D- and *z*-Values for *Listeria monocytogenes* and *Salmonella* Typhimurium in Packaged Low-Fat Ready-to-Eat Turkey Bologna Subjected to a Surface Pasteurization Treatment

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ABSTRACT The *D*-values of *Listeria monocytogenes* and *Salmonella* Typhimurium at various surface pasteurization temperatures were determined for low-fat turkey bologna. Four cm^2 meat squares were sterilized by irradiation prior to inoculation with 0.1 mL of a 10^8 cfu/mL culture, aseptically packaged in a linear low-density polyethylene pouch, and vacuum-sealed. Thermal treatments were administered by submerging packages in a heated water bath maintained at various set temperatures. At an 85°C water bath temperature, no *L. monocytogenes* cells were detected ($<10^2$) after 10 s of exposure, whereas at 61°C cells viable were detected ($>10^2$) up to 10 min of heating. No *S.* Typhimurium cells ($<10^2$) were detected after 10 s at 70°C, but cells survived up to 7 min at 60°C. The *D*-values for *L. monocytogenes* at 61 and 65°C were 124 and 16.2 s, respectively; whereas *S.* Typhimurium D-values were 278 s at 57 and 81 s at 60°C. Z-values were 4.44 and 5.56°C, respectively, for *L. monocytogenes* and *S.* Typhimurium. This study demonstrated that significant reductions in bacterial populations and complete inactivation of *S.* Typhimurium and *L. monocytogenes* cells can be achieved using an in-package thermal pasteurization process.

(*Key words*: D-value, z-value, in-package pasteurization, turkey bologna, *Listeria monocytogenes*)

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INTRODUCTION

Poultry products have come under scrutiny due to listeriosis outbreaks from cooked meat products composed of single and mixed meat species. The USDA has implemented a zero tolerance for *Listeria monocytogenes* in ready-to-eat meat products partly because this organism was the number one cause of USDA-recommended meat and poultry meat recalls from 1994 to 1998. There were 11 food recalls due to *L. monocytogenes* contamination in 2001. The expense for a recall alone, excluding liability costs, can amount to millions of dollars. Removal or destruction of pathogens on the outer surfaces of poultry products is an important link to the goal of producing pathogen-free products. Hence, new treatments to reduce bacterial populations inherent to poultry products are necessary to assure that such products reach the consumer in a wholesome state.

One aspect of the farm-to-table hazard analysis and critical control point program that requires greater attention is the control of bacterial pathogen proliferation and prevention of cross-contamination. The program should cover products through the processing plant, distribution, and marketing, especially at the retail and food service levels. For cooked products, growth of *L. monocytogenes* in vacuum-packaged, cooked meat products can occur prior to packaging or during storage and distribution. Once the meat surface is contaminated with *L. monocytogenes*, the bacterium may have a growth advantage. The advantage is due to the reduction of natural spoilage bacteria via cooking and to storage under refrigeration that limit competitive bacterial growth. To develop effective in-package thermal treatment processes for meat products, the rate of inactivation of target microorganisms (i.e., *L. monocytogenes*, *Salmonella*) at various processing temperatures must first be determined. To establish effective heat processing treatments for producing pathogen-free ready-to-eat meat products, decimal reduction values (*D*-values) and *z*-values must be determined. Thermal inactivation kinetics of *Salmonella* and *Listeria* have been examined in several poultry products including ground chicken breast (Murphy et al., 1999, 2000),

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Abbreviation Key: BHI = brain heart infusion; NAR = naladixic acid resistant; TS = tryptic soy.

chicken patties (Murphy et al., 2001, 2002), and chicken broth (Juneja et al., 2001). No information has been published on the surface heat inactivation of *Listeria* and *Salmonella* on ready-to-eat meats such as bologna. The objective of this study was to determine the surface thermal inactivation kinetic values (*D*- and *z*-values) of *Listeria monocytogenes* and *Salmonella* Typhimurium on packaged turkey bologna.

