pathogen.

⁵Time in min to achieve target 7.0 log reduction of specified pathogen.

⁶NI = not included because a 5.0 or 6.5 log reduction in a cooked poultry product would fail to comply with 9 CFR 381.150 which requires a minimum 7.0-log reduction of *Salmonella*.

ucts that states that a 6.5 to 7 log reduction of *Salmonella* is achieved instantaneously at 71.1°C in beef and in 0.4 min for 3% fat turkey (USDA – FSIS, 2005a, 2005b).

The D-values for *Salmonella* and STEC at temperatures less than 71.1°C were within ranges of val-

ues previously summarized (O’Bryan et al., 2006). However, differences in the methodologies of these prior studies (e.g. varying sample size, use of a single strain vs. multi-strain pathogen cocktail, or use of irradiated vs. non-irradiated meat) can limit meaningful

comparisons between studies where there are notable differences in reported thermotolerance. As an example, one study by Murphy et al. (2002) published a D-value of 8.09 min for *Salmonella* in a lean chicken patty product at 60.0°C, which is considerably longer than the 2.21 min reported for the turkey breast treatments in our study. That study included *Salmonella* Senftenberg in its strain cocktail during testing, the presence of which likely contributed to the greater heat tolerance. However, the larger D-value reported by Murphy et al. could also have been attributed to their use of a less selective enumeration medium [Tryptic Soy Broth (TSB) agar overlaid with TSB agar + 200 ppm nalidixic acid], which may have allowed for greater recovery of injured cells. Two different studies did not include *Salmonella* Senftenberg in their strain cocktail and still found higher D-values for *Salmonella* that ranged from 4.82 to 5.43 min at 60.0°C (Juneja and Eblen, 1999; Juneja et al., 2001a). In these studies, meat was irradiated to eliminate background microflora and *Salmonella* populations were enumerated on non-selective media. The impact of background microflora on thermal tolerance of pathogens is not well understood but the absence of such is not representative of the meat used in commercial production. Increased recovery, due either to lack of background competition or less stressful recovery conditions, could account for greater apparent thermotolerance of non-Senftenberg *Salmonella*. These comparisons to other reported D-values are needed to ensure that the D-values produced by this study fall within an acceptable range; but they are less helpful in the context of assessing the efficacy of Appendix A guidelines. Unlike those created in isothermal experiments, the conditions that impact the thermotolerance of pathogens during a commercial thermal process are dynamic and not limited to temperature. Because they are intended to serve as safe harbors for pathogen lethality, Appendix A guidelines must account for the multitude of factors that can influence pathogen survival. Validation in larger systems that mimic the conditions found in a meat product during commercial thermal processing helps to ensure that D-values accurately describe the thermotolerance of a pathogen outside a model system. Many reported D-values lack this type of validation, resulting in a need for greater caution when applying them to real thermal processes.

At 54.4°C, D-values for STEC were greater than those for *Salmonella* in all 3 product types, with the largest difference occurring in roast beef (Table 3). At temperatures $\geq 60.0^\circ\text{C}$, survivability decreased, and differences in thermotolerance across pathogens and

products diminished considerably. Figures 2 through 4 highlight the more rapid decline in D-values for STEC compared to the *Salmonella* and *L. monocytogenes* in all 3 products as temperatures increased beyond 60.0°C. This resulted in the low Z-values for STEC, which ranged from 4.38 to 4.86°C (Table 3). Kotrola and Conner (1997) reported similarly low Z-values for STEC in several processed turkey products, ranging from 4.40 to 4.78°C. In a turkey ham product with a similar formulation to the boneless ham investigated in this study (1.9% salt, 0.4% phosphate, 0.02% sodium nitrite), these researchers found that the thermal tolerance of STEC decreased considerably between 52.0 and 60.0°C with reported D-values of 60.4 and 1.0 min, respectively. Blackburn et al. (1997) also reported comparably low Z-values for STEC of 4.6 to 5.1°C in broth containing 0.5% NaCl. They also found that these Z-values increased to 5.8 to 7.0°C as salt concentration was increased to 8.5%, suggesting that rapid decline of thermal tolerance seen with STEC may be less severe in products with higher salt content. Our findings do not support the use of Appendix A for control of STEC at temperatures below 60.0°C, especially in products with salt content $\geq 3.0\%$, where the high salt content and low cooking temperature may result in greater thermotolerance.