MATERIALS AND METHODS

Meat Preparation

Fresh ready-to-eat, low-fat turkey bologna containing 14.2% fat, 10.7% protein, and <2% salt was obtained from a commercial processing plant and used for all experiments. An initial meat pH of 6.7 was determined by first homogenizing 10 g of meat in 100 mL of 0.1% sterile peptone water[2] and determined by placing the probe into the homogenate until the pH meter[3] was equilibrated. Meat slices of approximately 2.2-mm thickness were cut into 4-cm^2 pieces using sterile cutting templates. The meat samples were then frozen and irradiated using a Cobalt 60 source at 10 kiloGray for 79 min (conducted at Auburn University) to ensure product sterility before testing. Sterility was verified by conducting a total plate count on samples before inoculation. Samples were kept frozen and then thawed at 4°C prior to testing.

Listeria monocytogenes ATCC 15313 inoculum was cultured in screw cap tubes containing 9.9 mL of brain heart infusion (BHI) broth[2] and incubated aerobically at 37°C for 16 h with agitation at 200 revolutions per min.[4] *Salmonella* Typhimurium was an environmental isolate from the laboratory of Brian Sheldon located in the Poultry Science Department at North Carolina State University. This strain is resistant to 1,000 ppm nalidixic acid (NAR) and was cultured in screw cap tubes containing 9.9 mL of tryptic soy (TS) broth[2] for 16 h with agitation at 200 revolutions per min. A washed cell suspension of each strain was prepared by harvesting cells from the 9.9-mL culture by centrifugation at 10,000 × g[5] and washed twice with 10 mL of sterile 0.1% peptone water prior to diluting to achieve approximately 10^8 cells per mL of cell suspension. Cell populations were verified for each trial by plating on an appropriate culture medium (BHI for *L. monocytogenes* and TS for *S.* Typhimurium) using the pour plate method and incubated for 48 h at 37°C prior to enumeration.

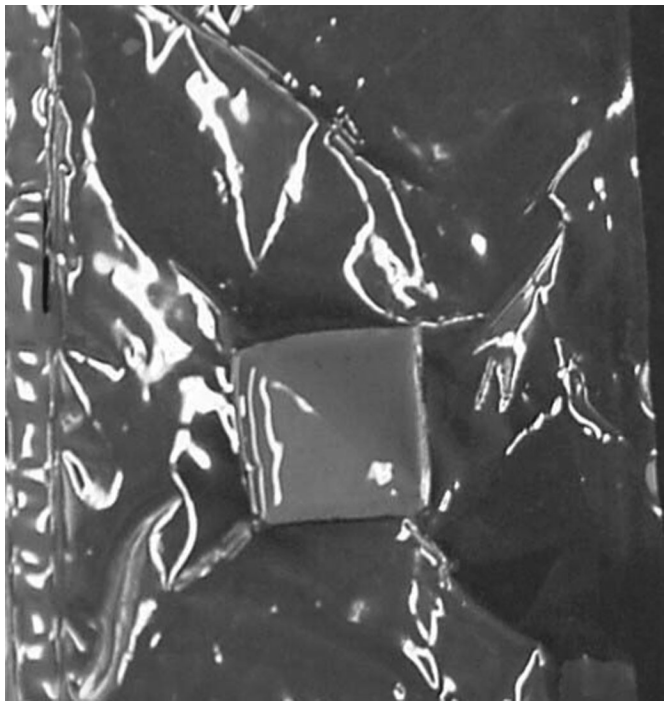

FIGURE 1. Inoculated turkey bologna meat sample sealed in a linear low-density polyethylene bag used for in-package thermal treatments.

D-Value and z-Value Determination

Prior to inoculation, sterile meat samples were removed from refrigeration and aseptically transferred to a sterile fume hood surface using sterile forceps. Samples were then inoculated evenly across the exposed surface with 0.1 mL of either *L. monocytogenes* or *S.* Typhimurium using a micropipetter to achieve approximately 10^8 cells per gram of meat. Meat samples were then sealed in a linear low-density polyethylene bag[6] using a vacuum sealer[7] (Figure 1). Packages were checked for leakers, which were discarded. Meat surface temperature was measured using K type Teflon insulated thermocouples[8] oriented on the meat surface inside the sealed vacuum bag. Time and temperature heating data were recorded using a channel datalogger[9] and thermal processing software.[10] Preliminary heating trials in a water bath were conducted at 95, 90, 85, 80, 75, 70, 65, and 60°C to determine the appropriate temperatures for deriving *D*-values. Based on the lack of recoverable cells at higher processing temperatures, 61 and 65°C were chosen for *L. monocytogenes* and 57 and 60°C for *S.* Typhimurium. Samples were submerged into a hot water bath,[11] and samples were removed at timed intervals after the target temperature was reached. A total of seven to 10 samples were taken for each replication at each process temperature to assure that the resulting thermal death curve plots would span over at least a three $\log_{10}$ reduction. Timing of the thermal treatments was started when the bologna surface reached the water bath

[2]Difco Laboratories, Detroit, MI.
[3]Orion model 420A, Orion Research, Inc., Boston, MA.
[4]Thermolyne Max-Mix III Type 68500, Barnstead/Thermolyne, Dubuque, IA.
[5]IEC HN-SII Centrifuge, International Equipment Company, Inc., Needham Heights, MA.
[6]Model P64x, Cryovac, Duncan, SC.
[7]Koch Model UV 250, Koch Supplies Inc., Kansas City, MO.
[8]Omega Engineering, Inc., Stamford, CT.
[9]CALPlex 32, TechniCAL, New Orleans, LA.
[10]CALSoft version 1.32, TechniCAL, New Orleans, LA.
[11]Microprocessor controlled 280 series water bath, Jouan, Inc., Winchester, VA.

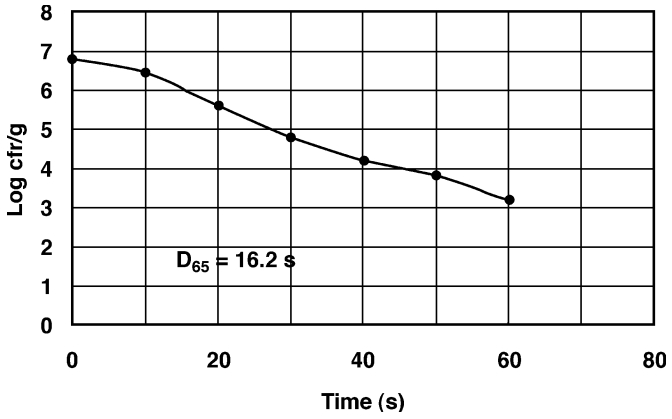

FIGURE 2. Surviving *Listeria monocytogenes* at 65°C. Each data point represents the mean of three experimental replications performed on separate days with duplicate plating of each sample. 0-time is the heating time when the water bath temperature was reached.

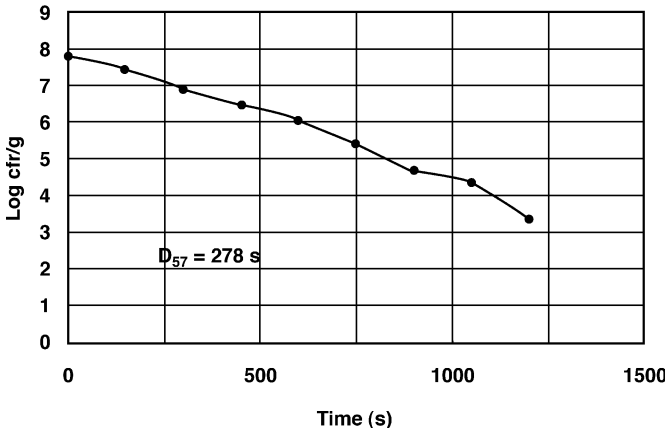

FIGURE 4. Surviving *Salmonella* Typhimurium at 57°C. Each data point represents the mean of three experimental replications performed on separate days with duplicate plating of each sample.

temperature. Total processing time and temperatures including the come-up and cool-down times were also recorded. The contribution of the come-up time to the death rate was estimated by calculating the average lethal rate recorded between temperatures during the come-up period. Lethal rate was calculated from

$$10_{r}^{(t-t)/z} \quad [1]$$

where t_r = reference temperature for each organism (i.e., temperature at which 1 log reduction occurs at 1 min hold time), t = meat surface temperature, z = z-value of the bacterium of interest (z used were those calculated from this study) (Fellows, 1990).