As expected, *L. monocytogenes* was found to be more heat tolerant than both *Salmonella* and STEC at all temperatures, including at 71.1°C, where lethality was not instantaneous (Table 3). For example, at 65.6°C, D-values for *L. monocytogenes* were 2 to 10 times longer than for either *Salmonella* or STEC for all three model products. The greater thermal tolerance of *L. monocytogenes* is consistent with other studies summarized by Doyle et al. (2001). The D-values for *L. monocytogenes* at 65.6°C, ranging from 0.62 to 1.71 min suggest that the use of Appendix A for target internal temperatures $\leq 65.6^\circ\text{C}$ would not provide 6.5 log reduction of *L. monocytogenes*. It is important to note, however, that while this pathogen is frequently detected in fresh meat and poultry there is little evidence to suggest that it is found at sufficiently high numbers prior to thermal processing to warrant a 6.5 log reduction (Jay, 1996; Lianou and Sofos, 2007). However, if the target reduction is lowered to 5.0 log, which is the required reduction for *Salmonella* and STEC in meat jerky (USDA - FSIS, 2014), cook processes following Appendix A would still be inadequate at any cook temperature. A greater understanding of the higher thermal tolerance of *L. monocytogenes* may prove valuable to better utilize thermal processing as part of a *Listeria* control program.

Comparison to Appendix A style tables

Table 4 shows hold times necessary to achieve 5.0-, 6.5-, and 7.0-log reductions for all three pathogens that were generated using the D-values derived in these experiments using a high humidity environment. Multiplying the experimental D-value by the target log-reduction produced the estimated hold times. It should be noted that while this estimation method does not account for the dynamic temperatures experienced by the bacteria during actual thermal processing in a product (integrated lethality), a conservative estimate of process lethality can be calculated by assuming that only time spent at the target temperature is relevant for lethality. Because Appendix A guidelines are intended to serve as a source of lethality “safe harbors” it is important to use the most reasonably conservative process lethality estimates available to evaluate their efficacy. The longer estimated hold times help to minimize the risk of overestimating the safety of Appendix A guidelines. If recommended hold times meet or exceed the times listed in Table 4, then it will likely provide adequate lethality in cook processes where conditions are less conducive to pathogen survival. However, there are processing conditions in which it is important to consider integrated lethality. One example is thermal processes that combine long come-up times with low cook temperature (e.g. 54.4°C), where prolonged exposure to sub-lethal heat could allow for a pathogen to thermally adapt, resulting in increased survivability at the target temperature (Stasiewicz, et al., 2008). Because the isothermal methodology used in this study did not account for a dynamic temperature profile by design, it is important to ensure that estimates derived using these methods are validated in representative commercial product thermal processes.

These data suggest that the times listed in Appendix A for temperatures $\geq 60.0^\circ\text{C}$ are conservative and therefore appropriate for continued use for controlling *Salmonella* and STEC when manufacturing product types similar to those tested in the current study. Appendix A values exceeded those calculated from our data for *Salmonella* and STEC in roast beef and ham when heated to temperatures $\geq 60.0^\circ\text{C}$. Similarly, values identified in the Poultry Time-Temperature tables (e.g. for 3% fat turkey) were conservative compared to our values for *Salmonella* and STEC in deli-style turkey breast at this temperature.

In contrast, when cooking to 54.4°C, hold times identified by Appendix A were shorter than those identified in our study for 5.0-, 6.5-, and 7.0-log reductions of STEC in all 3 meat matrices. Because this suggests that Appendix A guidelines may offer less lethality at temperatures $< 60.0^\circ\text{C}$, further research is necessary to determine

at which temperatures and under what conditions the time-temperature tables may need adjustment if they are to be used for controlling STEC at these temperatures. As noted above, times calculated for *L. monocytogenes* were longer in our study than Appendix A at all four temperatures in all three product types. The significantly greater thermal tolerance of *L. monocytogenes* was evident in the D-values and this table helps to elucidate the difficulty of controlling this pathogen via thermal processing.

These results support the USDA, FSIS Appendix A as an acceptable tool for *Salmonella* and STEC lethality for temperatures at or greater than 60°C. *L. monocytogenes* had greater thermal tolerance than *Salmonella* or STEC and a ≤ 5.0 log reduction would be expected if product were given a minimal process. It is important to note that this experiment only measured lethality upon immediate exposure to a specific temperature. In a commercial process, the physical dimensions of a product and the processing technology utilized may contribute to slower come-up times and longer overall heat exposures. Long come-up times at inactivation temperatures greater than 54.4°C will typically increase lethality. However, long exposure to sub-lethal temperatures can increase bacterial survival by providing the cells with time to adapt to the changing environment, which is the reason FSIS Salmonella Compliance Guideline (USDA - FSIS, 2017) recommends the time to reach final temperature should have no more than 6 h in the range of 10 to 55°C. The microbial environments at the surface and at the core of a product can differ significantly from each other, which can also influence the heat tolerance of an organism. Because actual thermal processing conditions differ significantly from this model system it is important then to consider integrated lethality when trying to fully describe the thermal tolerance of these pathogens and the safety of commercial thermal processing. Therefore, validation of these D-values took place in a series of follow-up experiments that examined integrated lethality, with the results published in a subsequent paper.

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