After removal from the water bath, samples were placed into an ice bath for 5 s to minimize any further thermal effects. The come-up and cool-down time periods were rapid enough that no additional lethality occurred based on the lethality calculations during these periods.

Each sample was subsequently removed from the vacuum pouches with sterile forceps and homogenized with 10 mL of sterile 0.1% peptone water by placing meat samples into a stomacher[12] for 2 min at 230 rpm. Homogenates were then 10-fold serially diluted and pour plated on BHI[2] and TS agar[2] containing 150 μg/mL of nalidixic acid to enumerate *L. monocytogenes* and *S.* Typhimurium (NAR), respectively. Plates were incubated at 37°C for 48 h before enumerating colonies.

D-values were determined from the linear portion of the survivor plots using linear regression analysis. *D*-values are reported in seconds and are defined as the time required to achieve a 1-log reduction in the bacterial population at a designated temperature. The z-values were determined by constructing decimal reduction time curves (mean log *D*-value versus temperature) and *z*-values calculated, where z = slope^{-1} (the temperature change necessary to effect a 10-fold change in the *D*-value (Holdsworth, 1997).

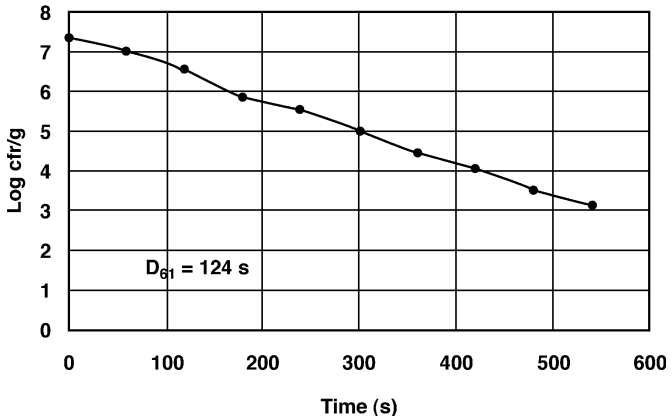

FIGURE 3. Surviving *Listeria monocytogenes* at 61°C. Each data point represents the mean of three experimental replications performed on separate days with duplicate plating of each sample. 0-time is the heating time when the water bath temperature was reached.

Statistical Analysis

Three separate replications per process temperature were conducted using each organism. Two meat pieces were sampled at each heating time. The variation in *D*-values between replications was determined by ANOVA of absolute deviations from group means and then by using the Levene's test for homogeneous variances and proc GLM command of SAS 2000.[13]

RESULTS AND DISCUSSION

Variation between replications for each of the water bath temperatures used was found to be nonsignificant ($P \leq 0.05$), and thus the survivor plot replications were averaged. Survivor curves for *L. monocytogenes* at 61 and 65°C and *Salmonella* Typhimurium at 57 and 60°C on the surface of low-fat, ready-to-eat turkey bologna are shown

[12]Seward Stomacher 400 Circulator, Seward, Inc., United Kingdom.
[13]Version 8.0, Statistical Analysis System, Cary, NC.

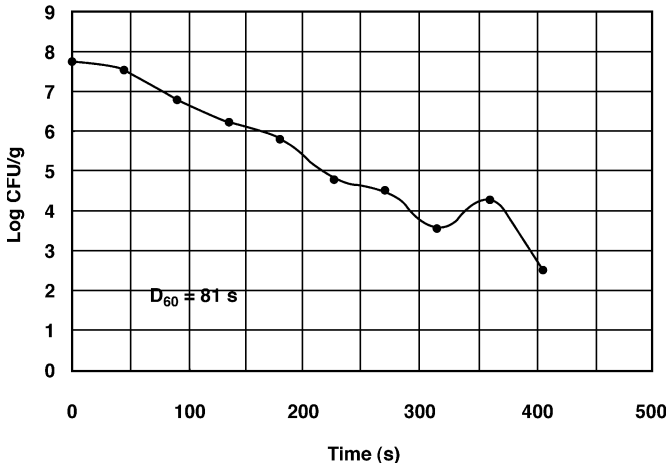

FIGURE 5. Surviving *Salmonella* Typhimurium at 60°C. Each data point represents the mean of three experimental replications performed on separate days with duplicate plating of each sample.

in Figures 2 to 5. Come-up times (the time for the thermocouple temperature at the meat surface to match the water bath temperature) of *L. monocytogenes* for the three replications (three thermocouple measurements for each replication for a total of nine measurements) at each temperature were approximately 60, 75, and 55 s at 65°C and 65, 55, and 70 s at 61°C, respectively. The three come-up times for *S.* Typhimurium were 75, 50, and 60 s at 57°C and 60, 55, 70 s at 60°C, respectively. Come-up heating times contribute to the overall killing effect of the thermal treatment. The accumulated lethality of the average come-up and cool-down periods was calculated using equation [1] by plotting the lethality and determining the area under the lethality plot. The reference temperatures (t_r) and *z*-values used were 62.42 (t_r), 4.44 (*z*) for *L. monocytogenes* and 60.72 (t_r) and 5.56°C (*z*) for *S.* Typhimurium, respectively. Come-up and cool- down thermal profiles of the bologna surface temperature are shown in Figure 6 for each processing temperature. The come-up times averaged approximately 60 s for all water bath temperatures, and the lethality plot of the come-up times for *L. monocytogenes* and *S.* Typhimurium are shown in Figures 7 and 8, respectively. The 65°C water bath temperature process had a calculated accumulated lethality of approximately 1 log, whereas all other processing temperatures were less than 0.5 log. The cool-down periods (not shown) were calculated to contribute to less than a 0.1-log reduction in population. The come-up periods were accounted for in the *D*-value calculations. The linearity observed in the survivor plots (Figures 2 to 5) shows that the calculations accurately accounted for any lethality during the come-up periods. If the lethality contributed to the come-up or cool-down period had not been accounted for correctly, the lethality of these periods would have been added to the initial and last sampling time, respectively. This would have increased the lethality of these portions of the thermal death plot causing the slope to change for these sampling times. Because the slope was linear across all of the sampling times, the come-up and cool-down were accounted for in the correct manner.

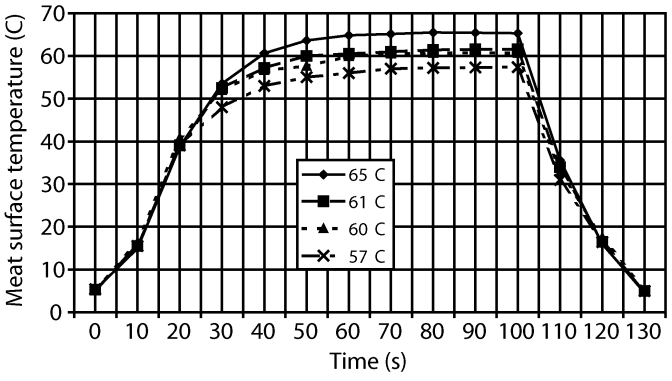

FIGURE 6. Temperature profiles for the bologna surface for each processing (water bath) temperature. Each line is an average of nine temperature profiles collected from three separate replications.

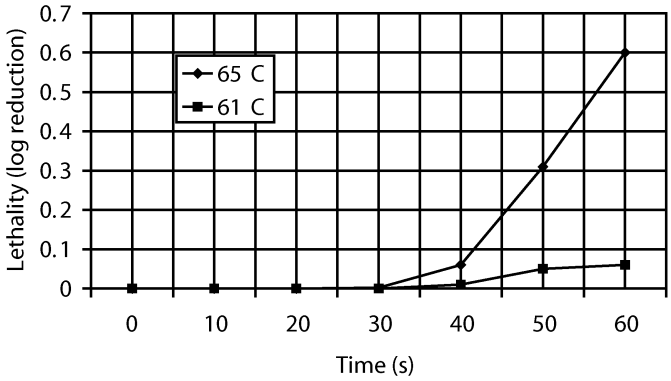

FIGURE 7. Lethality of the come-up periods for *Listeria monocytogenes* on the surface of vacuum-packaged low-fat turkey bologna submerged in 65 and 61°C water bath temperatures.

L. monocytogenes and *S.* Typhimurium cells exposed to temperatures greater than their optimum growth temperatures can lead to formation of heat-shock proteins that

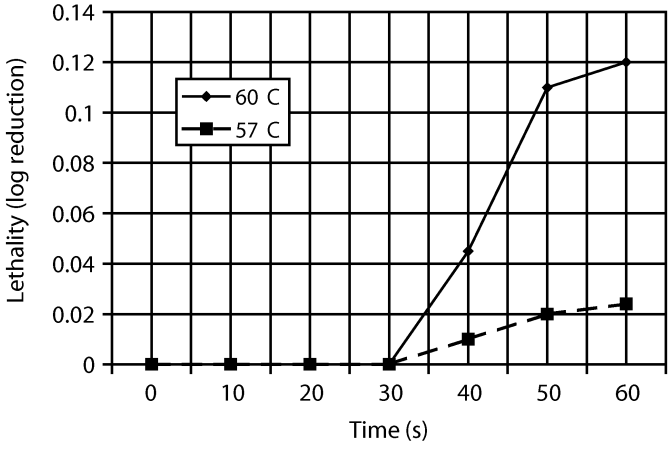

FIGURE 8. Lethality of the come-up periods for *Salmonella* Typhimurium on the surface of vacuum-packaged low-fat turkey bologna submerged in 60 and 57°C water bath temperatures.

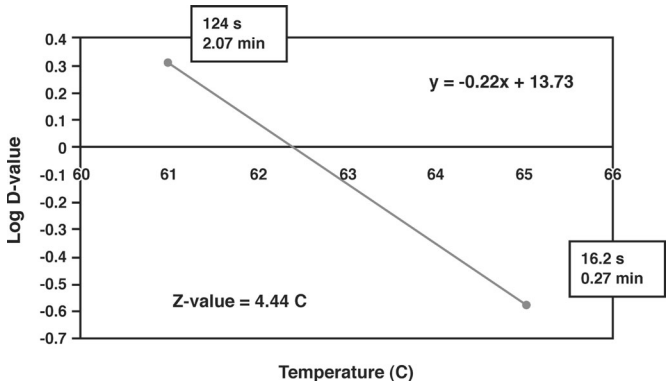

FIGURE 9. Plot of Log_{10} of D-values of *Listeria monocytogenes* at 61 and 65°C, respectively. z-value is the inverse slope of the linear trend line. Each point represents the mean of three experimental replications performed on separate days with duplicate plating.

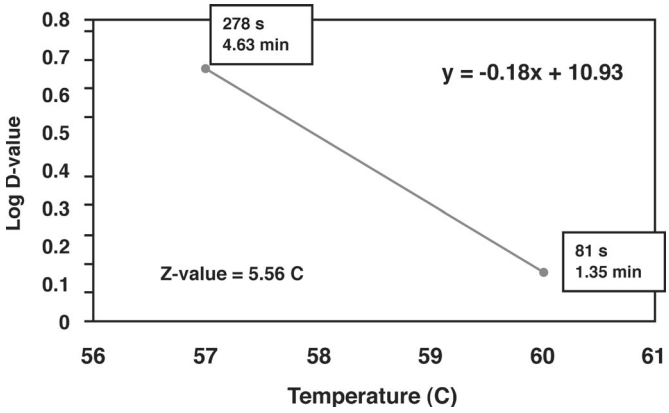

FIGURE 10. Plot of Log_{10} of the D-values for *Salmonella* Typhimurium at 57 and 60°C, respectively. The z-value is calculated from the inverse of the slope of the linear trend line. Each point represents the mean of three experimental replications performed on separate days with duplicate plating.

increase thermal tolerance. A time of 30 min has been used to heat-shock cultures to induce the production of heat-shock proteins. Thus, holding cells at higher than optimum temperatures for extended times can lead to the development of increased heat resistance (Mackey and Derrick, 1990; Doyle et al., 2001). Increases in thermal resistance can sometimes be viewed as tailing or shouldering effects on the survivor curve. The short come-up times (~ 1 min) of the thermal treatments evaluated in the present study were not apparently long enough to facilitate development of thermal resistance, which was verified by observing no visible tailing of the survivor curves, and their linear nature (average r value of 0.98). Linear survivor curves suggest that heat resistance was relatively uniform throughout the population.

The *D*-value for *L. monocytogenes* at 61°C was 124 s, but at 65°C the *D*-value decreased to 16.2 s. The Levene's test for homogeneity of variance revealed that the three replications did not differ for 61°C (*P* value = 0.97) or for 65°C (*P* value = 0.93). The z-value was 4.4°C, (Figure 9). Published data on the thermal resistance of *L. monocytogenes* in meat products show *D*-values that range from 0.75 to 28 min and *z*-values that range from 4.6 to 7.4°C (Carlier et al., 1996). In the present study, no *L. monocytogenes* cells were detected ($< 10^2$) in packaged meat submerged in 85°C water for 10 s; however cells were detected ($>10^2$) up to 10 min after submersion in a 61°C water bath.

D-values and *z*-values can be affected by a wide array of variables that should be considered when interpreting these values. Fat content can impact bacterial thermal resistance as *D*-values in foods high in fat have been reported to be four to eight times higher than those for low-fat foods (Doyle et al., 2001). The low-fat nature of meat used in this experiment probably explains why *D*-values were lower for this product than *D*-values for higher-fat foods that Doyle et al. (2001) found *D*-values ranging from 4.47 to 12.53 min at 65°C. Bacterial strain and growth phase of the organism also affects bacterial thermal resistance. Cells in stationary phase growth are sometimes more resistant than cells that are in the active phase of exponential growth (Doyle et al., 2001). Bacterial cultures used in this research were grown under aerobic conditions for 16 to 18 h prior to use. Cultures would be in late logarithm to early stationary phase of growth. This length of incubation was chosen to simulate similar conditions used in other research (Kalchayanand et al., 1992; Cutter and Siragusa, 1995; Cutter and Rivera-Betancourt, 2000; Thayer and Boyd, 2000). *D*-values can also vary between different strains of the same microorganism and different growth conditions under which the bacteria were cultured (Doyle et al., 2001).

D-values for *S.* Typhimurium at 57 and 60°C were 278 and 81 s, respectively. The Levene's test for homogeneity of variance did not differ for 57°C (*P* value = 0.85) or for 60°C (*P* value = 0.80). The z-value calculated using the *D*-values at these two temperatures was 5.6°C (Figure 10). There is substantial variation in published data on the *D*-values for different strains of *Salmonella*. However, there was general agreement between *D*-values cited in this research and with other published values. Juneja et al. (2001) reported a *D*-value of 4.16 min at 55°C in chicken broth and a *z*-value of 5.8°C between the temperatures of 58 to 62°C. The D_{57} value in the present study falls within the range of $D_{57.2}$ values (1.8 to 8.3 min) reported for a complex medium (D'Aoust, 1997). In the present study, no *S.* Typhimurium cells ($<10^2$) were detected after 10 s submersion at 70°C, yet cells survived up to 7 min of submersion at 60°C.

The overall goal of this study was to determine the thermal resistance of *S.* Typhimurium and *L. monocytogenes* in ready-to-eat, low-fat turkey bologna. *L. monocytogenes* had a D_{61} of 124 s and a D_{65} of 16.2 s, whereas *S.* Typhimurium had a D_{57} of 278 s and a D_{60} of 81 s. At higher water bath temperatures (70, 80, and 85°C), 10^8 cells were reduced to below detection levels within 1-min exposure (<10 s at 85 and 70°C for *L. monocytogenes* and *S.* Typhimurium, respectively). Determination of *D*-values using packaged food is a more accurate determination of a microorganism's thermal tolerance compared

to values determined in a nonfood environment. Food substrate, phase of growth, growth conditions, and strain of bacteria must all be considered when discussing bacterial *D*-values. Current food safety recommendations are focusing on process development to reduce the numbers of *Salmonella* spp. and *L. monocytogenes* in raw and processed meats. It is assumed that a process that ensures a reduction in *Salmonella* will also ensure that other vegetative organisms are reduced (USDA, 1999). Results of this research can be used to predict the time required to achieve a specified population reduction as required by the USDA Food Safety and Inspection Service to validate a thermal processing method. This data can help processors efficiently implement thermal processes that are effective in controlling *L. monocytogenes* and *S.* Typhimurium contamination in foods.

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